A DEVELOPMENTAL TOXICITY EVALUATION OF GOSSYPOL

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ABSTRACT

In utero development was analyzed in pregnancies that resulted from matings between gossypol-treated male rats and untreated female rats, and in pregnancies in which gossypol was administered to the pregnant rat only. Gossypol treatment of males had no effect on the outcome of pregnancy. There was no significant effect on resorption, fetal growth, or malformation rate. Similarly, gossypol administered to pregnant dams at stages during organogenesis had no observable effect on pregnancy. Under the conditions of this experiment, gossypol administered to either the breeding male rat or the pregnant female rat had no demonstrable adverse effect on development in utero.

INTRODUCTION

The antifertility effect of gossypol in males was first reported in China (1). Antifertility effects have been reported in rats (2,3), mice (4), hamsters (5), rabbits (6), and monkeys (7). Hamsters appear to be the most susceptible, and mice and rabbits the least susceptible, to the antifertility effects of gossypol (4,8).

Gossypol is reported to cause the deterioration of sperm culminating in the rapid loss of motility, often a separation of sperm head from sperm tail, and subsequently a reduction of sperm production in the testis (9). In sperm, gossypol induces ultrastructural abnormalities in the mitochondrial sheath of the mid-piece (10,11), uncoupling of oxidative phosphorylation with reduced ATP concentration (12), enzyme inhibition (13,14,15,16), and reduced steroidogenesis (17).

Considerable attention has been paid to the effects of gossypol on the morphology and biochemistry of the testes and spermatozoa, but relatively little information is available about the consequence of fertilization with sperm from gossypol-treated males. The present study was designed to investigate embryonic development from matings in which the male was undergoing gossypol treatment. Of special interest was the period during which the sperm were becoming abnormal and losing their capacity to fertilize, and the period during which normal sperm were

reappearing and fertility returned. The effect of gossypol on the pregnant rat was also investigated. Portions of these experiments have been published (18,19).

METHODS

Sprague-Dawley rats, purchased from Charles River (Kingston, NY) were used in this study. The animal rooms were maintained at a relatively constant temperature (72°F) with a 12-hour light/dark cycle. The rats were fed Purina Rodent Lab Chow 5001 and water ad libitum.

Gossypol acetic acid (95% pure) was obtained from Dr. Sheldon Segal of the Population Division, Rockefeller Foundation, New York. Gossypol was adminstered as its acetic acid or as gossypol prepared from its acetic acid according to the prodcedure described by Pons et al. (20). The results were identical regardless which form of gossypol was used and, therefore, they have been combined for presentation in this article. The maximum tolerated dose of gossypol in rats (strain not specified) is reported to be 15-20 mg/kg/day (Segal, personal communication). This dose induced infertility within 5 weeks. The no-effect dose is reported to be 5 mg/kg/day. The doses used in this experiment were 5, 10, and 20 mg/kg/day. A mixture of gossypol in sesame oil containing 0.5% gum tragacanth was prepared twice each week. The mixture was prepared so that the appropriate dose of gossypol was contained in 0.5 ml. Control rats received 0.5 ml of a sesame oil-gum tragacanth mixture.

Male rats were kept in individual cages, except when breeding. Female rats were kept in groups of five or six until they were bred. For breeding, a male was caged overnight with three females selected at random. The day of finding sperm in the vaginal smear was designated day 0 of pregnancy. Male rats of proven fertility were gavaged with either 10 or 20 mg gossypol/kg/body weight/day for six consecutive days each week. Males receiving the 20 mg/kg dose were treated for 5 or 6 weeks. Males receiving the 10 mg/kg dose were treated for 9 weeks, except for three males who continued to have successful matings and were treated for 16 weeks. Throughout the experiment, each male was placed with females each week until one successful copulation was achieved. Only occasionally did a male fail to achieve copulation each week (determined by the presence of sperm in the vaginal smear). During the period of infertility, spermatozoa were frequently seen with the sperm head separated from the sperm tail. The experiment was continued until each male had recovered his fertility and impregnated four females. Pregnant females were gavaged with a dose of either 10 mg or 20 mg gossypol/kg maternal body weight. Some pregnant rats received a single gavage treatment, on either day 6, 8,

10, 12, 14, or 16 of pregnancy, while others were gavaged daily from day 6 through day 15. Some rats in the latter group received a dose of 5 mg/kg.

