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Effects of Gossypol on Reproductive and Endocrine Functions of Male Cynomolgus Monkeys (Macaca Fascicularis)[1]

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ABSTRACT

Adult male cynomolgus monkeys were treated orally with 5 (n=4) or 10 (n=3) mg per kg per day of gossypol acetic acid (gossypol) for 6 months. A significant decrease in sperm concentration and motility, determined by evaluating semen ejaculates, was observed without any significant decrease in circulating levels of testosterone (T) among 10 mg per kg per day gossypol-treated animals. Similarly, there was no significant difference in plasma T levels after an intravenous bolus injection of luteinizing hormone releasing hormone (LHRH) (50 μg/animal) between control and gossypol-treated animals, further suggesting an adequate release of pituitary luteinizing hormone (LH) and normal Leydig cell function. Transient azoospermia was observed in 1 out of 4 and in 2 out of 3 animals after 4 months of gossypol treatment at 5 and 10 mg per kg per day, respectively. The effects of gossypol (10 mg per kg per day) were more dramatic and consistent on sperm motility. No striking abnormality of spermatozoa was observed by light microscopy, although there was an increase in the number of sperm with coiled or broken tail pieces and an occasional detached head and tail. However, at the ultrastructural level disruption of the axial complex was commonly observed with gossypol treatment. The effect was manifested at 5 mg per kg per day as a disruption of radial arms. At 10 mg per kg per day the entire axial complex was frequently destroyed, suggesting an impairment of sperm motility. No serious clinicopathologic side effects were observed except temporary diarrhea and anorexia among 10 mg per kg per day gossypol-treated animals during the initial stages of treatment. In addition, gossypol had a hypolipidemic effect which is a new significant entity for this compound. In conclusion, it is suggested that gossypol may be interfering with spermatogenesis and/or directly acting on the sperm itself within the testis or during its passage through the male reproductive tract in ways that affect both sperm production and sperm motility.

INTRODUCTION

Over the years a variety of antispermatogenic compounds have been found and some of their properties characterized (Jackson, 1966; Patanelli, 1975; Shandilya et al., 1979; National Coordinating Group on Male Infertility Agents, 1979). Of particular interest among these compounds is gossypol, a yellowish phenolic compound isolated from the seeds, stems and roots of the cotton plant.

The antispermatogenic effect of gossypol has been tested in experimental animals and humans in China and has been found to be effective in inhibiting spermatogenesis with only minimal side effects. The Leydig cells do not appear to be damaged by gossypol treatment and the serum levels of LH and T are unchanged and there is no reduction in libido. The semen of men given gossypol first showed a decrease in the percentage of motile spermatozoa, followed by an increase in malformed spermatozoa, and then finally a gradual drop in sperm concentrations until azoospermia was achieved (National Coordinating Group on Male Infertility Agents, 1979).

There have been several recent reports on the effect of gossypol in laboratory animals. In one of these studies (Nadakavukaren et al., 1979), there was a marked decrease in the number of sperm in the epididymis of the

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gossypol-treated rats. In the other study by Lin et al. (1980) gossypol treatment at a dose of 30 mg per kg per day was found to markedly diminish sperm production and the degree of decrease in sperm production was related to the treatment time. They also observed a decrease in serum levels of T and LH. In another study, Chang et al. (1980) observed sterility in male rats and hamsters given gossypol acetic acid at a dose level of 5 or 10 mg per kg per day for 12 weeks, while similar treatment in male rabbits for 5 to 14 weeks did not affect sperm concentration, although sperm motility was diminished during treatment. In another related study, Hoffer (1980) studied the effects of low and high doses of gossypol in male rats. At a low dosage (7.5 mg per kg per day), no differences in the morphologic effects of gossypol or gossypol monoacetate on the testis or epididymal sperm were observed. However, 5 different types of defects in sperm were observed at the ultrastructural level. At the higher dosages (20 and 30 mg per kg per day), a limited extent of testicular damage was observed while deleterious changes were much more apparent in epididymal sperm. On the other hand, Bardin and associates (1980) found the male rhesus monkey resistant to the antifertility effects of gossypol (20 mg per animal per day × 3 months). No changes were seen in testosterone or gonadotropin levels. Sperm counts were normal, although decapitated sperm were frequent.

Because of an increasing need for and interest in the development of suitable male contraceptives, we undertook our study on nonhuman primates in an effort *a*) to confirm and extend the studies done in China and in the United States and *b*) to provide further evidence that gossypol treatment is not associated with demonstrable toxic side effects. In this paper we report the effects of orally administered gossypol on some aspects of reproductive and endocrine functions of male cynomolgus macaques (*Macaca fascicularis*).

MATERIALS AND METHODS

Animals

Adult male cynomolgus monkeys (*Macaca fascicularis*) were purchased through Primate Imports, Inc., Port Washington, NY, and were housed in individual cages under controlled environmental conditions (temperature 25°C, 10L:14D schedule). The animals had free access to food and water. They were fed a diet prepared in our laboratory that was designed to mimic the diet usually consumed by North American humans (Table 1).

Dosage and Treatment Scbedule

Gossypol acetic acid was obtained from the Southern Regional Research Center of the United States Department of Agriculture. We suspended the gossypol in a small quantity of corn oil (50 mg/ml). Initially, gossypol was administered orally with a syringe for 2 months; but subsequently, we were able to administer the drug in a small slice of apple for the remaining period of the experiment. The animals were divided into 3 groups: the first group consisted of 4 monkeys which received corn oil only and served as controls; the second group of 4 monkeys received gossypol at a dose level of 5 mg per kg per day for 3 months and later the dose was increased to 10 mg per kg per day for the next 3 months since no significant effect on sperm concentration was observed at the 5 mg per kg per day dose level; the third group consisted of 3 animals who were treated with 10 mg per kg per day of gossypol. The study was divided into 2 phases: a pretreatment phase of 2 months and a treatment phase of 6 months.

