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Ultrastructure of Monkey *(Macaca Radiata)* Spermatozoa: Effect of Gossypol in Vivo

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Summary. The present study examines the ultrastructure of ejaculated spermatoza from bonnet monkey, *Macaca radiata* under noraml conditions, with gossypol treatment and during recovery from such treatment. Monkeys were fed orally with gossypol acetic acid (GAA) for 3 months (4 mg/monkey/5 days a weak). Semen samples collected by electroejaculation, and the spermatozoa were examined using both light and electron microscopy. The degree of motility was also noted by Kalla et al. [12]. Ejaculated spermatoza were immotile 90 days after GAA treatment, but little evidence for any abnormality in the spermatozoa could be seen by light microscopy. Some ultrastructural changes were observed, but not to the extent previously reported in spermatozoa of *Macaca fascicularis* [23]. After termination of treatment, semen samples were obtained every 5th day until sperm count and motility recovered to the normal level. After 90 days only a small proportion of spermatozoa showed abnormal structure. We conclude that in a subhuman animal model gossypol induced effects on sperm motility and morphology are reversible.

Key words: Monkey spermatozoa, Gossypol effect, In vivo examination, Ultrastructure.

Introduction

The discovery by Chinese workers that gossypol was a male antifertility agent aroused considerable interest among workers in reproductive biology [16]. The observation that gossypol has a considerable antifertility effect in males has been verified in laboratory animals by a number of workers [10, 19, 20]. However the precise mechanism of action remains unknown.

We have reported previously that gossypol inhibits motility of bonnet monkey spermatozoa and reduces sperm density [11, 12]. Decrease in these parameters was also observed by Hang et al. [7], Coutinho et al. [3], Frick et al. [5]. These authors used males, which had been given 20 mg gossypol daily for 12–50 weeks. Other in vivo studies have consistently revealed prominent defects in midpiece mitochondria of rat spermatozoa [6, 8, 15, 17]. Distinct changes in the axial filament complex of the sperm tail have also been reported by these authors.

Here we describe the effect of low dose orally administered gossypol on the ultrastructure of *Macaca radiata* spermatozoa.

Material and Methods

Four monkeys, each about 9 kg body weight were fed with gossypol acetic acid (GA-19-3 – 98.55%) obtained from Dr. A. V. Graci, U.S.D.A., New Orleans, USA, suspended in Tonoferon^R at a dosage of 4 mg per animal 5 days week for 90 days. Four control animals were given a vehicle dose by the same route. Semen samples were obtained every 5–7 days by the electroejaculation method of Settlage [22]. The semen volume, sperm motility and sperm density were determined together with the citric acid and fructose levels in the seminal plasma [11, 12].

Electron Microscopy

Semen samples collected before initiation of drug treatment, on the 80th day of treatment and on the 55th day after termination of treatment were fixed and embedded using the following procedure. Ejaculated spermatozoa were washed twice with phosphate buffer pH 7.4, centrifuged at 1,500 g/10 min. The pellet was fixed with 2.5% glutaraldehyde in 0.16 M collidine buffer pH 7.4 for 2 h at 4 °C. After fixation the spermatozoa were washed and postfixed with 1% buffered OsO_4 for 1 h at room temperature followed by washing and centrifugation. The samples were then dehydrated in a graded series of ethanol treated propylene oxide as an intermedium and embedded in Epon 812. 0.5 μm semithin sections stained with 1% Azur II – 1% Methylene blue in 2% Borax and ultrathin sections stained with 1% uranyl acetate in methanol and lead citrate were prepared. Sections were examined under Philips EM 300.

Observations were made on the morphology of the spermatozoa with particular reference to the head (acrosome, nucleus), the mitochondrial sheath and the axial filament complex.

Results

Under the light microscope the spermatozoa show a typical flagellate pattern as known from other species of the suborder *Simiae* (Fig. 1).

