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Ultrastructural, Fertility, and Spermicidal Studies with Isomers and Derivatives of Gossypol in Male Hamsters[1]

ANITA P. HOFFER,[2,3] ASHOK AGARWAL,[3]
PETER MELTZER,[4] PATRICIA HERLIHY,[4] REHAN H. NAQVI,[5]
MARJORIE C. LINDBERG,[5] and STEPHEN A. MATLIN[6]

Harvard Program in Urology (Longwood Area)[3]
Department of Anatomy and Cellular Biology
Harvard Medical School
Boston, Massachusetts 02115

H. G. Pars Pharmaceutical Laboratories, Inc.[4]
Cambridge, Massachusetts 02138

E. G. & G. Mason Research Institute[5]
Worcester, Massachusetts 01608
and
Department of Chemistry[6]
City University
Northampton Square
London, United Kingdom

ABSTRACT

The effects of fourteen new, orally administered synthetic analogs of gossypol on testicular ultrastructure and fertility in hamsters and the spermicidal properties of these compounds, as well as of the optical isomers of gossypol against hamster and human sperm in vitro, are reported in this study. Test compounds were administered to adult male hamsters by daily gavage for 9 weeks at doses ranging from 15 to 50 mg/kg. The results of this study have demonstrated that the fourteen new gossypol analogs evaluated herein are not effective as male antifertility agents and their in vitro activity or lack of activity as spermicides is unrelated to their in vivo contraceptive potential. In addition, the results of the study suggest that (1) the isopropyl moiety of the gossypol molecule, like the aldehyde group, is essential for its mechanism of action and (2) the pathognomonic defect in the mitochondrial sheath induced by gossypol appears to be related to its unique activity as a male antifertility agent. The significance of these findings is discussed.

INTRODUCTION

Considerable attention has been devoted to the effects of gossypol on male fertility and on the morphology and biochemistry of the testes, epididymides, and epididymal spermatozoa (Prasad and Diczfalusy, 1982; Qian and Wang, 1984). However, relatively little information is available on the effects of optical isomers and chemical analogs of gossypol on these same parameters. The present study reports the effects of fourteen new, orally administered synthetic analogs of gossypol on testicular ultrastructure and fertility in hamsters and the spermicidal properties of these compounds, as well as of the optical isomers of gossypol against hamster and human sperm in vitro.

MATERIALS AND METHODS

Biology: Fertility Studies

Adult male golden Syrian hamsters (n=140) obtained from Engle Laboratory Animals (Farmersburg, IN) were administered test compounds by daily

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[2] Reprint requests: Dr. Anita P. Hoffer, Medical Research Building–4th Floor, Brigham & Women's Hospital, Harvard Medical School, 75 Francis Street, Boston, MA 02115.

TABLE 1. In vitro and in vivo effects of gossypol and related experimental compounds on sperm motility and fertility in male hamsters.

Compound	In vitro activity[b] % Motility at 10 min	20 min	40 min	60 min	Dose, mg/kg/day	In vivo activity Week 7 F/C/T	Week 7 Embryos/dam Normal	Abnormal	Week 10 F/C/T	Week 10 Embryos/dam Normal	Abnormal
1	100	100	100	100	–	9/9/10	9.1	0.5	8/10/10	10.7	0
2	0	–	–	–	16	0/10/10	0	0	0/4/8	0	0
3	0	–	–	–	16	0/4/10	0	–	0/2/10	0	–
4	–	–	–	–	40	10/10/10	9.8	0.4	10/10/10	10.6	0.1
5	93	79	75	74	30	7/7/10	10.7	0.1	7/9/10	8.5	0
6	95	90	90	90	32	9/9/10	11.1	0.2	8/8/10	11.4	0
7	100	97	95	100	15	10/10/10	8.9	0	8/9/10	7.8	0
8	88	77	87	88	28	8/8/10	11.5	0.9	9/9/10	8.4	0.8
9	100	100	68	66	32	8/9/9	8.3	0.2	9/9/9	9.5	0.1
10	0	–	–	–	16	9/10/10	7.0	0	8/9/10	11.1	0
11	0	–	–	–	30	9/9/10	8.6	0.6	9/9/10	12.0	0.1
12	42	35	30	35	40	10/10/10	9.9	0.1	10/10/10	11.0	0
13[c]					32	9/10/10	10.3	0.05	10/10/10	11.2	0.3
14	77	–	63	59	32	10/10/10	10.9	0.1	10/10/10	12.8	0
15	0	–	–	–	50	8/10/10	9.9	0.4	5/6/10	9.2	0
16	90	73	0	–	16	10/10/10	11.2	0.1	9/10/10	10.0	0.1
17	25	26	27	30	27	9/10/10	11.4	0.05	8/8/10	11.3	0.3

[a] See Figure 1 for chemical names and structures of compounds.

[b] All compounds tested at a concentration of 50 μM.

[c] Not tested in vitro.

gavage for 9 wk at doses ranging from 15 to 50 mg/kg (Table 1); 10 males were used for each compound tested. All drugs were suspended in steroid-suspending vehicle (SSV) and protected from the light; they were prepared fresh weekly. Each male was caged with 2 adult virgin females during the 7th week and at the end of the 9th week of treatment. Females were checked daily for vaginal sperm and were killed 1 wk after mating or 1 wk after termination of cohabitation for determination of the number of normal fetuses and implantation sites. All conceptuses that were half or less than half normal size were considered to be abnormal and reabsorbing. Since only females that mated would be expected to become pregnant, the mean number of normal and abnormal embryos per dam (Table 1) was calculated on the basis of the number of females found to be sperm positive and not the total number cohabited.

Spermicidal Assays

Hamster. For studies with hamster sperm, the spermicidal properties of each of the experimental compounds were assayed in vitro, as described earlier (Manmade et al., 1983), by incubating sperm with the compounds at concentrations of 50 μM in Hank's balanced salt solution (HBSS), and measuring the percent motility of the sperm as a function of time. Prior to dilution in HBSS, all compounds were dissolved in 1 ml ethanol, or in the case of Compounds 8, 10, and 14, in 1 ml acetone. The 50-μM data presented in Table 1 have been normalized to 5–10 min (the time of complete inhibition of sperm motility by gossypol acetic acid) and are reported at 10-, 20-, 40- and 60-min intervals after addition of the spermatozoa to the test solutions.

Human. To express the results of the in vitro assay with human sperm in terms comparable to those of the hamster spermicidal assay, it was necessary to establish a baseline for the minimum concentration of gossypol needed to produce 100% inhibition of human sperm motility within 5–10 min of exposure. Gossypol was dissolved initially in 1 ml of abso-

TABLE 2. In vitro spermicidal activity of gossypol acetic acid against human sperm.

Concentration of gossypol acetic acid	% Motility 0 min	10 min	20 min	40 min	60 min
50 μM	91	78	–	–	55
100 μM	81	50	48	33	18
200 μM	1	0			
250 μM	0				

TABLE 3. Evaluation of the in vitro effect of solvent used to dissolve gossypol analogs.

Item	% Motility				
	0 min	10 min	20 min	40 min	60 min
Ejaculate in BWW*	100	–	94	102	96
Ejaculate in BWW + ethanol	100	–	94	101	90
Ejaculate in BWW	100	–	–	108	108
Ejaculate in BWW + acetone	100	–	–	101	108

*BWW = Biggers, Whitten, and Whittingham's medium.

lute ethanol and aliquots were added to decreasing amounts of Biggers, Whitten and Whittingham's medium (BWW) to achieve concentrations of 50, 100, 200, and 250 μM; motility observations were carried out to 60 min of exposure (Table 2). Controls were performed to evaluate the independent effect, if any, of trace amounts of ethanol on human sperm motility (Table 3).

The optical isomers of gossypol and all test compounds were subsequently compared to gossypol acetic acid as the standard (Table 4). They were evaluated at a concentration of 250 μM, the concentration of gossypol acetic acid that was found to be necessary for complete inhibition of washed human spermatozoa within 5 min. Prior to dilution in BWW, Compounds 8, 10, and 14 were dissolved in 1 ml acetone; the rest were dissolved in ethanol. Control experiments to compare the effects of BWW containing comparable trace amounts of acetone and ethanol on sperm motility were also performed (Table 3).

