Ann. Rev. Pharmacol. Toxicol. 1984. 24:329–60

GOSSYPOL: A POTENTIAL ANTIFERTILITY AGENT FOR MALES

Shao-Zhen Qian

Department of Basic Medical Research, Jiangsu Family Planning Institute, 123 Tian Fei Xiang, Nanjing 210005, People's Republic of China

Zhen-Gang Wang

Department of Pharmacology, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences, 5 Dong Dan San Tiao, Beijing, People's Republic of China

INTRODUCTION

Gossypol is a yellowish phenolic compound occurring naturally in certain species of cotton plants of the family Malvaciae, mostly in the seeds and root bark. At one time, gossypol was considered only a toxic waste in the processing of cottonseed products (1). In 1957, however, Liu (2) reported that Wang village in Jiangsu, China, had not had a single childbirth for as long as 10 years, from the 1930s to the 1940s, while before and after this period of collective infertility the villagers had been fecund. It was found that in these unprolific years the villagers had switched for economic reasons from soybean oil to crude cottonseed oil for cooking purposes. In the face of this evidence, Liu suggested that gossypol, the biologically active substance in cottonseed, might cause female infertility, particularly since many of the female villagers suffered menstrual disturbances at the same time. Later, the Hubei Provincial Group (3) documented the antispermatogenic effects of crude cottonseed oil in rats and monkeys, and since then a number of workers have investigated the active principle(s) in cottonseed and cotton root bark. Several groups (4–11) almost simultaneously demonstrated the male antifertility effect of gossypol.

The work on gossypol as an antifertility agent, initiated by Liu (2) and pursued actively by these groups, has stimulated nationwide attention; after the

0362-1642/84/415-0329$02.00

publication of a review article by the National Coordinating Group (12), it attracted universal interest. Various aspects of the subject have been reviewed by many authors (13–22). The present paper is a general review of gossypol as a potential male contraceptive, focusing on the pharmacological features not fully discussed in previous review articles.

THE CHEMISTRY OF GOSSYPOL

Gossypol has a molecular weight of 518.54 and a structure of (2,2'-binaphthalene)-8,8'-dicarboxaldehyde-1,1',6,6',7,7'-hexahydroxy-5,5'-diisopropyl-3,3'-dimethyl, as documented by Edwards (23) through total synthesis of the molecule. If gossypol is recrystallized in different solvents three crystalline substances with different melting points can be obtained. These substances have different optical properties and crystalline forms but show no weighty differences in their chemical and spectral behavior, suggesting not much dissimilarity in their chemical structures. However, many reactions of gossypol cannot be explained by this structure.

In an attempt to clarify the multiplicity of reactions of gossypol, Adams & Geissman (24) proposed that gossypol exists in three tautomeric forms: the aldehyde, the ketonoid, and the hemiacetal (Figure 1). Although the presence of the three tautomers of gossypol can be authenticated by the preparation of their respective derivatives, substantial concentrations of the hemiacetal or ketonoid forms have not yet been ascertained. Recently, the use of NMR to

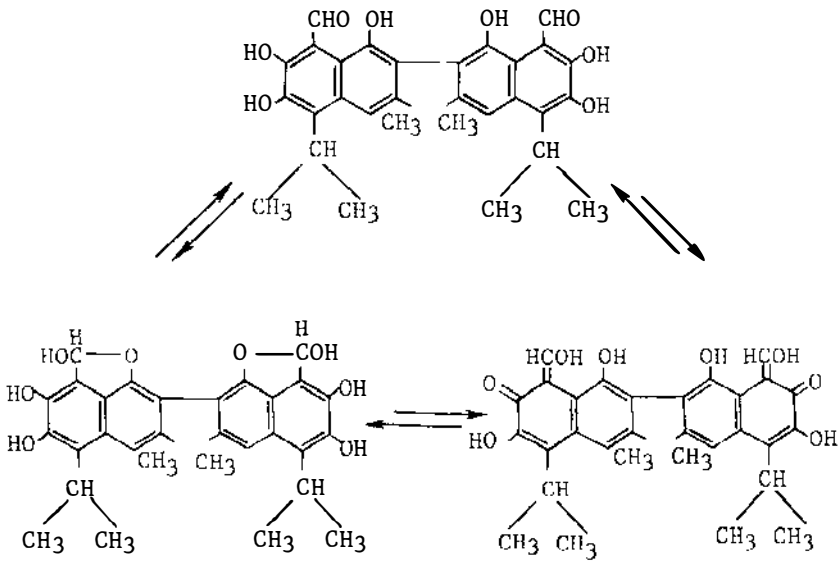

Figure 1 Tautomeric forms of gossypol

study the chemical shifts of gossypol in various solutions has shed further light on its structural changes. In ordinary inert solvents, gossypol exists mainly in aldehyde form, while in polar solvents, such as DMSO, the hemiacetal form occurs in dynamic equilibrium with the aldehyde form (25).

There are two optical isomers of gossypol. Initially, gossypol was only isolated from the Gossypium species as a racemate. Not long ago, the (+)−isomer was isolated first from Thespesia populnea, then from the Gossypium species (26, 27). The spectral characteristics and melting points of the (+)−isomer and the racemate are identical, but the former is more soluble in ordinary organic solvents than the latter. The melting points of the corresponding derivatives of the (+)−isomer and the racemate are different. Resolution of the racemate has recently been achieved (27a). It has been suggested that the optical activity of gossypol is the consequence of atropisomerism, i.e. the restriction of rotation of the two naphthalene units about the interlinking C-C bond.

THE ANTIFERTILITY EFFECT OF GOSSYPOL

Three forms of gossypol, gossypol, gossypol acetic acid, and gossypol formic acid, have been used in laboratory investigations and clinical trials. They are very much the same in their biological activities; therefore, they will be discussed as a whole under the generic name gossypol. Unless specified otherwise, the term refers to the racemate administered orally.

There are pronounced differences among animal species in their sensitivity to the antifertility action of gossypol. Among the laboratory animals tested, hamsters seemed to be the most sensitive, followed by rats, monkeys, and dogs in decreasing order, while rabbits and mice appeared to be insensitive (7, 12, 28–31). The effective dose for hamsters was 5–10 mg/kg per day, given for 6–12 weeks; recovery of fertility occurred 4–14 weeks after withdrawal of the drug (29, 30, 32, 33).

The effective dose for rats ranged from 10 to 30 mg/kg per day, given for 3–10 weeks. Onset of infertility was dose-dependent; the recovery of fertility after treatment ceased was also related to the dose, occurring 3–12 weeks after withdrawal (5, 7, 9, 12, 34–43). Gossypol at a dose of 7.5 mg/kg per day administered for longer period of time could also induce infertility in rats (5, 42, 45), but 3 mg/kg per day given for 16 weeks was without effect (5). With regard to the minimal effective dose, reports are inconsistent. 5 mg/kg per day given for 6 weeks (7) or 4 weeks (46) led to infertility, but 6 mg/kg per day given for 5 weeks was said to be ineffective (39). The sensitivity of rat testes toward gossypol showed marked individual variation; long-term treatment might cause complete atrophy of the seminiferous epithelium in some of the animals, and sterility is the likely consequence (47, 48) of this condition.

In dogs, gossypol appeared to be barely antispermatogenic, although toxic doses of gossypol could more or less inhibit spermatogenesis (28, 49). In rabbits, gossypol did not induce infertility. 10mg/kg per day given for 14 weeks hardly affected the sperm concentration and motility in the ejaculate; the pregnancy rate and the implantation sites of female rabbits inseminated with spermatozoa from the treated males did not differ significantly from the control (29). When rabbits were given gossypol at a dose of 10 mg/kg per day for 77 to 250 days, both the sperm data and fertility were not significantly affected despite severe toxicity resulting in eventual death (31). Gossypol did not inhibit spermatogenesis in mice. An oral dose of 15–30 mg/kg per day did not significantly affect the motility of epididymal and vasal spermatozoa (7, 50); the same was true with toxic doses (7, 30). It is interesting to note that, although gossypol did not affect spermatogenesis in mice, it inhibited pregnancy in female mice during the first two weeks of gestation (30).

Monkeys are moderately sensitive to the antispermatogenic action of gossypol. When rhesus monkeys were given a dose of 4 mg/kg per day for two years, spermatogenesis was completely inhibited in two of the three animals; in the third a few normal spermatids and spermatozoa could still be found in some of the seminiferous tubules (28). In cynomolgus monkeys, gossypol at a dose of 10 mg/kg per day given for as long as 6 months only decreased the sperm count and motility in the ejaculate (51).

It may be worth mentioning that it is gossypol itself and not the impurities present in the crude preparation that is antispermatogenic (32), and that the (+)−isomer isolated from Thespesia populnea is not antispermatogenic in rats (7, 52) and hamsters (52a) at dose levels higher than the effective dose of the racemate. Both the (+)−isomer and the racemate inhibited implantation in female rats, the suggested mechanism being inhibition of histamine release (53) or blocking of the LH effect (54). Gossypol inhibited sperm motility in vitro and in vagino (44, 55–61a), as well as when it was injected into the periepididymal fat (62) or directly into the epididymis (63). The spermicidal effect in vitro of the water-soluble coprecipitate of gossypol-polyvinylpyrolidone was more powerful than that of gossypol (57). Gossypol also reduced the ability of human sperm to penetrate both cervical mucus and zona-free hamster ova (61a).

THE METABOLISM OF GOSSYPOL

Gossypol is absorbed through the intestine as well as through the epithelial lining of the stomach (64). In this connection, it is interesting to note that, in an attempt to mitigate the gastric side effects of gossypol, one group of clinicians adopted enteric-coated tablets for the clinical trial. They found that both the antifertility effect and the systemic side effects of these tablets were much less than those of ordinary tablets (R. A. Zhang, personal communication). Apparently the differing response was due to decreased gastric absorption of

gossypol caused by the enteric coat. Fecal excretion was the major route by which gossypol, administered either orally or parenterally, was removed from the animal body. Most of the absorbed gossypol was excreted via the bile, suggesting biliary circulation of gossypol between the liver and the intestine (64–73). High concentrations of gossypol in the bile are in harmony with the tentative conclusion that compounds of high molecular weight containing polar anionic groups and two or more aromatic rings tend to be excreted into the bile.

In rats given a single oral dose of (^{14}C) gossypol (64) in a 13-day experimental period, 77.4% of the ingested radioactivity was recovered from the feces, 12.1% from the expired CO_2, and 3.1% from the urine. The accumulation of radioactivity in the tissues was relatively low, totaling 12.5% of the administered dose one day after administration. Besides the gastrointestinal contents, which contained the highest radioactivity, the tissues examined one day after administration arranged according to their specific activity in decreasing order were as follows: gastrointestinal tract, liver, heart, kidney, spleen, lung, blood, muscle, adipose tissue, testis, and brain. After 24 hours, the activities of all these tissues gradually decreased, with minor fluctuations, and on the 13th day total tissue activity was only 0.28% of the administered dose. The peak of radioactivity in almost all the tissues examined was on the first day after administration. Reabsorption of gossypol from the renal tubule through the mechanism of nonionic absorption might account for the low urinary excretion of radioactivity.

A relatively large quantity of the ingested activity was recovered from the expired CO_2; for this reason, the investigators believed that decarbonylation was a major route of gossypol biodegradation in rats. The biological half-life of radioactivity in the body was 48 hours. Skutches & Smith indicated that the binaphthalene nucleus of the gossypol molecule was not degraded and only the formyl carbon was metabolized to CO_2 (74). More or less similar results were obtained by Xue et al (70, 71), but they showed that after a single oral administration of (^{14}C) gossypol to rats, the bioliogical half-life in the body was 60 hours, and that in many of the tissues examined, including heart, spleen, kidney, adrenal, pituitary, muscle, and testis, the peak of radioactivity occurred in the fourth to ninth days post-administration. They also indicated that the pituitary, adrenal, and thyroid glands exhibited very high specific activity, even outstripping that of the liver on the second, fourth, and ninth days. The results of repeated dosing of (^{14}C) gossypol were very much the same as those of the single-dose experiment (70, 71). Wang et al (72) pointed out that the distribution and excretion of gossypol in rats and monkeys were quite similar, and that in rats the half-life of gossypol in the gastrointestinal tract was 9.6 hours, indicating a low rate of absorption. Tang et al (73) compared the metabolism of (^{14}C) gossypol in mouse, rat, dog, and monkey and found that the pattern of distribution of activity in various tissues following a single dose of gossypol was much alike in the four species. Among them, the specific

activity of the heart was the highest in the dog, that of the testis was the highest in the rat; the circulatory half-life was the longest in the dog, and the fecal excretion was higher in monkey and rat than in the other two species. Sang et al (28) indicated the greater excretion and lesser absorption of gossypol in monkey than in dog. These two groups of authors believed that the discrepancies in the metabolism of different species might have important bearing on their differential responses to the antifertility and toxic effects of gossypol. The fact that antifertility doses of gossypol selectively damaged the spermatogenic cells of rats, leaving the vital organs, which contained much higher concentrations of gossypol than did the testes, unaffected (12, 17), speaks strongly in favor of a specific vulnerability of the testicular cells to the action of gossypol. In rats treated orally with gossypol at a dose of 40 mg/kg per day for 8 weeks, the concentrations of free and bound gossypol in the liver, spleen, kidney, lung, and testis were found to increase gradually over the experimental period (75).