All pregnant rats were kept in individual cages. At day 20, they were given an overdose of ether, and the uteri were exposed and examined for resorption sites. The fetuses were recovered, weighed, and fixed in Bouin's fluid or 95% alcohol. In experiments in which the male was treated, the fetuses in each litter were apportioned between Bouin's fluid and alcohol in a ratio of approximately 4:1. In experiments in which the pregnant female was treated, whole litters were fixed in either Bouin's fluid or alcohol in a ratio of approximately 3:2. The fetuses fixed in Bouin's fluid were subsequently examined for external malformations and then free-hand sectioned with a razor blade. Fetuses fixed in alcohol were prepared for staining with alizain red S for visualization of the skeleton.

Four gossypol-treated males (20 mg/kg) and two sesame oil-treated males were randomly selected to mate with untreated females who were allowed to deliver and rear their pups. There were nine such matings in the gossypol group and ten matings in the sesame oil group. Ninety viable pups (11.2/litter) were delivered from the gossypol matings and 115 pups (11.5/litter) from the sesame oil matings, two of which died. Each litter was culled to three male pups and three female pups. The pups in each litter were tested for postnatal maturation according to procedures outlined by Zbinden (21). The results for fetal weight and resorption were analyzed by the Student t-test for independent samples (22). Because of the very few numbers of malformations, the incidence was not analyzed statistically.

RESULTS

Table I summarizes the weight gain of male rats treated with 20 mg/kg gossypol. Twenty-one male rats were gavaged with the gossypol mixture for six consecutive days each week for 5 or 6 weeks. Two males died from complications resulting from the procedure. Three males became sick during treatment (listless, rough coat, anorexic) but recovered completely following cessation of treatment. The remaining males appeared healthy throughout the experiment. Thirteen males were used as controls and received the sesame oil-gum tragacanth mixture for 5 or 6 weeks. These males were healthy throughout the experiment. In each experiment, a significant weight loss was observed during the period of gossypol treatment. In the period following treatment, the gossypol-treated males resumed their weight gain, at times exceeding the weight gained by the controls. For

TABLE I

WEIGHT GAIN IN MALE RATS

Duration of Treatment	Dosage of Gossypol	Number Males Treated/ Survived	WEIGHT GAIN (g±SD) During Treatment Period	Following Treatment Period	Throughout Experiment
Experiment #1					
6 weeks	20 mg/kg/day	6/5	2.5±39.5 (P=0.001)	120.4±35.3 (P=0.02)	122±39.5 (P=0.001)
	0 mg/kg/day	5/5	101.0±16.7	77.6±8.4	176.4±13.3
Experiment #2					
5 weeks	20 mg/kg/day	7/7	2.5±42.3 (P=0.001)	114.2±41.8 (P=0.002)	114.1±33.3 (P=0.05)
	0 mg/kg/day	3/3	77.3±12.2	88.3±22.1	165.7±16.8
Experiment #3					
5 weeks	20 mg/kg/day	8/7	41.1±26.1 (P=0.005)	89.8±13.4 (P=0.1)	131.0±28.5 (P=0.3)
	0 mg/kg/day	5/5	80.8±12.2	73.0±29.2	141.8±40.3

the duration of the experiment, gossypol-treated males tended to gain less weight than their control counterparts.

Seventeen males were gavaged for six consecutive days each week with 10 mg/kg gossypol. One male died from complications of the procedure. One male became sick during treatment, but recovered when the treatment was discontinued. The remaining males appeared healthy throughout the experiment. Eight males were gavaged with sesame oil-gum tragacanth and served as controls. The results with the 10 mg/kg dose paralleled those with the 20 mg/kg dose. There was a statistically significant (P=0.02) reduction in weight gain by the experimental males only during treatment. Following treatment, weight gain resumed and at the end of the experiment there was no significant difference in weight gain between experimental and control males.