All of the experimental compounds were tested against semen samples of the same normal male volunteer to eliminate donor variation. After liquefaction at room temperature for 15–30 min, semen was centrifuged at 600 × *g* for 10 min in BWW without bovine serum albumin (BSA), and the pellet was resuspended and diluted to a concentration of 8–10 million/ml in serum-free BWW. Appropriate concentrations of the experimental contraceptives were then added to the sperm and percent motility was determined using an Olympus phase microscope.

Light and Electron Microscopic Studies. Adult male golden Syrian hamsters (CD-Charles River) ranged in weight from 95 to 110 g. After treatment with selected experimental contraceptives or the control vehicle for 10 wk by daily gavage, they were killed for morphological studies. The dose levels varied from 16 mg/kg/day, the amount of gossypol acetic acid administered, to 50 mg/kg/day. Dosage was determined according to the quantity of test compound available. Animals used in this study were maintained in accordance with the guidelines of the Committee on Care and Use of Laboratory Animals

TABLE 4. In vitro spermicidal activity of gossypol and its analogs against human sperm.*

Compounds**	% Motility				
	0 min	10 min	20 min	40 min	60 min
(+) Gossypol	0				
(−) Gossypol	0				
(±) Gossypol	0				
Compound 5	88	69	57	–	37
Compound 7	74	–	44	15	12
Compound 8	94	–	97	65	16
Compound 10	90	–	2	0	
Compound 11	0				
Compound 12	84	75	60	–	40
Compound 14	100	–	77	48	44
Compound 15	0				
Compound 17	93	–	51	0	

*All compounds were tested at a concentration of 250 μM.

**All compounds dissolved in absolute ethanol except for the last three, which were dissolved in absolute acetone.

of the Institute of Laboratory Animal Resources, National Research Council (DHEW publication No. [NIH]78–23, revised 1978).

At the end of each experiment, hamsters were anesthetized with either pentobarbitol (Nembutal, Abbott Laboratories, Chicago, IL) or urethane. An incision was made in the scrotum, and the testes and epididymides were exposed and gently dissected free. The spermatic cord was ligated twice, approximately 0.5 cm apart, and the cord in between was severed. The testis was transferred to a 10-ml beaker filled with saline, and the testicular artery was cannulated with a 25-gauge needle attached to a 25-cm piece of polyethylene tubing (PE 60, Clay-Adams, Parsippany, NJ). This tubing was connected to a 3-way valve and then to a standard 140-cm-high perfusion system. The testis was flushed briefly with 0.9% saline and then perfused with 5% glutaraldehyde in 0.1 M collidine buffer (pH 7.4). Immediately after the saline entered the testicular artery, the spermatic cord was severed proximal to the ligature to provide a route for the effluent. After the testis blanched (about 10 s), the saline was replaced with fixative and the perfusion continued for 15 min. The testis remained immersed in fixative for an additional 30–60 min thereafter. The tissue was then cut into 1-mm-thick blocks, rinsed briefly in buffer, and postosmicated in 1.33% OsO_4 buffered with 0.1 M collidine buffer for 2–3 h. The tissue was dehydrated in a series of increasing concentrations of cold acetone and embedded in Araldite. Sections showing pale gold interference colors were cut with a diamond knife on a Porter-Blum MT-2 microtome and stained with uranyl acetate (1:1 solution of saturated uranyl acetate and acetone) followed by lead citrate (Venable and Coggeshall, 1965). For light microscopy, sections were cut from the same blocks at 0.5 to 1.0 μm and stained with toluidine blue. Identification of the stages of spermatogenesis in the hamster was based on the classification of Clermont (1954).

Chemistry

The test compounds and their structures evaluated in this study are shown in Figure 1. The isomeric forms of gossypol, (−), (+) gossypol, were prepared by the resolution of racemic gossypol, as reported previously (Matlin and Zhou, 1984). Gossypol and gossypol acetic acid were generously provided by the Contraceptive Development Branch, NICHD. All of the remaining test compounds were provided by Dr. Meltzer at H. G. Pars Pharmaceutical Laboratories, Cambridge, MA. The synthesis of these analogs will be published elsewhere.

RESULTS

In Vitro Studies with Hamster and Human Spermatozoa

The effects of gossypol acetic acid, gossypol, and related compounds on the motility of hamster sperm were evaluated at fixed time intervals of 10, 20, 40 and 60 min after their addition to the spermatozoa at a concentration of 50 μM (Table 1). Of the synthetic analogs tested, the 3 compounds that showed inhibitory activity comparable to that of gossypol acetic acid itself were Compounds 10, 11, and 15, a hemigossypol derivative. Another compound, 16, also produced complete inhibition of sperm motility, but not until after 40 min of incubation. Immobilization of hamster sperm was not achieved by any of the other compounds tested.

For the comparable testing of gossypol acetic acid and gossypol-related compounds against washed human sperm, all test compounds were evaluated at a concentration of 250 μM (Tables 2 and 4). Suitable controls demonstrated that neither the vehicle alone nor either of the solvents used to prepare solutions of the test compounds had significant independent effects on human sperm motility (Table 3). In most cases, results similar to those with hamster spermatozoa were obtained with the test compounds and human sperm (Tables 4 and 5). Both Compounds 11 and 15 produced complete inhibition of human sperm motility within the first 10 min of incubation; Compound 10 also inhibited the motility of all the spermatozoa, but not until 40 min of incubation. On the other hand, one compound (17) that failed to achieve complete inhibition of sperm motility during the 60-min test period was somewhat more effective as a human spermicide, producing 100% inhibition of sperm motility within 40 min of incubation.

No differences in the antimotility effects of the racemic vs the "+" or "−" isomeric forms of gossypol were observed against human sperm under the experimental conditions employed (Table 4). The optical isomers were not available in sufficient quantities for testing with hamster as well as human sperm.

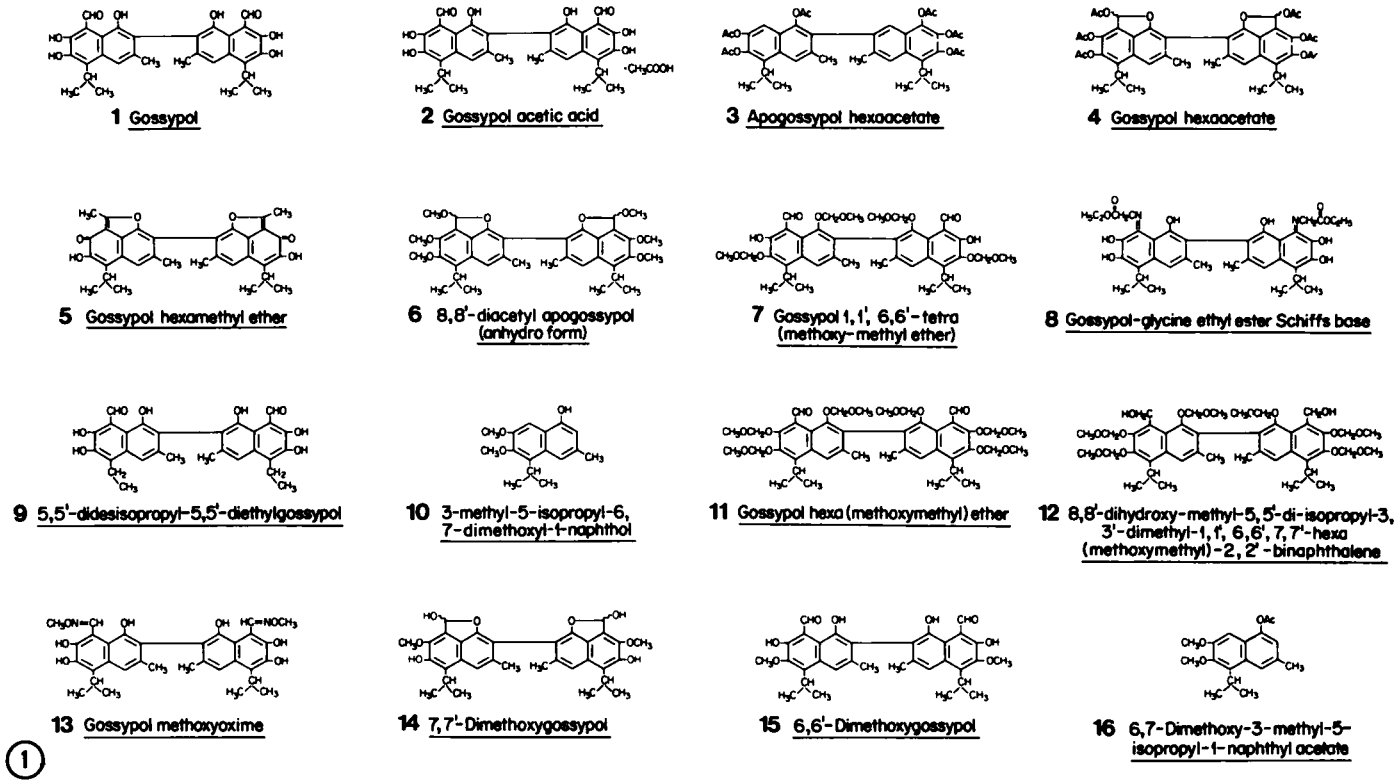

FIG. 1. Chemical names and structures of gossypol and related compounds.