In pigs, the metabolism of (^{14}C) gossypol was similar to that of rats, but the tissue deposit of radioactivity was higher and the recovery from the expired CO_2 was lower. The total tissue deposit one day after administration was 32.9% in pigs (12.5% in rat), and the total recovery from expired CO_2 in a 20-day experiment was 2.1% of the dose ingested (12.1% in rats in a 13-day experiment). Low recovery from expired CO_2 indicated that decarbonylation was not an important route of gossypol biodegradation in pigs. The researchers also opined that the higher toxicity of gossypol in pig than in rat might be related to the difference in tissue deposition and decarbonylation of gossypol in these species (69).

Iron and protein form nonabsorbable Fe-gossypol chelate and protein-gossypol complex in the gastrointestinal tract; therefore, they are used to detoxify gossypol present in the cottonseed feed for livestock (1). In rats, the addition of ferrous salts to the feed reduced tissue deposition, accelerated fecal excretion, and shortened the half-life of (^{14}C) activity in the body; it also increased the respiratory elimination of (^{14}C), which might be explained by the postulation that iron catalyzes the decarbonylation process of gossypol (64). Protein supplements to the diet increased fecal excretion and decreased tissue deposition of (^{14}C) gossypol (65). In passing, it may be worth mentioning that Mg-gossypol complex has been shown to be antispermatogenic in rats, with a relatively low toxicity compared with gossypol (76).

THE TOXICITY OF GOSSYPOL

Single-Dose Toxicity

The single-dose oral LD_{50} of gossypol suspended in water is listed for some animal species in Table 1; if the drug is given in oil, the values will be 10% less

Table 1 Single-dose oral LD_{50} of gossypol (mg/kg) in water for several species

Rat	Mouse	Rabbit	Guinea Pig	Pig
2400–3340	500–950	350–600	280–300	550

[see(1)]. In rats, the single-dose oral LD_{50} of gossypol in peanut oil was 2590 ± 310 mg/kg (9).

Repeated-Dose Toxicity

The manifestations of repeated-dose oral toxicity of gossypol in some laboratory animals are outlined in Tables 2, 3, 4, and 5.

After reviewing the prolific literature relevant to the study of the toxicity of gossypol, including those not cited in this article, we have drawn the following conclusions:

1. Tolerance to gossypol varies greatly in different species. Among mice, rabbits, rats, guinea pigs, dogs, pigs, and monkeys, rats and the monkeys seem to be the most tolerant, dogs and rabbits the least. Preliminary results indicate that hamsters are even more tolerant than rats (29); female hamsters appear to be less tolerant to gossypol than males (77).
2. In rats, ordinary antifertility doses of gossypol seem to be nontoxic; only if the total dose is elevated to about 5–9 times the proposed minimal effective dose (7.5 mg/kg per day, given orally for 12 weeks), i.e. 20 mg/kg per day for 39 weeks or 30 mg/kg per day for 16 weeks, do minor lesions occur first in the liver, then in the heart or the kidneys in a few of the treated animals. Rats, as mentioned above, are sensitive to the antispermatogenic action of gossypol.
3. Monkeys tolerate the toxic effect of gossypol but are only moderately sensitive to its antispermatogenic action.
4. Dogs are very sensitive to the toxic effect but barely sensitive to the antispermatogenic effect of gossypol. Almost every dog died at each of the dose levels tested. The organs most seriously damaged are the heart and the liver: low doses seem to damage the heart more, leading to acute cardiac failure and sudden death; high doses seem to damage the liver more, causing cachexia and death. Fatal doses of gossypol induce only slight to moderate inhibition of spermatogenesis.
5. Rabbits are sensitive to the toxic but not the antispermatogenic effects of gossypol. Although the toxic manifestations are very conspicuous after gossypol administration, leading to fatality, the animals are still fertile shortly before death.

Table 2 Repeated-dose oral toxicity of gossypol in rats

Regimen (mg/kg/d)	Weeks	Manifestations	References
7.5	52	SGPT, BUN, ECG normal; histology of heart, liver, kidney normal; bone marrow and blood picture normal. Fertility suppressed, mating normal; germinal epithelium damaged but with no gross degeneration. Leydig cells unaffected.	78
7.5	12	Histology of organs normal; majority infertile, mating normal.	45
10	12	ECG normal; SGPT and gamma globulin slightly increased, but recovered after withdrawal of gossypol; in 1 out of 12 rats, SGPT highly increased with focal necrosis of liver, but histology of kidney and heart normal; for the rest, liver, kidney, heart normal. All infertile, Leydig cell unaffected.	7
10	26	Bone marrow and blood picture normal; histology of vital organs normal; no change in oil red "O" staining, G6Pase, G6PDH, ATPase, AKPase, ACPase, RNA, and DNA of liver; oil red "O" staining, G6PDH, ATPase, AKPase, ACPase of kidney; oil red "O" and Sudan Black staining and 3-OH-steroid dehydrogenase of adrenal gland. Infertile, mating normal.	79
15	10	Histology of heart, lung, kidney, liver, spleen, stomach and small intestine normal; bone marrow picture normal.	5
20	39	MAO, AKPase, PAS and oil red "O" staining of liver normal; individual animal showed slight degenerative changes in liver cell and increased SDH activity.	17
30	10	No effect on body weight and weight of accessory sex glands; hCG binding in testis normal; SDH and ATPase of testis and liver normal. Infertile; germinal epithelium damaged in part of animals.	43
30	16	No effect on body weight and weight of accessory sex glands; histology of heart, liver, lung, kidney and spleen normal; occasional minor focal inflammatory infiltration in different organs. Infertile since fifth week, damage to germinal epithelium began at second week; no effect on Leydig cell seen under electron microscope.	80

THE GENETIC EFFECT OF GOSSYPOL

Offspring Observation

Gossypol-treated rats were mated with untreated females after recovery of fertility. On gross examination, the offspring of the F_1 and F_2 generations were normal (7). Of the 8806 human volunteers taking gossypol, the spouses of 266

Table 3 Repeated-dose oral toxicity of gossypol in rabbits

Regimen (mg/kg/d)	Days	Manifestations	References
16	14–140	SGPT, NPN and blood picture normal; bradycardia and ECG changes in part of animals. 6 out of 10 died 14–140 days after dosing began	81
80	8–17	Loss of weight, loss of appetite, dyspnea, hind limb paralysis, collapse. Died 8–17 days after dosing began. Autopsy: congestion of liver and lungs. Fertility and semen spermatozoa normal shortly before death.	31
40	23–35	Similar to above. Died 23–35 days after dosing began	31
20	35–84	Similar to above. Died 35–84 days after dosing began	31
10	77–250	Similar to above. Died 77–250 days after dosing began	31

subjects conceived after withdrawal of gossypol or during the regimen. 53 gave birth to apparently normal babies; the rest of the fetuses were artificially aborted (18).

Dominant Lethal Mutagenic Effects

Male rats were given 20 mg/kg of gossypol per day for 4 weeks to make them infertile, then the animals were allowed to mate with untreated females for three rounds on days 37–40, 47–50, and 57–60 post-regimen. On the thirteenth day of pregnancy, the researchers sacrificed the females and recorded the numbers of live and dead fetuses and the number of implantation sites. They found that after the first and second rounds of mating, the ratio of dead fetuses to the number of implantation sites was significantly higher in the treated than in the control animals. However, after the third round, the ratio of the treated animals dropped to a level that did not differ significantly from that of the control. Results suggest that gossypol may damage the genetic material but the effect is transient and may decrease with time (87).

Embryotoxicity and Teratogenicity

In Wistar rats and long-haired rabbits, gossypol in dose levels 5- or 30-fold the clinical dose did not show significant embryotoxicity or teratogenicity (88).

Ames Test

De Peyster & Wang (89) indicated that gossypol was not mutagenic to the five standard tester strains of Salmonella typhimurium either with or without the inclusion of a rat-liver metabolic enzyme fraction. Similar results were obtained by many other researchers (90–94). It may be worth mentioning that

Table 4 Repeated-dose oral toxicity of gossypol in dogs

Regimen (mg/kg/d)	Days	Number of animals	Manifestations	References
1.5	60–141	4	Loss of weight, loss of appetite, weakness, caddy stool, dyspnea. All animals died suddenly 60–141 days after dosing began. Autopsy: cardiac dilatation; edema, lysis and atrophy of myocardium; congestion of liver; cloudy swelling and hyaline degeneration of renal tubules; pulmonary congestion and edema. Cause of death: acute cardiac failure. Germinal epithelium: slight to moderate damage.	49
3.0	51–64	4	Similar to above, except liver showed additional slight fatty degeneration; germinal epithelium slightly damaged.	49
30	18–28	4	Severe anorexia, loss of weight, weakness, nausea, vomiting, tarry stool, anaemia, cachexia; died 18–28 days after dosing began. Autopsy: similar to above, except liver showed moderate fatty degeneration and pachy necrosis. Cause of death: cachexia. Germinal epithelium damage: slight to moderate.	49
1.0	129–130	2	Anorexia, loss of weight, weakness, tachycardia. ECG, NPN, SGPT normal. Died suddenly 129 and 130 days after dosing began. Autopsy: myocarditis and endocarditis.	82
5.0	73	2	One dog died on day 73 of dosing, manner of death unnoticed; another sacrificed on the same day. Other manifestations similar to above.	82
1.0–3.0	25–131	12	Anorexia, vomiting, diarrhea, weakness, bradycardia, ECG abnormalities (flattening of T and prominant U). Some dogs died on day 25, 43, 60, 63, and 131 of dosing. Autopsy: cardiac dilatation and hypertrophy, endocarditis, congestion of kidney and spleen, fatty degeneration and necrosis of liver, edema and hemorrhage of lungs. Inhibition of spermatogenesis: not obvious.	17

Table 5 Repeated-dose oral toxicity of gossypol in monkeys

Regimen (mg/kg/d)	Months	Number of animals	Manifestations	References
1–2	14	3	Renal function, histology of heart and kidney, renal LDH and ATPase normal. Liver showed temporary ultrastructural changes (distension of endoplasmic reticulum, increase in lysosomes).	17
4.0	24	3	Serum Na, K, Mg, Cl, creatinine, LDH, NPN and SGOT normal. Urinary Na, K, Mg, Cl, creatinine, LDH, SGOT, AKP normal; urine concentrating ability normal. Cellular K normal, cellular Na increased. Myocardium: slightly congested, LDH-1,2 decreased, LDH-3,4,5 increased, ATPase unchanged, SDH increased or unchanged. Ultrastructure essentially normal. Liver: hepatic sinusoid slightly distended, partial vacuolation in central zone of lobule; temporary changes in ultrastructure (distension and vacuolation of endoplasmic reticulum, lysosomes increased, ribosomes decreased, mitochondrial damage, decrease of cristae); LDH, G6PDH, ATPase and RNA unchanged. Kidney: cloudy swelling of proximal tubules, mitochondrial damage; ATPase and ACPase decreased, G6PDH and juxtaglomerular cell granules unchanged. Histology of spleen, stomach, intestine and adrenal normal. Germinal epithelium markedly damaged in two animals, but in the third a few normal spermatids and spermatozoa still present.	17, 28, 83–86a
8.0	4	2	Myocardial striation obscure with occasional cloudy swelling and acidocytosis. Liver: slight cloudy swelling, fatty infiltration, focal inflammation and acidocytosis. Cloudy swelling of renal tubule.	17
5 or 10	6		No serious clinico-pathological side effects. Sperm count and motility depressed.	51

in mice skin-painting tests, gossypol showed tumor-inducing or promoting activity (95). Lifetime carcinogenicity data so far are unavailable.

Chromosomal Observations

The effect of gossypol on the frequency of occurrence of sister chromatid exchange (SCE), micronuclei, and chromosomal aberrations has been investi-

gated repeatedly by different groups and the results are roughly consistent: low (5-fold clinical dose) or medium (around 10-fold clinical dose) dose levels did not appear to damage the genetic material in vivo, while high doses (more than 30-fold clinical dose) did. The same dose-dependent relationship also seems to hold true with in vitro experiments. In humans, routine clinical dosages did not seem to affect genetic material. The results reported by different workers are listed in Table 6.

THE ENDOCRINE EFFECT OF GOSSYPOL

Gossypol does not demonstrate androgenic, antiandrogenic, estrogenic, or antiestrogenic activity, but it does potentiate the androgenicity of methyl testosterone (30). In regard to the effect of gossypol on the endocrine glands or tissues, three main kinds of investigations have been carried out, including morphological studies, in vitro studies, and studies of the effect on blood hormone levels. The results of these investigations will be discussed separately in the following paragraphs.

Morphological Studies

THE EFFECT ON THE HYPOTHALAMUS In gossypol-treated rats, the cell size, the volume of cytoplasm, and the number of cytoplasmic granules in the neurons of the paraventriculus nucleus were decreased, while the nuclei of these cells were distended, assuming a vesicular appearance (108).

