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Gossypol contraception and mechanism of action

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Gossypol is a yellowish polyphenolic compound found in the pigment glands of the seed, leaf, stem and root of the cotton plant, genus *Gossypium*, of the family Malvaceae. It was initially noted from its toxicological effects on non-ruminant animals when the livestock were fed with cottonseed meal as food supplement (Withers *et al.*, 1915). There was renewed interest in gossypol human physiology during 1956–1959, primarily because of the potential for using the high-protein cottonseed flour, Incaparina, as a protein supplement for malnourished children and pregnant women in developing countries. Results indicated that small amounts of gossypol in food was safe for human consumption (Bressani *et al.*, 1980). The elixir extracted from cotton root bark which contains a high concentration of gossypol has long been used traditionally in some countries as an abortifacient and menses inducer (Slocumb, 1980). Antitumour activity of gossypol on some ascites carcinoma and solid tumours was also cited (Vermel *et al.*, 1963). Much of the information indicated that the utilization of gossypol by humans around the world has a long history.

In the 1960s, Chinese workers discovered through mass investigation in some districts in China that cooking with crude cottonseed oil led to infertility in human males (Hubei provincial Hancuan 'Disease of Burning sensation', Treatment and Prevention group 1970). Large-scale animal experiments carried out in 1970s showed that the active ingredient associated with cottonseed oil which induced infertility was gossypol (National Coodinating Group on Male Antifertility Agents, 1978). Following an extensive series of studies using purified gossypol, gossypol-acetic acid and gossypol-formic acid on the antifertility effect, site and mechanism of action, pharmacokinetics, metabolism and toxicity in several species of animals (Wu, 1972; Wang and Lei, 1972; Jiangsu Provincial Cooperation Group on Male Contraception, 1972; Shangtung Provincial Herbaceous Contraceptive Group, 1972; Dai *et al.*, 1972, 1975; Xue *et al.*, 1973, 1975; Tang, 1980), clinical studies of gossypol as a male contraceptive agent were suggested. The first clinical trial of gossypol was started in 1972, and the above three types of gossypol tablet were tested in 14 districts of China. Over 10 000 men used the drugs for a period ranging from 6 months to 8 years, the overall efficacy

is 99.07% as estimated by sperm examination. Besides an incidence of 0.75% hypokalaemic paralysis which may be related to dietary intake of potassium, no serious toxic side-effects were observed provided the dosage was kept at the antifertility level. The discovery of gossypol's antifertility effect has aroused worldwide attention and interest among andrologists and in the field of population and family planning, because it is a new non-steroid drug which has been tested on more than 10000 subjects and for far longer periods than has any other agent. It constitutes a major lead in the search for male contraceptive agents from phenolic compounds, and represents the only approach to have a reasonable chance of being tested on a large-scale before the end of this decade.

CHEMISTRY, PHYSIOLOGICAL PROPERTIES AND METABOLISM OF GOSSYPOL

Gossypol exists mainly as the binaphthalene aldehyde form among its three tautomeric chemical structures (Fig. 11.1), with a molecular weight of 518.54, and an empirical formula $C_{30}H_{30}O_8$. Both the phenolic and carbonyl groups of gossypol are very reactive, they could react with acids, bases, oxygen and many other kinds of biochemical groups. Especially available are ε-amino groups of lysine, which bind, react and lead to a reduction in the availability of free protein and of protein quality (Lyman *et al.*, 1959). Gossypol can affect enzymes by reacting with the substrate or by combining with the enzyme itself thus inhibiting activity (Tankesly *et al.*, 1970; Abou-Donia, 1976), disturbing ion metabolism (i.e. depletion of intracellular potassium in some tissues), causing histological damage and

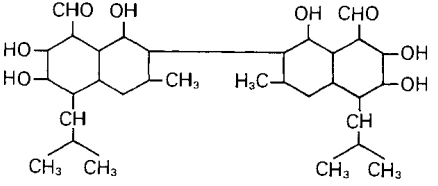

Fig. 11.1 Tautomeric structure of gossypol

inducing physiological toxic effects (Quan *et al.*, 1979). Gossypol might chelate ferrous ion, thereby interfering with the normal utilization of iron in blood in the synthesis of haemoglobin and lead to iron deficiency (Skutches *et al.*, 1973), haemolytic anaemia and malnutrition (Ridgon *et al.*, 1958). But the precise toxicological action is not yet clear.