Antifertility Studies with Gossypol Acetic Acid and Related Compounds in the Hamster

Fertility studies were carried out in male hamsters during Weeks 7 and 10, as shown in Table 1. Of the 14 synthetic analogs tested, none appeared to have a significant effect on the incidence of successful matings or on fertility. In general, treated males produced normal numbers of implantation sites in

TABLE 5. Comparison of the in vitro spermicidal activity of gossypol and its analogs against human and hamster sperm.

Compound #		Effect on sperm motility*	
		Hamster	Human
1	Vehicle control	–	–
2	Gossypol	+	+
3	Gossypol acetic acid	+	+
4	Apogossypol hexaacetate	●	●
5	Gossypol hexaacetate	–	–
6	Gossypol hexamethyl ether	–	●
7	8, 8'-Diacetyl apogossypol (anhydro form)	–	–
8	Gossypol 1, 1', 6, 6'-tetra (methoxy-methyl ether)	–	–
9	Gossypol-glycine ethyl ester Schiffs base	–	●
10	5,5'-Didesisopropyl-5, 5'-diethylgossypol	+	+/–
11	3-Methyl-5-isopropyl-6, 7-dimethoxyl-1-naphthol	+	+
12	Gossypol hexa (methoxymethyl) ether	–	–
13	8, 8'-Dihydroxy-methyl-5, 5'-diisopropyl-3, 3'-dimethyl-1, 1', 6,6', 7,7'-hexa (methoxymethyl)-2, 2'-binaphthalene	●	●
14	Gossypol methoxyoxime	–	–
15	7,7'-Dimethoxygossypol	+	+
16	6,6'-Dimethoxygossypol	+/–	●
17	6,7-Dimethoxy-3-methyl-5-isopropyl-1-mapthyl acetate	–	+/–

* + = Complete inhibition of motility within the first 10 min of incubation; +/– = complete inhibition of motility within 40 min of incubation; – = failure to achieve complete inhibition of motility within a 60-min incubation period; ● = only tested in vivo due to limited compound availability.

mated females, although a slight suppression of mating behavior was noted in males treated with Compound 5 for 6 and 9 wk. An increased proportion of abnormal embryos was produced by males treated with Compound 8, although the overall fertility rate of these animals did not appear to be affected.

Histological and Ultrastructural Effects of Gossypol and Related Compounds on the Seminiferous Tubule in Vivo

The morphological effects of selected derivatives on the hamster seminiferous epithelium were compared with those of the parent compound to identify any similarities that might be indicative of antifertility potential or shared mechanism(s) of action. The compounds analyzed were gossypol and gossypol acetic acid (Compounds 2 and 3), the diethyl analog of gossypol (Compound 10), 6,6′-dimethoxygossypol (Compound 16), and 8,8′-diacetyl apogossypol (Compound 7). Gossypol and gossypol acetic acid were evaluated together to (1) establish a morphological baseline for the effects of this group of compounds on hamster testis and (2) determine whether cocrystallization with acetic acid alters the biological properties or morphological effects of gossypol. The diethyl analog of gossypol was studied to evaluate the effects on activity and testicular ultrastructure, if any, of the minor modification of isopropyl groups to ethyl groups at the 5 and 5′ positions. Dimethoxygossypol was chosen to analyze the consequences of blocking one of the three phenolic groups in each napthol. Diacetyl-apogossypol was tested to determine the importance of the aldehyde groups (in the 8 and 8′ positions) for biological activity. The results are summarized below.

The testes of adult male hamsters made infertile by daily treatment with 16 mg/kg of gossypol or gossypol acetic acid for 9 wk were examined with the light and electron microscopes. No differences were observed between the effects of these two substances on the hamster testis. Therefore, the following observations apply equally to both groups of animals. As in rats made infertile with gossypol (Hoffer, 1983), the effects on hamster seminiferous tubules were variable with some showing more severe defects than others. Approximately 40% of tubules were normal in appearance viewed with the light microscope. Affected tubules (Fig. 3) showed a variety of defects, including increased numbers of degenerating mid-pachytene spermatocytes and round spermatids, decreased or absent elongating spermatids in Stages X, XI and XII, frequent premature exfoliation of Step 16 and 17 spermatids, and an overall decrease in tubular diameter Moderate to severe vacuolization of Sertoli cells was noted in many tubules (Fig. 3). At the ultrastructural level, a defect in the midpiece of late spermatids, pathognomonic for gossypol, could also be observed and was similar in all respects to that found in gossypol-treated rats. In hamsters, the mitochondrial sheaths of Step 16 and 17 spermatids were affected, exhibiting numerous discontinuities along their length and severely degenerated mitochondria (Fig. 4). Interestingly, the axonemal complexes of late spermatid flagella are not damaged by gossypol (Fig. 5). Defective midpiece mitochondria were never observed in late spermatids of control animals (Fig. 6).

The diethyl analog of gossypol was administered by gavage to adult male hamsters at a dose of 16 mg/kg/day for 9 wk. Although the structure of the diethyl analog differs very little from that of gossypol, its morphological effects are strikingly different. Using the light microscope, we detected none of the effects seen with gossypol—such as an overall decrease in some tubular diameters, Sertoli cell vacuolization, premature exfoliation of late spermatids, or increased numbers of degenerating germ cells—with the diethyl analog (Fig. 7). At the ultrastructural level, the pathognomonic defect in the mitochondrial sheath of late spermatids, which is observed in the testes of gossypol-treated hamsters, could not be observed after treatment with the diethyl compound (Figs. 8, 9).

6,6′-Dimethoxygossypol and 8,8′-diacetyl apogossypol were both administered orally at a dose of 15 and 16 mg/kg/day, respectively, for 9 wk. Neither compound produced any of the histological or ultrastructural changes that were observed after treatment with gossypol and gossypol acetic acid, and therefore the photomicrographs shown apply equally for both compounds. Germ cells and Sertoli cells, as well as interstitial cells, were all normal in appearance at both the light- and electron-microscope levels (Figs. 10, 11).

DISCUSSION

In the present study, the ultrastructural, antifertility, and spermicidal effects of gossypol acetic acid and gossypol, its levo- and dextrorotatory isomers and 14 synthetic analogs were evaluated.