THE EFFECT ON THE PITUITARY In rats, gossypol reduced the number of cytoplasmic granules in the gonadotrophs (108), increased the number of active-stage gonadotrophs, decreased the number of quiescent-stage gonadotrophs, and led to the appearance of cells similar to castration cells (109). It may bring about hydropic degeneration of β_1 cells and more or less affect all types of pars distalis cells (110).

THE EFFECT ON THE LEYDIG CELL A majority of the researchers working on the question reported that gossypol does not affect the morphology of Leydig cells (7, 28, 80, 109, 111, 112). However, in testicular biopsy specimens from sterile men with a history of using crude cottonseed oil, Leydig cells were reduced in number and showed early signs of degeneration (113). Moreover, in rats treated with gossypol, the cell size, the volume of cytoplasm, and the smooth endoplasmic reticulum of Leydig cells were reduced, with increased number of lysosomes and occasional vacuolization (113a).

In Vitro Studies on Testicular Steroidogenesis

Lin et al (114) found that adding 10^{-5}M and 10^{-7}M of gossypol to rat Leydig cell culture reduced LH-stimulated production of testosterone (T) in the culture;

Table 6 Chromosomal observations[a]

Species	Dose (μg/ml in vitro; mg/kg/d per os)	Chromosomal aberration (cell type)	SCE (cell type)	Micronuclei (cell type)	References
Hamster	0.23–2.3 in vitro		Negative (CHO)		96
Hamster	0.2–2.0 in vitro		Negative (CHO)		97
Rat	20, oral 9 days	Negative (SG, SC)		Negative (LP)	98
ICR mice	4, oral 4 days		Negative (BM)		99
ICR mice	1–10, oral 19 days	Negative (SG, SC)	Negative (SG)		100
ICR mice	3 and 8, oral 9 days		Negative (SG)		101
ICR mice	20–50, oral 19 days		Increased (SG, SC)	Increased (SG)	100
ICR mice	20, oral 9 days		Increased (SG)		101
Kunming mice	4, oral 14 days	Negative (SG)	Negative (SG)		102
Man	10 in vitro		Negative (LP)		92
Man	1–9 in vitro	Negative (LP)	Increased (LP)		103
Man	0.2–2.0 in vitro		Negative (LP)		97
Man	0.005–0.1 in vitro	Negative (LP)			104
Man	Routine 2 years		Negative (LP)	Negative (LP)	105
Man	20 mg/day 78–85 days	Negative (LP)			106
Man	Routine 34–50 months	No significant effect on number of nucleolar organizing regions and occurrence of acrocentric chromosome association in peripheral lympocytes.			107

[a]BM = bone marrow cell; CHO = Chinese hamster ovary cell; LP = peripheral lymphocyte; SC = spermatocyte; SG = spermatogonium; routine = 20 mg/day for 60–70 days, followed by a maintenance dose of 40–50 mg/week orally.

they also discovered that LH-stimulated T production by Leydig cells from rats treated with gossypol was significantly lower than that by Leydig cells from control rats. Therefore, these authors concluded that gossypol depressed testicular steroidogenesis. Several other groups obtained similar results in rats (41, 115, 116) and in rabbits (31). However, Zhuang (117) indicated that these concentrations of gossypol did not decrease hCG-induced T production per 10^6 Leydig cells of rat and that T concentration in the culture media was lowered as a consequence of the decreased number of Leydig cells, apparently due to the addition of gossypol. The (^{125}I) hCG binding in testis homogenate of gossypol-treated (25–30 mg/kg/d over 10 weeks) rats was similar to that of untreated animals, suggesting noninterference of gossypol in hormone action at the target level (43).

In connection with these in vitro experiments, it is advisable to note that the actions of gossypol in vitro and in vivo are remotely different in many respects. For example, in isolated rabbit heart, Qian et al (118) found that gossypol 0.5 or 1.0 μg/ml in Locke's solution completely inhibited ventricular contractility, while free gossypol 2.13–2.25 μg/ml in blood (donated by other rabbits fed gossypol at a dose of 30 mg/kg per day for 8–12 days) did not inhibit it at all. It has been reported that most, if not all, gossypol in the body is conjugated with different molecules of the organism and that so-called free gossypol is actually conjugates of gossypol with micromolecules. Their actions will naturally differ from those of gossypol in vitro. Therefore, special care should be taken in the interpretation of in vitro experimental results.

Effect on Blood Hormone Levels

As can be seen from Table 7, the results from various experiments are largely inconsistent, particularly in regard to the effect of gossypol on T level, which may be due to differences in animal species (strains), drug purity and dosage, or other experimental conditions employed.

CLINICAL TRIALS OF GOSSYPOL

The initial clinical trials of gossypol as a male antifertility agent were carried out by Qian et al (11) in 1972. Although customarily undertaking clinical trials so soon after documentation of an antifertility effect in animals would be unseemly, in this case the trial was completely justified. In the first place, humans have used gossypol and gossypol-containing drugs and foods for a long period of time with few adverse consequences (123–127). Second, generally accepted standards have been set for gossypol consumption by humans, being 450 mg/kg in food in the United States and 600 mg/kg as recommended by international groups (128). Moreover, before the discovery of the antifertility action of gossypol, Qian's group had documented gossypol as the active

Table 7 Effect of gossypol on blood hormone levels

Species	Regimen (mg/kg/d)	Weeks	T	LH	FSH	References
Rat			Negative	Negative	Negative	12
Rat	7.5	12	Negative	Negative	Negative	42, 45
Rat	12	6	Negative			7
Rat	15	12	Negative	Negative	Negative	42, 114
Rat	25	10	Negative	Negative		43
Rat	30	5		Negative		119, 120
			Response to LHRH normal			
Rat	30	5 or 6	Lowered	Lowered	Negative	41, 42, 114
Rat	30	5	Lowered	Negative	Negative	40
Rat	20	4	Lowered	Negative		113a
Young rat	20	8	Lowered	Negative		113a
Hamster	10 or 15	5	Negative	Negative		33
Hamster	10 or 15	10, 8	Lowered	Negative		33
Rabbit	20 or 10	12–20	Lowered			31
Monkey	2	13	Negative	Negative	Negative	45
Monkey	4	39–87	Negative			7
Monkey	8	7	Negative	Negative		45
Monkey	8	17	Negative			7
Monkey	10	36	Negative			51
Man	Routine		Negative	Negative	Negative	12, 17, 18
			Response to LHRH normal			143, 145
Man	Routine	36–52	Negative	Negative		121
			Response to LHRH and hCG normal			
Man	Routine		Negative	Negative		146
Man	Routine total dose 3–17 gm		25% increased	Negative	50% increased	122

principle of cotton root bark, a Chinese folk medicine for the treatment of chronic bronchitis and cough, and had carried out clinical trials of gossypol on bronchitic patients.

Qian et al (11) found that gossypol given orally at a dose of 60–70 mg per day for 35–42 days caused a gradual increase in the percentage of nonmotile spermatozoa in the ejaculate, followed by oligospermia, necrospermia, and azoospermia in all 25 volunteers. Interestingly, sperm motility decreased markedly as early as the second week of administration, suggesting that gossypol may act on epididymal or testicular spermatozoa. Recovery occurred around three months after withdrawal. The side effects of this dosage were reversible and generally of mild degree, mainly including decrease or increase in appetite, fatigue, dryness of mouth, diarrhea, inconsiderable elevation of

SGPT, and a tendency to sleepiness; individual cases suffered slight oedema of the eyelid, seemingly decreased libido and potency, and insignificant depression of serum potassium levels. When the dose was decreased to 24–35 mg per day and the duration of treatment appropriately prolonged, the side effects were much reduced and the antifertility effect was retained. In all subjects except three who had trivial complaints, health status was good one year after the gossypol regime ended. The investigators concluded that gossypol was an effective antispermatogenic agent in men, but that the significance of the side effects necessitated further toxicological studies (11).

After a series of toxicological studies, second- and then third-phase trials were carried out in several parts of China. Until 1980, the total number of volunteers had amounted to 8806 (18). Optimal or routine loading and maintenance doses were determined to be 20 mg/day for 60–70 days and 40–50 mg/week respectively. With this dosage level, antifertility efficacy was 99.07%. The common side effects in men taking routine doses were similar to those reported by Qian et al (11); additionally, an infrequent but important side effect, hypokalemic paralysis, was uncovered during the expanded trials, the overall occurrence being 0.75% (18), although in certain districts it was as high as 4.7% (129). Hypokalemic paralysis was always preceded by a prodromal stage characterized by muscular weakness and/or severe fatigue, and the use of potassium salt at this stage could prevent paralysis (129). The problem of hypokalemia will be discussed separately below.

Changes in sperm count and motility in subjects taking the optimal doses of gossypol were similar to those in men taking 60–70 mg/day, although these subjects took longer to show evidence of necrospermia and azoospermia. Recovery ensued in most subjects after cessation of gossypol treatment, but around 10% of the volunteers remained azoospermic six months to 4.5 years post-regimen, indicating the possibility of irreversibility of fertility (18). The recovery rate was much higher in subjects taking gossypol for less than two years than in those taking it for more than two years. Quantitative histological studies of testicular biopsy specimens of infertile men taking gossypol-containing crude cottonseed oil also indicated the possibility of irreversibility of spermatogenesis if large amount of the oil had been used, particularly for a long period of time (113).

The pattern of exfoliated cells in human semen has been studied by several groups (131–134). Zong (131) indicated that in men taking routine doses of gossypol, exfoliated cells, mainly mid- and late-stage spermatids and a few double- or multinucleated cells, could be seen at the end of the first month, followed by gradually increasing numbers of primary spermatocytes and spermatogonia. At the end of fifth month, the semen pictures showed wide individual variations. In general, the following three patterns could be differentiated: (*a*) no-cell semen, (*b*) semen with spermatids predominant, (*c*) semen

with primary spermatocytes predominant. Zong believed that the second was the most ideal pattern. In this case both the spermatogonia and the spermatocytes were capable of division, giving rise to an incessant number of new spermatids. In no-cell semen, all cell types were seriously inhibited and sterility would have resulted if treatment had continued. In these cases, suspension of gossypol treatment for some period of time may obviate this possibility.

Shi et al (132) studied changes in the pattern of exfoliated cells in human semen during and after gossypol treatment. They found, in addition to what Zong reported (131), that the peak of exfoliation of spermatids, spermatocytes, and spermatogonia occurred in the sixth, twenty-fourth, and thirty-sixth months of maintenance dosing respectively, and that after withdrawal of gossypol the recovery of fertility seemed to be related to the prominent cell type exfoliated at the time of withdrawal. If the cell type was spermatogonia, the chances for recovery were less. As a result of these discoveries, these researchers also advocated the examination of exfoliated cells in semen as a means of decreasing the occurrence of gossypol-induced sterility. Chen & Li indicated that, in men taking gossypol, the LDH-X activity of spermatozoa decreased dose-dependently, which might serve as another index for the individualization of the dosage (130). Later, several groups documented the inhibitory effect of gossypol on LDH-X activity (135–139a) and considered it to be gossypol's target of action. Gossypol did not induce autoimmune reaction to sperm in men (140).

Investigators in Shandong province recommended the use of gossypol formic acid for clinical trials because, they claimed, the side effects appeared fewer than those associated with gossypol and gossypol acetic acid (36, 141, 142).

There is no clear evidence on whether all the side effects associated with gossypol treatment, such as decreased libido, can be ascribed entirely to the drug. Factors other than gossypol may cause these symptoms as well. For the resolution of this problem, well-controlled double-blind clinical studies have been initiated (143, 144). Coutinho carried out the first clinical trial outside China in 8 men for periods of 6–12 months (121). Treatment consisted of oral administration of 20 mg daily for 4 months, followed by 20 mg every other day. Reduction in sperm count occurred after 45 days and azoospermia at the end of 4 months of treatment. No change in semen volume was detected. None of the subjects reported loss of libido or potency. Blood chemistry, serum potassium, and testosterone levels were normal throughout the treatment. Response to LHRH and hCG was normal. There were no significant changes in blood pressure and body weight. Recovery of semen values was faster in men who took the drug for short periods of time. These results are consistent with those obtained by Chinese scientists.

HYPOKALEMIC PARALYSIS

Hypokalemic paralysis is an infrequent side effect of gossypol, but for the time being it is the most important stumbling block to the general application of the drug as an antifertility agent. Hypokalemic paralysis associated with gossypol administration usually occurs in March, when vegetable food is in short supply, and in September, when people sweat a great deal. Nothing particular has been found in the history of the subjects, no family or past history of paralysis or thyrotoxicosis. The clinical picture is that of hypokalemic syndrome: fatigue, muscular weakness, followed by flaccid paralysis starting from the lower extremity and gradually spreading upward, but usually not affecting the respiratory muscles. The principal laboratory findings are hypokalemia with corresponding ECG changes; 24-hour urinary potassium increased; renal concentrating and diluting ability, renal acidifying power and renogram normal; blood pH, aldosterone level, and thyroid function normal. Recovery is prompt and complete after potassium repletion in most cases, but a few may remain hypokalemic for a long time after cessation of gossypol treatment. These cases require intermittent potassium therapy; otherwise, paralysis may recur.