The following observations were made at monthly intervals during the pretreatment and treatment phases of the study.

Semen Evaluation

Semen specimens were collected by electroejaculation using a rectal probe (Weisbroth and Young, 1965). The semen was examined for sperm concentration, motility and morphologic characteristics. The sperm motility was evaluated subjectively by the same observer and recorded as percent motile sperm. Three months following gossypol treatment at 5 and 10 mg per kg per day, semen samples for electron microscopy

TABLE 1. Composition of the experiment diet.[a]

Ingredients	Gm/100 g
Casein, USP	8.0
Lactalbumin	8.0
Wheat flour	36.0
Dextrin	6.0
Sucrose	5.0
Applesauce	4.5
Lard	6.0
Butter	3.0
Beef tallow	7.0
Dried egg yolk	3.0
Safflower oil	4.0
Complete vitamin mixture (devoid of vitamin D)	2.5
Alphacel	1.0
Hegsted salts mixture	4.0
D_3 in corn oil	[b]
NaCl (table salt)	2.0
	100.0

[a]The diet contains 456.81 Kcal/100 g. The cholesterol content of all diets was 0.19 mg/Kcal and 43% of calories were provided from a mixture of fat.

[b]To provide 2.5 IU/g.

were collected into a balanced salt solution, washed to remove extraneous proteins and then fixed overnight at 3°C in 2.5% glutaraldehyde buffered to pH 7.2 with 0.1 M phosphate. Subsequent to primary fixation the sperm were washed by centrifugation and resuspension in the same buffer and then secondarily fixed for 1 h at room temperature in buffered 1% osmium tetroxide. The specimens were then centrifuged into a pellet, dehydrated with a graded series of ethanol through propylene oxide and embedded in epoxy resin. Silver to gray sections obtained with a diamond knife in a Sorvall MT-2B ultramicrotome (DuPont Instruments, Newton, CT) were double stained with lead citrate and methanolic uranyl acetate prior to observation in a Philips EM-400 electron microscope (Philips Electronics, Mahwah, NJ). General observations were made of all regions of the sperm including the acrosome/nucleus, neck or connecting piece, the midpiece with its mitochondrial sheath and the flagellum; primary emphasis in all samples was placed upon organization of the axial filament complex in the midpiece and flagellum.

Testicular Endocrine Function Evaluation

Plasma levels of testosterone were measured every month throughout the study. Blood samples from fasted monkeys were collected in the morning between 0900 and 1100 h to avoid the complicating effects of circadian variation. A LHRH stimulation test was done to evaluate in vivo pituitary-gonadal function among gossypol-treated animals. Synthetic LHRH (prepared by Dr. Folkers, San Antonio, TX, for The Population Council) was dissolved in sterile, pyrogen-free saline to a concentattion of 50 μg/ml. Each of the 4 control monkeys was given 1 ml saline and all of 7 gossypol-treated monkeys were given 50 μg LHRH in 1 ml saline. All monkeys were lightly sedated with Ketamine (15 mg per kg per animal i.m.) and the experiments started at 0900 h with the drawing of the first blood sample. Blood samples were obtained at −30 and 0 min in relation to the time of an intravenous bolus injection of LHRH. Blood samples then were collected at 0.5, 1, 2 and 4 h following LHRH administration. Plasma was separated and kept frozen at −20°C until assayed.

Plasma levels of T were determined according to the procedure of Perachio et al. (1977) which requires extraction but not chromatographic separation. All samples were analyzed in duplicate utilizing 25−50 μl plasma volumes. The sensitivity of the assay was 10 pg/tube. The intra- and inter-assay coefficients of variation were 5% and 18%, respectively.

Clinicopathologic Studies

Throughout the experiment the animals' health was evaluated regularly. In order to ascertain whether gossypol treatment caused any alterations in metabolic and key organ functions of the animals, the following investigations were done at variable intervals before and during the treatment. Body weights were recorded at monthly intervals and indirect blood pressures were determined with a Dinamap Research Monitor (Model 1245 from Applied Medical Research). The systolic and diastolic blood pressures were recorded on monkeys sedated with Ketamine (15 mg/kg i.m.).

Plasma total cholesterol (TPC) concentrations were measured by the AutoAnalyzer II method of Rush et al. (1971). The manganese precipitation method as described in detail in the Manual of Laboratory Operations: Lipid Research Clinics Program (1974) was used for high density lipoprotein-cholesterol (HDL-Chol) determinations. Low density lipoprotein (LDL) and very low density lipoprotein (VLDL) concentrations were calculated as the difference between TPC and HDL-Chol. All plasma lipids were determined in our Lipid Analytic Laboratory that is in complete compliance with the Cooperative Lipid Standardization Program of the Center for Disease Control. Plasma concentrations of sodium and potassium, serum glutamic-oxaloacetic transaminase (SGOT) serum glutamic-pyruvic transaminase (SGPT) and serum gamma glutmyl transpeptidase (γGT), serum protein, serum creatinine, blood urea nitrogen, erythrocytes, leuckocytes, hematocrit, and prothrombin time were measured in our Clinical Pathology Laboratory according to the established procedures.

Statistical analyses of the data were done by the Student's *t* test.

RESULTS

Clinicopathologic Findings

Throughout the study period no adverse clinical findings were observed in any of the monkeys treated with gossypol except the temporary diarrhea and loss of appetite among the monkeys treated at 10 mg per kg per day of gossypol during the initial stages of treatment. The animals recovered when the treatment was suspended for a brief period of time (1 week). The mean body weight of the animals in various groups did not change throughout the period of treatment.

