

Effects of Diazacholesterol Dihydrochloride (SC-12937), an Avian Antifertility Agent, on Rat Testis

AMIYA P. SINHA HIKIM AND J. CHAKRABORTY

The present study was undertaken to evaluate the effectiveness of an avian chemosterilant, 20, 25-diazacholesterol dihydrochloride (SC-12937), on the rat testis. Adult male rats were injected intraperitoneally with 10 mg (Group 1) or 30 mg (Group 2) of SC-12937/kg/d or with vehicle alone (Group 3) for 10 days, and were killed 24 hours after the last injection. A wide range of variation in the appearance of affected seminiferous tubules was observed in the testis of SC-12937-treated rats at both dose levels. This ranged from apparently normal-looking seminiferous tubules to almost completely atrophied tubules with no cells. Affected tubules exhibited intrapithelial vacuoles of varying size, multinucleated giant cells, germ cell exfoliation, and tubular atrophy. The presence of severely damaged and entirely normal seminiferous tubules adjacent to one another in the same section was noteworthy. The changes appeared to be dose-related. A greater number (34.6%) of affected tubules were observed in rats receiving 30 mg of SC-12937 compared with the ones receiving 10 mg of this compound (19.6%). The Sertoli cells also were affected by this drug and exhibited cytoplasmic vacuolation, a marked increase in the accumulation of lipid droplets and myeloid bodies. Necrotic Sertoli cells also were observed in the severely affected tubules. The possible mechanism of antispermatogenic action of SC-12937 in rats has been discussed briefly.

Key words: testis, germ cell, Sertoli cell, antifertility agent, SC-12937, rat.

J Androl 1986; 7:277-284.

This study was supported by a grant from the Rockefeller Foundation.

Reprint requests: J. Chakraborty, Ph.D., Professor, Department of Physiology, Medical College of Ohio, C.S. 10008, Toledo, Ohio 43699.

Current address for Dr. Sinha Hikim: Medical Research Building, 4th Floor, Harvard Medical School, Brigham and Women's Hospital, 75 Francis Street, Boston, Massachusetts 02115.

Submitted for publication October 14, 1985; revised version received March 27, 1986; accepted for publication April 2, 1986.

*From the Department of Physiology,
Medical College of Ohio,
Toledo, Ohio*

A variety of chemicals have been reported to cause spermatogenic disruption in rats and in various other mammalian species, including mice, dogs, monkeys, and men (Patanelli, 1975; Gomes, 1977; Davies and Meanock, 1981; Gould et al, 1983). Such compounds are of special interest because of their potential application as male contraceptive agents in humans and animals. These agents also are important for mammalian pest control.

One such compound, 22, 25 diazacholesterol-dihydrochloride (SC-12937), a hypocholesterolemic agent, first was used by Elder (1964) as a female oral contraceptive in pigeons. Lofts et al (1968) tested this drug on male pigeons and found a dose-dependent action in the pigeon testis. According to Lofts et al (1968), at a low dosage, SC-12937 markedly "interfered with the continued proliferation of spermatogonia and spermatocytes," while "at the higher dosage level, in addition to the above effects, many primary spermatocytes were pycnotic and there had been an interference of the germ-cell coordination in the germinal epithelium." Sinha Hikim (1985) first demonstrated that a single subcutaneous injection of SC-12937 (100 or 200 mg/kg body weight) caused testicular damage in bandicoot rats, a rodent species in India. Damage included focal disorganization of the seminiferous epithelium with exfoliation of premature germ cells in the lumen, germ cell hypoplasia, and the presence of occasional tubules showing loss of advanced germ cells beyond the first layer of primary spermatocytes.

The present investigation was undertaken to assess spermatogenic function in a laboratory animal model by treating the Sprague-Dawley rat daily with 10 or 30 mg/kg of SC-12937 for 10 days and evaluating whether this drug can be used effectively as a chemosterilant for rat control. The bandicoot rat, for example, is a pest that destroys an enormous amount of food grain in many developing countries.