Pharmacokinetic studies indicate that species differences exist between animals in the absorption, distribution, excretion and metabolism of gossypol within the body. The half-life of gossypol in the blood of mice and dogs after a single administration of [^{14}C]gossypol was longer than that in rats and monkeys (Tang *et al.*, 1980), and the accumulation of radioactive

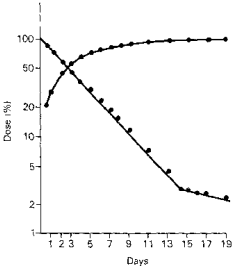

Fig. 11.2 Excretion of [^{14}C]gossypol from the rat body after a single oral dose of 20 μCi/7.5 mg. Half-excretion time = 60 h. (o) Total excretion; (0) Radioactivity in body

gossypol in tissues was also higher. This species difference with regard to the accumulation of gossypol in the blood and tissues might provide an explanation of the differences in toxic response in these animals. After a single oral dose of [^{14}C]gossypol to rats (20 μCi/7.5 mg/animal), the half-life for the elimination from the body was 60 h (Fig. 11.2), it took 19 days to eliminate 97.74% of the dose from the body (Xue *et al.*, 1975, 1979a,b). Excretion of gossypol has been shown to be mainly through the bile-faecal pathway. The amounts excreted in 19 days were 83.5% in the faeces, 11.7% in exhaled CO_2 and 2.5% in the urine (Xue *et al.*, 1979b). Whole-body autoradiography demonstrated that one to two days after oral administration, the radioactivity was located mainly in the gastrointestinal tract and liver. By day four there was a general increase in radioactivity in all tissues, and radioactivity in the main visceral organs rapidly reached a peak con-

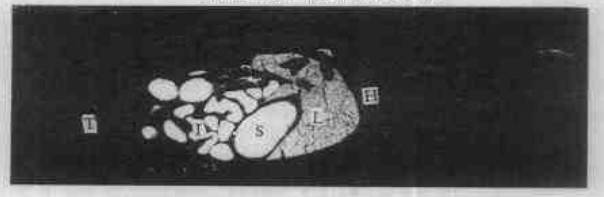

Fig. 11.3 Whole body autoradiograph showing the distribution of [^{14}C]gossypol (white area) in various tissues of rat one day after a single oral dose of labelled gossypol (20 μCi/7.5 mg). Note the high radioactivity in the gastrointestinal tract and liver, and the negligible amount in testis. H, heart; I, intestine; L, liver; S, stomach; T, testis; K, kidney

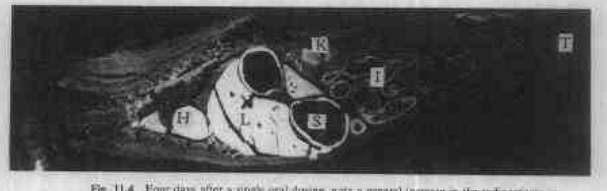

Fig. 11.4 Four days after a single oral dosing, note a general increase in the radioactivity in all tissues. Very low radioactivity could be detected in testis

Fig. 11.5 High radioactivity in testis is shown in the whole body autoradiograph by day 9 after a single oral dose of labelled gossypol

centration by day 9 (Figs 11.3, 11.4 and 11.5). Two weeks after administration, the radioactivity in all tissues and organs was markedly decreased, and by day 19, most of the tissues had no detectable radioactivity (Xue *et al.*, 1979a). It is likely that continued administration would lead to accumulation in the body and toxic effects. The pattern of gossypol distribution in rats, mice, rabbits, dogs and monkeys was essentially similar, the highest concentrations of gossypol were found in the liver, followed by spleen, kidney, heart, lung, pancreas, muscle, adipose tissue, testis and brain (Table 11.1) (Xue *et al.*, 1979a,b). The low concentration of gossypol in the testis might be due to the blood–testis barrier, and in a similar manner, gossypol was undetectable in the brain and spinal cord apparently due to blood–brain barrier.

