

# REVERSIBLE CHEMICAL STERILIZATION : EFFECTS OF CYCLOHEXANOL ADMINISTRATION ON THE TESTES AND EPIDIDYMIDES OF MALE RABBIT

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**Summary:** Cyclohexanol administration (25 mg/kg/day orally for 40 days) produced a brief period of infertility in rabbits by inhibiting the process of spermatogenesis at the spermatocyte and spermatid levels. Seminiferous tubule and Leydig cell nuclear dimensions were reduced. The lumen of epididymides and ductus deferens were devoid of spermatozoa.

Cyclohexanol administration reduced the concentrations of RNA, protein, sialic acid and glycogen in the testes and epididymides, whereas the total cholesterol concentration of the testes was elevated. Depletion of adrenal ascorbic acid was conspicuous. Moderate elevation of serum cholesterol, phospholipids, triglycerides, bilirubin, and pyruvate transaminase were recorded.

Histopathological examination of liver did not show any damage. Leydig cell impairment and decreased production of RNA and sialic acid in the testes returned to subnormal values after cessation of cyclohexanol treatment for 70 days. Normal spermatogenesis was seen after 10 weeks of recovery period.

**Key words:** cyclohexanol inhibition spermatogenesis  
RNA and sialic acid lipid metabolism reversible action

## INTRODUCTION

Alkylating chemicals, particularly aziridine and esters of methanesulphonic acid have shown rapid actions on the spermatogenic process in rodents (8).

The simple 3 carbon compound,  $\alpha$ -chlorohydrin (3-chloro-1, 2-propanediol) had been the most effective and most potent epididymal antifertility compound. Niridazole another non-steroidal compound is toxic to the gonads of Schistosomes. Low doses of  $\alpha$ -chlorohydrin inhibit spermatogenesis and higher doses induce testicular necrosis (1).

In the present investigation, cyclohexanol is being screened for its antispermatogenic activity in male rabbit, since it is proposed to synthesize a large number of compounds bearing chlorine atom and a hydroxyl group at adjacent carbon atom.

## MATERIALS AND METHODS

Cyclohexanol diluted with olive oil was administered orally in daily doses prorated per kilogram of body weight. Fifteen healthy male rabbits weighing 1.5-2 kg were allotted to groups of five each. Group 2 and 3 rabbits received cyclohexanol (25 mg/kg/day) for a period of 40 days. Group 2 rabbits were allowed to recover for a period of 70 days after cessation of cyclohexanol treatment. Group 1 animals received the vehicle alone and served as controls.

Twenty-four hr after the administration of the final dose of cyclohexanol, the right testes and epididymides were removed surgically. The testes and epididymides were fixed in Bouin's fluid after recording their weights. Paraffin sections were prepared and stained with haematoxylin and eosin. Blood was obtained from the femoral vein. The left testes and epididymides were frozen and total RNA, protein, sialic acid, glycogen, acid phosphatase and adrenal ascorbic acid contents were determined later (16,12,26,15,7,21).

One hundred seminiferous tubules appearing circular in section were traced with camera lucida drawing at 80 X. Two perpendicular diameters of each tracing were measured, averaged and expressed in terms of mean tubular diameter. Student's 't' test was applied in comparing means. The measurement of the diameter of the 100 Leydig cell nuclei were carried out on 4 sections from each testicle with camera lucida drawings at 800 X.

Besides these, routine laboratory studies, such as haemoglobin, packed cell volume, serum proteins, cholesterol, phospholipids and triglycerids were made. Hepatic function was followed by determination of SGPT, acid/alkaline phosphatase, blood sugar and bilirubin. The serum transaminase (SGPT) estimation was made according to Mohun and Cook (14). Serum analysis and hematological studies were done by routine clinical techniques used in this laboratory (25). Statistical evaluation of the data for each group included calculations of the mean, standard deviation and standard error of the mean.