No changes were observed in most of the metabolic and key organ functions of the animals as evidenced by the monthly measurements of hematocrit, erythrocytes, leukocytes, serum protein, serum creatinine, blood urea nitrogen, prothrombin time, SGOT, SGPT and serum γGT. Plasma levels of sodium and potassium remained within normal range (Fig. 1) and so was the blood pressure of the animals in all groups (Fig. 2).

Among the monkeys treated with 10 mg per kg per day of gossypol we had an unexpected finding, i.e. gossypol had a hypolipidemic effect. Since there was a significant decrease ($P<0.005$) in total plasma cholesterol concentrations, we sought to determine whether the decreases were in HDL-Chol or in the cholesterol concentrations of LDL + VLDL. No significant differences were observed in HDL-Chol concentrations between control and gossypol-treated animals throughout the experiment, but levels of LDL + VLDL choles-

terol were significantly decreased ($P<0.001$) after 2 months of treatment among the 10 mg per kg per day gossypol-treated monkeys and these difference remained statistically significant throughout the study. The details of the hypolipidemic effect of gossypol have been reported elsewhere (Shandilya and Clarkson, 1982).

Seminal Fluid Analyses

Sperm concentration. Because of the small number of animals in each group and the inherent variability of the various seminal fluid parameters, the pretreatment observations were compared with all the observations in the treatment period.

Table 2 shows the sperm concentration among the gossypol-treated monkeys throughout the study period. Although there were some erratic and unpredictable fluctuations in the control group, adequate enough suppression of sperm production was observed in some gossypol-treated monkeys. In Group II, during the first 2 months of gossypol-treatment (5 mg per kg per day), a definite downward trend could be seen in 3 out of 4 animals but this was quickly reversed in 2 animals. When the dose of gossypol was increased to 10 mg per kg per day, transient azoospermia developed in 1 animal (#1040) and a significant decrease in sperm concentration in the other 2 animals (#1038 and #1039).

Among the Group III (10 mg per kg per day, gossypol-treated) animals, a dramatic decrease in sperm concentration was seen in 2 out of 3 animals after 2 months of treatment and transient azoospermia was observed in 1 animal

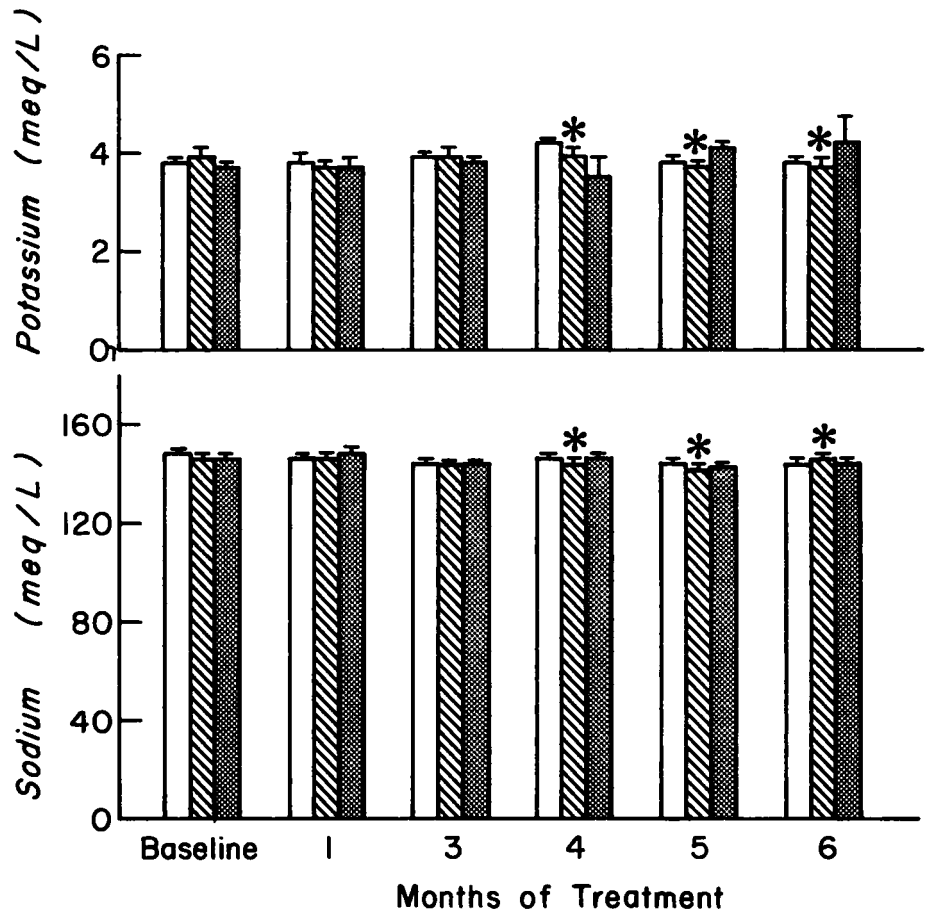

FIG. 1. Effect of gossypol treatment on plasma concentrations of sodium and potassium (mean ± SEM). ▫ Control, ▫ Gossypol-treated (5 mg per kg per day), ▫ Gossypol-treated (10 mg per kg per day), *Dose increased to 10 mg per kg per day.