ANTIFERTILITY EFFECT OF GOSSYPOL AND ITS TARGET CELL TYPES

A large number of papers have reported the antifertility effect of gossypol in various species of animals including rats, mice, hamsters, guinea pigs, rabbits, dogs, pigs and monkeys (National Coordinating Group, 1978; Xue *et al.*, 1979c; Dai *et al.*, 1978; Chang *et al.*, 1980; Zatuchni and Osborn, 1981; Shantung Institute of Traditional Medicine and Chinese Academy of Medical Science, 1975). There are species differences between animals in terms of antifertility effect. These differences are mainly attributed to the differences in sensitivity to gossypol damage. Among them the golden hamster, rats, monkeys and humans are more sensitive to the antispermatogenic effect of gossypol, whereas rabbits, mice, dogs, guinea pigs and pigs seem to be insensitive. Toxic effects are usually manifested before the occurrence of damage to germinal epithelium. Quantitative determination and whole-body autoradiographic studies in rats revealed tissue and cell-type differences in response to gossypol. We found that even though the testis had a lower concentration of gossypol than many visceral organs (i.e. the peak concentration of liver, spleen, kidney, heart and testis are 1192, 716, 708, 398 and 372 d min^{-1} g^{-1} respectively, Table 11.1), it showed the most severe damage (Xue *et al.*, 1979b). The damage to testicular germ cells usually occurred before any toxic reaction could be detected in the somatic cells of organs such as liver, heart, and kidney as well as the interstitial and Sertoli cells in testis. The difference between somatic and germ cell response to gossypol and the fact that the testis was damaged at low dosage of gossypol suggests that the selective action of gossypol on the testis is not due to its local concentration but to a higher sensitivity and vulnerability of the germ cells to the drug (Xue *et al.*, 1979a,b, 1981).

Gossypol–acetic acid administered orally at 15–30 mg kg^{-1} body weight day^{-1} to male rats became infertile in approximately 4–5 weeks, the onset of infertility seemed to be dose related. The earliest damage was seen in the metamorphosing spermatids and pachytene spermatocytes 2–3 weeks after drug treatment (Xue *et al.*, 1979a, 1980), showing different degrees of pathological changes such as pyknosis, nuclear vacuolation, swelling or displace-

Table 11.1 The specific radioactivity in various tissues of rat following a single dose of [^{14}C]gossypol (20 μCi/7.5 mg)

Tissue	*Specific radioactivity**						
	12 h	1 day	2 days	4 days	9 days	14 days	19 days
Heart	250	292	316	398	298	316	94
Liver	978	1192	970	400	287	67	70
Spleen	313	388	494	718	546	360	170
Lung	160	274	300	304	358	280	130
Kidney	314	420	536	708	360	100	86
Pancreas	192	232	244	348	268	110	72
Muscle	192	346	298	302	214	204	204
Testis	270	294	256	284	372	116	78
Gastro-tract content	2560	1760	520	562	206	148	38
Small intestine content	2770	2196	884	658	106	56	46
Large intestine content	3216	9256	1028	826	122	36	62
Adrenal	334	378	608	878	500	408	112
Thyroid	224	908	662	536	370	248	136
Pituitary	258	492	1272	1352	396	296	286
Hypothalmus	52	54	128	92	116	82	60
Medulla oblongata	34	70	86	110	132	76	30

* d min $^{-1}$ g fresh tissue^{-1}, each value is an average of determinations from three animals. Quench corrections were made utilizing standard [^{14}C]hexadecane

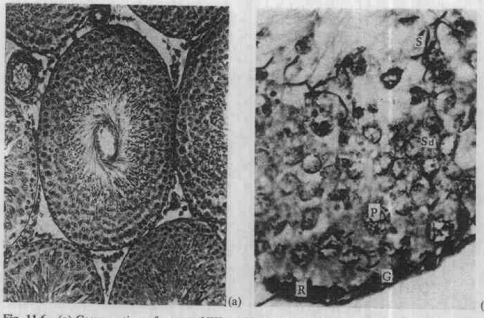

Fig. 11.6 (a) Cross section of a stage VIII seminiferous tubule of normal rat testis, showing the normal structure of the germinal epithelium. G, spermatogonia; R, resting spermatocyte; Sd, spermatids; S, spermatozoa. PAS-haematoxylin. × 225 (b) Same stage of rat seminiferous tubule after gossypol treatment (30 mg kg^{-1} day^{-1}) for 3 weeks, showing vacuolation and karyorrhexis of spermatids (Sd); pyknosis or karyolysis of pachytene spermatocytes (P). Note a few cells are exfoliated from the germinal epithelium. PAS-haematoxylin. × 675