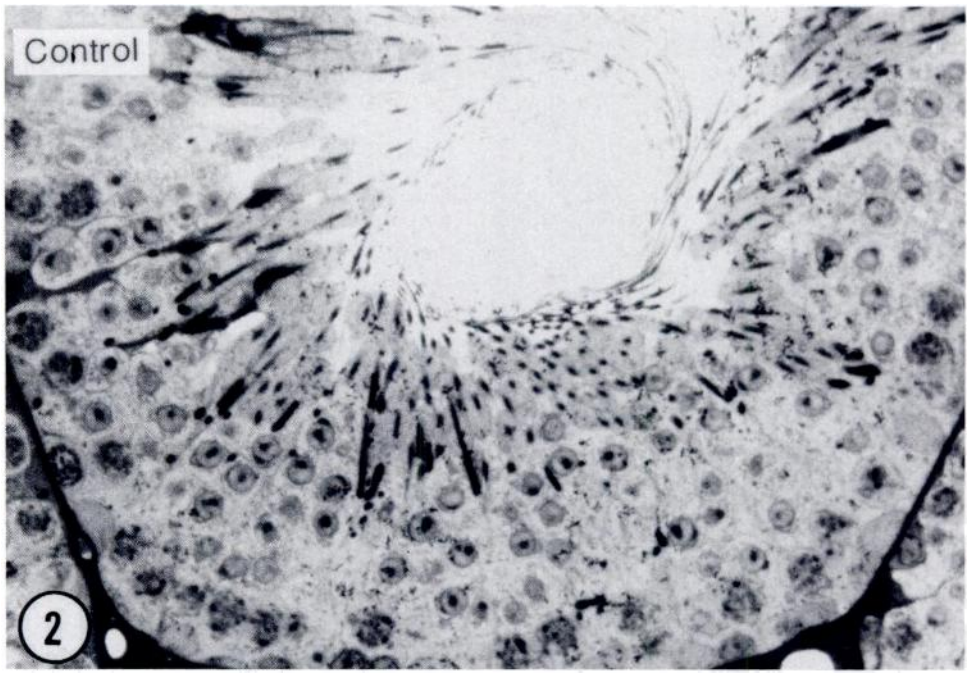

FIG. 2. Light micrograph of testis from a control hamster administered vehicle for 9 wk. A seminiferous tubule in Stage VII is shown. The seminiferous epithelium looks normal; no histological damage due to vehicle administration can be detected. ×750.

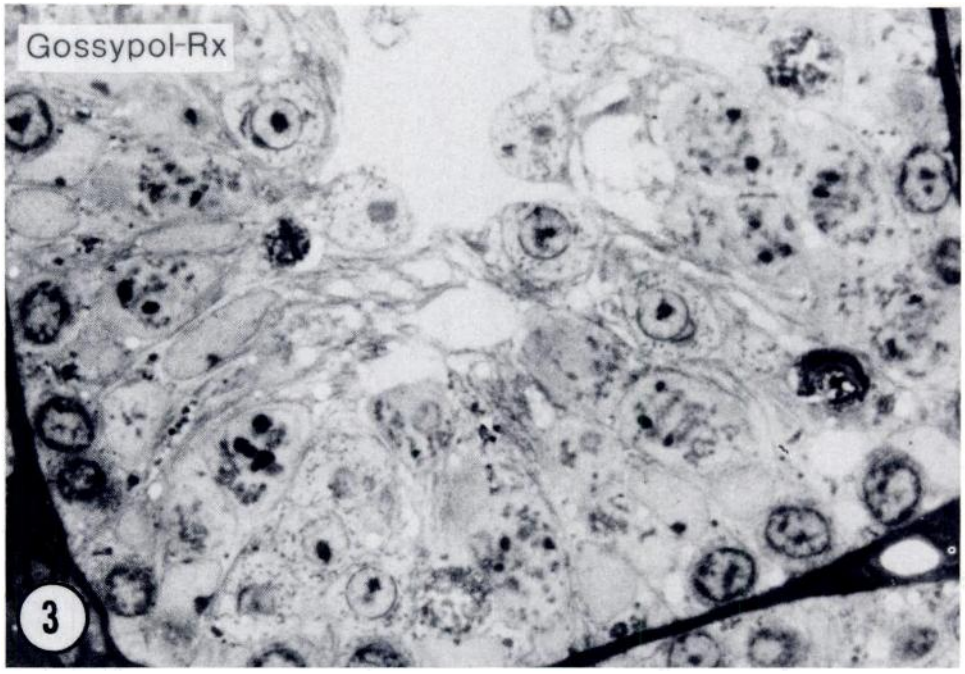

FIG. 3. Light micrograph of testis from hamster treated orally with racemic gossypol (16 mg/kg/day for 9 wk). The seminiferous tubules exhibit various degrees of degenerative changes including intraepithelial vacuolization, premature exfoliation of Step 16 and 17 spermatids (compare with Figure 2) and the presence of several degenerating spermatocytes (not shown here). ×1400.

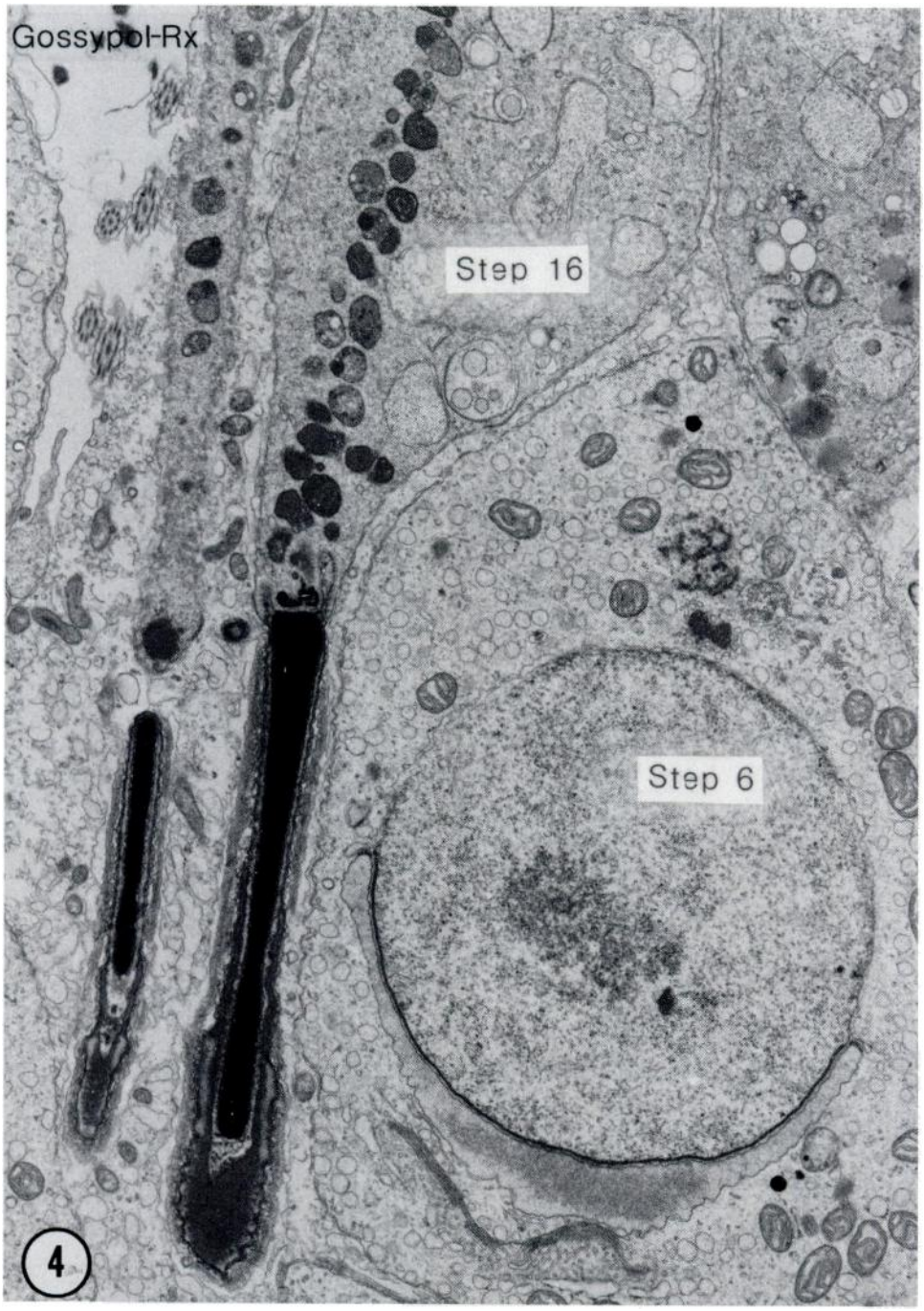

FIG. 4. Electron micrograph of a Stage VI seminiferous tubule from a hamster treated orally with racemic gossypol (16 mg/kg/day for 9 wk). The mitochondrial sheath of this Step 16 spermatid exhibits varying degrees of degenerative changes including vacuolization, disorganization, and frequent deletions. X10,800.