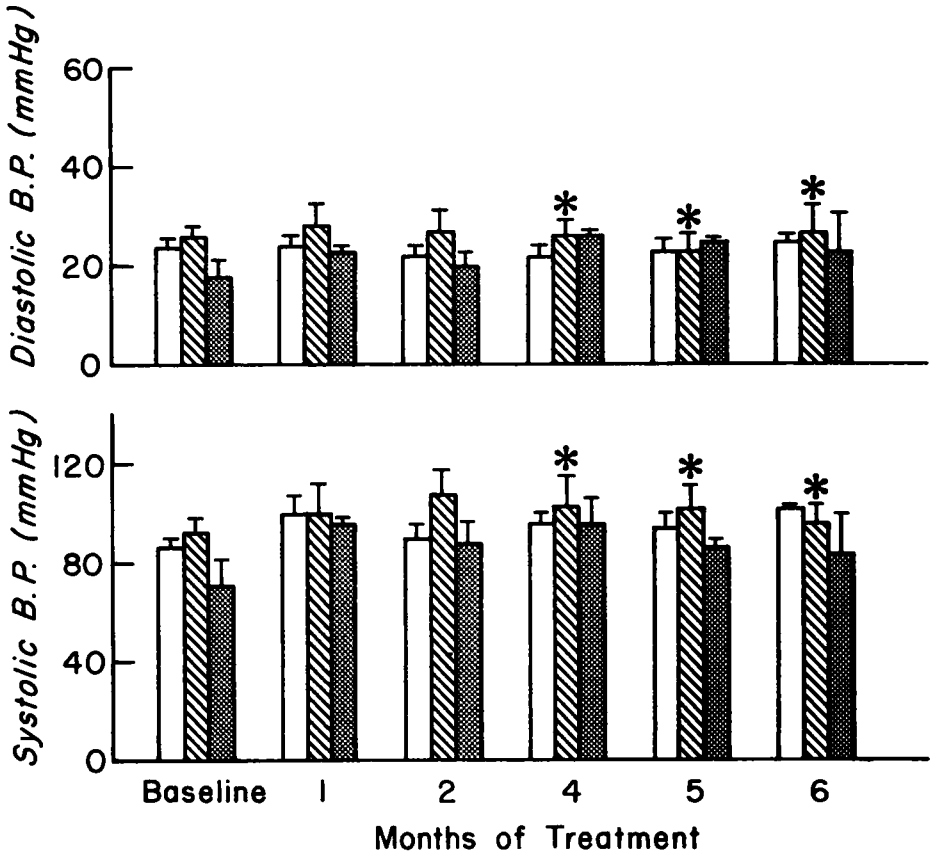

FIG. 2. Effect of gossypol treatment on blood pressure (mean ± SEM). □ Control, □ Gossypol-treated (5 mg per kg per day), □ Gossypol-treated (10 mg per kg per day), *Dose increased to 10 mg per kg per day.

(#1037) after 4 months and in the other animal (#1033) after 6 months of treatment. In the third animal (#1031), there was no tendency to continued suppression of sperm production until 4 months of treatment. However, by the sixth posttreatment month, sperm count had decreased to 562 x 10^6/ml as compared to the pretreatment concentration of 2716 x 10^6/ml.

Sperm motility. Figure 3 shows the percentage of motile sperm in each group and demonstrates that in the control group, sperm motility ranged between 30–90% during the 8-month period. After treatment with gossypol (5 mg per kg per day) for 3 months, the percentage of mean sperm motility decreased slightly but the difference was not significant ($P<0.10$) when compared to control values. No significant effect on percent mean motility was observed following an increase in the gossypol dose to 10 mg per kg per day. However, there was a dramatic decrease in the percent mean sperm motility among the Group III gossypol-treated (10 mg per kg per day) animals.

Sperm morphologic characteristics. In general, no striking abnormalities of spermatozoa were observed by light microscopy, although there was an increase in number of sperm with coiled or broken tail pieces, midpiece cytoplasmic droplets and occasional detached heads and tails. However, distinct differences at the ultrastructural level were observed in spermatozoa among gossypol-treated monkeys.

Figure 4 is a series of micrographs illustrating the general morphology of cynomolgus monkey sperm and the organization of the axial filament complex. This axial filament complex similar to spermatozoa from other mammalian (Phillips, 1975) and nonmammalian species (Burton, 1973) consisted of a central pair of microtubules surrounded by 9 doublet tubules. This axial complex originated in the distal portion of the connecting piece and extended throughout

TABLE 2. Effect of gossypol on sperm concentration ($\times 10^6$ per ml) in *Macaca fascicularis*.

Group and monkey no.	Baseline[a]	Months of treatment 1	2	3	4	5	6
I Control							
1032	881 (872–890)	33	112	2502	492	168	485
1034	355 (40–670)	72	40	288	172	15	215
1035	49 (38–60)	118	100	545	22	502	28
1036	919 (300–1538)	1560	920	1555	667	1810	760
II Gossypol-treated (5 mg per kg per day)							
1038	1869 (2107–1631)	3358	1560	628	1360[b]	3048[b]	598[b]
1039	1102 (1235–969)	755	880	1092	1567[b]	538[b]	145[b]
1040	1512 (2925–99)	165	208	588	0[b]	30[b]	890[b]
1042	1335 (2465–205)	295	745	2440	1142[b]	2028[b]	2185[b]
III Gossypol-treated (10 mg per kg per day)							
1031	2716 (3890–1542)	3422	1508	2370	3047	–	562
1033	2772 (4375–1169)	2755	662	15	102	50	0
1037	2588 (2015–3161)	1088	64	8	0	98	12

[a]Mean values (range).

[b]Dose increased to 10 mg per kg per day.