ment of head cap, karyorrhexis, and cytolysis. Exfoliated cells, ghost cells, multinucleated giant cells and necrotic spermatozoa with their heads and tails separated were seen in the lumen of the tubules (Fig. 11.6). By the end of 4–5 weeks, the severely damaged tubules become atrophic and depopulated to a marked degree, spermatids and spermatocytes of the mid- and late stages disappeared almost completely, and only a single layer of cells consisting of Sertoli cells and spermatogonia are left in the tubules (Fig. 11.7). Gossypol target cells were designated the spermatozoa, spermatids and spermatocytes of mid- and late stages based on the basis that: (1) they are the most susceptible and vulnerable to gossypol damage (i.e. the first to show a detectable change and the first to degenerate); (2) gossypol greatly inhibited the incorporation of labelled amino acids into these classes of cells; (3) calculated according to the kinetics of target cell types, the time span of onset of infertility obtained was in good agreement with the time calculated theoretically for chemical agents affecting testicular function (Xue *et al.*, 1979c, 1981, 1982).

The site of cellular damage and the sequence of pathological changes in testes of rhesus monkeys (Shantung Institute of Traditional Medicine and Chinese Academy of Medical Sciences, 1975; Bardin *et al.*, 1980) following the administration of gossypol were essentially identical with that of rats (Fig. 11.8). Hamsters also show a similar pattern of damage but appear to be more sensitive than rats to the effects of gossypol (Chang *et al.*, 1980).

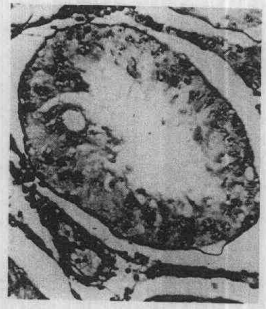

Fig. 11.7 Section of rat testis 4 weeks after gossypol treatment, showing the atrophic tubules with marked depopulation. PAS-haematoxylin. × 375

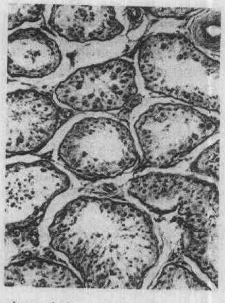

Fig. 11.8 Section of monkey testis 4 months after gossypol treatment (4 mg kg^{-1} day^{-1}), showing the atrophic tubules with marked depopulation. PAS-haematoxylin. × 120

In human volunteers the testicular biopsies and exfoliated cell types found in semen were consistent with the pattern of cellular damage seen in rats and monkeys. This indicated that the target cell types and the site of gossypol action were exactly alike in rats, monkeys, and men (Xue, 1981).

No detectable damage to Leydig cells, Sertoli cells or the epididymal epithelium were observed in rats fed at antifertility dosages. Neither hormone levels, histochemical reactions nor morphology along the reproductive axis underwent significant changes (Xue *et al.*, 1979c; Xue, 1981). However, decrease in serum LH and testosterone levels in gossypol-treated rats have been reported recently by Hadley and some other workers (Hadley *et al.*, 1981).

Of the spermatogenic elements the spermatogonia are the least susceptible to gossypol, usually remaining unaffected and maintaining their abilities for mitosis (Xue *et al.*, 1979, 1981).

THE TARGET ORGANELLE AND SUBCELLULAR SITE OF ANTISPERMATOGENIC EFFECT OF GOSSYPOL

The target organelle

Since results of ultrastructural damage of rat testicular mitochondria following gossypol teatment were reported in 1973 (Xue *et al.*, 1973, 1979; Dai, 1973, 1978), similar mitochondrial and ultrastructural changes in rat testis (Hadley *et al.*, 1981; Hoffer, 1980) and human seminal (Hang *et al.*,

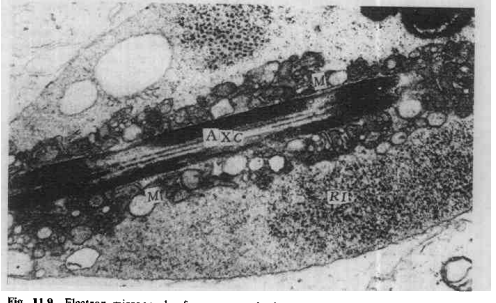

Fig. 11.9 Electron micrograph of a metamorphosing spermatid embedded in Sertoli cell cytoplasm from a rat after 6 weeks' treatment with gossypol, showing free ribosome granules (RI) and lysosome-like bodies (L) in residual cytoplasmic remnants (R) and derangement, swelling and vacuolation of the spiral sheath mitochondrial (M) × 19 800