For several years during the past decade, the problem of whether gossypol can induce hypokalemia had been the subject of dispute. Attempts to produce hypokalemia by administering gossypol to various experimental animals have not been successful. Next, although the clinical and laboratory findings on hypokalemia associated with gossypol are undeniably different from those of the more familiar forms of hypokalemia, such as familial periodic paralysis (thyrotoxic or non-throtoxic), it is difficult to differentiate hypokalemia associated with gossypol from hypokalemia of unknown diagnosis (147). One would naturally think that hypokalemia associated with gossypol administration might simply be hypokalemia of unknown diagnosis.

In 1975, Qian et al (148) showed that gossypol decreased the potassium content of myocardium in isolated rabbit heart. A little later the same group (149) found that gossypol reduced the intracellular potassium ion concentration in low-K–fed rats but not in regularly fed rats. Similar results were obtained in isolated skeletal muscles by Xu & Qian (150). These were the first experimental evidences documenting a definite effect of gossypol on potassium metabolism. Moreover, Qian et al (15, 129) pointed out that the incidence of hypokalemic paralysis was astonishingly higher in subjects taking gossypol than in the control population and that among the gossypol takers hypokalemic paralysis occurred only in those with a relatively low K intake, a phenomenon curiously similar to what happened in rats (149). Putting all these facts together, one may conclude that hypokalemic paralysis in gossypol takers is not a casual coexistent hypokalemia of unknown diagnosis but is related to the

administration of gossypol. In regard to the mechanism of development of gossypol-induced hypokalemia, Qian et al (148) suggested that gossypol might inhibit the activity of Na-K-ATPase or some other Mg-dependent enzyme systems associated with energy metabolism. This hypothesis is based on the following facts: (*a*) the potassium-depleting effect of gossypol on isolated heart can be reversed by Mg ion (148), which is a cofactor for Na-K-ATPase; (*b*) the effect of gossypol on K metabolism is apparent only in rats and men having a low-K intake (15, 149), and it is well known that Na-K-ATPase is more susceptible to specific inhibitors in a low-potassium environment (152).

The effect of gossypol on Na-K-ATPase activity has been investigated by several groups. Gossypol inhibited renal Na-K-ATPase activity in guinea pigs fed a relatively low K diet and in renal slices cultured in a low K medium; the addition of K to the diet or the medium could mitigate this inhibition (153). In rats, guinea pigs, rabbits, and monkeys fed regular diets, ordinary antifertility doses of gossypol did not significantly affect renal Na-K-ATPase activity (154–156; H. P. Lei, personal communication). When large doses were given to rats and guinea pigs, the enzyme was inhibited (156, 157), however. In guinea pigs, large doses of gossypol also inhibited the Na-K-ATPase activity of the skeletal muscle (157). Na-K-ATPase activity in rat brain synapses (158), in guinea-pig renal cortex (158a), and in spermatozoa of sea urchin (159) was inhibited by gossypol in vitro in a dose-dependent manner. Na-K-ATPase is the principal enzyme system responsible for the maintenance of a high intracellular K ion concentration (152). Inhibition of the activity of this enzyme will inevitably lead to a decrease in intracellular K content, renal K loss, and depletion of body K, all of which happened in hypokalemic patients administered gossypol (129, 145, 160). In gossypol-treated rats, the urinary excretion of (^{42}K) was increased in certain phases after administration of (^{42}K) (161). Recently, it has been shown that gossypol decreases the K ion concentration of spermatozoa (162).

Hypokalemia is a low-incidence side effect of gossypol administration. Most of the subjects taking gossypol do not show hypokalemia and the body K level of these subjects are normal (151). The production in animal models of low-incidence side effects is a very difficult problem in toxicology. As has been mentioned, attempts to produce hypokalemia were not successful in all the experimental animals tested. In rats, gossypol did not significantly affect the urinary and fecal excretion of K (149, 163), or the (^{42}K) distribution in tissues (161, 164), nor did it aggravate hypokalemia produced by deoxycorticosterone acetate (165). Plaa (166) stressed that, in the development of such low-incidence side effects, factors other than the drug may play a contributing role; if these factors are not operational, the effects can not be produced. K deficien-

cy may be one of the contributing factors in gossypol-induced hypokalemia (15, 149). Recently, Wang et al (167) indicated that the in vitro inhibitory effect of gossypol on vas deferens, uterus, and ileum is exaggerated in a low K medium.

In the two cases of gossypol-induced chronic hypokalemia that occurred in Jiangsu, Qian (15) found that the urinary prostaglandin E_2-like substances (PGEL) was greatly increased, reaching 1599 and 2416 ng/24 hours respectively, more than 3- to 5-fold the normal value in his laboratory (168). In the case with higher urinary PGEL, potassium repletion for one month did not elevate the serum K level nor ameliorate the hypokalemic symptoms. Addition of indomethacin brought about a dramatic short-term therapeutic effect, with normalization of serum K and urinary PGEL levels (15). This finding strongly suggests the participation of prostaglandin in the development of chronic hypokalemia induced by gossypol. It has been known that prostaglandin of the E series increases urinary K excretion (169) and that K deficiency could enhance renal PGE and F biosynthesis (170). On the basis of these evidences, Qian postulated an explanation for the mechanism of development of chronic hypokalemia induced by gossypol treatment (15). Gossypol might initiate a sequence of events, including inhibition of Na-K-ATPase, leakage of intracellular K extracellularly, and renal loss of K, resulting in hypokalemia. K deficiency would augment the renal biosynthesis of PGE, which in turn would lead to a greater loss of K from the kidney. A vicious circle is thus formed. In this regard it is interesting to note that both PGE (171) and low extracellular K level (152) are inhibitory to Na-K-ATPase activity, and that gossypol itself may stimulate PG biosynthesis (15, 172). All these relationships may prompt the formation of a hypokalemia-causing cycle, as can be seen in Figure 2. The cycle, once established, seems to continue even after the withdrawal of the

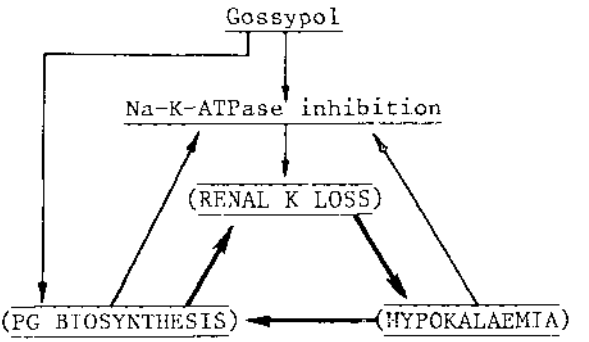

Figure 2 Possible mechanism for the development of chronic hypokalemia

causative factor. The addition of indomethacin to the cycle may block the PG biosynthesis link.

THE SITE AND MECHANISM OF ACTION

Several groups of researchers reported that, in the spermatogenic series, the cell types most sensitive to gossypol are the late spermatids and pachytene spermatocytes (17, 80, 112, 173–175). This view is consistent with the results of epididymal ligation experiments indicating that the target organ of gossypol action is the testis and not the epididymis (34). However, damage to epididymal spermatozoa (5, 176), testicular and epididymal spermatozoa, and epididymal epithelium (177) has been shown to be the earliest finding discernible after gossypol administration. Moreover, in young guinea pigs, gossypol interfered with the formation of the tight junction of Sertoli cells (178); in man and animal, gossypol might cause degenerative changes in Sertoli cells (112, 174, 179, 180). In gossypol-treated rats, one of the earliest signs of gossypol action is Sertoli cell damage, including vacuolization and breakage of tight junctions (177). In vitro studies indicated that gossypol causes marked morphological changes in Sertoli cells with decreased formation of androgen-binding protein (181). Therefore, the initial action of gossypol on these cellular sites should also be considered. In regard to the effect of gossypol on the Sertoli cells, it has been reported that gossypol does not affect the permeability of the blood-testis barrier in adult rats (182) and the protein synthesis of Sertoli cells (183).

Gossypol has long been known as an uncoupler of oxidative phosphorylation (1). It uncouples spermatozoal oxidative phosphorylation (184), first stimulates and then inhibits respiration (59, 185), and reduces ATP production (55, 184, 186, 187). In rats given (^{14}C) gossypol, the mitochondrial fraction of the testis homogenate revealed the highest radioactivity compared with other fractions (188). Among the cellular organelles of the spermatogenic cells, the earliest and most conspicuous damage caused by gossypol occurred in the mitochondria (17, 37, 80, 112, 133, 134, 145, 173, 176, 189–191). Therefore, Xue and his colleagues (17, 192) postulated that the mitochondria of spermatogenic cells may be the subcellular target of gossypol action. As indicated above, gossypol inhibits Na-K-ATPase and LDH-X activity. The former has been suggested as the molecular site of the gossypol effect causing hypokalemia (15, 148, 153) and the latter as the molecular site causing infertility (135, 136). Xue et al (192) noted that both of these enzymes are present in the mitochondria. Another mitochondrial enzyme, pyruvate dehydrogenase, is also inhibited by gossypol (159). However, Tso & Lee (162) indicated that, although gossypol lowers the K content of spermatozoa apparently as a consequence of the inhibition of membrane Na-K-ATPase activity, this inhibition does not seem to be the cause of decreased sperm motility.

Qian (15, 172) proposed that prostaglandins might participate in the antispermatogenic mechanism of gossypol. This hypothesis is based on the following facts: (*a*) the antispermatogenic but not the toxic effect of gossypol can be reversed by aspirin, a prostaglandin synthetase inhibitor, and augmented by K deficiency (15, 193, 194); (*b*) in rats fed a normo- or low-K diet, gossypol increases the plasma, renal, and testicular PG levels (193; Y. Xu, S. Z. Qian, et al, unpublished data); (*c*) systemic administration of PGE damages similar cell types in rat testes, as does gossypol (195). In another report gossypol was shown to lower the plasma PGF_{2a} level but to leave the PGE level unaffected (196).

In regard to the antagonism of gossypol action, it may be worth mentioning that Smilax reverses the toxicity but not the antifertility effect of gossypol (197). Wang et al (167) suggested that the cyclic nucleotide system might participate in the mechanism of gossypol action. They indicated that gossypol increases the ratio of cAMP/cGMP, which is known to inhibit the motility, respiration, and metabolism of spermatozoa. Yu et al (198) indicated that the Zn content of atrophied testis and epididymis of gossypol-treated rat decreased and suggested that chelation of Zn by gossypol might be related to its antispermatogenic activity. Kalla & Vasudev pointed out that gossypol does not affect the Zn content of human sperm (199).

Gossypol has been shown to interfere with the transition in the synthesis of nuclear histone from lysine-rich to arginine-rich ones in the late spermatid, as shown by Chen et al (200). These researchers believed that this inhibition of transition might play a role in the antispermatogenic mechanism of gossypol, as it is well known that the transition is indispensable to the fertility of spermatozoa. Spermatozoal acrosin, proacrosin, LDH-X, NAD-isocitrate dehydrogenase, succinyl-CoA synthetase, and fumarase are highly sensitive to gossypol; acrosin has the lowest inhibition threshold (201, 202). Gossypol also inhibits testicular ATPase (203) and testis-specific LDH-X (130, 135–139a) activity, inhibits the utilization of fructose by spermatozoa (204), and is cytotoxic in vitro (104). All these factors may participate in the mechanism of gossypol action.

Results are inconsistent concerning the effect of gossypol on DNA synthesis (46, 119, 205–208). Pathways involved in the metabolism of succinate have been shown to be more sensitive to gossypol than those of pyruvate and maleate, and the succinate-to-cytochrome C segment is the most sensitive in the chain. However, as this inhibition threshold is higher than either the uncoupling threshold or the concentration inhibiting sperm motility, the segment might not be the main target of gossypol effect (209). In hamsters, substantial reduction in sperm population occurred before the suppression of serum T level (33), and in rabbits gossypol significantly lowered the serum T level while the animals remained fertile (31). Therefore, it has been suggested that the antifertility effect of gossypol does not seem to be mediated through its suppressive effect on testicular steroidogenesis.

Several miscellaneous effects of gossypol are worth mentioning, including:

1. Lengthening of pentobarbital sleeping time (210) and inhibition of glutathione-S-transferase activity (136), indicating a harmful influence on detoxification mechanisms.
2. Mitochondrial degeneration of the skeletal muscle (211, 211a), depression of catechol-O-methyltransferase activity (212), blocking of neuromuscular transmission, and lowering of the cholinergic responses in vitro (213–215). However, Wang et al (145) indicated that gossypol does not notably affect the vegetative nervous system.
3. Decrease of Ca absorption (216) and lowering of low-density lipoprotein level of blood (51, 217).
4. Interferon induction (218), antitumor, antimicrobial, and antiviral activities (1, 219, 220).

Gossypol does not appear to affect the function and morphology of the adrenal cortex (108, 221, 222). It was shown to inhibit the endometrial cells and has been used clinically for the treatment of functional bleeding, endometriosis, and leiomyoma with promising results (223–227).