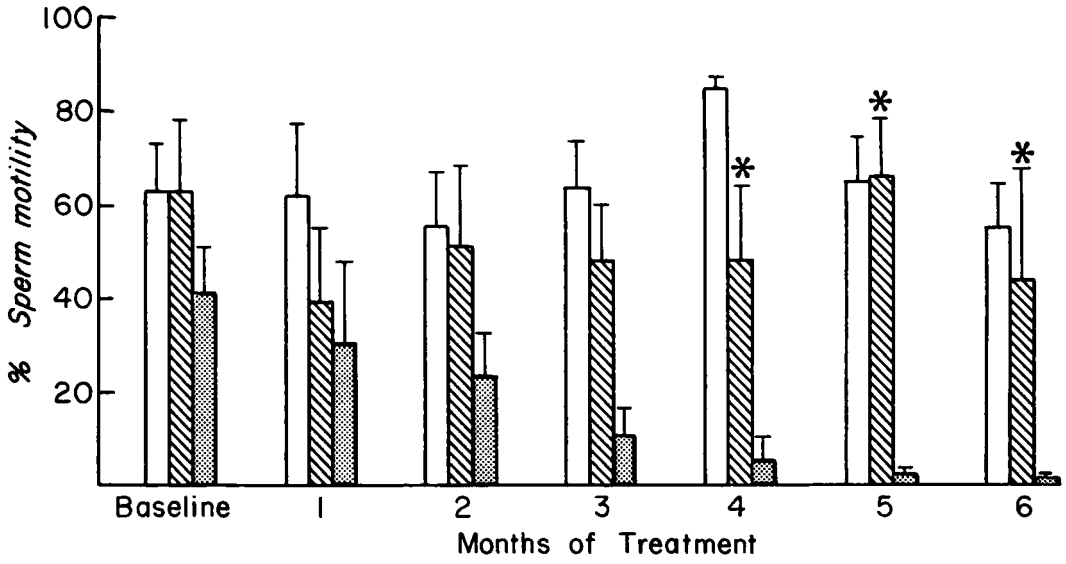

FIG. 3. Effect of gossypol treatment on sperm motility in cynomolgus monkeys (mean ± SEM). ▫ Control, ▫ Gossypol-treated (5 mg per kg per day), ▫ Gossypol-treated (10 mg per kg per day), *Dose increased to 10 mg per kg per day.