Gossypol induced infertility in all males treated with the 20 mg/kg/day dose, although its onset and duration were variable (Table II). Sesame oil did not interfere with fertility. Infertile matings were observed as early as the third week of treatment and as late as the last week of treatment (sixth week). With the 10 mg/kg dose, infertility began as early as the fourth week and as late as the eighth week of treatment, except that three males continued to have fertile matings even after 16 weeks of gossypol treatment. The duration of infertility ranged between 3 and 7 weeks (average = 5 weeks) at the high dose and between 2 and 7 weeks (average = 4.4 weeks) at the low dose. These figures are not exact, however, because, for one reason or another, males occasionally failed to mate during a given week.

Table III summarizes the results of breeding males prior to, during, and following treatment with gossypol. There were 19 males treated with 20 mg/kg/day gossypol and 16 males treated with 10 mg/kg/day gossypol. Twenty males received sesame oil. Gossypol administered to males had no effect on day 20 fetal weight, or on the number of implantation sites, with the possible exception of implantations in pregnancies resulting from matings during the treatment period with males treated with 10 mg/kg/day. The number of resorptions did not increase significantly during or following gossypol treatment, and gossypol treatment did not increase the number of resorptions over the number found following sesame oil treatment. In five of the six groupings there were more resorptions in the sesame oil-treated groups than in the gossypol-treated groups. The incidence of resorption in Sprague-Dawley untreated rats can be expected to be around 6% (Schardein, personal communication). A total of 2,524 fetuses in pregnancies from sesame oil-treated males were examined for soft tissue defects. Three of these (0.1%)

TABLE II

GOSSYPOL AND FERTILITY

Gossypol Treatment	Week of last fertile mating[1]	Week fertility returned[1,2]	Duration of infertility[1]
19 males	3rd - 3 males	9th - 1 male	3 weeks - 1 male
	4th - 9 males	10th - 8 males	4 weeks - 4 males
20 mg/kg/day	5th - 5 males	11th - 8 males	5 weeks - 8 males
	6th - 2 males	12th - 1 male	6 weeks - 4 males
			7 weeks - 1 male
16 males	4th - 2 males	12th - 1 male	2 weeks - 1 male
	5th - 6 males	13th - 6 males	3 weeks - 1 male
10 mg/kg/day	6th - 4 males	15th - 6 males	4 weeks - 7 males
	8th - 1 male	16th - 4 males	5 weeks - 2 males
			7 weeks - 1 male[3]
	3 males did not lose fertility		

[1]Times for some males can only be approximate because some males failed to mate each week.

[2]One male at each dose discarded because they were inadvertently not bred for several weeks.

[3]Failed to breed during week 5 and 6.

TABLE III
GOSSYPOL-TREATED MALES AND THE OUTCOME OF PREGNANCY

Treatment[1]	Number Females Pregnant	Fetal Weight(g±SD)	Number Implantation Sites	Sites/ Litter	% Resorbed	% Survivors Malformed
Pretreatment						
GP-20	21	3.65±0.38	270	12.8	3.7	0
Sesame	11	3.58±0.20	144	13.1	5.5	0
GP-10	20	3.67±0.30	260	13.0	5.3	0.4
Sesame	8	3.51±0.21	105	13.1	5.6	0
During Treatment						
GP-20	72	3.72±0.27	920	12.7	4.0	0
Sesame	59	3.81±0.49	783	13.2	7.4	0.1
GP-10	83	3.64±0.50	954	11.5[2]	7.0	0.2
Sesame	37	3.52±0.31	525	14.1	9.0	0.4
Post-treatment						
GP-20	91	3.72±0.53	1125	12.3	7.3	0.3
Sesame	64	3.62±0.40	870	13.5	4.5	0
GP-10	76	3.58±0.33	937	12.3	4.6	0.4
Sesame	20	3.52±0.27	270	13.5	4.8	0

[1]GP-20 = 19 males treated with gossypol 20 mg/kg/day for 5 or 6 weeks. GP-10 = 16 males treated with gossypol 10 mg/kg/day for 7-16 weeks. Sesame = 20 males treated with sesame oil (13 for GP20; 7 for GP10).