Electron Microscopy

Normal Sperm Ultrastructure. The ultrastructure of bonnet monkey spermatozoa appears to follow the pattern described for other spermatozoa of subhuman species.

The nucleus is elongated and contains condensed homogenous chromatin material, without any apparent vacuolization or special regions (Fig. 2). The acrosomal cap covers the greater part of the nucleus. In the neck region the tail is joined directly to the basal plate (Fig. 2). A proximal centriole is present (Fig. 4) enclosed by segmented columns (Fig. 6). The mid-piece is characterized by the presence of the mitochondrial sheath. The mitochondria are highly variable in size, density and orientation. Two types of mitochondria exist in sections of control spermatozoa, with light (Fig. 5) and dense (Fig. 6) internal structure respectively.

In light mitochondria the inner mitochondrial compartment is frequently vacuolated (Figs. 3, 4). The principal piece of the tail region consists of moderately dense homogenous material arranged in longitudinal strands (Figs. 8, 9, 10). The structure of the axoneme of the axial filament complex does not differ from that of axonemes of other mammalian spermatozoa, the tubules being arranged in a 9 + 2 pattern. The dynein arms are visible at high mignification (arrow Fig. 9). A cytoplasmic droplet was observed in different regions of the tail which contained amorphous material and vesicles of different sizes. It is bound by cytoplasmic membrane (Fig. 5).

The cytoplasmic membrane appears often swollen in the head region, while in the tail region it is commonly present with the exception of a few spermatozoa (Fig. 3).

Ultrastructure of GAA Treated Spermatozoa. No abnormalities were observed in sperm head region after gossypol treatment (Fig. 11). Greater numbers of spermatozoa with disrupted plasma membranes in the midpiece region were found. The mitochondrial structure did not differ from that of controls; in control sections light and dense forms were present (Fig. 11).

The most remarkable finding is shown in Figs. 11, 12 and 15. In transverse sections two or usually more tails surrounded by cytoplasmic material were enclosed in a single membrane (Fig. 12).

The axoneme, however, shows a normal 9 + 2 pattern and does not exhibit a disordered arrangement (arrow, Fig. 14).

Ultrastructure After Recovery from Gossypol Treatment. During the recovery phase a greater number of spermatozoa showed normal architecture. Few spermatozoa with bent tails were seen in the semen sample collected 55 days after termination of GAA treatment.

Discussion

The general morphology of bonnet monkey spermatozoa observed in this study coincides with other investigations on mammalian spermatozoa [9, 13, 18].

No ultrastructural alterations were observed in the sperm head region after gossypol treatment. This confirms the findings of Chinese workers using a cytophotometric method, which suggest that gossypol has no effect on the human spermatozoal DNA content [24].

The mitochondrial sheath of the spermatozoa has been reported to be the most sensitive structure responding to GAA treatment. Quite contrary to observations made in rat [6, 8, 15] we have not observed distinct mitochondrial damage in the monkey spermatozoa. In our own in vivo experiment with rats, however (dosage 20 mg/kg body weight/day for 16 weeks), we did find such changes, disarrangement and/or swollen mitochondria, frequently disrupted, great difference in size, often vacuoles in place of cristae [unpublished]. This discrepancy in our monkey experiment may be due to the extremely low dosage of gossypol used. Shandilya et al. [23] also reported effects on the mitochondria of monkey spermatozoa, when they used a dosage of 10 mg/kg body weight/monkey daily for 6 months, while these effects were absent when they administered only 5 mg/kg body weight.

The phenomenon of two or more tail slices within a single cytoplasmic membrane noted on transverse sections may be explained by curving and coiling of tails. Such a phenomenon has also been observed in human spermatozoa under pathological conditions [21] and in monkey spermatozoa after 1(2.4-dichlorobenzyl)-indozole-3-carboxylic (DIC 8) treatment [14].