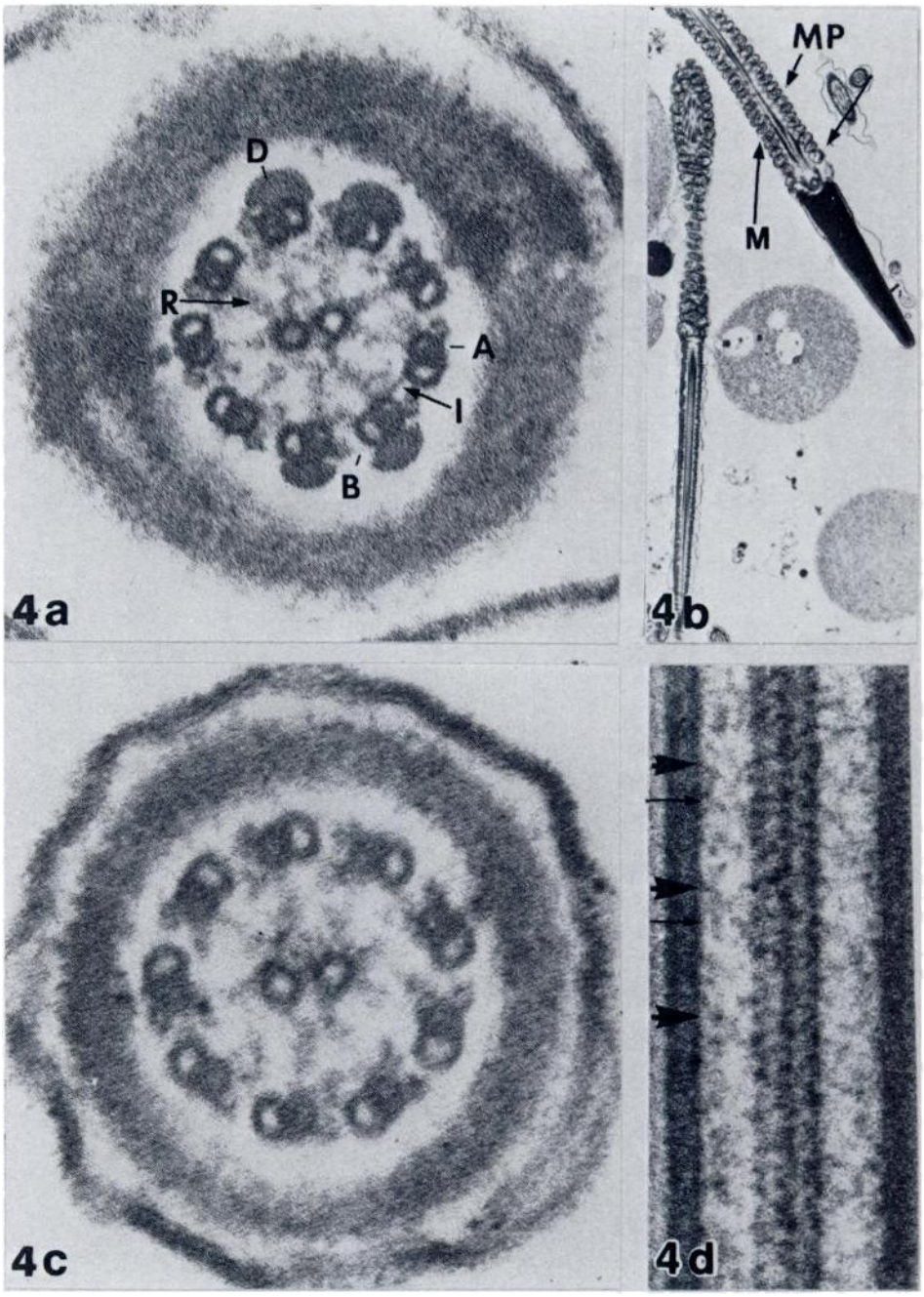

FIG. 4. Transmission electron micrographs of sperm from control animals: *4a*) Transverse section illustrating the axial complex in the distal portion of the sperm midpiece. The central sheath is connected to the doublet A tubules (*A*) by spoke-like radial links (*R*). Inner arms (*I*) of the A tubules establish circumferential continuity when connected to the adjacent doublet B tubule (*B*). Distal portions of the midsection dense fibers (*D*) are also shown. X 190,000 *4b*) Low magnification micrograph illustrating the origin (*arrow*) of the axial complex in the connecting piece region. The complex extends through the midpiece (*MP*) with its circumferential mitochondria (*M*) into the flagellum. X 3500 *4c*) Transverse section through the distal flagellum. As shown the basic structure described for the axial complex in the midpiece is conserved throughout the flagellum. X 190,000 *4d*) Longitudinal section through the axial complex showing the radial link periodicity. The *large arrows* mark major period reference points and the *small arrows* mark the minor period spacings. X 200,000

the flagellum. Circumferential and radial continuity within the complex was maintained by the extension of delicate filamentous proteins among the constituent tubules. Among the 9 doublets the filamentous proteins extend from the inner arm of 1 A tubule to the inner aspect of the adjacent B tubule. Radial links consisting of moderately opaque material enshrouded the central pair of tubules and extended in a uniform spoke fashion to the B tubules of the peripheral doublets (Figs. 4a and c). This arrangement was consistent from the midpiece to the flagellum tip. In longitudinal section the radial links occur predictibly along the axis with an overall major periodicity of 900–950 Å. This major period is comprised of minor distances of 350 Å and 600 Å (Fig. 4d).

The gross morphology of sperm from low dose gossypol-treated animals (5 mg per kg per day) was not altered; however, subtle alterations in the axial filament complex were consistently observed. As shown in Fig. 5, gossypol treatment disrupted the radial links resulting in a coarse aggregation of the otherwise delicate flocculent material. This radial arm disruption appears to have been associated with doublet disintegration, since the A tubules were frequently dissociated in the gossypol-treated animals. Gossypol treatment at 10 mg per kg per day resulted in total sperm disruption. Ejaculates obtained from these animals were characterized by sperm having grossly distorted midpiece regions with mitochondrial breakdown (Fig. 6a). Within the axial filament complex, total disruption was often observed (Fig. 6b). Even in sperm containing some intact microtubules the filamentous radial links and the interdoublet strands were replaced by a random coarse preciptate (Fig. 6c).

Testicular Endocrine Function

In Fig. 7 the plasma levels of T before and during gossypol treatment are presented. Although there were some changes in mean plasma T levels for the various groups, no statistically significant differences could be seen between control and gossypol-treated monkeys. However, there was a slight decrease in mean T levels after 6 months of treatment among Group III monkeys (10 mg per kg per day, gossypol-treated) as compared to controls but the difference was not statistically significant ($P>0.10$). The Leydig cell response, as estimated by T production, to LH released in response to LHRH administration was similar between control and gossypol-treated animals (Fig. 8).

DISCUSSION

The results of our present study are in agreement with those reported by other investigators (National Coordinating Group on Male Infertility Agents, 1979; Nadakavukaren et al., 1979; Lin et al., 1980; Chang et al., 1980) with regard to decreases in sperm production and motility after gossypol treatment. The effects of gossypol were more pronounced and consistent on sperm motility and were directly related to treatment time among 10 mg per kg per day treated animals. On the other hand, no significant effect on percent mean motility was observed even after increasing the gossypol dose to 10 mg per kg per day for next 3 months among Group II monkeys (5 mg per kg per day). We are unable to explain the refractoriness of these monkeys. Further, we have observed the "rebound" in sperm production after attaining azoospermia in 2 monkeys (#1037 and #1040) given the same dosage of gossypol (10 mg per kg per day), which seems to be in contrast to the Chinese report (National Coordinating Group on Male Infertility Agents, 1979) indicating continued low sperm count (below 4×10^6/ml) by administering low maintenance dosage of gossypol in humans. Whether a still higher dosage can prevent "rebound" in sperm production in monkeys cannot be ascertained at present since gossypol treatment at 15 mg per kg per day or higher dosages resulted in prolonged diarrhea and anorexia. More work is needed to evaluate the fertility risk of "rebound" in sperm counts especially when the sperm motility is very poor.

It is noteworthy in our studies that reduction in sperm motility observed with gossypol treatment are correlated to alterations in the axial filament complex. In addition to radial link disorganization, gossypol at low doses in our experiments caused a breakdown of the A tubules in the doublets. Specificity of low dose gossypol for the A tubule and its inner arm was not expected, however other investigators have demonstrated differential susceptibility of microtubules to cold (Behnke and Forer, 1967) and in the case of doublet tubules to pronase digestion (Shay, 1972). Unfortunately, the temporal relationships among disorganization of the radial links, dissociation of the doublet A