1980) and testicular biopsy changes (Hei *et al.*, 1981) have been reported too. The damage to the germinal epithelium was found to begin with the spermiogenic spermatids around the seminiferous lumen and was characterized by prominent ultrastructural changes of mitochondria. Damage to the sperm tail spiral sheath mitochondria included derangement, swelling, cristae depletion, vacuolation and breakdown of the intact mitochondria (Fig. 11.9). The earliest sign of drug effect and the earliest organelle damage

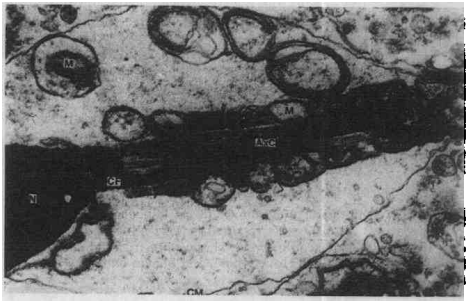

Fig. 11.10 Electron micrograph of a spermatozoon from a volunteer 30 days after administration of gossypol (20 mg/day). Note the spiral sheath mitochondria (M) are deranged and vacuolated. AXC, axial complex; AC, acrosome; CM, cell membrane; N, nucleus × 23 000

was observed in the germinal epithelium (Xue *et al.*, 1979c, 1982). Similar damage was observable in ejaculated spermatozoa 30–50 days after gossypol treatment (Fig. 11.10) (Hang *et al.*, 1980). With increased duration of drug administration, alteration in the spermatid head-cap acrosome system including the distortion and lysis of the acrosomal vesicles and granules, swelling and oedema of Golgi complex and pyknotic nucleus also were observed. No pathological damage could be detected in the Sertoli cell, except occasional, exhibition of some adaptive changes associated with the phagocytic activity (Xue *et al.*, 1982).

LDH-X enzyme inhibition

We found in 1977 that the mitochondrial marker enzyme LDH-X of human spermatozoa was markedly decreased after gossypol treatment (Chen *et al.*, 1978). The homogeneous blue-purple granules of LDH-X formazan in normal sperm mitochondria became coarse and decreased 30–50 days after

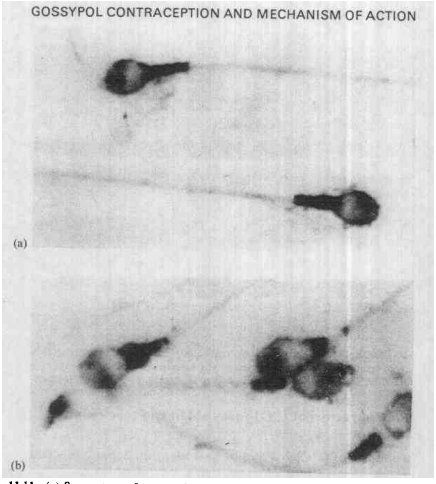

Fig. 11.11 (a) Spermatozoa from a volunteer before gossypol treatment, showing the LDH-X formazan deposits located homogeneously in the spiral sheath mitochondrial. Preston's method. × 2000. (b) Staining reaction of spermatozoa from a volunteer 50 days following gossypol treatment. Note that the formazan granules became coarse, unevenly distributed, lysed and decreased. Preston's method. × 2000

gossypol administration (Fig. 11.11). Six colour bands representing lactate dehydrogenase fractions (LDH-1, -2, -3, LDH-X, -4, -5) could be identified after electrophoretic separation on cellulose acetate strips prepared from the sperm homogenate of volunteers before treatment. The activity of LDH-X and LDH-1 decreased obviously (despite individual deviation) after gossypol treatment (Fig. 11.12). The mean amount of LDH-X as a percentage of the total LDH decreased from 42.6% before treatment to 31.1%, 26.3% and 10.2% respectively on days 30, 50 and 100 post-treatment (Chen *et al.*, 1978; Xue *et al.*, 1982). Lee and Malling (1981) also showed selective inactivation of LDH-X and LDH-5 from mouse and human by gossypol and regarded it as the target enzyme. Recently, we used [^{14}C]gossypol to test whether gossypol bound specifically to LDH-X. Preliminary results showed an electrophoretic pattern where [^{14}C]gossypol bound more heavily to LDH-4 than LDH-X.