## RESULTS

*Organ weights* : Cyclohexanol administration did not cause loss in body weight, whereas a significant reduction was noticed in the weights of testes and epididymides. Adrenal gland weights did not change (Table I).

Microscopic examination of the testes presented marked degenerative changes. The changes consisted of loss of type A spermatogonia, spermatocytes, spermatids and

TABLE I : Changes in the weight of adrenal, testes, epididymides together with seminiferous tubule and Leydig cell nuclear diameter of rabbit after cyclohexanol treatment.

Treatment	Body wt kg	Adrenal	Testes mg/kg body wt.	Epididymides	Seminiferous tubule diam. ( $\mu$ m)	Leydig cell nuclear diam. ( $\mu$ m)
Control (5)	1.5 $\pm$ 0.3	210 $\pm$ 35	1305 $\pm$ 132	409 $\pm$ 25	178 $\pm$ 13	9.3 $\pm$ 0.1
Cyclohexanol (25 mg/kg/day for 40 days) (5)	1.3 $\pm$ 0.7	237 $\pm$ 25	448 $\pm$ 55 <sup>+</sup>	281 $\pm$ 13 <sup>+</sup>	130 $\pm$ 7 <sup>+</sup>	6.5 $\pm$ 0.2 <sup>+</sup>
Recovery for 70 days (5)	1.6 $\pm$ 0.3	219 $\pm$ 13	910 $\pm$ 76 <sup>@</sup>	370 $\pm$ 49 <sup>@</sup>	205 $\pm$ 3 <sup>@</sup>	8.7 $\pm$ 0.1 <sup>@</sup>

<sup>+</sup>  $p < 0.01$  compared with controls;

<sup>@</sup>  $P < 0.01$  compared with group 2;

Figures in parentheses represent the number of rabbits examined.  
All figures  $\pm$  S.E.M.

spermatozoa (Fig 1 and 2). A large number of spermatids showed morphological changes. Cytolysis and chromatolysis were common. Leydig cells were shrunken with scant cytoplasm, their nuclei were reduced in diameter (Table I,  $P < 0.01$ ).

*Epididymides and ductus deferens*: Histological examination of the epididymides showed that the luminal epithelium was reduced and the stereocilia were scanty. The lumen of the cauda epididymides and ductus deferens were devoid of spermatozoa. A few tubules showed the presence of degenerating cells.

*Recovery phase*: The effects of cyclohexanol on the testes and epididymides were reversible. Normal spermatogenesis was seen after 70 days of cessation of drug administration. The organ weights, seminiferous tubule and Leydig cells nuclear dimensions were restored to normal (Table I).

*Histological preparation of liver* - did not show any damage except the degranulation of the hepatoplasm. There was no liver necrosis.

TABLE II : Changes in total protein, RNA, sialic acid, cholesterol, adrenal ascorbic acid, glycogen and enzyme phosphatase activity of testes and epididymides of rabbit after cyclohexanol treatment.

	Protein ( $\mu\text{g}/\text{mg}$ )		RNA ( $\mu\text{g}/\text{mg}$ )		Sialic acid ( $\mu\text{g}/\text{mg}$ )		Glycogen ( $\text{mg}/\text{g}$ )	Choles- terol ( $\text{mg}/\text{g}$ )	Acid Phos. ( $\mu\text{g P}/\text{mg}/\text{hr}$ )	Adrenal ascorbic acid ( $\text{mg}/\text{g}$ )
	T	E	T	E	T	E	T	T	T	
Control	316 $\pm$ 24	218 $\pm$ 19	3.5 $\pm$ 0.2	2.9 $\pm$ 0.2	4.3 $\pm$ 0.5	3.8 $\pm$ 0.2	3.1 $\pm$ 0.2	6.7 $\pm$ 0.8	3.4 $\pm$ 0.2	2.6 $\pm$ 0.2
Cyclohexanol 25 mg/kg)	184 $\pm$ 12+	134 $\pm$ 23+	2.3 $\pm$ 0.1+	1.7 $\pm$ 0.3+	2.5 $\pm$ 0.2+	2.3 $\pm$ 0.5+	1.6 $\pm$ 0.1+	10.1 $\pm$ 0.5+	1.5 $\pm$ 0.3+	0.33 $\pm$ 0.1+
Recovery for 70 days	259 $\pm$ 17*	270 $\pm$ 15*	3.1 $\pm$ 0.5*	2.5 $\pm$ 0.1*	3.9 $\pm$ 0.3*	2.7 $\pm$ 0.3	3.5 $\pm$ 0.2*	7.8 $\pm$ 0.3*	2.7 $\pm$ 0.1	1.7 $\pm$ 0.1