Sperm abnormalities of coiled and recurved tails can be induced in vitro by changing the osmolarity of the seminal plasma or ionic concentration of the media [2, 4]. Under these experimental conditions swelling of the sperm cell is evident and bending of the mid-piece (hairpin bend) or coiling of the tip of the tail (pigtail bend) is common. It has been further suggested that disulphydryl linked structures in the spermatozoa prevent the coiling phenomenon as the spermatozoa from the cauda epididymis show less frequency of coiling in comparison to the spermatozoa from the caput region of the epididymis [2]. It may be noted that disulphydryl linked groups in the spermatozoa have been implicated in the mechanism of action of gossypol [1]. The origin of these coiled tails is difficult to explain. One is a direct effect on spermiogenesis or maturation in the epididymis the other one is a change in the secretory products which in turn induces this effect. Absence in control samples rules out the possibility of artefact. Recovery of spermatozoon motility after GAA treatment depends on dosage and duration of treatment [25]. In our study [11, 12] moti-

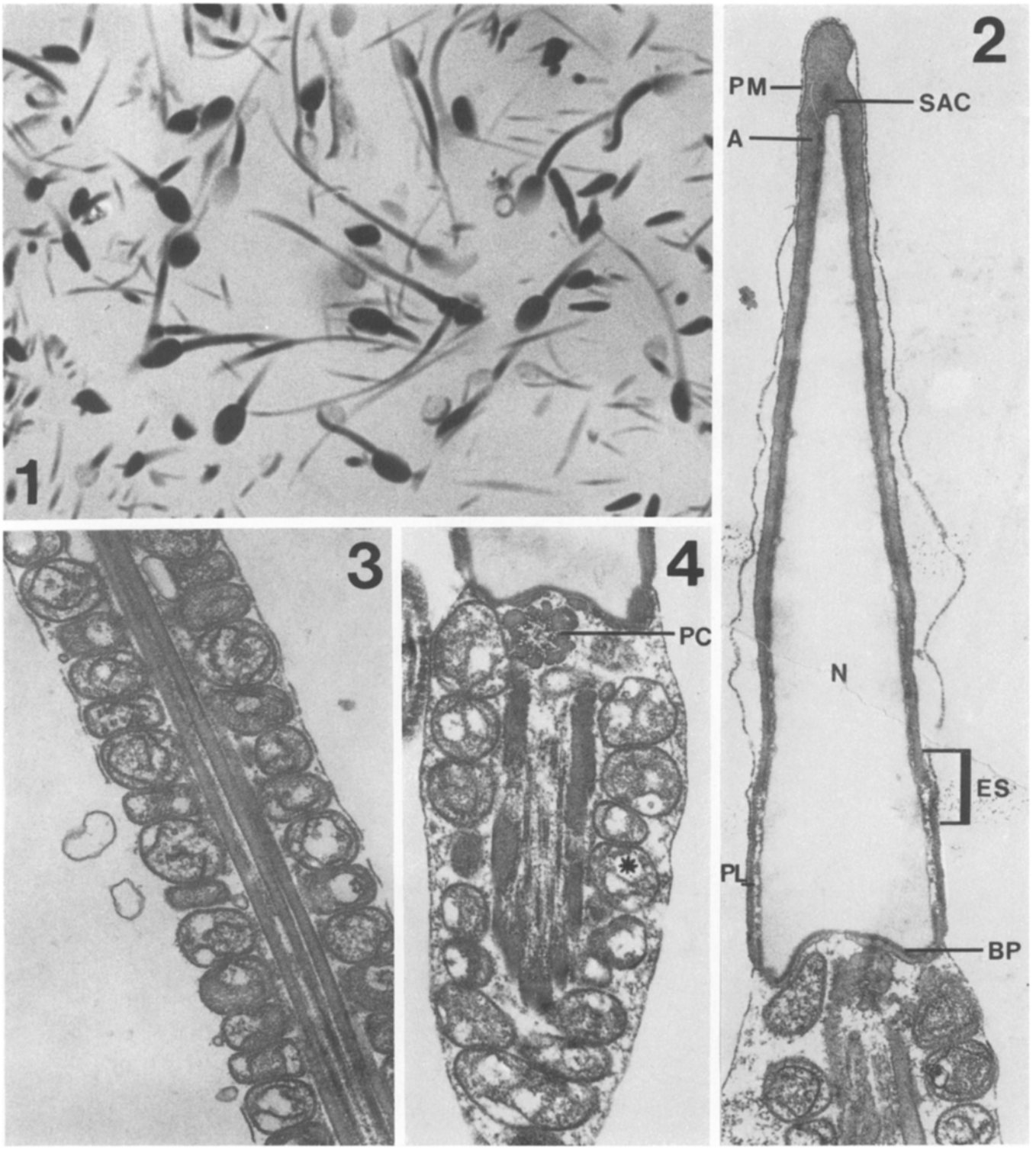

Fig. 1. Semithin section of control spermatozoa showing typical pattern from species of the suborder *Simiae*. Magn. x1,730

Fig. 2. Sperm head – the acrosome (*A*) with subacrosomal cleft (*SAC*) covers the greater part of the nucleus (*N*); the plasma membrane (*PM*) is slightly swollen (fixation artefact); distal portion of the equatorial segment (*ES*) the plasmalemma (*PL*) are present. The tail is joined to the basal plate (*BP*). Magn. x34,600

Fig. 3. Longitudinal section of the tail, of an untreated spermatozoa. Note the apparent absence of the plasma membrane. Magn. x42,250

Fig. 4. Proximal centriole (*PC*) in the connecting piece of the neck region. Notice the vacuoles in the mitochondria of control spermatozoa (*). Magn. x43,100