[2]P = 0.001

were malformed. Ten malformed fetuses were found in a total of 4,231 fetuses examined for soft tissue defects in matings from gossypol-treated males (0.2%). The incidence in untreated Sprague-Dawley rats can be expected to be about 0.5% (based on observations of 21,186 fetuses at a commercial laboratory). There was no significant alteration in the sex ratio of the fetuses following treatment of the male parent with gossypol.

Table IV lists the malformations observed. In the gossypol-treated groups, one malformed fetus occurred in a litter from a mating prior to treatment and two malformed fetuses occurred in litters from matings during treatment. The remaining seven malformed fetuses were found in litters from matings after treatment. No litter contained more than one abnormal fetus. No single abnormality was common to all malformed fetuses, and no dose response was observed. In the sesame oil-treated matings, all malformations occurred in litters from matings during the treatment period.

Table V compares the appearance of ossification centers in selected bones in fetuses from dams mated with 20 mg/kg/day gossypol-treated or sesame oil-treated males. Matings occurred prior to treatment, during treatment, and folloiwng treatment. A total of 541 fetuses from the experimental matings and 398 fetuses from the control matings were examined. The percentages given in the table for each observation will not always total 100% because not all categories are listed for each observation, and sometimes a portion of a fetus was lost during preparation.

Cervical Vertebral Centra: The ossification centers of the cervical vertebral centra are among the last vertebral ossification centers to appear. Relatively few are present in the day 20 fetuses. Gossypol had no effect on ossification in the cervical vertebrae.

Sternebrae: The ossification centers for sternebrae 5 and 6 are the last to appear. Gossypol had no apparent affect on their ossification.

Forelimbs: The ossification centers in the phalanges begin to appear around day 20. All phalangeal ossification centers were missing in about 70% of both experimental and control fetuses. There was no consistent effect of gossypol on the appearance of the phalangeal ossification centers. About 70-80% of all fetuses lacked only the ossification center in the first metacarpal. The more primitive condition, absence of both the #1 and #5 ossification center appeared to be more prevalent in the fetuses from sesame oil-treated matings.

TABLE IV

MALFORMATIONS IN FETUSES FROM MATINGS WITH GOSSYPOL-TREATED MALES

Treatment	Dosage	Male No.	Malformation	Time of Occurrence
Gossypol	20 mg/kg/day	43	one fetus:hydronephrosis	7 weeks after end of treatment
		27	one fetus:tailless,imperforate anus,fused kidney	8 weeks after end of treatment
		32	one fetus:right-sided aortic arches	6 weeks after end of treatment
	10 mg/kg/day	43	one fetus:right-sided aortic arches	8 weeks after end of treatment
		46	one fetus:gastroschisis	3 weeks after end of treatment
		58	one fetus:right-sided aortic arches	1st week of treatment
			one fetus:tailless,imperforate anus	6 weeks after end of treatment
		60	one fetus:hydronephrosis	6 weeks after end of treatment
		62	one fetus:hydronephrosis	1 week before treatment
			one fetus:hydronephrosis	3rd week of treatment
		10 fetuses malformed of 4,213 surviving fetuses (0.2%)		
Sesame Oil		38	one fetus:anophthalmia	5th week of treatment
		45	one fetus:cleft palate	4th week of treatment
		54	one fetus:microphthalmia	9th week of treatment
		3 fetuses malformed of 2,524 surviving fetuses (0.1%)		