T = Testis, E = Epididymides

+P<0.01 compared with controls

All figures : Mean  $\pm$  S.E.

\*Not significant compared with controls.

Biochemical estimation : Means of six determinations;

TABLE III : Serum analysis of rabbit after cyclohexanol treatment.

	Protein	Choles- terol ( $\text{mg}/100\text{ ml}$ )	SGPT (Reitman- Frankel Unit)	Phospho- lipids ( $\text{mg}/100\text{ ml}$ )	Trigly	Phosphatase activity ( $\mu\text{g P}/\text{ml}/\text{hr}$ )		Blood sugar urea ( $\text{mg}/100\text{ ml}$ )	Bilirubin ( $\text{mg}/100\text{ ml}$ )	
						Alk.	Acid			
Control	11800 $\pm$ 217	68 $\pm$ 11	18.6 $\pm$ 2.5	6.5 $\pm$ 0.5	73 $\pm$ 3.7	6.8 $\pm$ 0.5	2.6 $\pm$ 0.2	92 $\pm$ 2.0	38 $\pm$ 1.2	0.329 $\pm$ 0.01
Cyclohexanol (25 mg/kg/day for 40 days)	9840 $\pm$ 156+	164 $\pm$ 18+	31.4 $\pm$ 1.5+	9.2 $\pm$ 0.3+	96 $\pm$ 2.1+	10.5 $\pm$ 0.7+	2.0 $\pm$ 0.3*	98 $\pm$ 3.5*	39 $\pm$ 2.6*	0.513 $\pm$ 0.03+
Recovery for 70 days.	10500 $\pm$ 127	117 $\pm$ 29+	25.7 $\pm$ 2.3*	8.1 $\pm$ 0.2+	79 $\pm$ 2.5*	11.3 $\pm$ 1.2+	1.9 $\pm$ 0.2	94 $\pm$ 3*	41 $\pm$ 2*	0.471 $\pm$ 0.02+

+P<0.01 compared with controls.

\*Not significant compared with controls.

Biochemical estimations : means of six determinations.

*Biochemical changes* : Total RNA, protein, sialic acid and glycogen contents were low in the testes and epididymides of cyclohexanol treated rabbits (Table II). The testicular cholesterol was increased significantly, whereas there was a reduction in the acid phosphatase enzyme activity of the testes. The adrenal ascorbic acid contents were low (Table II). These changes returned to subnormal values after cessation of cyclohexanol treatment for 70 days (Table II).