In the mating studies, none of the newly synthesized test compounds exhibited antifertility activity after 9 wk of treatment at the various doses employed. Although one compound, 7,7′-dimethoxygossypol, suppressed mating, as indicated by the absence of spermatozoa from vaginal washings at 10 wk, 5/6 of the males that did mate after treatment with this compound sired normal numbers of embryos. The closely related compound, 6,6′-dimethoxygossypol, appeared to be without any effect on mating behavior. Perhaps the most intriguing observation of the mating studies, however, was the absence of any antifertility

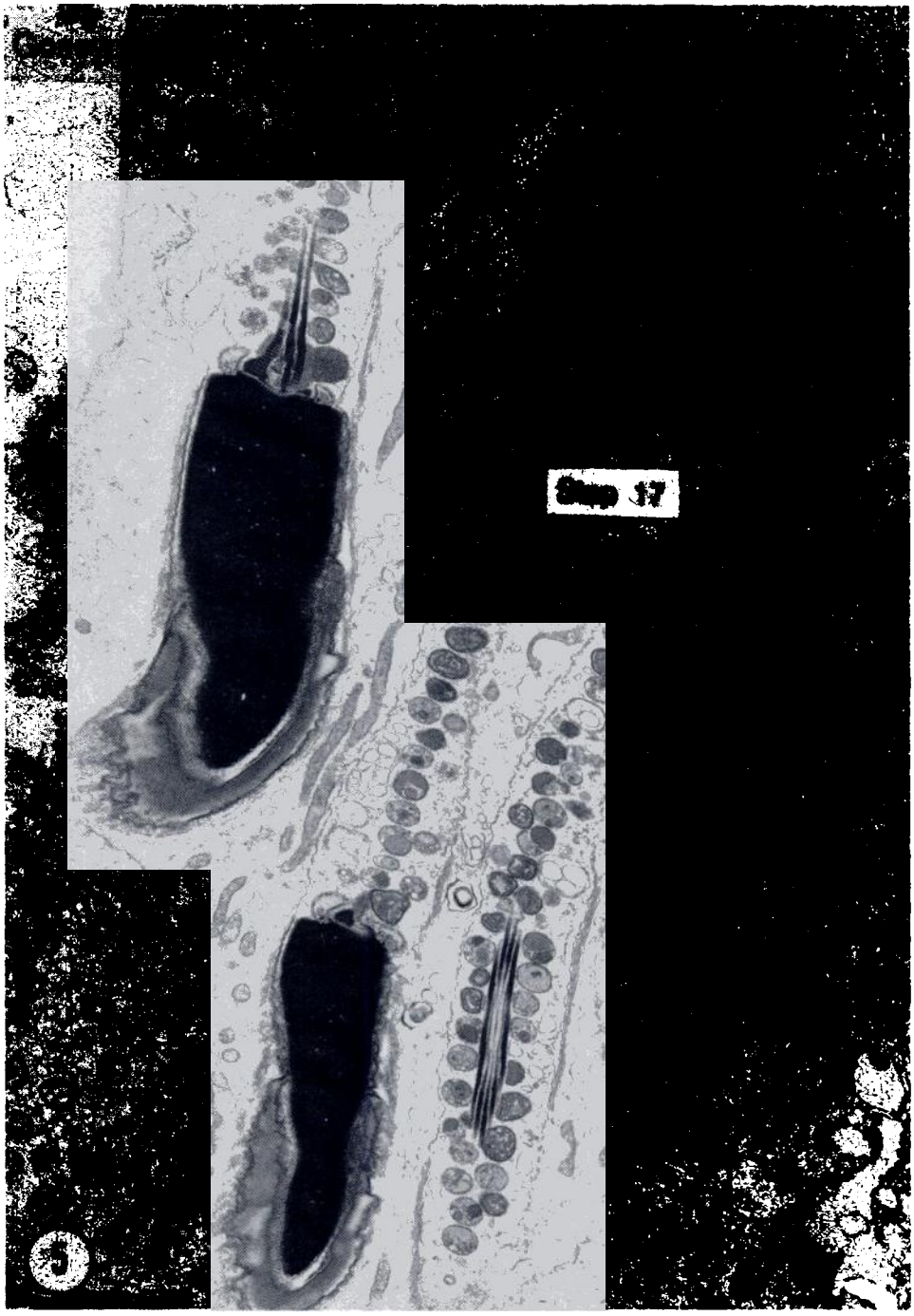

FIG. 5. The gossypol-induced pathognomonic defects in the mitochondrial sheath are shown here in a Stage VII seminiferous tubule. The mitochondrial profiles of this Step 17 spermatid include ruptured cristae, vesicles and granules of varying size and density, and a homogeneous mitochondrial matrix. X10,000.

effect of the diethyl analog of gossypol. This compound, which differs from gossypol by only one methyl group, was synthesized to explore the role of the isopropyl moiety in gossypol's biological activity (Meltzer et al., 1985). The lack of contraceptive activity of the diethyl compound emphasizes the unique quality of the parent compound, gossypol, and suggests that an intact isopropyl group may be related to the mechanism of action of this highly specific male contraceptive. On the other hand, the

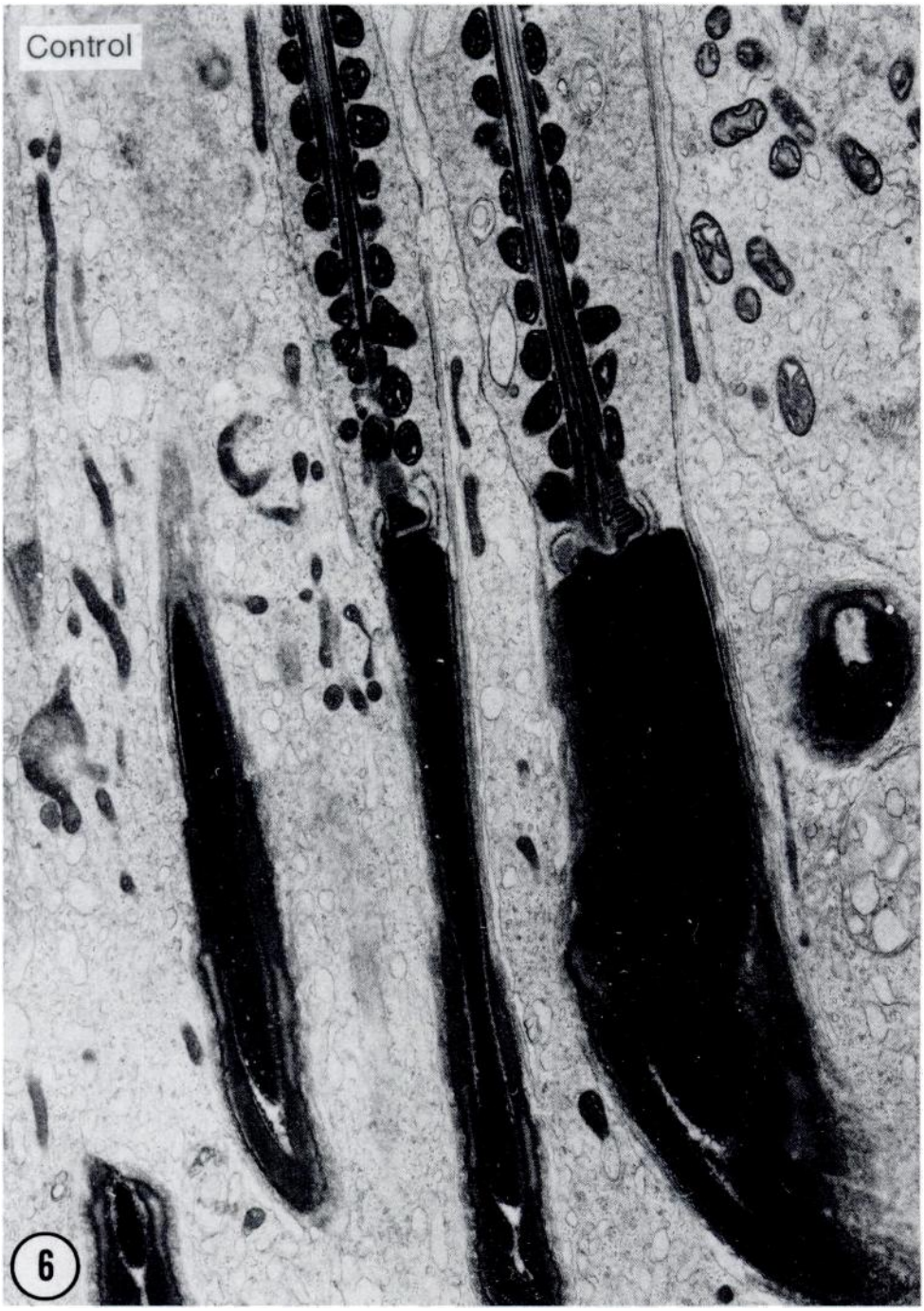

FIG. 6. Electron micrograph of a testis from a control hamster after administration of vehicle for 9 wk. The mitochondrial sheaths are completely normal in appearance in the late spermatids (Steps 16 and 17). No damaged mitochondria can be detected. ×21,000.