TABLE V

GOSSYPOL-TREATED MALES (20 mg/kg) AND FETAL SKELETAL DEVELOPMENT

	Pretreatment		During Treatment		Post-treatment	
	gossypol	sesame oil	gossypol	sesame oil	gossypol	sesame oil
Number of fetuses examined	60	39	216	166	265	193
MISSING OSSIFICATION CENTERS (%)						
1. Cervical vetebrae centra						
C1-6	95.8	97.0	94.1	89.7	91.3	94.6
C7	71.5	56.4	55.0	53.6	57.3	55.4
2. Sternebrae						
#5	28.3	30.7	28.2	16.2	25.6	25.9
#6	13.3	15.3	13.4	9.4	13.5	22.7
3. Forelimb phalanges						
#1-5	68.4	64.1	67.5	63.2	73.9	81.3
metacarpals						
#1	85.0	76.9	75.4	85.5	70.1	65.8
4. Hindlimb phalanges						
#1-5	98.3	100	99.0	96.3	96.2	99.4
metatarsals						
#1	98.3	100	99.0	96.3	93.9	97.4
5. Basioccipital bone	71.6	84.6	73.6	64.4	76.9	65.8
6. Hyoid bone	10.0	0	7.8	3.0	7.1	6.7

Hindlimbs: Very nearly 100% of all fetuses from both experimental and control matings lacked the ossification centers in all phalanges, and nearly all lacked the ossification center in the first metatarsal.

Head: There was no marked difference between experimental and control fetuses in the absence of the ossification center for the basioccipital bone; it was absent in approximately 70% of all fetuses. The hyoid bone, on the other hand, was present in almost all fetuses.

Another way to attempt to quantify effects on ossification is to look for reductions in the size of the ossification centers (Table VI).

13th rib: Occasionally the extent of ossification of the 13th rib was reduced, but, as the table shows, this was a rare occurrence.

Double Centra: Double centra indicate a delay in maturation, but relatively few were seen in this study and, during treatment, greater numbers were found in fetuses from sesame oil-treated matings.

Sternebrae: The ossification centers of the fifth and sixth sternebrae were often found to be quite small, but there was no consistent difference between experimental and control fetuses.

Head: The supraoccipital, interparietal, basioccipital, and hyoid bones were sometimes deficient in their ossification, but there were no consistent differences between the control and experimental fetuses.

Extra rib: Rarely, an extra rib was found, but there was no difference between gossypol and sesame oil fetuses.

Wavy rib: Wavy ribs were found in greater numbers in fetuses from sesame oil-treated matings than in fetuses from gossypol-treated matings.

Skeletal features were also examined in fetuses from dams mated with 10 mg/kg gossypol-treated males. A total of 466 fetuses were examined from the experimental matings and 188 fetuses from the control matings. The results did not differ significantly from those observed in the 20 mg/kg group. There were minor differences between ossification in the experimental and control fetuses, but no trend was established and no dose response was demonstrated.

Table VII presents the results observed in behavioral tests of the offspring from matings between untreated females and either gossypol-treated or sesame

TABLE VI

GOSSYPOL-TREATED MALES (20 mg/kg) AND FETAL SKELETAL DEVELOPMENT

	Pretreatment		During Treatment		Post-treatment	
	gossypol	sesame oil	gossypol	sesame oil	gossypol	sesame oil
Number of fetuses examined	60	39	216	166	265	193
REDUCED OSSIFICATION CENTERS (%)						
1. 13th rib	1.6	7.6	2.7	1.8	1.5	3.1
2. Double centra	3.3	0	0.9	6.6	2.6	1.0
3. Sternebrae						
#5	70.0	64.1	58.3	71.6	68.3	62.1
#6	41.6	41.0	25.9	30.7	43.0	29.5
4. Supraoccipital bone	11.6	15.2	11.4	3.6	12.6	9.7
5. Interparietal bone	20.0	15.3	11.5	10.2	12.8	11.9
6. Basioccipital bone	28.3	15.3	26.8	34.9	21.8	33.0
7. Hyoid bone	6.6	15.3	11.0	12.6	4.4	7.2
EXTRA 14th RIB	0	0	0.9	0.6	1.8	1.5
WAVY RIBS	0	2.5	0.4	0	0.3	1.5