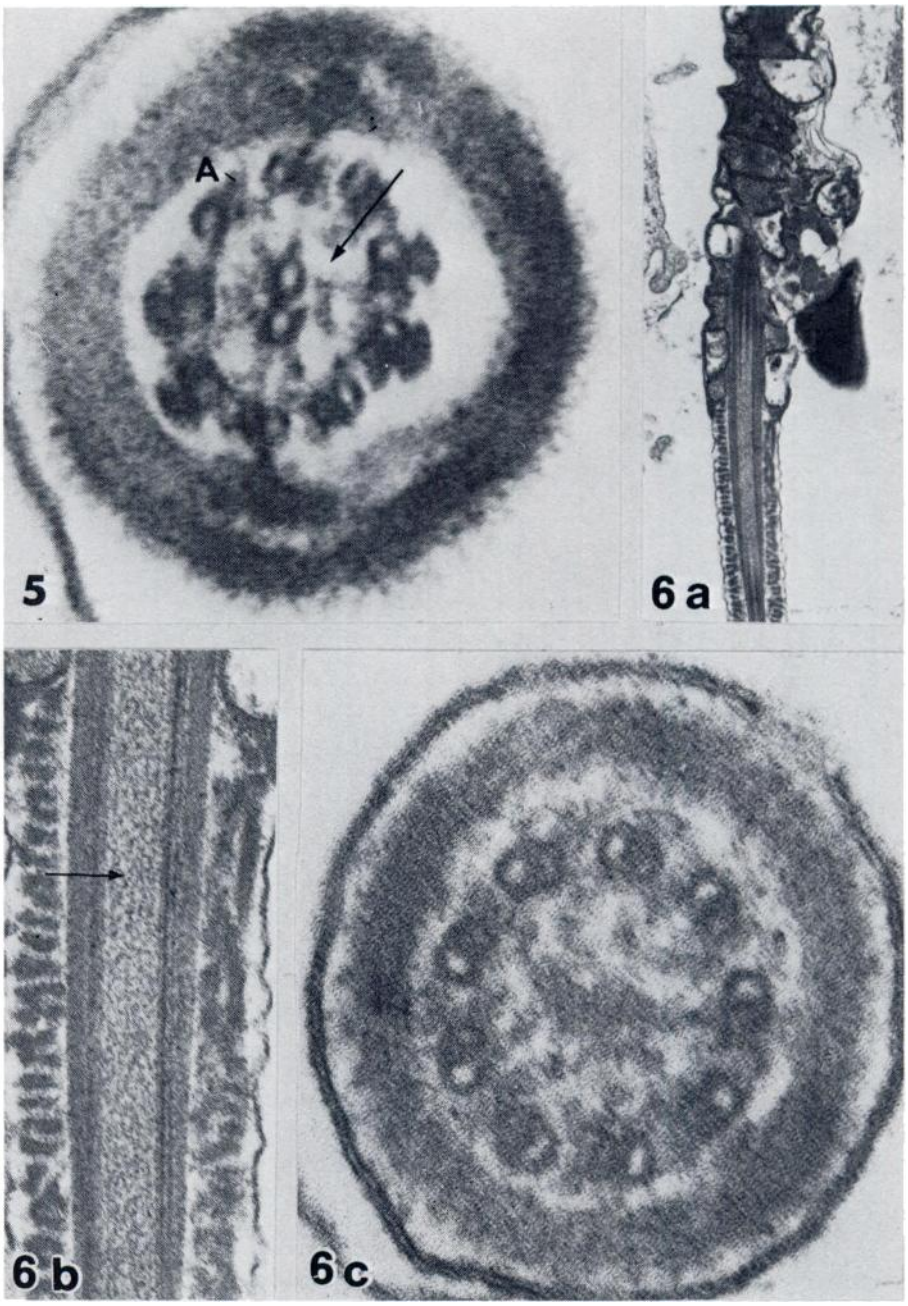

FIG. 5. Transverse section through the axial complex of sperm from a low dose gossypol (5 mg per kg per day) treated animal. The disruption of the radial links (*arrow*) and dissociation of doublet A tubules (*A*) were frequently observed in these specimens. X 190,000

FIG. 6. Micrographs of sperm from a high dose (10 mg per kg per day) gossypol-treated animal: *6a*) Low magnification micrograph illustrating the dramatic gross damage which occurs in sperm with high dose gossypol. X 14,500 *6b*) Longitudinal section through the axial complex of sperm. Note the randomization (*arrow*) of proteins which normally comprise the orderly radial links. X 60,000 *6c*) Transverse secretion through an axia complex similar to that illustrated in *6b*. Nonspecific granular material has replaced the radial arm periodicity. X 190,000