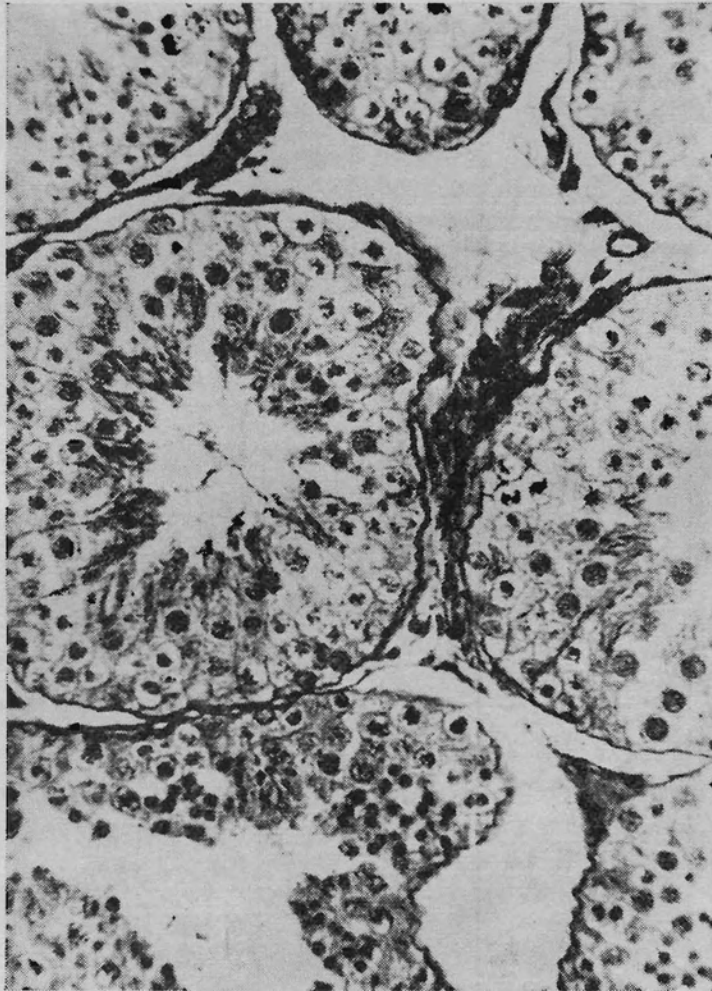


Fig. 1 : Section of testis from a control rabbit. The seminiferous tubules show full spermatogenesis.  
X 100 H.F.

tase enzyme activity of the testes. The adrenal ascorbic acid contents were low (Table II). These changes returned to subnormal values after cessation of cyclohexanol treatment for 70 days (Table II).

*Serum and blood analysis :*

Cyclohexanol administration for a period of 40 days depleted the serum protein contents, whereas an elevation of serum cholesterol, phospholipids, triglycerides, bilirubin,