CONCLUDING REMARKS

Gossypol is an effective antispermatogenic agent for certain susceptible animals and for humans. The source of the drug, the cotton plant, is abundant and the preparation inexpensive. Prasad & Diczfalusy (20) wrote: "The discovery of gossypol and the demonstration of its antifertility action mark an important milestone in the search for a new male antifertility agent. . . . For the present, gossypol represents the only approach which has a reasonable chance to reach the stage of large scale clinical testing before the end of this decade." These words appropriately reflect the views of most of the researchers in this field. However, quite a lot of work, particularly in regard to its toxicology, must be done before it (or one of its analogues) can be developed into a practical male antifertility drug. The following points appear to indicate the main directions for future research in the scope of pharmacology and toxicology.

1. Hypokalemia: exploration of the contributing factor(s), means to prevent its occurrence, mechanism(s) of development, production of animal model, etc.
2. Irreversibility: exploration of the contributing factor(s), measures to detect impending sterility, etc.
3. General toxicological assessment: effects on the endocrine system, liver, heart, and kidney; lifetime carcinogenicity studies, etc.
4. Studies on the mechanism(s) of action. These studies may also be helpful in the development of gossypol analogue(s) exhibiting satisfactory antifertility and minimal toxic effects.

ACKNOWLEDGMENTS

We are greatly indebted to Mmes. S.Q. Chen, Y. Xu, H. Q. Ying, and Z. R. Zhang, and Drs. J. H. Ding, X. H. Wang, and C. Q. Zhong for their assistance in the preparation of this manuscript.

Literature Cited

1. Abou-Donia, M. B. 1976. Physiological effects and metabolism of gossypol. *Residue Rev.* 61:124–59
2. Liu, B. S. 1957. A tentative idea of the use of cooking cottonseed oil for fertility control. *Shanghai J. Chin. Med.* 6:43–47
3. Hubei Provincial Epidemic Prevention Station. 1967. Prevention and treatment of "Hanchuan Fever" and "Xinzhou Paralysis". Restricted publication
4. Wu, X. R. 1972. *Study on the antifertility effect of cottonseed and gossypol.* Presented at 1st Natl. Conf. Male Antifertil. Agents, Sept., Wuhan
5. Wang, N. G., Lei, H. P. 1972. *Antifertility effect of gossypol acetic acid on male rats.* Presented at 1st Natl. Conf. Male Antifertil. Agents, Sept., Wuhan. Republished 1979 in *Natl. Med. J. China* 59:402–5
6. Dai, R. X., Pang, S. N., Lin, X. K., Ke, Y. B., Liu, Z. L., Dong, R. H. 1972. *A study of antifertility of cottonseed.* Presented at 1st Natl. Conf. Male Antifertil. Agents, Sept., Wuhan. Republished 1978 in *Acta Biol. Exp. Sinica* 11:1–10
7. Wang, Y. E., Luo, Y. D., Tang, X. C. 1972. *Studies on the antifertility action of cottonseed meal and gossypol.* Presented at 1st Natl. Conf. Male Antifertil. Agents, Sept., Wuhan. Republished 1979 in *Acta Pharm. Sinica* 14:662–69
8. Shandong Coord. Group Antifertil. Plants. 1972. *Animal screening of antifertility effective constituents of cottonseed.* Presented at 1st Natl. Conf. Male Antifertil. Agents, Sept., Wuhan
9. Zhang, Y. G., Shi, Q. X. 1972. *Antifertility effect of gossypol on male rats.* Presented at 1st Natl. Conf. Male Antifertil. Agents, Sept., Wuhan. Republished 1980 in *Zhejiang J. Med.* 2:56–57
10. Jiangsu Coord. Group Male Antifertil. Agents. 1972. *Antifertility effect of gossypol on male rats.* Presented at 1st Natl. Conf. Male Antifertil. Agents, Sept., Wuhan
11. Qian, S. Z., Hu, J. H., Ho, L. X., Sun, M. X., Huang, Y. Z., Fang, J. H. 1972. *The first clinical trial of gossypol on male antifertility.* Presented at 1st Natl. Conf. Male Antifertil. Agents, Sept., Wuhan. Republished 1980 in *Clinical Pharmacology and Therapeutics,* ed. P. Turner, pp. 489–92. London: MacMillan. 576 pp.
12. Natl. Coord. Group Male Antifertil. Agents. 1978. Gossypol—A new male antifertility agent. *Chin. Med. J.* 91:417–28
13. Qian, S. Z. 1972. *The pharmacology and clinical application of gossypol.* Presented at 1st Natl. Conf. Male Antifertil. Agents, Sept., Wuhan
14. Qian, S. Z. 1975. *Effect of gossypol on the energy metabolism.* Presented at 4th Natl. Conf. Male Antifertil. Agents, Oct., Suzhou. Republished 1977 in *Acta Acad. Med. Wuhan* 1:71–74
15. Qian, S. Z. 1981. Effect of gossypol on potassium and prostaglandin metabolism and mechanism of action of gossypol. In *Recent Advances in Fertility Regulation,* ed. C. F. Chang, D. Griffin, A. Woolman, pp. 152–59. Geneva: Atar. 399 pp.
16. Qian, S. Z., Xu, Y. 1981. The effect of gossypol on potassium and prostaglandin metabolism. *Reprod. Contracep. China* 1:6–10
17. Xue, S. P. 1981. Studies on the antifertility effect of gossypol, a new contraceptive for males. See Ref. 15, pp. 122–46
18. Liu, Z. Q., Liu, G. Z., Hei, L. S., Zhang, R. A., Yu, C. Z. 1981. Clinical trial of gossypol as a male antifertility agent. See Ref. 15, pp. 160–63
19. Ying, H. Q. 1981. The isomerism of gossypol. *Reprod. Contracep. China* 4:8–13
20. Prasad, M. R. N., Diczfalusy, E. 1982. Gossypol. *Int. J. Androl. Suppl.* 5:53–70
21. Lei, H. P. 1982. Review and prospect of gossypol research. *Acta Pharm. Sinica* 17:1–4

21a. Lei, H. P. 1983. Prospect of gossypol as a pill for males. *Acta Pharm. Sinica* 18:321–24

22. Gu, Z. P. 1983. A glimpse of studies of gossypol in foreign countries. *Reprod. Contracep. China* 1:8–10
23. Edwards, J. D. Jr. 1958. Total synthesis of gossypol. *J. Am. Chem. Soc.* 80: 3798–99
24. Adams, R., Geissman, T. A. 1960. Gossypol, a pigment of cottonseed. *Chem. Rev.* 60:555–74
25. Baram, N. I., Ismailov, A. I., Kamaev,

F. G., Leont'ev, V. B. 1976. NMR spectroscopic study of different samples of gossypol. *Khim. Prir. Soedin.* 2:249–52

26. King, T. J., de Silva, L. B. 1968. Optically active gossypol from Thespesia populnea. *Tetrahedron Lett.* 3:261–63
27. Dechary, J. M., Pradcl, P. 1971. The occurrence of (+)−gossypol in Gossypium species. *J. Am. Oil Chem. Soc.* 48:563–64

27a. Si, Y. K., Zhou, J., Huang, L. 1983. Resolution of racemic gossypol. *Bull Sci. China* 28:640

28. Sang, G. W., Zhang, Y. G., Shi, Q. X., Shen, K. Y., Lu, F. Y., Zhao, X. J., Wang, M. Q., Liu, X. L., Yuan, Y. Y. 1980. Chronic toxicity of gossypol and the relationship to its metabolic fate in dogs and monkeys. *Acta Pharmacol. Sinica* 1:39–43
29. Chang, M. C., Gu, Z. P., Saksena, S. K. 1980. Effect of gossypol on the fertility of male rats, hamsters and rabbits. *Contraception* 21:461–69
30. Hahn, D. W., Rusticus, C., Homn, R., Johnson, A. N. 1981. Antifertility and endocrine activities of gossypol in rodents. *Contraception* 24:97–105
31. Saksena, S. K., Salmonsen, R., Lau, I. F., Chang, M. C. 1981. Gossypol: Its toxicological and endocrinological effects in male rabbits. *Contraception* 24:203–14
32. Waller, D. P., Fong, H. H. S., Cordell, G. F., Soejarto, D. D. 1981. Antifertility effects of gossypol and its impurities on male hamsters. *Contraception* 23:653–60
33. Saksena, S. K., Salmonsen, R. A. 1982. Antifertility effects of gossypol in male hamster. *Fertil. Steril.* 37:686–90
34. Dai, R. X., Dong, R. H. 1973. *Studies on the antifertility effect of gossypol. I. An experimental analysis by epididymal ligature.* Presented at 2nd Natl. Conf. Male Antifertil. Agents, Aug., Qingdao. Republished 1978 in *Acta Biol. Exp. Sinica* 11:15–22
35. Shandong Coord. Group Antifertil. Plants. 1973. *Studies on the antifertility effect of gossypol on male rats.* Presented at 2nd Natl. Conf. Male Antifertil. Agents, Aug., Qingdao
36. Yuan, J. Y. 1979. Gossypol formic acid, a male contraceptive. *J. Shandong Med. Drug* 2:36–37
37. Nadakavukaren, M. J., Sorensen, R. H., Tone, J. N. 1979. Effect of gossypol on the ultrastructure of rat spermatozoa. *Cell. Tissue Res.* 204:293–96
38. Weinbauer, G. F., Rovan, E., Frick, J. 1982. Antifertility efficacy of gossypol acetic acid in male rats. *Andrologia* 14:270–75
39. Shi, Q. X., Zhang, Y. G., Yuan, Y. Y. 1981. Studies on the antifertility effect of gossypol. II. Effect on spermatogenesis in rats. *Acta Zool. Sinica* 27:22–28
40. Hoshiai, H., Uehara, S., Nagaike, F., Momono, K., Mori, R., Suzuki, M., Lin, Y. C. 1981. Action mechanism of gossypol on rats as male contraceptive agent: *In vivo* study. *Jpn. J. Fertil. Steril.* 26:35–39
41. Hadley, M. A., Lin, Y. C., Dym, M. 1981. Effect of gossypol on the reproductive system of male rats. *J. Androl.* 2: 190–99
42. Chang, C. C., Gu, Z. P., Tsong, Y. Y. 1982. Studies on gossypol. I. Toxicity, antifertility, and endocrine analysis in male rats. *Intl. J. Fertil.* 27:213–18
43. Kalla, N. R., Foo, T. W. J., Sheth, A. R. 1982. Studies on the male antifertility agent gossypol acetic acid. V. Effect on the fertility of male rats. *Andrologia* 14:492–500
44. Zhejiang Acad. Med. Sci. 1975. *In vitro spermicidal action of gossypol.* Presented at 4th Natl. Conf. Male Antifertil. Agents, Oct., Suzhou
45. Bartin, C. W., Sundaram, K. S., Chang, C. C. 1980. *Toxicology, endocrine and histopathologic studies in small animals and Rhesus monkeys administered gossypol.* Presented at PARFR Workshop on Gossypol, March, Chicago
46. Kalla, N. R., Vasudev, M., Arora, G. 1981. Studies on the male antifertility agent gossypol acetic acid. III. Effect on rat testis. *Andrologia* 13:242–49
47. Zhou, L. F., Lei, H. P. 1981. Recovery of fertility in rats after gossypol treatment. See Ref. 15, pp. 147–51
48. Dai, R. X., Pan, S. Y. 1980. Studies on the antifertility effect of gossypol. VI. Observations of testicular atrophy of rats administered gossypol for long terms. *Acta Exp. Biol. Sinica* 13:192–99
49. Jiangsu Coord. Group Male Antifertil. Agents. 1972. *Studies on the repeated-dose toxicity of gossypol in dogs.* Presentcd at 1st Natl. Conf. Male Antifertil. Agents, Sept., Wuhan
50. Shi, Q. X., Zhang, Y. G. 1980. Studies on antifertility effect of gossypol. I. Effect of gossypol on androgen-dependent organs. *Acta Zool. Sinica* 26:311–16
51. Shandilya, L. H., Clarkson, T. B., Adams, M. R., Lewis, J. C. 1982. Effect of gossypol on reproductive and endocrine functions of male cynomolgus monkey. *Biol. Reprod.* 27:241–52
52. Yao, X. Y., 1981. Studies on the isola-

tion and the antifertility effect of (+)−gossypol. *Reprod. Contracep. China* 2:51–52

52a. Waller, D. P., Bunyapraphatsara, N., Martin, A., Vournazos, C. J., Ahmed, M. S., Soejarto, D. D., Cordell, G. A., Fong, H. H. S. 1983. Effect of (+)−gossypol on fertility in male hamsters. *J. Androl.* In press

53. Murthy, R. S. R., Basu, D. K., Murti, V. V. S. 1981. Antifertility effect of (+)−gossypol. *Curr. Sci.* 50:64–66

54. Yuan, Q. X., Gao, D. W., Li, C. Z. 1983. Effects of gossypol on the implantation of female rats and its possible mechanism. *Reprod. Contracep. China* 2:25–30