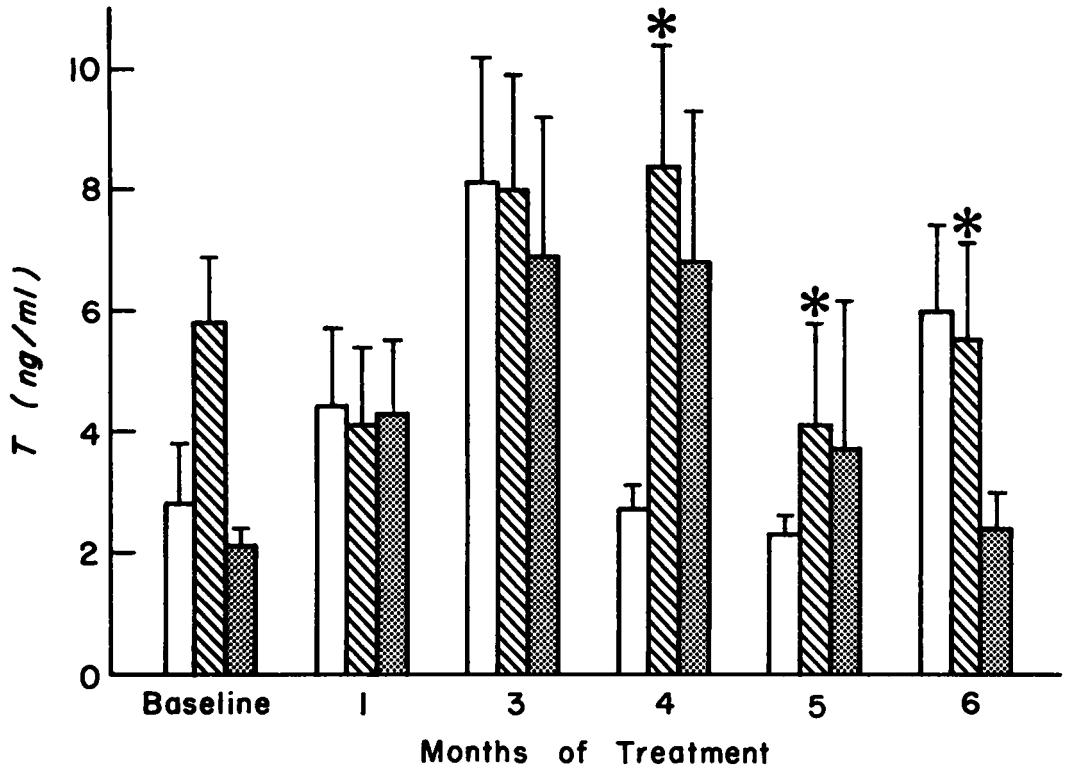

FIG. 7. Plasma testosterone concentrations among gossypol-treated monkeys (mean ± SEM). □ Control, □ Gossypol-treated (5 mg per kg per day), □ Gossypol-treated (10 mg per kg per day),*Dose increased to 10 mg per kg per day.

tubules and loss of sperm motility could not be determined in our study, but our observations with low dose gossypol suggest that radial arm breakdown is one of the earliest events in the spermato-pathology of gossypol.

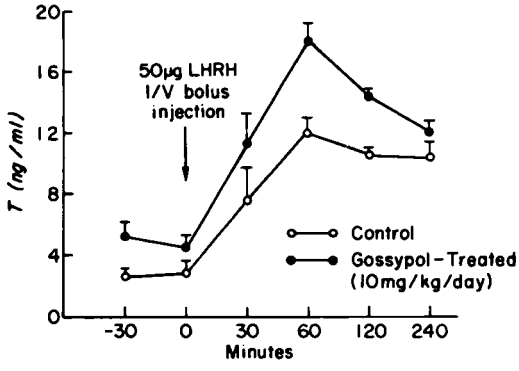

FIG. 8. Plasma levels of testosterone (mean ± SEM) in male cynomolgus monkeys after the administration of 50 μg LHRH.

Other investigators (Nadakavukaren et al., 1979; Hoffer, 1980) have documented an adverse effect of gossypol on plasma membranes, circumferential mitochondria and the axonemal complex of sperm. The observations of these previous investigations as recently reviewed by Zatuchni and Osborn (1981) include: supernumary or displaced outer dense fibers; missing outer dense fibers or doublets in the principal pieces; early signs of mitochondrial degeneration in the midpiece; and a profile containing only outer dense fibers. Our observations differ from those reported by these previous investigators in that no ultrastructural changes of a consistent nature were observed in either mitochondria or the plasma membranes with low doses of gossypol. Conceivably, the discrepancies in low-dose observations have resulted from either dose differences, 5 mg/kg in our experiments versus 7.5 mg/kg in Hoffer's

studies, or species susceptibility, cynomolgus monkey versus rat. Consistent with the observations of these other investigators are the effects observed when higher doses of gossypol were given. Under these conditions dramatic mitochondrial and plasma membrane changes were observed. In addition, the higher dosage in our experiments typically resulted in total disruption of axial filament integrity and often involved sperm destruction. It is unlikely, in view of this observation that either the plasma membrane or mitochondrial alterations in our experiments represent specific gossypol effects.

Although there were some changes in mean testosterone concentrations for the various groups associated with the season of the year, no significant differences can be seen between control and gossypol-treated monkeys. These results are in accordance with those reported by other investigators (National Coordinating Group on Male Infertility Agents, 1979; Bardin et al., 1980; Hoffer, 1980) who did not find any change in plasma T concentrations among gossypol-treated men, rhesus monkeys and rats, respectively, but are in contrast to the other study done in rats (Lin et al., 1980) where a significant decrease in T concentrations has been observed after 5 weeks of gossypol treatment at much higher dosage (30 mg per kg per day) than what we used in our present experiment (10 mg per kg per day). Similarly, there was no significant difference in plasma T levels after an i.v. bolus injection of LHRH between control and gossypol-treated monkeys. Although we did not measure plasma LH levels per se after LHRH administration, an increase in plasma T concentrations provide direct in vivo evidence for adequate pituitary LH release and normal Leydig cell function among gossypol-treated monkeys.

The results of the clinicopathologic findings have been published separately in complete detail. In brief, gossypol had a hypolipidemic effect. This is a therapeutic property of the compound that has not been reported previously. The principal differences in the total plasma cholesterol concentrations between the control animals and the animals treated with 10 mg per kg per day of gossypol are in the cholesterol concentrations of the LDL's. The difference in HDL-Chol between the control and the treated groups is not statistically significant, while the differences in plasma concentrations of LDL-Chol are highly significant. The results of our study did not show any changes in plasma levels of sodium and potassium nor in blood pressure among gossypol-treated animals, while in one of the studies done in China, slight disturbances in potassium metabolism were observed among men given gossypol, however, a cause and effect relationship was not established (National Coordinating Group on Male Infertility Agents, 1979). A further period of observation will be necessary to confirm the long-term safety of this compound.

In conclusion, we have shown in this limited pilot study that there is a significant suppression of sperm production and motility with a daily oral dose of 10 mg per kg per day of gossypol without any significant changes in plasma levels of T. Only minimal side effects were observed, however, in our studies, TPC and LDL + VLDL cholesterol showed significant decrease which warrants further study. More work is needed to understand the exact mechanism and site of action of gossypol in achieving its antifertility and hypolipidemic effects.

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