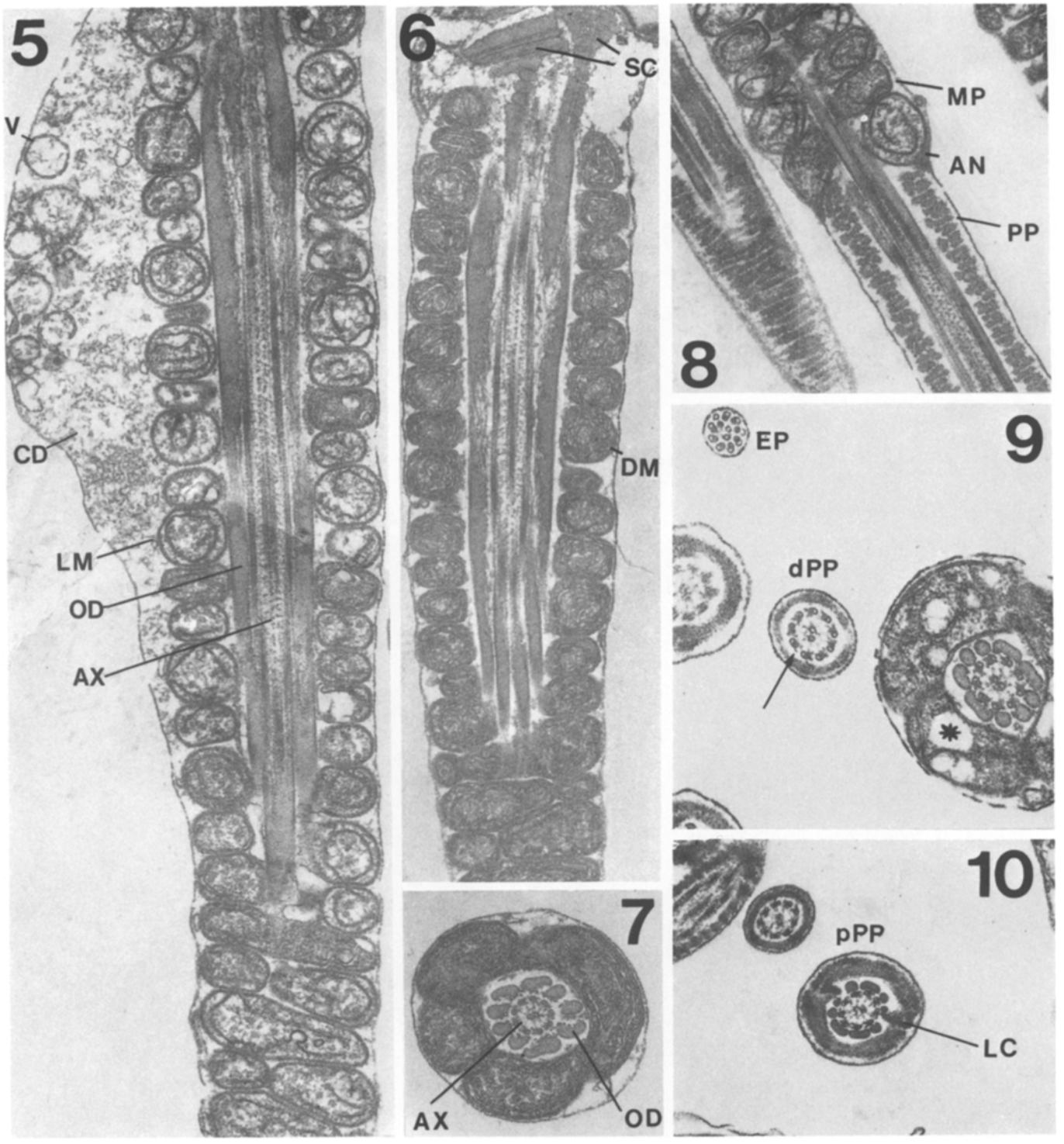

Fig. 5. Middle piece of the tail with light mitochondria (*LM*). A small cytoplasmic droplet (*CD*) was often found in this region. Magn. x33,800

Fig. 6. Middle piece of the tail with dense mitochondria (*DM*). The segmented columns (*SC*) enclose the proximal centriol in the connecting piece of the neck region. Magn. x33,800

Fig. 7. Cross section through the middle piece of the tail showing the 9 outer dense fibres (*OD*) and the axial filament complex with the 9 + 2 tubules pattern. Magn. x43,100

Fig. 8. The annulus (*AN*) characterizes the transition from midpiece (*MP*) to principal piece (*PP*). Magn. x29,000

Fig. 9. Cross sections through the tail – middle piece, distal principal piece (*dPP*), end piece (*EP*). The dynein arms of the axoneme marked by the *arrow*. Magn. x51,700

Fig. 10. Tail cross section through proximal principal piece (*pPP*) with the longitudinal columns (*LC*). Magn. x39,900