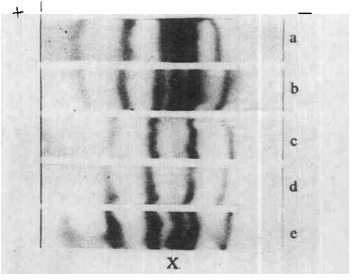

Fig. 11.12 Electrophoretic patterns of sperm homogenate LDH of volunteers before and after gossypol treatment, 6 bands represented the corresponding lactate dehydrogenase fractions (LDH-1, -2, -3, LDH-X, -4, -5). a,b, before treatment; c,d,e, after treatment. Note the inhibition of LDH-X (X) in c and d, and with individual deviation in e

High incorporation of [^{14}C]gossypol in the testicular mitochondria

The distribution of [^{14}C]gossypol in testicular and hepatic subcellular fractions has also been studied (Liang *et al.*, 1981; Xue *et al.*, 1982). Wistar adult male rats received an oral dose of 25 μCi/1.95 mg [^{14}C]gossypol (specific activity 12.7 μCi/mg) on the 1st and 4th day consecutively. Testis and liver were removed on days 7, 9 and 11 after treatment, and various subcellular fractions prepared for radioactivity determination by Beckman Liquid Scintillation Spectrometer. Additional intratesticular injection of [^{3}H]ouabain 1 h before death was performed in 7 animals for double tracing experiments. The results are shown in Tables 11.2, 11.3.

The distribution of [^{14}C]gossypol in all the five fractions reached their peak by day 9 (Table 11.2). The mitochondrial unit radioactivity was 2–3 times significantly higher than the other four subcellular fractions ($p<0.001$–0.05). The high affinity of [^{14}C]gossypol for testicular mitochondria was reproducible and independent of dosage. The distribution of [^{14}C]gossypol in liver subcellular fractions had a different order with the highest radioactivity in the microsome and cell membrane fractions, followed by the mitochondrial fraction (Table 11.2). This result was similar to that found by Abou-Donia (1970). The deviation in intracellular distribution of [^{14}C]gossypol between hepatic and spermatogenic cells provide further evidence that the patterns of gossypol incorporation and intracellular localization are different according to their cellular structures and to different cell types.

Table 11.2 Distribution of [^{14}C]gossypol in testicular and hepatic subcellular fractions of rats

Organ	*Dosage*	*Days after dosing*	*Radioactivity* (d min^{-1} mg protein^{-1})*				
			Mitochondria	*Nuclear fraction*	*Plasma membrane*	*Microsome*	*Soluble fraction*
Testis	50 μCi/ 3.9 mg	7(4)*	692 ± 129	170 ± 27	224 ± 24	98 ± 15	45 ± 8
		p**		p < 0.05	p < 0.05	p < 0.01	p < 0.01
		9(5)	1071 ± 274	261 ± 68	245 ± 59	141 ± 38	36 ± 9
		p**		p < 0.05	p < 0.05	p < 0.01	p < 0.01
		11(4)	462 ± 29	148 ± 28	139 ± 8	75 ± 8	42 ± 13
		p**		p < 0.001	p < 0.001	p < 0.001	p < 0.001
	25 μCi/ 1.95 mg	9(3)	311 ± 21	71 ± 5	98 ± 1	68 ± 28	15 ± 4
		p**		p < 0.01	p < 0.01	p < 0.01	p < 0.001
Liver	20 μCi/ 1.56 mg	2(7)	665 ± 194	520 ± 141	1423 ± 156	1708 ± 448	443 ± 48
		p**	p < 0.05	p < 0.05	p < 0.05		p < 0.05

* Expressed as mean value for animals indicated in ().

** *p* values expressed as the difference in radioactivity between mitochondria and the four subcellular fractions of testis and liver respectively

Table 11.3 The distribution of [^{14}C]gossypol and [^{3}H]ouabain in subcellular fractions of rat testes

Fraction	*Radioactivity* (d min^{-1} mg protein^{-1})									
	Mitochondria		*Nuclear fraction*		*Plasma membrane*		*Microsome*		*Soluble fraction*	
	^{3}H	^{14}C	^{3}H	^{14}C	^{3}H	^{14}C	^{3}H	^{14}C	^{3}H	^{14}C
Mean value	3668	481	1349	100	2038	164	835	79	2758	41
±SE (7*)	±373	±45	±171	±12	±245	±21	±112	±9	±396	±4
p**			<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.05	<0.001

* Number of animals

** p value represents difference in radioactivity of ^{14}C and ^{3}H respectively of mitochondria from those of the other four subcellular fractions

The double tracer experiments demonstrated that the distribution of [^{14}C]gossypol and [^{3}H]ouabain in mitochondria was the highest among the five subcellular fractions. These results suggest that the testicular mitochondria might be the gossypol sensitive target organelles and the mitochondrial protein (including Na-K-ATPase which binds to [^{3}H]ouabain) might be the binding site. Receptor assays for gossypol using [^{3}H]gossypol (specific activity 2 Ci/nmol) are now in progress to test this hypothesis.