possibility that the modification of the hydrophobic domain results in poor bioavailability to the testis and hence poor contraceptive activity can not be excluded on the basis of the data currently available. The data from the present study also indicate that the aldehyde group is particularly important for the antifertility activity of gossypol. Finally, the optical isomers, (+) and (−) gossypol were available in limited quantity and were not evaluated for their antifertility activity in this laboratory. However, others have already demonstrated that the (+) and (−) isomers are inactive and active, respectively, as

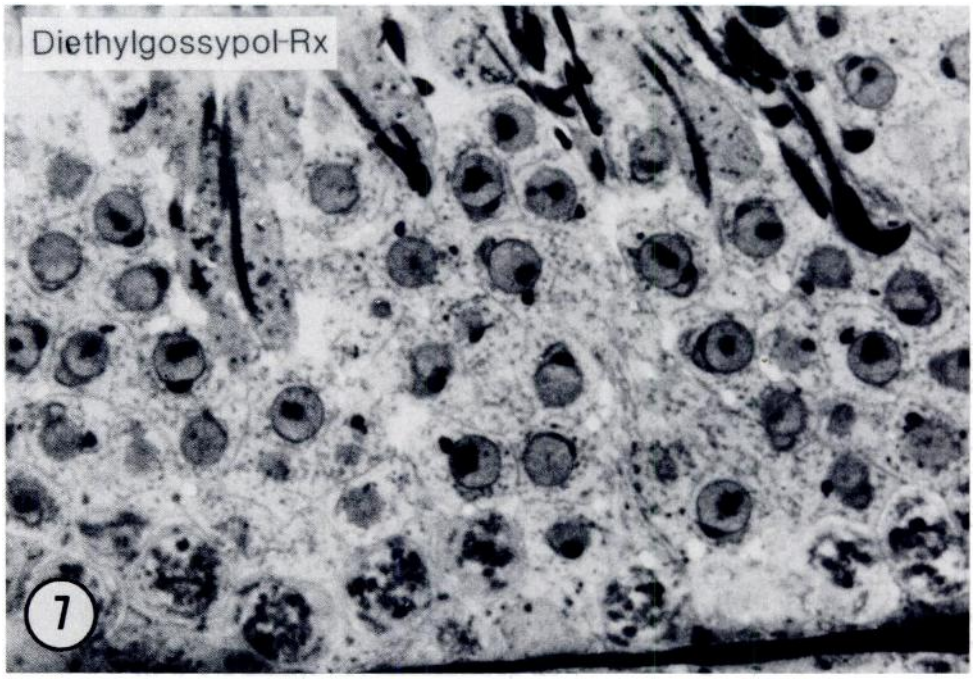

FIG. 7. Seminiferous tubules of a hamster treated orally with diethylgossypol (16 mg/kg/day for 9 wk). The epithelium looks normal, and no histological defects due to administration of diethyl gossypol can be recognized (compare with Figure 2). X1400.

antifertility agents in male hamsters (Waller et al., 1983; Matlin et al., 1985). Interestingly, (+) gossypol has been shown to have an antifertility effect in female hamsters (Murthy et al., 1981).

Spermicidal activity against hamster sperm comparable to that of gossypol itself was exhibited by the hemigossypol derivative (Compound 11), the diethyl analog (Compound 9) and 7,7′-dimethoxygossypol (Compound 15). However, it is evident that in vitro inhibition of motility does not necessarily correlate with decreased fertility following oral administration, except in the case of gossypol and gossypol acetic acid. These results are not surprising. Others have suggested, in a variety of contexts, that the effects of gossypol observed in vitro may not be relevant to its effects in vivo. For example, Qian and Wang (1984) reported that gossypol in Locke's solution (1.0 μg/ml) completely inhibited ventricular contractility, whereas free gossypol (2.13–2.25 μg/ml) in blood donated by other rabbits fed gossypol at a dose of 30 mg/kg/day for 12 days did not inhibit it at all. The observation led them to conclude that most of the gossypol in the body, including so-called free gossypol, is conjugated with different molecules and micromolecules of the organism and that their actions will naturally differ from those of gossypol in vitro. Similarly, discrepancies between in vitro and in vivo effects of gossypol on Sertoli and Leydig cells led Zhuang et al. (1983) to cite binding of gossypol to protein in vivo, chemical alterations of gossypol in vivo, secondary effects on Leydig cells mediated via effect(s) on other cell types, length of exposure to the compound, or different effective concentrations in in vivo and in vitro studies as possible reasons for such differences. Finally, Kim et al. (1984) showed that the spermicidal effect against hamster sperm of (+) gossypol is equal to that of racemic or (−) gossypol but that (+) gossypol lacks in vivo antifertility activity. Our results are consistent with these observations and extend them to demonstrate that the spermicidal effects of gossypol derivatives and analogs in vitro are also not predictive of antifertility activity in vivo.

In vivo studies on the effects of diethyl gossypol and 6,6′-dimethoxygossypol on LDH-C_4 (the testis-specific isozyme of LDH) in monkey sperm have been reported by Whaley et al. (1986). One compound, 6,6′-dimethoxygossypol, was found to be a weaker inhibitor of LDH-C_4 than gossypol, whereas the diethyl analog proved to be a more potent inhibitor of this enzyme than gossypol. These results suggest that, as with spermicidal activity, the in vitro inhibition of LDH-C_4 is also a poor predictor of in vivo antifertility activity.