55. Kalla, N. R., Vasudev, M. 1980. Studies on the male antifertility agent gossypol acetic acid, *in vitro* study on the effect on human spermatozoa. *IRCS Med. Sci.: Libr. Compend.* 8:375–76

56. Burgos, M. H., Chang, C. Y., Nelson, L., Segal, S. J. 1980. Gossypol inhibits motility of Arbacia sperm. *Biol. Bull.* 159:467–68

57. Waller, D. P., Zaneveld, L. J. D., Fong, H. H. S. 1980. In vitro spermicidal activity of gossypol. *Contraception* 22:183–87

58. Ridley, A. J., Blasco, L. 1981. Testosterone and gossypol effects on human sperm motility. *Fertil. Steril. Suppl.* 35:244

59. Tso, W. W., Lee, C. S. 1981. Effect of gossypol on boar spermatozoa in vitro. *Arch. Androl.* 7:85–88

59a. Tso, W. W., Lee, C. S. 1982. Cottonseed oil as a vaginal contraceptive. *Arch. Androl.* 8:11–14

60. Williams, W. L. 1980. New antifertility agents active in rabbit vaginal contraception method. *Contraception* 22:659–72

61. Cameron, S. M., Waller, D. P., Zaneveld, L. J. D. 1982. Vaginal spermicidal activity of gossypol in Macaca arctoides. *Fertil Steril.* 37:273–74

61a. Aitken, R. J., Liu, J., Best, F. S. M., Richardson, D. W. 1983. Analysis of the direct effect of gossypol on human spermatozoa. *Int. J. Androl.* 6:157–67

62. Hadley, M. A., Burgos, M. H. 1981. *Inhibition of rat epididymal sperm by gossypol.* Presented at NY Acad. Sci. Meet. Cell Biol. Testis, April

63. Yu, Z. H. 1982. Spermatocidal action of some fat-soluble chelating agents in pouch of cauda epididymides in rats. *Acta Pharmacol. Sinica* 3:260–63

64. Abou-Donia, M. B., Lyman, C. M., Dieckert, J. W. 1970. Metabolic fate of gossypol: The metabolism of gossypol-^{14}C in rats. *Lipids* 5:938–46

65. Lyman, C. M., Cronin, J. T., Trant, M. M., Odell, G. V. 1969. Metabolism of gossypol in the chick. *J. Am. Oil Chem. Soc.* 46:100–4

66. Abou-Donia, M. B., Lyman, C. M. 1970. Metabolic fate of gossypol: The metabolism of [^{14}C] gossypol in laying hens. *Toxicol. Appl. Pharmacol.* 17: 160–73

67. Albrecht, J. E., Clawson, A. J., Smith, F. H. 1972. Rate of depletion and route of elimination of intravenously injected gossypol in swine. *J. Animal Sci.* 35: 941–46

68. Abou-Donia, M. B., Dieckert, J. W. 1974. Urinary and biliary excretion of (^{14}C) gossypol in swine. *J. Nutr.* 104:754–60

69. Abou-Donia, M. B., Dieckert, J. W. 1975. Metabolic fate of gossypol: The metabolism of (^{14}C) gossypol in swine. *Toxicol. Appl. Pharmacol.* 31:32–46

70. Xue, S. P., Zhou, Z. H., Liu, Y., Wu, Y. W., Zong, S. D. 1975. *The pharmacokinetics of (^{14}C) gossypol acetic acid in rats. I. Whole body and microautoradiographic studies on the distribution and fate of (^{14}C) gossypol in rat body.* Presented at 4th Natl. Conf. Male Antifertil. Agents, Oct., Suzhou. Republished 1979 in *Acta Biol. Exp. Sinica* 12:179–94

71. Xue, S. P., Liu, Y., Fei, R. R., Han, S. M., Su, S. Y. 1975. *The pharmacokinetics of (^{14}C) gossypol acetic acid in rats. II. Quantitative studies on the kinetics of distribution, excretion and metabolism of (^{14}C) gossypol acetic acid in rat body.* Presented at 4th Natl. Conf. Male Antifertil. Agents, Oct., Suzhou. Republished 1979 in *Acta Biol. Exp. Sinica* 12:275–87

72. Wang, N. G., Li, G. X., Chen, Q. Q., Lei, H. P. 1973. *The metabolism of gossypol in vivo.* Presented at 2nd Natl. Conf. Male Antifertil. Agents, Aug., Qingdao. Republished 1979 in *Natl. Med. J. China* 59:596–99

73. Tang, X. C., Zhu, M. K., Shi, Q. X. 1980. Comparative study on the absorption, distribution and excretion of (^{14}C) gossypol in four species of animals. *Acta Pharm. Sinica* 15:212–17

74. Skutches, C. L., Smith, F. H. 1974. Metabolism of gossypol, biosynthesized from methyl (^{14}C)- and carbosyl (^{14}C)-labeled sodium acetate in rat. *J. Am. Oil Chem. Soc.* 51:413–15

75. Jensen, D. R., Tone, J. N., Sorensen, R. H., Bozek, S. A. 1982. Deposited pat-

tern of the antifertility agent, gossypol, in selected organs of male rats. *Toxicology* 24:65–72

76. Shi, C. Z., Ding, W. P., Yu, M. Q., Wu, X. R. 1981. Preparation, antifertility effect and toxicity of Mg-gossypol. *Acta Acad. Med. Wuhan* 4:6
77. Wu, Y. M., Chappel, S. C., Flickinger, G. L. 1981. Effect of gossypol on pituitary-ovarian endocrine function, ovulation and fertility in female hamster. *Contraception* 24:259–68
78. Zhou, L. F., Chen, Q. Q., Wang, N. G., Lei, H. P. 1975. *Observation on the effect of prolonged administration of gossypol acetic acid to rats*. Presented at 4th Natl. Conf. Male Antifertil. Agents, Oct., Suzhou. Republished 1980 in *Natl. Med. J. China* 60:343–44
79. Zhou, L. F., Lei, H. P., Gao, Y., Liu, Y., Wang, N. Y., Guo, Y. 1982. Further observation on the effect of prolonged administration of gossypol acetic acid to rats. *Acta Pharm. Sinica* 17:245–52
80. Xue, S. P., Zong, S. D., Su, S. Y., Wu, Y. W., Lin, Y., Zhou, Z. H., Ma, X. X. 1973. *Antifertility effect of gossypol on the germinal epithelium of the rat testis. A cytological, autoradiographical and ultrastructural observation*. Presented at 2nd Natl. Conf. Male Antifertil. Agents, Aug., Qingdao. Republished 1980 in *Sci. Sinica* 23:642–57
81. Shandong Coord. Group Male Antifertil. Plants. 1973. *Repeated-dose toxicity of gossypol in rabbits*. Presented at 2nd Natl. Conf. Male Antifertil. Agents, Aug., Qingdao
82. Inst. Materia Medica, Chinese Acad. Med. Sci. 1973. *Repeated-dose toxicity of gossypol in dogs*. Presented at 2nd Natl. Conf. Male Antifertil. Agents, Aug., Qingdao
83. Shi, Q. X., Zhang, Y. G., Sang, G. Y., Wang, M. Q., Shen, K. Y., Lu, F. Y. 1979. Effect of long-term administration of gossypol on the potassium metabolism and renal function of rhesus monkey. *Bull. Pharm. Sinica* 14:89–90
84. Feng, Z. Q. 1980. *Electronmicroscopic observation on the effect of long-term administration of gossypol on the structure of liver in rhesus monkey*. Presented at Ann. Meet. Chin. Anat. Soc., Chengdu
85. Liu, Y., Su, S. Y., Chen, X. M., Xue, S. P. 1980. Elcctronmicroscopic observation on the ultrastructure and histochemical changes of LDH and SDH of cardiac muscle in male monkeys. *Acta Anat. Sinica* 11:428–32

85a. Zhuang, Y. Z., Xu, M. Y., Zhao, X. J., Liu, Y., Su, S. Y. 1981. Electronmicroscopic observation of the effect of gossypol on the liver and kidney of rhesus monkey. *Zhejiang J. Med.* 1:27

86. Feng, Z. Q. 1982. *Electronmicroscopic observation on the effect of long-term administration of gossypol on the structure of kidney in monkey*. Presented at Ann. Meet. Chin. Anat. Soc., Guilin
87. Zhang, Z. S., Pan, X. X., Wang, M. M., Yao, Y. L. 1979. Dominant lethal mutagenic effect of gossypol in male rat. *Med. Industry* 7:20–22
88. Tan, Y. B., Zheng, H. Z., Zhang, Z. S., Wang, M. M., Yao, Y. L. 1982. Embryotoxicity and teratogenicity of gossypol acetic acid. Restricted publication
89. de Peyster, A., Wang, Y. Y. 1979. Gossypol, proposed contraceptive for men passes the Ames test. *N. Engl. J. Med.* 301:275–76
90. Colman, N., Gardner, A., Herbert, V. 1979. Non-mutagenicity of gossypol in the Salmonella mammalian-microsome plate assay. *Environ. Mutagen* 1:315–20
91. Wuhan Med. College. 1980. Assaying of mutagenicity of gossypol by Ames test. *Acta Acad. Med. Wuhan* 1:87
92. Li, C. B., Ding, F., Ma, Z. R., Zhao, S. Y. 1981. Determination of the genetic safety of gossypol by Ames test and SCE frequency. *Acta Fu Dan Univer.* 20:361–65
93. Zhang, H. J., Wei, Z. W., Zhu, Y. Z. 1982. Determination of the mutagenicity of gossypol by Ames test. Restricted publication
94. Majumdar, S. K., Thatcher, J. D., Dennis, E. H., Cutrone, A., Slockage, M., Hammond, M. 1982. Mutagenic evaluation of two male contraceptives: 5-thio-D-glucose and gossypol acetic acid. *J. Hered.* 73:76–77
95. Haroz, R. K., Thomasson, J. 1980. Tumor initiating and promoting activity of gossypol. *Toxicol. Lett. Suppl.* 6:72
96. Lei, S. P., Xu, S. W., Wang, Y. C. 1980. Effect of gossypol acetic acid on SCE of Chinese hamster ovary K-1 cell. *Acta Biol. Exp. Sinica* 13:426
97. Zhang, Z. S., Zheng, H. Z., Ding, Y. N., Jiang, X. R. 1982. In vitro effect of gossypol acetic acid on human lymphocyte SCE. Restricted publication
98. Zhang, Z. S., Pan, X. X., Wang, M. M., Yao, Y. L. 1981. Genetic studies on gossypol. I. Comparative study of the cytogenetic effects of gossypol acetic acid on male germ cell and lymphocytes in rats. *Reprod. Contracep. China* 1:33–36

99. Zhang, Z. S., Wang, M. M., Zheng, H. Z., Tan, Y. B., Lu, Q., Yao, Y. L., Jiang, X. R., Wang, R. L. 1981. Genetic studies on gossypol. II. The effect of gossypol acetic acid on sister chromatid exchange in mouse. *Reprod. Contracep. China* 2:42–44
100. Zhuo, J. M., Lu, Q. J., Jiang, S. H. 1982. Effect of gossypol on germ cell chromosomes in mice. *Reprod. Contracep. China* 3:49–52
101. Wang, R. L., Wang, M. M., Lu, Q., Zheng, H. Z., Yao, Y. L., Jiang, X. R., Zhang, Z. S. 1982. Genetic studies on gossypol. III. Mutagenic effect of gossypol acetic acid on SCE of mice spermatogonia. Restricted publication
102. Yang, Y. H., Shieh, S. P. 1982. Studies on the gossypol effect on chromosome aberration and SCE in mice. *Acta Anat. Sinica* 13:215–20
103. Cai, Y. Y., Liu, Y., Xu, G. L., Shieh, S. P. 1981. Effect of gossypol on frequency of chromosomal aberration and SCE in human peripheral lymphocyte in vitro. *Acta Anat. Sinica* 12:293–98
104. Ye, W. S., Liang, J. C., Hsu, T. C. 1983. Toxicity of a male contraceptive, gossypol, in mammalian tissue. *In Vitro* 19:53–57
105. Zhang, Z. S., Lu, Q., Tan, Y. B., Jiang, X. R., Zheng, H. Z., Wang, M. M., Yao, Y. L. 1983. SCE and micronucleus test in subjects taking gossypol formic acid. *Acta Genet. Sinica* 10:157–60
106. Zhang, Z. S., Lu, Q., Tan, Y. B., Jiang, X. R., Zheng, W. Z., Wang, R. L. 1983. Chromosome aberration studies in gossypol formic acid users. *Reprod. Contracep. China* 1:56–57
107. Huang, T. H., Fan, Y. S., Chen, L. F., Li, P., Miao, Y. S., Li, C. C. 1983. Effect of gossypol acetic acid on function of rRNA and acrocentric chromosome association in human cells. *Natl. Med. J. China* 63:97–100
108. Yuan, D. X., Liu, X. Y., Gao, Y. H., Wang, J. Y., Fu, Z. L. 1980. Histochemical observations on pituitary, adrenal cortex and hypothalamus in rats after gossypol administration. *Acta Anat. Sinica* 11:331–36
109. Ye, S. J., You, M. M., Shieh, S. P. 1982. Ultrastructural observations on the gonadotrophic cells of adenohypophysis and Leydig cell in gossypol treated rats. *Acta Anat. Sinica* 13:206–10
110. Zhou, J. Y., Zhung, Y., Zhang, S. X., Bo, A. H., Han, S. Q. 1981. A histological study on the effect of gossypol on anterior lobe of pituitary of castrated hogs. *Acta Anat. Sinica* 12:101–6
111. Luo, Y., Dong, Z. Q., Feng, Y. Z. 1980. Effect of gossypol acetic acid on histology of human testis. *Chin. J. Urol.* 1:198–99
112. Hoffer, A. P. 1982. Ultrastructural studies of the seminiferous and epididymal epithelium and epididymal sperm in rats treated with gossypol. *Arch. Androl* 9: 35
113. Wang, Y. F., Wu, M. Z., Wang, Z. X., Gu, D. Q., Gu, J. Z., Wu, Q. H. 1982. Quantitative histological investigation of testes from normal adults and men infertile after taking raw cottonseed oil. *Reprod. Contracep. China* 24:31–34