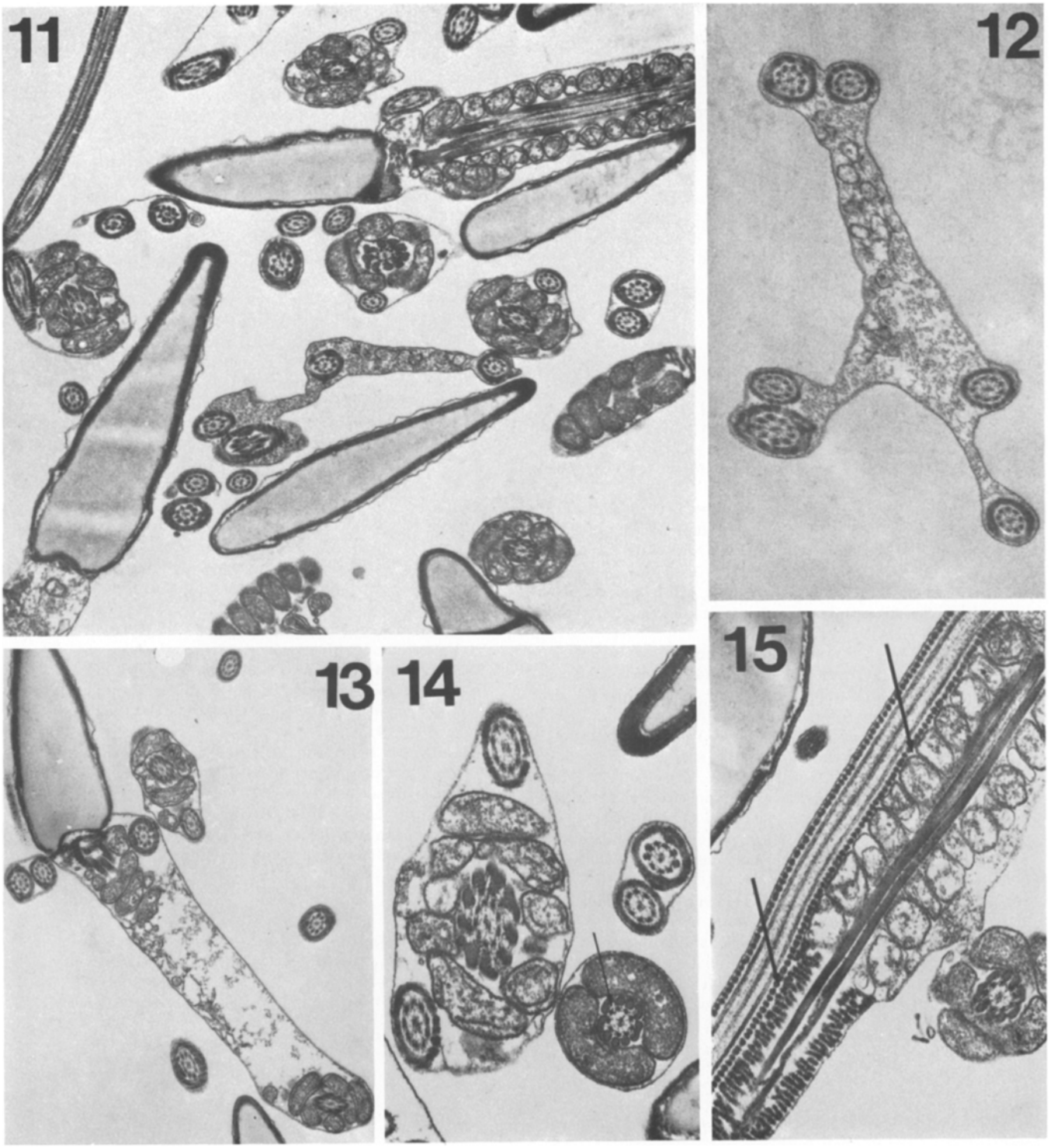

Fig. 11. A typical area of an ultrathin section of ejaculated spermatozoa of a treated animal. The head with the acrosome appears normal, a great number of distinctly bent and curved tails is visible. Magn. x17,500

Fig. 12. Principal piece enclosed in residual cytoplasmic droplet. Magn. x25,100

Fig. 13. A typical photograph of a spermatozoon with bent tail. The bending occurs directly after the basal plate in the neck region. Magn. x17,500

Fig. 14. Cross section through midpieces and principal pieces of the tail, both light and dense mitochondria are also visible, the axial filament complex appears normal, the dynein arms are present (*arrow*). Magn. x29,700

Fig. 15. Longitudinal section through a bent tail. Notice the missing plasma membrane between the middle and the principal piece (*arrows*). Magn. x29,700

lity, sperm density and semen volume of the gossypol treated monkeys returned to normal 80 days after termination of treatment. This explains that on the 55th day after termination of treatment few bent tails are still present in the ultrathin section while the majority of the spermatozoa appeared normal.

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