Effect of gossypol on the respiration and oxidative phosphorylation of isolated rat testicular mitochondria

The effects of gossypol on the respiration and oxidative phosphorylation of isolated testicular mitochondria were determined by the Warburg manometric method and are shown in Fig. 11.13. Low concentrations of gossypol (20–40 μmol/l) stimulate mitochondrial respiration, but as gossypol concentrations increase the respiration declines sharply. Respiration was completely inhibited as the concentration reached 300 μmol/l. Inorganic phosphate (P_i) steadily decreased following the addition of gossypol, and oxidative phosphorylation was inhibited completely at about 80 μmol/l (Xue *et al.*, 1982).

The functions of isolated mitochondria determined by the oxygen electrode polarographic method also gave a similar result. Figure 11.14 and Table 11.4 show that the rate of oxygen consumption decreased steadily with the increase in gossypol concentration, and was inhibited completely at a concentration of 300–400 μmol/l (Xue *et al.*, 1982).

These results show that gossypol can uncouple oxidative phosphorylation and respiratory chain of testicular mitochondria, in a dose-dependent way. Similar results were obtained for the P/O value of testicular mitochondria isolated from rats that had been administered gossypol previously. The mean P/O ratio of the control group mitochondria was 3.75, whereas the groups treated with gossypol *in vivo* at dosages of 6, 10, and 15 mg decrease to 2.84, 2.60 and 2.58 respectively (Xue *et al.*, 1982). The difference between the treated and control groups was highly significant ($p < 0.001$). In addi-

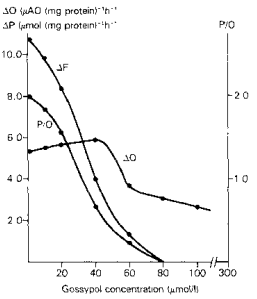

Fig. 11.13 Gossypol effect on the oxidative phosphorylation of isolated testicular mitochondria of rats. Each point represents mean values of three experiments each with three animals

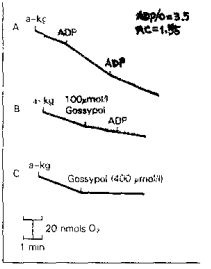

Fig. 11.14 Effect of gossypol on the respiration of isolated rat testicular mitochondria. Curve A shows a typical polarographic trace of oxygen consumption by mitochondria. Curve B shows the inhibition of mitochondrial respiration by gossypol (100 μmol/l). Curve C shows the complete inhibition of mitochondrial respiration at a final concentration of 400 μmol/l of gossypol. The inhibition both in B and C curves was not reversed by ADP. Numbers indicate nmol O_2 mg protein^{-1} min^{-1}, RC., respiratory control ratio: α-kg, α-ketoglutarate

Table 11.4 Effect of gossypol on the respiration of isolated rat testicular mitochondria

Gossypol concn (μmol/l)	Oxygen consumption (nmol O_2 mg protein^{-1} min^{-1} ± SE)*	
	*Gossypol added before ADP***	*Gossypol added after ADP***
0	8.05 ± 1.02	16.76 ± 1.49
2	10.48 ± 0.96	16.03 ± 0.25
5	9.37 ± 0.95	11.79 ± 0.90
10	6.58 ± 0.51	6.66 ± 0.14
40	5.07 ± 0.49	2.23 ± 0.47
100	4.02 ± 0.65	1.05 ± 0.11
200	1.60 ± 0.13	0.76 ± 0.23
300	0.53 ± 0.32	0.00 ± 0.00
400	0.00 ± 0.00	0.00 ± 0.00

* Data are mean values of 5 replicates ± SE

** Experiments were conducted at 30°C in 2 ml of reaction medium (pH7.5) in which α-ketoglutarate was used as substrate. Mitochondria (1.5 mg protein) were added, when a steady state of respiration was recorded, gossypol (in 0.5 mol/l NaOH solution, adjusted to pH7.5) and ADP (160 μmol/l) as phosphate receptor were added respectively to the table listed

tion, experiments on guinea pig renal cell mitochondria demonstrated that previous gossypol treatment *in vivo* would increase the sensitivity of isolated mitochondria to gossypol *in vitro*.