113a. Liang, S. X., Pang, S. N., Dong, R. H., Dai, R. X. 1981. Radioimmunoassay of serum T and LH in male rats administered gossypol. *Acta Biol. Exp. Sinica* 14:191–97

114. Lin, Y. C., Hadley, M. A., Llingener, D., Dym, M. 1980. Effect of gossypol on the reproductive system of male rats. *Biol. Reprod.* 22(Suppl. 1):95A
115. Lin, T., Murono, E. P., Osterman, J., Nankin, H. R., Ciulson, P. B. 1981. Gossypol inhibits testicular steroidogenesis. *Fertil. Steril.* 35:563–66
116. Hoshiai, H., Uehara, S., Nagaike, F., Momono, K., Hoshi, K., Suzuki, M. 1982. Action mechanism of gossypol as a male contraceptive agent: In vitro study on Leydig cell of rat. *Jpn. J. Fertil. Steril.* 27:156–60
117. Zhuang, L. Z. 1981. Effect of gossypol on the growth and function of Leydig and Sertoli cells in culture. *Biol. Reprod.* 24(Suppl. 1):229
118. Qian, S. Z., Xu, Y., Zhang, X. L. 1974. *Effect of gossypol on the isolated rabbit heart*. Presented at 3rd Natl. Conf. Male Antifert. Agents, Feb., Beijing
119. Wang, Z. G. 1979. *Some pharmacological studies on gossypol*. Presented at the Int. Coop. Contraceptive Res. (ICCR), Population Council, May, New York
120. Wang, H. Y., Xu, Y. S., Jiang, L. G., De, S. L., Shi, Y. Q., Zhou, Z. M. 1979. Effect of sex steroid hormone and gossypol on LH secretion of rat pituitary by RIA. *Acta Physiol. Sinica* 31:337–42
121. Coutinho, E. M. 1982. Clinical studies with gossypol. *Arch. Androl.* 9:37–38
122. Zhang, G. Y., Zhou, X. Y., Gao, S. M., Guo, Z. S., Liu, G. Z., Cao, J. 1983. Comparison of changes of serum gonadotropins and steroid hormones in azoospermic men caused by gossypol and other factors. *Reprod. Contracep. China* 2:31–35
123. Bressani, R., Aguifre, A., Scrimshaw, N. S. 1959. All vegetable protein mixture

for human feeding. *J. Nutr.* 65:351–55

124. Bydagyan, F. E., Vladimirev, B. D., Levitskii, L M., Shchurov, K. A. 1947. Influence of prolonged consumption of small amount of cottonseed meal on the human organism. *Gigiena Sanit.* 7:28–33
125. Cook, E. F., Martin, E. W. 1948. *Remington's Practice of Pharmacy,* p. 798. Mack
126. Erynimov, L. C. 1966. Treatment of tumor of urinary bladder with gossypol and ionol in combination with surgery. *Vop. Onkol.* 2:29–35
127. Harper, G. A., Smith, K. J. 1968. Status of cottonseed protein. *Econ. Bot.* 22:63–72
128. Beradi, L. C., Goldblatt, L. A. 1969. Gossypol. In *Toxic Constituent of Plant Foodstuff,* ed. I. E. Liener, pp. 211. New York: Academic. 500 pp.
129. Qian, S. Z., Jing, G. W., Wu, X. Y., Xu, Y., Li, Y. Q., Zhou, Z. H. 1977. *Gossypol-related hypokalaemia, clinicopharmacologic studies.* Presented at 5th Natl. Conf. Male Antifertil. Agents, Aug., Qinhuangdao. Republished 1980 in *Chin. Med. J.* 93:477–82
130. Chen, X. M., Li, H. Y. 1978. *Effect of gossypol on LDH-X of human spermatozoa.* Proc. Ann. Meet. Chin. Anat. Soc., Guilin, p. 127
131. Zong, S. D. 1977. *Studies on the exfoliated cells in semen of men taking gossypol.* Presented at 5th Natl. Conf. Male Antifertil. Agents, Aug., Qinhuangdao. Republished 1979 in *Med. Res. Commu.* 2:24
132. Shi, Q. X., Qiu, J. X., Zhang, G. Y. 1981. Analysis on exfoliated cells in human semen after oral administration of gossypol acetic acid. *Acta Pharmacol. Sinica* 2:262–66
133. Tung, S. M., Zhou, X. H., Zhou, Y. X. 1980. Studies on antifertility effect of gossypol: Cytological observation of human semen. *Acta Acad. Med. Prim. Shanghai* 7:19–26
134. Shao, T. S., Zhang, B. C., Ye, W. S., You, M. M. 1982. Cytological, cytochemical and ultrastructural observation on the human sperm following gossypol administration. *Acta Anat. Sinica* 13: 201–5
135. Lee, C. Y., Malling, H. V. 1981. Selective inhibition of sperm-specific LDH-X by an antifertility agent, gossypol. *Fed Proc.* 40:718
136. Maugh, T. H. 11. 1981. Male "pill" blocks sperm enzyme. *Science* 212:314
137. Tso, W. W., Lee, C. S. 1982. LDH-X: An isoenzyme particularly sensitive to gossypol inhibition. *Int. J. Androl.* 5: 205–9
138. Lee, C. Y. G., Moon, Y. S., Gomel, V. 1982. Inactivation of LDH-X by gossypol. *Arch. Androl.* 9:34–35

138a. Giridharan, N., Bamji, M. S., Sankaram, A. V. B. 1982. Inhibition of rat testis LDH-X by gossypol. *Contraception* 26:607–15

139. Lee, C. Y. G., Moon, Y. S., Yuan, J. H., Chen, A. F. 1982. Enzyme inactivation and inhibition by gossypol. *Mol. Cell. Biochem.* 47:65–70

139a. Eliasson, R., Virji, N. 1983. Effect of gossypol acetic acid on the activity of LDH-C_4 from human and rabbit spermatozoa. *Int. J. Androl.* 6:109–12

140. Xie, W. Y., Ni, Y. X., Jiang, Y., Wang, Y. X., Wu, Y. L. 1981. Observation on sperm immunity to gossypol. *Reprod. Contracep. China* 4:35–37
141. Shandong Coord. Group Antifert. Plants. 1975. *Preliminary report on the experimental study and clinical trial of gossypol formic acid.* Presented at 4th Natl. Conf. Male Antifert. Agents, Oct., Suzhou
142. Shandong Coord. Group Antifert. Plants. 1977. *A concluding report on the clinical trial of gossypol for male fertility control, 1312 cases.* Presented at 5th Natl. Conf. Male Antifert. Agents, Aug., Qinhuangdao
143. Liu, G. Z. 1982. Double-blind study of gossypol: The loading phase. *Arch. Androl.* 9:38–39
144. Lyle, K. C. 1982. Controlled clinical trial of gossypol: Methodological considerations. *Arch. Androl.* 9:38
145. Wang, Z. G., Zhou, Z. M., Shi, Y. Q., Liu, J. S., Li, Y. M. 1975. Preliminary report on the clinical trial of gossypol in peasants. Restricted publication
146. Frick, J., Danner, C., Köhle, R., Kunit, G. 1981. Male fertility regulation. In *Research on Fertility and Sterility,* ed. J. Cotes-Prieto, A. Campos-da-Pas, M. Neves-e-Castro, pp. 291. Lancaster: MTP. 456 pp.
147. Kjerulf-Jensen, K., Krarup, N. B., Warming-Larsen, A. 1951. Persistent hypokalaemia requiring constant K therapy. *Lancet* 260:372–75
148. Qian, S. Z., Xu, Y., Jing, G. W. 1975. *The K-depleting effect of gossypol on isolated rabbit heart and its possible mechanism.* Presented at 4th Natl. Conf. Male Antifert. Agents, Oct., Suzhou. Republished 1979 in *Acta Pharm. Sinica* 14:116–19
149. Qian, S. Z., Xu, Y., Chen, Z. C., Cao, L. M., Sun, S. G., Tang, X. C., Wang,

Y. E., Shen, L. Y., Zhu, M. K. 1977. *Influence of gossypol on the K metabolism of rats and effect of some possible contributing factors (low-K and low-Mg intake).* Presented at 5th Natl. Conf. Male Antifert. Agents, Aug., Qinhuangdao. Republished 1979 in *Acta Pharm. Sinica* 14:513–20

150. Xu, Y., Qian, S. Z. 1981. The influence of gossypol on the potassium content of isolated skeletal muscle. *Bull. Pharm. Sinica* 16:78
151. Kuming Med. College 1st Hospital. 1981. K balance study on subjects taking gossypol acetic acid. *Chin. J. Urol.* 2:143–44
152. Schwartz, A., Lindenmayer, G. E., Allen, J. C. 1975. Na-K-ATPase, pharmacological, physiological and biochemical aspects. *Pharmacol. Rev.* 27:1–134
153. Bi, X. F., Zheng, Y. J., Yang, H. F., Zhang, Z. Y. 1980. Effect of gossypol on renal Na-K-ATPase activity. *Sci. Sinica* 9:914–19
154. Fu, Y. F., Zhao, R. S., Liu, J. G. 1979. Effect of gossypol acetic acid on renal Na-K-ATPase activity of rabbit and rat. *Nature China* 2:724–25
155. Wu, Y. W., Wang, N. Y., Tong, D. S., Shieh, S. P. 1981. Ultra-cytochemical observations on the effect of gossypol on renal Na-K-ATPase of rat and guinea pig. *Acta Anat. Sinica* 12:289–92
156. Fei, R. R., Liang, D. C., Gao, Y., Liu, Y., Guo, X. Y., Zhou, Z. H., Xue, S. P. 1982. Gossypol effect on the activity of renal cell membrane Na-K-ATPase of rats and guinea pigs. *Reprod. Contracep. China* 1:42–45
157. Su, S. Y., Liu, Y., Zhou, Z. H., Shieh, S. P., Zhao, X. J., Xu, M. Y., Zhuang, Y. Z. 1982. Relationship between gossypol administration and activity of Na-K-ATPase in animal. *Acta Anat. Sinica* 13:85–91
158. Feng, B. Y., Xu, M. Y. 1982. Effect of gossypol on the ATPase activity of the brain synapses. *Bull. Sci. Sinica* 17: 1072–75

158a. Ye, Y. X., Zheng, Y. J., Yang, H. F. 1983. Effect of gossypol on ATPase activity and (^{3}H)-ouabain binding in kidney cortex membranes. *Chin. J. Nucl. Med.* 3:38–41