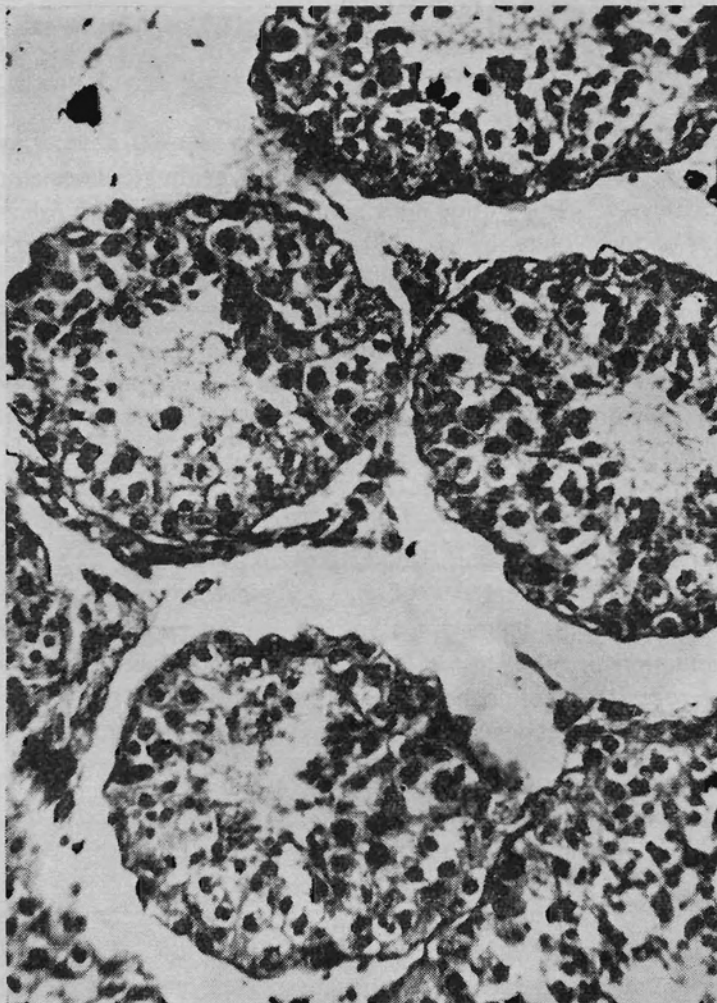


Fig. 2 : Testis after cyclohexanol treatment (25 mg/kg/day for 40 days).  
Note the degenerative changes and the absence of spermatozoa.  
X 100 HE

pyruvate transaminase and alkaline phosphatase activity was recorded. Blood sugar and blood urea remained in normal range (Table III).

Serum transaminase, triglycerides and protein contents showed a tendency to return towards normalcy after 70 days of cessation of drug administration. Whereas, the total cholesterol, phospholipids, bilirubin and phosphatase enzyme activity levels, mostly remain unaltered as compared with treatment period (Table III). Haematological studies of cyclohexanol treated rabbits were in normal range.

## DISCUSSION

Cyclohexanol in daily oral doses (25 mg/kg/day for 40 days) produced a brief period of infertility in rabbit by inhibiting the process of spermatogenesis at the spermatocyte and spermatid levels. This action was similar to that observed following nitro'uran (17), thiophene (24) and  $\alpha$ -chlorohydrin (23).

Low levels of RNA, protein and sialic acid in the testes and epididymis is probably due to an inhibition of spermatogenesis and suppressed Leydig cell function. The glycogen content of the testes of cyclohexanol-treated rabbits was reduced. Reduced glycogen content of the testes may be due to impaired glycolysis which results in an antispermatogenic action of the drug (13).

Davis and Coniglio (2) reported an increase in cholesterol concentration after one month of cryptorchidism in rat testis. Cholesterol concentration was increased similarly in the testes of cyclohexanol-treated rabbits. An increase and decrease of testicular cholesterol has been considered important, since it is implicated in the inhibition/and stimulation of sperm formation in the testes (10) and is the primary substrate for androgen synthesis (3). Decreased acid phosphatase enzyme activity in the testes is an indication of decreased activity of spermatogenesis and correlates well with the pathological changes (18).

Sayer *et al.* (22) reported that adrenocorticotrophic hormone (ACTH) injections in rats and guinea pigs lowers the adrenal ascorbic acid activity which was correlated with an elevated pituitary gonadotropin release (28).

The results of total serum protein, triglycerides and transaminase enzyme activity were comparable with those of controls after recovery period. Elevated levels of serum cholesterol, phospholipids and reduced phosphatase enzyme activity, after 70 days of cessation of drug administration suggests metabolic disorder at intracellular level (27) and an interference in the genesis of adrenal steroids (11).

Increased concentration of alkaline phosphatase, pyruvic transaminase and bilirubin in the serum has been ascribed to leakage of these enzymes/secretions from the damaged

liver tissue (5,9,4). However, histological examination of liver biopsies did not show much damages. Such lack of correlation between the histological picture and enzyme levels has been observed previously (20). Persistence of elevated levels of alkaline phosphatase, SGPT and bilirubin even after 70 days of recovery period is somewhat alarming.

Normal spermatogenesis was obtained after 70 days of recovery period. The site of action of cyclohexanol is not known. The action may depend on its structural similarity to  $\alpha$ -chlorohydrin and its analogues. In  $\alpha$ -chlorohydrin, the antifertility action was shown to be dependent on the carbon atom adjacent to the hydroxyl group bearing chlorine (6). The absence of chlorine in cyclohexanol, makes the study more interesting and suggests further probing into the structure-activity relationship with respect to antifertility action.

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