Abou-Donia (1974) reported that gossypol inhibited rat liver mitochondrial respiration *in vitro*. These results demonstrated further that gossypol could uncouple oxidative phosphorylation and respiratory chain in rat testicular mitochondria. But this does not mean that gossypol would affect hepatic cells *in vivo* at a concentration similar to that which caused germ cell damage, as the incorporation and intracellular distribution of gossypol are tissue specific. The concentration of gossypol in mitochondria of hepatic and testicular mitochondria are obviously different. Clearly under *in vivo* conditions the testicular mitochondria are vulnerable target organelles for gossypol.

THE MOLECULAR MECHANISM OF ANTIFERTILITY ACTION OF GOSSYPOL

Direct cytotoxic action of gossypol on spermatogenic cells

Gossypol has been shown to be a cytotoxic substance capable of reducing the activity of oxidative enzymes; interfering with oxidative metabolism (Myers *et al.*, 1966); uncoupling the respiratory chain and oxidative phosphorylation at high concentration (Abou-Donia, 1974; Xue *et al.*, 1982); reducing energy-linked enzyme activities (Adeyemo *et al.*, 1981; Burgos *et al.*, 1980; Kalla *et al.*, 1981; Tso *et al.*, 1982a); affecting Na-K-ATPase (Bi, 1980); blocking the action of proteolytic enzymes, pepsinogen and acrosin (Tanksly *et al.*, 1970; Tso, 1982b); and inactivating LDH-X, malate dehy-

drogenase and glutathione *S*-transferase (Lee *et al.*, 1981, 1982). Gossypol fed to male rats at antifertility dosages induced infertility but did not cause significant effects on the body weight, seminal vesicle and prostate weight, nor changes in the interstitiual cells of Leydig, libido and steroid hormone levels—plasma testosterone, dihydrotestosterone, serum LH and FSH (Bardin *et al.*, 1980; Xue *et al.*, 1979, 1981; Hoffer, 1980). No significant effect on the blood chemistry, bone marrow, serum sodium, calcium, chloride iron and seminal plasma fructose (National Coordinating Group, 1978). However, it is feasible to inhibit spermatogenesis by intereference with certain metabolic steps in mitochondria, thereby decreasing the incorporation of labelled amino acids with concomitant selective damage in target germ cells (Xue *et al.*, 1979c, 1980). In contrast to most sex hormone drugs, gossypol works locally at the level of the seminiferous tubules. A dose-dependent cytotoxic effect on the number of viable cells and mitotic index was demonstrated with both Chinese hamster ovary (CHO) cells and human lymphocytes after the cultures received treatment of gossypol (5 μg/ml) for various durations (Ye *et al.*, 1983). Cellular DNA, RNA and protein synthetic activities were also reduced, but did not seem to induce chromosome breakage. Inhibition of the motility of sea urchin (Adeyems *et al.*, 1981) and human spermatozoa (Kalla *et al.*, 1981; Tso *et al.*, 1982a) *in vitro* is due to the impairment of the ATPase activity in the sperm. The *in vitro* fertilization capacity of gossypol-treated human spermatozoa showed an obvious decrease in penetration rate into golden hamster ova (Young *et al.*, personal communication). Gossypol blocked acrosin activity of boar spermatozoa *in vitro* (Tso *et al.*, 1982b). All the data confirmed the direct effect of gossypol on sperm and spermatogenic cells. For this reason the use of gossypol as a vaginal spermicidal contraceptive has been suggested.

CONCLUDING REMARKS

Gossypol has been shown to be an effective male antifertility agent of relatively safety at the antifertility dosage. It has provided a new lead in the search for drugs for male fertility control from phenolic compounds, and represents the only approach to appear to have a reasonable chance to reach large-scale clinical tests in the near future. However, it possesses several disadvantages, especially hypokalaemia and the danger of sterility after long-term administration.

Gossypol damaged testicular mitochondrial ultrastructure, uncoupling mitochondrial respiration and oxidative phosphorylation, inhibited specifically mitochondrial energy-linked enzymes, ion-regulating enzymes and sperm marker enzyme LDH-X. The concomitantly high affinity of [^{14}C]gossypol for mitochondria suggests that testicular mitochondria might be the sensitive target organelle. On the basis of these findings, the author suggests that mitochondrial protein (enzyme) might be the binding and active site of gossypol thus impairing sperm motility and interrupting spermatogenesis.