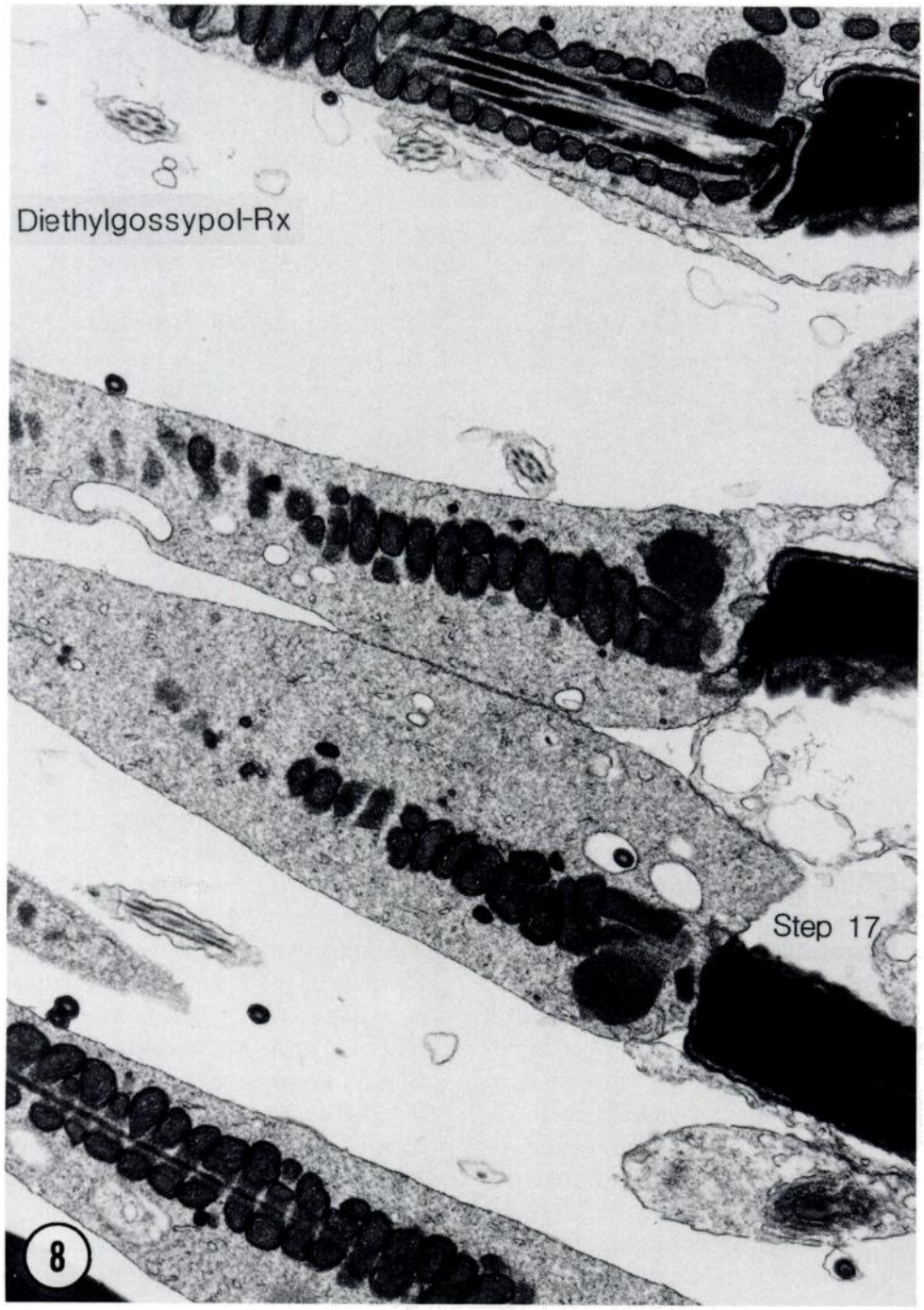

FIG. 8. Electron micrograph showing Step 17 spermatids of a hamster treated orally with diethylgossypol (16 mg/kgday for 9 wk). Notice the absence of vacuolated and degenerating mitochondria in these mid-pieces. X18,000.

In vivo studies with gossypol acetic acid show both LDH-C_4 inhibition and antifertility effects in rats (Olgiati et al., 1984), but similar in vivo experiments using chemical analogs of gossypol have not yet been reported.

The spermicidal properties of (±), (+), and (−) gossypol against human spermatozoa were also analyzed in the present study. At a concentration of 250 μM, no differences could be detected in the inhibition of sperm motility by these compounds

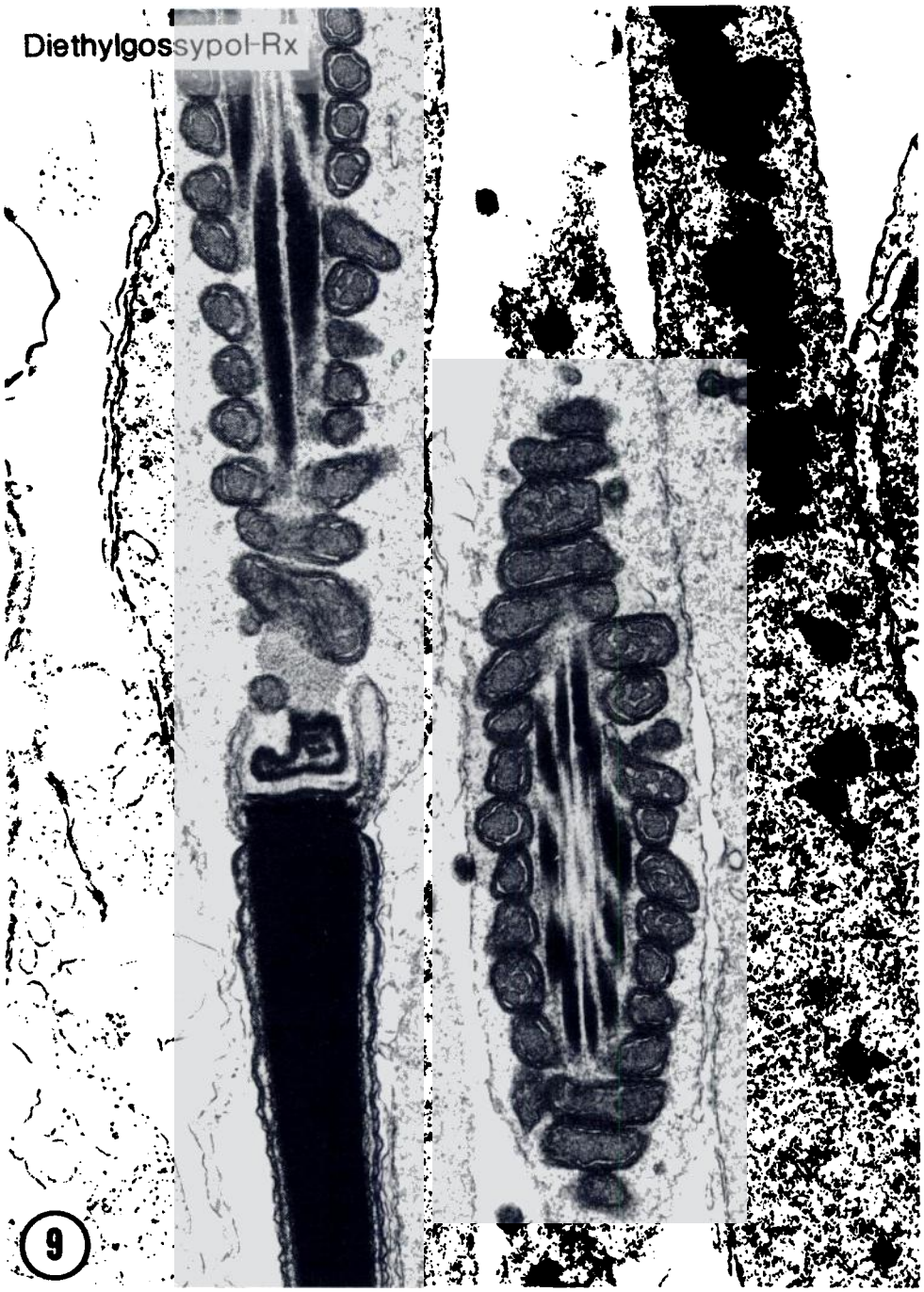

FIG. 9. The normal-appearing mitochondrial sheaths of Step 17 spermatids are shown to better advantage at higher magnification in this electron micrograph from a hamster treated orally with diethylgossypol (16 mg/kg/day for 9 wk). No damaged mitochondria can be detected. X27,500.

under the conditions of the assay used in this study. Similar results were obtained using PVP (polyvinylpyrollidone)-coprecipitates of these compounds by Kim et al. (1984), although the concentrations of optical isomers used in their assay (1–25 mg/ml) were significantly higher than those reported here.