TABLE VII

BEHAVIORAL STUDIES

F1 Generation

Test	Day of Occurrence	Percent Positive gossypol[1]	Percent Positive sesame oil[2]
Righting Reflex	1	95	100
	2	5	0
Pina Detachment	2	9	60
	3	73	40
	4	18	0
Negative Geotaxis	4	58	30
	5	27	52
	6	15	14
	7	0	4
Downy Hair Present	5	86	100
	6	14	0
Palmar Grasp	6	89	88
	7	11	12
Fur Present	9	100	100
Auditory Startle Reflex	11	38	52
	12	47	43
	13	15	5
Full Eye Opening	14	36	48
	15	30	35
	16	34	17
Free-Fall Righting Reflex	17	77	100
	18	23	0
Testes Descent	24	20	35
	25	60	61
	26	20	4
Vaginal Opening	30	25	25
	31	0	38
	32	25	13
	33	50	12
	34	0	12

[1]50 pups tested. [2]61 pups tested.

oil-treated males. The events occurring during the first week of postnatal life (righting reflex, pina detachment, negative geotaxis, presence of downy hair, and attainment of the palmar grasp) had relatively little variation between the experimental group and the control group. During the second week of postnatal life, observations were made for the presence of fur, auditory startle reflex, full eye opening, and the free-fall righting reflex. The final two events evaluated, descent of the testes and opening of the vagina, occur during the 5th and 6th postnatal week. Minor discrepancies were found between experimental and control pups, but they could be accounted for by age differences at the time of testing. Deliveries were not always observed, especially at night and during weekends and, therefore, the exact ages of the pups was sometimes unknown.

Tables VIII and IX summarize the effects of gossypol when administered to pregnant dams as a single gavage dose during one day of pregnancy. The days of treatment were gestation days 6, 8, 10, 12, 14, or 16. All viable day 20 fetuses were fixed in Bouins' fluid and subsequently sectioned. Neither the 10 mg/kg dose nor the 20 mg/kg dose had any adverse effect in the dams. Their pregnancies were uneventful, and the dams remained healthy throughout the experiment. The fetuses were examined for gross and soft tissue malformations only. Gossypol had no significant effect on the number of resorptions. Because the highest dose had no effect, very few rats were tested at the low dose. Fetal weight appeared unaffected by gossypol treatment. A possible decrease in fetal weight following the 10 mg/kg dose at day 10 and 16 is indicated in the table. However, only three dams were dosed at each day, and the decreased weight may be spurious. There were very few malformations seen in either the experimental or the control fetuses. Of 1,266 fetuses from gossypol-treated dams examined for gross and soft tissue malformations, only three fetuses were found to be abnormal (0.2%). A dam treated at day 10 had one fetus with anophthalmia and cleft palate, a dam treated at day 14 had one fetus with microphthalmia, and another fetus with anophthalmia. Of 648 fetuses examined from sesame oil-treated dams, two were abnormal (0.3%). Both abnormal fetuses were found in litters of dams treated with sesame oil at day 12. One fetus was agnathic and the other had right-sided aortic arches. The incidence of malformations was very low in both experimental and control litters, less than the expected spontaneous incidence for the Sprague-Dawley rat (0.5%). No dose response was demonstrated.

Table X gives the results of the effect of gossypol on pregnancy when administered throughout the period of organogenesis (day 6-15). There was no adverse effect of

TABLE VIII

PREGNANCY OUTCOME FOLLOWING MATERNAL TREATMENT WITH GOSSYPOL

Day of Treatment	Dosage mg/kg	Number Gavaged/ Survived	Number Implant. Sites	Sites/ Litter	% Resorbed	% Survivors Malformed	Fetal Weight (g) means±SD
6	10	3/3	41	13.6	2.4	0	3.62±0.25
	20	15/15	206	13.7	8.2	0	3.75±0.25
	0	10/10	107	10.7	11.2	0	3.65±0.42
8	10	3/3	39	13.0	5.1	0	3.77±0.30
	20	14/14	194	13.8	3.1	0	3.71±0.24
	0	10/10	126	12.6	9.5	0	3.49±0.53
10	10	3/3	41	13.6	4.8	0	3.42±0.15
	20	17/17	225	13.2	4.4	0.4[1]	3.75±0.49
	0	10/10	141	14.1	6.3	0	3.87±0.33

[1]One fetus with anophthalmia and cleft palate.