159. Adeyemo, O., Chang, Y. C., Segal, S. J., Koide, S. S. 1982. Gossypol action on the production and utilization of ATP in sea urchin spermatozoa. *Arch. Androl.* 9:343–49
160. Bi, X. F., Ye, Y. X., Yang, H. F., Zhang, Z. R. 1981. Preliminary study on gossypol in causing hypokalaemia. *Acta Acad. Med. Sinica* 3:175–78
161. Xue, S. P., Liang, D. C., Shao, T. S., Wu, Y. W., Liu, Y., Zhou, Z. H., Wang, N. Y. 1979. Studies on the effect of gossypol on (^{42}K) metabolism of rat. *Acta Anat. Sinica* 10:78–87
162. Tso, W. W., Lee, C. S. 1982. K leakage: Not the cause of gossypol induced antimotility in spermatozoa. *Int. J. Androl. Suppl.* 5:53–70
163. Sun, Y. B., Chen, Q. Q., Wang, Y., Su, M. 1982. Effect of gossypol on K excretion of male rat. *Acta Acad. Med. Sinica* 4:126–27
164. Wang, Z. G., Zhou, Z. M., Shi, Y. Q., Liu, J. S., Li, Y. M. 1975. Effect of gossypol on the distribution of [^{42}K] in rats. Restricted publication
165. Yu, T. H., Zhang, X. D., Wang, Z. H. 1981. Gossypol did not affect the hypokalaemia by DOCA in rat. *Reprod. Contracep. China* 2:39–41
166. Plaa, G. L. 1978. The problem of low-incidence response. In *Proc. 1st Int. Congr. Toxicol,* ed. G. L. Plaa, W. A. M. Duncan, pp. 207–19. New York: Academic. 670 pp.
167. Wang, Z. G., Gao, H. Q., Hu, A. Z., Wu, T., Zhang, Z. R., Li, Y. M., Shen, R. G. 1983. Studies on the mechanism of gossypol action. *Bull. Sci. China.* In press
168. Cheng, D. D., Li, Y. Q., Ye, G. C., Qian, S. Z. 1981. A simplified method for solvent extraction of PGE_2-like substances from human urine. *Reprod. Contracep. China* 2:57–59
169. Shea-Donohni, P. T., Bolger, P. M., Eisner, G. M., Slotkoff, L. M. 1979. Effect of PGE_2 on electrolyte and fluid excretion: Evidence of direct tubular effect. *Can. J. Physiol. Pharmacol.* 57:1448–52
170. Düsing, R., Attallah, A. A., Presyna, A. P., Lee, J. B. 1978. Renal biosynthesis of PGE_2 and F_{2a}, dependence on extracellular potassium. *J. Lab. Clin. Med.* 92:669–72
171. Mozsik, G., Kutas, J., Nagy, L., Nemeth, G. 1974. Inhibition of Na-K-ATPase system from human gastric mucosa by PGE_1 and PGE_2. *Eur. J. Pharmacol.* 29:133–37
172. Qian, S. Z. 1982. Participation of prostaglandin in the mechanism of action of gossypol. *Arch. Androl.* 9:36–37
173. Dai, R. X., Pan, S. N., Liu, Z. L. 1975. *Studies on the antifertility effect of gossypol. II. A morphological analysis of the antifertility effect of gossypol.* Presented at 4th Natl. Conf. Male Antifert. Agents,

Oct., Suzhou. Republished 1978 in *Acta Biol. Exp. Sinica* 11:27–30
174. Hei, L. S., Cai, G. Z., Ying, G. H., Lei, J. Z. 1981. Electronmicroscopic observation of the effect of gossypol on spermatogenesis in cancer patients. *Natl. Med. J. China* 61:527–29
175. Ye, W. S., Lin, T. S., Liu, Z. H., You, M. M., Guo, Y. 1982. Effect of gossypol on protein and nucleic acid synthesis in spermatogenic cells of rat in vivo and in vitro. *Acta Anat. Sinica* 13:92–96
176. Hoffer, A. P. 1982. Ultrastructural studies of spermatozoa and the epithelial lining of the epididymis and vas deferens in rats treated with gossypol. *Arch. Androl.* 8:233–46
177. Gu, Z. P., Zong, S. D., Chang, C. C. 1983. Morphological changes in testes and epididymides of rat after gossypol. *Acta Pharmacol. Sinica* 4:40–45
178. Pelletier, R. M., Friend, D. S. 1981. Effect of gossypol on the postnatal seminiferous epithelium and Sertoli cell junction of guinea pig. *Biol. Reprod.* 24(Suppl. 1):25
179. Wang, D. X., You, M. M., Xue, S. P. 1982. Ultrastructural observation of gossypol effect on the Sertoli cells in rat. *Acta Anat. Sinica* 13:211–14
180. Oko, R., Hrudka, F. 1982. Effect of gossypol on spermatozoa. *Arch. Androl.* 9:39–43
181. Zhuang, L. Z., Phillips, D. M., Gunsalus, G., Bardin, C. W., Mather, J. P. 1983. Effect of gossypol on rat Sertoli and Leydig cells in primary culture. In press
182. Yang, Y. Z., Wu, D., Wang, N. Y., Chen, X. M. 1982. Studies on the effect of gossypol on blood-testis barrier in rat. *Reprod. Contracep. China* 22:52–55
183. Zhou, X. M. 1982. Gossypol effect on protein synthesis in Sertoli cells in rats. *Reprod. Contracep. China* 1:39–41
184. Tso, W. W., Lee, C. S., Tso, M. Y. W. 1982. Effect of gossypol on boar spermatozoal ATP metabolism. *Arch. Androl.* 9:319–32
185. Tso, W. W., Lee, C. S. 1982. Gossypol uncoupling of respiratory chain and oxidative phosphorylation in ejaculated boar spermatozoa. *Contraception* 25: 649–56
186. Ke, Y. B. 1982. Studies on the mechanism of antifertility effect of gossypol. *Jiangsu J. Med. Pharm.* 8:23–24
187. Ke, Y. B., Tso, W. W. 1982. Variations of gossypol susceptibility in rat spermatozoa during spermatogenesis. *Int. J. Fertil.* 27:42–46
188. Liang, D. C., Fei, R. R., Liu, Y., Yang, S. L., Su, S. Y., Guo, X. Y., Liu, Z. H., Zhou, Z. H., Xue, S. P. 1981. Studies on the distribution of (^{14}C) gossypol in subcellular fractions of rat testis and site of gossypol action. *Acta Acad. Med. Sinica* 3:153–57
189. Feng, Z. Q. 1975. *Effect of gossypol on human sperm, a electronmicroscopic study*. Presented at 4th Natl. Conf. Male Antifert. Agents, Oct., Suzhou
190. Hang, Z. B., Wang, Y. P., Gan, D. Q., Liu, Y., Zong, S. D. 1980. Electronmicroscopic observation of the effect of gossypol on human spermatozoa. *Acta Anat. Sinica* 11:299–302
191. Oko, R., Hrudka, F. 1982. Segmental aplasia of the mitochondrial sheath and sequclae induced by gossypol in rat spermatozoa. *Biol. Reprod.* 26:183–86
192. Xue, S. P., Liang, D. C., Fei, R. R., Chen, X. M., Ye, S. J., Wu, Y. W., You, M. M., Guo, X. Y. 1982. Subcellular target site of antifertility effect of gossypol and its hypothetical action mechanism. *Sci. Sinica B* 12:1095–108
193. Xu, Y., Wang, W. H., Qian, S. Z. 1983. Antagonism of antifertility effect of gossypol by aspirin. *Acta Pharmacol. Sinica* 4:122–24
194. Qian, S. Z., Xu, Y., Wu, S. Y. 1983. The antispermatogenic effect of gossypol in K-deficient rats. *Acta Pharmacol. Sinica*. 4:183–85
195. Ye, G. C., Xu, Y., Qian, S. Q., Jiang, X. J., Sun, W. K., Tang, X. C. 1983. Effect of large dose of PGE on spermatogenesis and tissue PG levels in rats. *Acta Pharm. Sinica* 18:406–10
196. Wang, Z. G., Cheng, J. X., Yang, W. 1980. Effect of gossypol on plasma PG levels in rats. *Bull. Sci. China* 25: 720
197. Wang, W. H., Yu, J. H., Zhou, Z. R., Chen, Z. W., Zhao, N. J., Li, Y. Q. 1982. Antagonism of gossypol toxicity by Smilax. *Bull. Chin. Materia Med.* 7:32–34
198. Yu, T. H., Chang, H. T., Hsieh, P. L. 1981. The in vivo effect of gossypol on Zn, Fe, and Mn concentration in rats. *Acta Physiol. Sinica* 33:17–23
199. Kalla, N. R., Vasudev, M. 1981. Studies on the male antifertility agent gossypol acetic acid. II. Effect on motility and ATPase activity of human spermatozoa. *Andrologia* 13:95–98
200. Chen, X. M., Zhou, W. Y., Ma, X. X., Feng, J. B., Xue, S. P. 1982. Gossypol effect on synthesis and turnover of basic nucleoprotein in spermatids of rat. *Acta Anat. Sinica* 13:193–200
201. Tso, W. W., Lee, C. S. 1982. Gossypol:

An effective acrosin blocker. *Arch. Androl.* 8:143–47
202. Tso, W. W., Lee, C. S., Tso, Y. W. 1982. Sensitivity of various spermatozoal enzymes to gossypol inhibitor. *Arch. Androl.* 9:31–32
203. Kalla, N. R., Wei, J. F. T. 1981. Effect of gossypol acetic acid on respiratory enzymes in vitro. *IRCS Med. Sci: Biochem.* 9:792
204. Pösö, H., Wichmann, K., Jänne, J., Luukkainen, T. 1980. Gossypol, a powerful inhibitor of human spermatozoal metabolism. *Lancet* 1:885–86
205. Shandong Coord. Group Antifert. Plants. 1976. *DNA content of human sperm in subjects taking gossypol.* Presented at 5th Natl. Conf. Male Antifert. Agents, Aug., Qinhuangdao
206. Wu, K., Liu, H. M., Zhang, X. L., Zhou, W. Y. 1980. Effect of gossypol on DNA content of human sperm. *Acta Sichuan Med. College* 11:127–29
207. Zhou, W. Y., Xue, S. P. 1981. Effect of gossypol acetic acid on DNA content of primary spermatocytes of rat. *Reprod. Contracep. China* 3:26–29
208. Ye, Y. X., Liang, D., Gao, H. Y., Ye, G. Y. 1982. Effect of gossypol on the incorporation of (^{3}H) thymidine in liver, testis and small intestine of experimental animals. *Acta Pharm. Sinica* 16:390–93
209. Tso, W. W., Lee, C. S. 1981. Variation of gossypol sensitivity in boar spermatozoal electron transport chain segment. *Contraception* 24:569–76
210. Lei, H. P., Li, G. X., Wang, N. G., Chen, Q. Q., Guan, M. Z. 1979. Effect of gossypol acetic acid on the liver. *Natl. Med. J. China* 59:330–32
211. Yu, M. Q., Chen, W. J., Wu, X. R. 1979. Histochemical study on the effect of gossypol acetic acid on some enzymic activity of neuro-muscular junction, skeletal and cardiac muscles. *Acta Acad. Med. Wuhan* 2:93
211a. Su, S. Y., Liu, Y., Zou, Y. H. 1983. Electronmicroscopic observation of gossypol effects on gastronemius muscle in laboratory animals. *Acta Anat. Sinica* 14:98–103
212. Tang, F., Tsang, A. Y. F., Lee, C., Wong, Y. P. D. 1982. Inhibition of catechol-O-methyltransferase by gossypol: The effect of plasma (serum) protein. *Contraception* 26:515–19
213. Ma, R. H., Yu, D. Q., Wu, X. R. 1979. Inhibitory effect of gossypol acetic acid on neuro-muscular system. *Acta Acad. Med. Wuhan* 3:71–72
214. Shu, H. D., Yang, Q. Z., Xu, K. 1982. Effect of gossypol acetic acid on neuromuscular transmission. *Acta Pharmacol. Sinica* 3:17–21
215. Ma, R. H., Jiang, C. S., Li, F., Wu, X. R. 1980. Effect of gossypol on some functions of autonomic nervous system. *Acta Acad. Med. Wuhan* 1:65–67
216. Liu, X. M., Zhao, Q. R., Lin, H. 1982. Radiotracer study on the effect of gossypol acetic acid on Ca metabolism of rat. *Chin. J. Nucl. Med.* 2:175–77
217. Clarkson, T. B. 1982. Hypolipidemic effect of gossypol in cynomolgus monkey (Macaca fascicularis). *Lipids* 17:285–90
218. Khadzhibaeva, G. S., Pogodina, V. V., Laterpova, R. V., Bil'ner, L. M. 1978. Interferonogenic activity of low-molecular weight gossypol. *Antibiotiki* 23: 365–68
219. Aizikov, M. I., Kurmukov, A. G., Isamukhamedov, I. 1977. Antimicrobial and wound-healing effect of gossypol. *Dokl. Akad. Nauk Uzb SSR* 6:41–42
220. Wichman, K., Valteri, A., Luukkainen, T. 1982. Inhibiting herpes simplex virus type 2 infection in human epithelial cells by gossypol, a potent spermicidal and contraceptive agent. *Am. J. Obstet. Gynecol.* 142:593–94
221. Zhang, N. Z., Fu, Y. F., Dou, S. Y. 1981. The effect of gossypol acetic acid on adrenocortical function. *Natl. Med. J. China* 61:412
222. Ye, S. J., You, M. M., Xue, S. P. 1982. Ultrastructural effect of gossypol on adrenal cortex of rats. *Acta Anat. Sinica* 13:324–27
223. Han, M. L. 1980. Gossypol treatment for menopausal functional bleeding, leiomyoma and endometriosis, a preliminary report. *Acta Acad. Med. Sinica* 2:167–70
224. Cheng, K. F., Wu, W. Y., Tang, M. Y., Chu, P. T. 1980. Endometrial changes after administration of gossypol for menorrhagia. *Am. J. Obstet. Gynecol.* 138:1227–29
225. Liu, Y. X., Jia, X. Z., Zou, A. M., Zhao, M. 1981. Effect of gossypol on the amount of receptors of sex steroid hormone in human endometrium. *Chin. J. Obstet. Gynecol.* 16:129–31
226. Wuhan Med. College. 1981. Clinical observation on the use of gossypol in gynecology. *Chin. J. Obstet. Gynecol.* 16:132–36
227. Zhou, S. W. 1981. A concluding report on the use of gossypol in gynecology clinic. *Chin. J. Obstet. Gynecol.* 16:137–41