No ultrastructural defects were observed in the seminiferous epithelium of hamsters treated orally with 6,6′-dimethoxygossypol, 8,8′-diacetyl apogossypol, or the diethyl analog. By contrast, both gossypol and gossypol acetic acid produced vacuolated and degenerating mitochondria in the midpieces of late

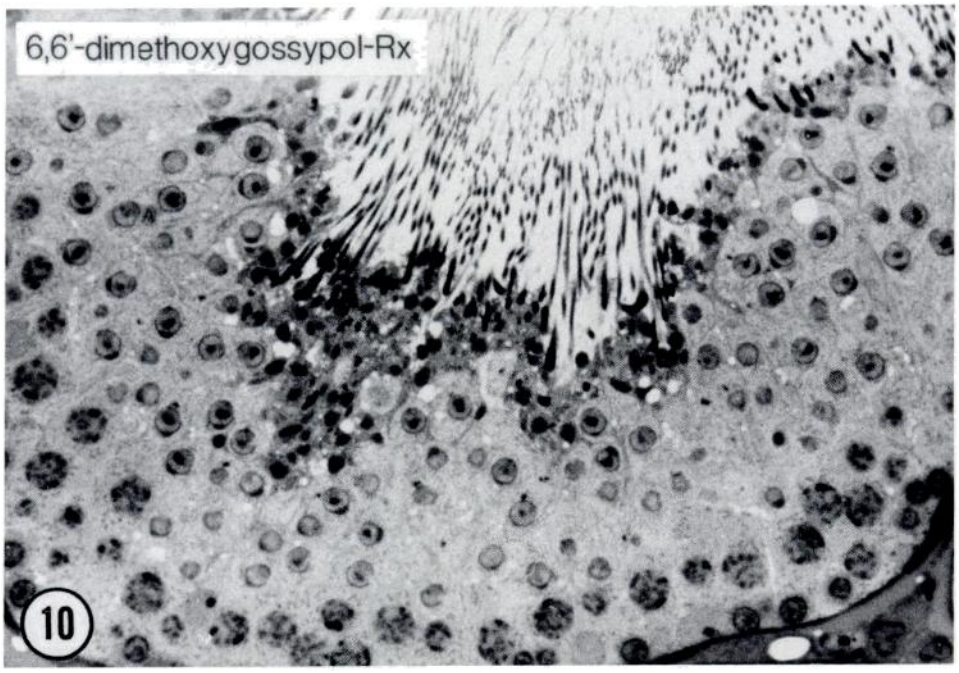

FIG. 10. Testis from hamster treated orally with 6,6′-dimethoxygossypol (16 mg/kg/day for 9 wk). No damage to seminiferous tubules can be detected; germ cells, Sertoli cells, and interstitial cells are all normal in appearance at the light-microscope level (compare with Fig. 2). ×750.

spermatids. The fact that gossypol acetic acid exerts a pathognomonic effect on the ultrastructure of mitochondria in late spermatids in both rats and hamsters is well documented (Hoffer, 1983, 1985). However, the role of this defect in the mechanism of the antifertility activity of gossypol has not been conclusively demonstrated. In undertaking the ultrastructural portion of this study, it was thought that a valuable clue to the significance of the pathognomonic defect in the antifertility mechanism of gossypol might be obtained if a new compound were found that was active as a contraceptive and also produced mitochondrial sheath damage similar to that caused by gossypol and gossypol acetic acid. Conversely, the presence of the pathognomonic defect in animals not rendered infertile by treatment with the test compounds would indicate that the mitochondrial sheath defect is not essential for the antifertility action of gossypol. None of the males treated with 6,6′-dimethoxygossypol, 8,8′-diacetyl apogossypol or the diethyl analog developed specific defects in the mitochondrial sheath of Step 16 and 17 spermatids, the last two steps of spermiogenesis in the hamster. These data suggest, but do not prove, that the gossypol-induced pathognomonic defect in the midpieces of late spermatids is related to its mechanism of antifertility action.

In summary, the results of this study have shown that (1) the fourteen new gossypol analogs evaluated herein are not effective as male antifertility agents; (2) their in vitro activity or lack of activity as spermicides is unrelated to their in vivo contraceptive potential; (3) the isopropyl moiety of the gossypol molecule, like the aldehyde group, may be essential for its mechanism of action; and (4) the pathognomonic defect in the mitochondrial sheath induced by gossypol appears to be related to its unique activity as a male antifertility agent, but the exact nature of this relationship is not well understood. To date, gossypol is the only nonsteroidal male antifertility agent that has been evaluated in large scale clinical trials. Although the preliminary results from these studies were encouraging (National Coordinating Group, 1978; Frick et al., 1981; Hoshiai et al., 1982; Coutinho et al., 1984), problems with reversibility and with infrequent but potentially dangerous side effects (hypokalemia) remain (Qian and Wang, 1984). It is hoped that a synthetic analog of gossypol will

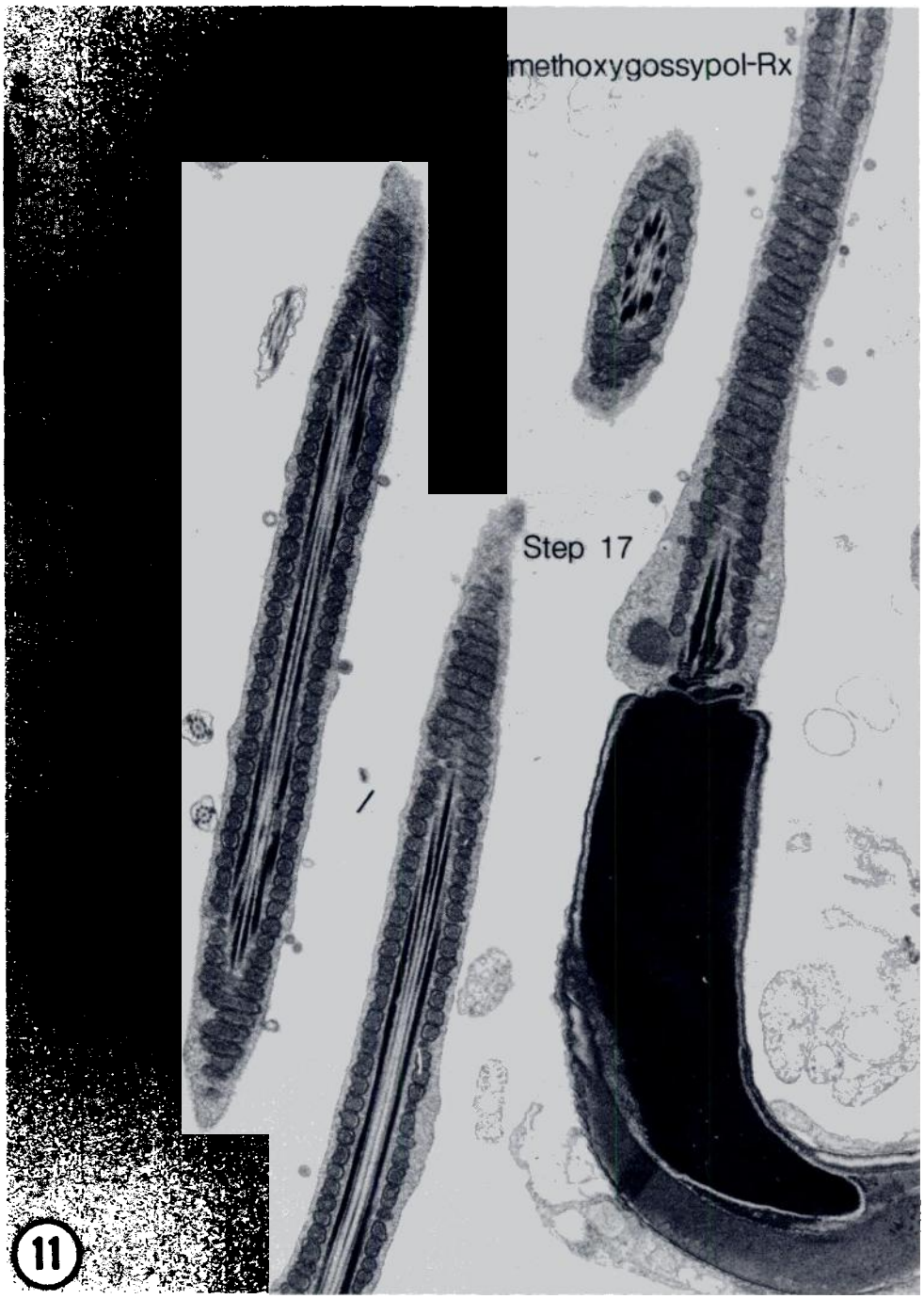

FIG. 11. Electron micrograph of the tubular lumen in a hamster testis after oral treatment with 6,6'-dimethoxygossypol (16 mg/kg/day for 9 wk). The mitochondrial sheaths of Step 17 spermatids shown here are free of the pathognomonic defects that are characteristic of gossypol treatment. Sperm heads (nuclei and acrosomes) are also normal in appearance. ×11,000.

eventually be found that retains its antifertility activity while eliminating its pharmacologically undesirable properties.

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