TABLE IX

PREGNANCY OUTCOME FOLLOWING MATERNAL TREATMENT WITH GOSSYPOL

Day of Treatment	Dosage mg/kg	Number Gavaged/ Survived	Number Implant. Sites	Sites/ Litter	% Resorbed	% Survivors Malformed	Fetal Weight (g) mean±SD
12	10	3/3	43	14.3	0	0	3.62±0.07
	20	16/16	224	14.0	9.8	0	3.79±0.40
	0	10/10	118	11.8	7.6	1.8[1]	3.73±0.31
14	10	3/3	32	10.6	6.2	3.1[2]	4.05±0.43
	20	10/10	112	11.2	3.5	0.9[3]	4.05±0.38
	0	9/9	104	11.5	4.8	0	3.77±0.36
16	10	3/3	36	12.0	5.5	0	3.19±0.71
	20	12/12	152	12.7	7.2	0	3.67±0.27
	0	8/8	98	12.2	4.0	0	3.68±0.23

[1]One fetus with agnathia, one fetus with right-sided aortic arches; [2]One fetus with microphthalmia; [3]One fetus with microphthalmia.

TABLE X

PREGNANCY OUTCOME FOLLOWING MATERNAL TREATMENT WITH GOSSYPOL

Day of Treatment	Dosage mg/kg	Number Injected/ Survived	Number Implant. Sites	Sites/ Litter	% Resorbed	% Survivors Malformed	Fetal Weight mean±SD
6-15	5	15/15	213	14.2	7.0	0.5[1]	3.48±0.67
	10	15/15	213	14.2	6.5	0	3.83±0.26
	20	15/15	188	12.5	3.7	1.1[2]	3.65±0.30
	0	24/24	342	14.2	4.9	0.3[3]	3.71±0.30

[1] One fetus with agnathia.

[2] One fetus with anophthalmia and micrognathia; one fetus with anophthalmia, exencephaly, and umbilical hernia.

[3] One fetus with umbilical hernia.

gossypol on the health of the pregnant dam. The number of implantation sites was somewhat reduced in litters of dams receiving the highest dose of gossypol. It is unlikely that this reduction was caused by the gossypol treatment. Implantation is probably complete at the time of the first treatment at day 6, therefore, any reduction in implantation sites should be reflected in a corresponding increase in resorptions. This was not observed. The incidence of resorption in the 20 mg/kg group was less than that found in the two other gossypol groups, and also less than that in the sesame oil control group. Therefore, it is unlikely that a causal relationship exists between the administration of gossypol and the incidence of resorption. The weight of the day 20 fetus was unaffected by gossypol treatment. Of 578 fetuses examined from gossypol-treated dams, 3 were malformed (0.5%). One fetus in the 5 mg/kg group was agnathic. One fetus in the 20 mg/kg group had anophthalmia and micrognathia, and one fetus had anophthalmia, exencephaly, and umbilical hernia. Of 325 fetuses examined from dams treated with sesame oil, one was malformed (0.3%). The abnormal fetus had an umbilical hernia. The incidence of malformations was very low in both experimental and control fetuses, no higher than the expected spontaneous incidence for the Sprague-Dawley rat.

Skeletal examination of fetuses from dams treated with gossypol or sesame oil at one day of pregnancy, day 6, 8, 10, 12, 14, or 16, revealed no significant differences in development at any day of treatment. A total of 466 fetuses were examined from sesame oil-treated dams, 645 fetuses were examined from dams treated with 20 mg/kg gossypol, and 158 fetuses examined from dams treated with 10 mg/kg gossypol. There were no significant differences in skeletal development between the experimental and control fetuses of fetuses from dams treated daily throughout the organogenetic period (days 6-15).

DISCUSSION

Gossypol can induce both morphological and biochemical alterations in spermatozoa. As long as spermatozoa retain their ability to fertilize, however, there is apparently no adverse effect on the outcome of that fertilization. The high dose of gossypol (20 mg/kg/day) used in this experiment markedly interfered with weight gain during the period of treatment. Subsequently, the gossypol-treated males resumed weight gain, but at the termination of the experiment they still had not always caught up with the sesame oil-treated controls. The low dose of gossypol (10 mg/kg/day) had a much less dramatic effect on weight gain in the treated males. A dose-correlated suppression of weight gain is a

common finding with gossypol treatment.

Based on the results of the present study, it can be concluded that gossypol treatment of male rats does not influence the outcome of pregnancies resulting from conception during the treatment period or after the treatment period. There were no observable effects on fetal weight or on the incidence of resorption or malformation. The results demonstrate that there are individual variations in the response of males to gossypol treatment with respect to the time of onset and the duration of infertility. This experiment did not permit the distinction between variations inherent in the male and variations induced by gossypol. All males recovered their fertility, however. The antifertility effect of gossypol was obtained without any marked toxicity to the males, in contrast to the report by Weinbauer et al. (23).

The incidence of malformations was very low in this experiment, lower than that expected to occur spontaneously in a colony of untreated Sprague-Dawley rats. Regardless of treatment, there was never more than one malformed fetus in any given litter. Each malformation observed in both experimental and control fetuses has been described as a spontaneous malformation in the Sprague-Dawley rat. A dose-response was not demonstrated, and there was no distinct or reproducible syndrome of malformations. These observations suggest that the malformations are not due to the treatment of males with gossypol.

A comparison of the results of the skeletal studies reveals no significant pattern to the variation in ossification between fetuses from gossypol-treated matings and fetuses from sesame oil-treated matings. There was no consistent dose-response demonstrated. In many cases, there was little or no change in ossification within each dose group when the results were compared between pretreatment, treatment, and post-treatment periods. Overall, there is nothing in the results obtained to suggest gossypol treatment of males has an adverse effect on the development of the fetal skeleton.

Minor variations in the timing of the events associated with postnatal maturation occurred between pups from gossypol-treated matings and pups from sesame oil-treated matings. However, the variation in time rarely exceeded 24 hours and, therefore, could easily be caused by differences in age of the pups at the time of testing. The exact age of all the pups was not known because not all births were observed.

The effect of gossypol treatment of the female has not been studied extensively. Because of its physical

properties, gossypol is assumed to reach the embryo (3). Hahn et al. (4) found that doses of gossypol up to 90 mg/kg administered for the three consecutive days prior to ovulation had no effect on ovulation in the rat. They also reported that treatment of pregnant mice from day 1-13 of pregnancy resulted in increased numbers of nonviable fetuses. However, the authors raised serious questions about the unhealthy condition of the mice used in their experiment. Wu et al. (24) examined gossypol-treated hamsters for effects on endocrine function, ovulation, and fertility. They reported a rise in serum FSH, a fall in pituitary FSH, and a higher serum and ovarian concentration of estrone and estradiol in gossypol-treated hamsters compared with controls. The estrous cycle remained normal and there was no effect on ovulation. Wu et al. (24) also reported that gossypol failed to alter the pregnancy rate or affect the outcome of pregnancy; all fetuses appeared normal with no retardation of growth. However, only five pregnant hamsters were examined. Barcellona et al. (25) treated pregnant rats throughout organogenesis (day 6-15) with gossypol in dosages of 12.5 to 100 mg/kg/day. They concluded gossypol was not teratogenic and was embryocidal only at concentrations that were toxic to the dam.

In the present experiment, gossypol administered to pregnant dams, either on a single day during gestation or throughout the organogenetic period, had little observable effect on the outcome of pregnancy. There was no effect on fetal weight at day 20 of gestation, and there was no increase in resorptions. Maturation of the pups during the first month of postnatal life was unaffected by gossypol treatment. Examination of the fetal skeleton revealed no significant difference between experimental and control fetuses. The incidence of malformations was lower in this experiment than the spontaneous incidence expected in a colony of Sprague-Dawley rats (0.5%). This observation, together with the lack of a demonstrable dose-response and the lack of a distinct or reproducible syndrome of malformations indicates that, at the doses used in this experiment, gossypol is not teratogenic in the pregnant rat.

Under the conditions of these experiments, gossypol administered to either the breeding male rat or the pregnant female rat had no significant adverse effect on the outcome of conception.

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