Materials and Methods

Experimental Design

Fifteen male Sprague-Dawley rats (Charles River Labs, Portage, MI), weighing between 300 and 380 g, were divided into three groups and individually housed in standard facilities with controlled temperature and light-dark cycles. Food and water were provided *ad libitum*. Rats in groups 1 and 2 were injected intraperitoneally with 10 and 30 mg/kg/day of SC-12937 (20,25 diazacholesterol dihydrochloride; Lot SCOTT II-106A, G.D. Searle, Skokie, IL), respectively, for ten days. SC-12937 was prepared in sterile distilled water immediately before the administration. Rats in group 3 were given an equal volume of distilled water (vehicle) and served as controls. All animals were killed 24 hours after the last injection and subjected to gross examination. Body weight and weights of the liver, kidneys, spleen, adrenals, and testis were recorded. Each testis was removed and divided into five parts. One piece from each part, ie, a total of five pieces from each testis, were processed for epon embedding.

Specimen Preparation for Light and Electron Microscopy

Tissues collected from the rats were fixed immediately by immersion in 2.5% glutaraldehyde in 0.1 M cacodylate buffer for 12 to 16 hours, postfixed in 1% OsO₄ in 0.1 M s-collidine buffer for 1 hour, dehydrated, and embedded in epon (Chakraborty and Jhunjunwala, 1982). Semithin (1- μ m) sections were cut from the epon-embedded blocks and stained with 1% toluidine blue for light microscopic examination. For electron microscopic studies, thin sections (90-100 nm) were cut from well-preserved regions of the blocks with a diamond knife (Dupont Instruments, Wilmington, DE) on a LKB ultramicrotome (LKB Instru-

ments, Gaithersburg, MD), stained with lead citrate, and examined under a Philips 300 electron microscope.

Estimation of Damage to the Seminiferous Tubule

Damage to seminiferous tubules was estimated by using 1- μ m semithin sections stained with toluidine blue. Eight to 10 sections from each rat (1 section/block) were examined under a Nikon Optiphot microscope (Nikon Inc., Garden City, NY). For evaluation of the degree of damage in the seminiferous epithelium, the tubules were classified as follows: 1) tubules exhibiting mild intraepithelial vacuolization; 2) presence of multinucleated "giant cell" in the tubular wall; 3) tubules lined with mainly Sertoli cells with few or no germ cells; and 4) atrophied tubules filled with cellular debris. Sixty to 80 tubules from each rat of each group were examined. The number of damaged seminiferous tubules in each of the above categories was determined and the percentages were calculated. To obtain the diameter of the seminiferous tubules, 10 randomly selected, round seminiferous tubules were directly measured under the light microscope from each rat, using an ocular micrometer and 20 \times objective (Chakraborty et al, 1985; Jhunjunwala et al, 1986).

Result

Body Weight and Organ Weights

No significant change in the average body weight and organ (liver, kidney, spleen, adrenal, and testis) weight was recorded between control and SC-12937-treated rats (Table 1).

Light Microscopic Observation

Control Rats: No difference in testicular morphology was observed between the normal and distilled water-injected control rats. The seminiferous tubules contained normal Sertoli cells and all types of germ cells, that is, spermatogonia, spermatocytes, and spermatids at different stages of development. Tubular profiles showed layers of differentiating germ cells indicative of different stages of the cycle of the seminiferous epithelium (Fig. 1). Leydig cells, blood and lymphatic vessels and connective tissue elements also were normal in appearance.

SC-12937 Treated Rats: At the light microscope level, a wide range of variation in the appearance of the seminiferous tubules was noticed in the affected testes at both dose levels. The degree of damage ranged from tubules with mild intraepithelial vacuoles (Fig. 2a) to flattened seminiferous epithelium lined by a single layer of cells consisting of Sertoli cells and few spermatogonia (Fig. 2b). Tubules with multinucleated giant cells containing approximately two to 16 nuclei (spermatid) enclosed in a common mass of cytoplasm (Figs. 3a,b) were frequently ob-

TABLE 1. Body and Organ Weights of Control and SC-12937-Treated Rats

	Treatment		
	Group 1 (10 mg/kg of SC-12937)	Group 2 (30 mg/kg of SC-12937)	Group 3 (Vehicle Only)
Body	367 \pm 19*	364 \pm 7	372 \pm 11
Liver	15.58 \pm 0.41	15.86 \pm 0.66	15.99 \pm 0.94
Kidneys	2.56 \pm 0.08	2.61 \pm 0.05	2.73 \pm 0.13
Spleen	0.94 \pm 0.08	0.94 \pm 0.11	0.84 \pm 0.03
Adrenals	0.06 \pm 0.004	0.06 \pm 0.004	0.06 \pm 0.001
Testis	1.45 \pm 0.04	1.48 \pm 0.07	1.62 \pm 0.08

*Mean \pm SEM.

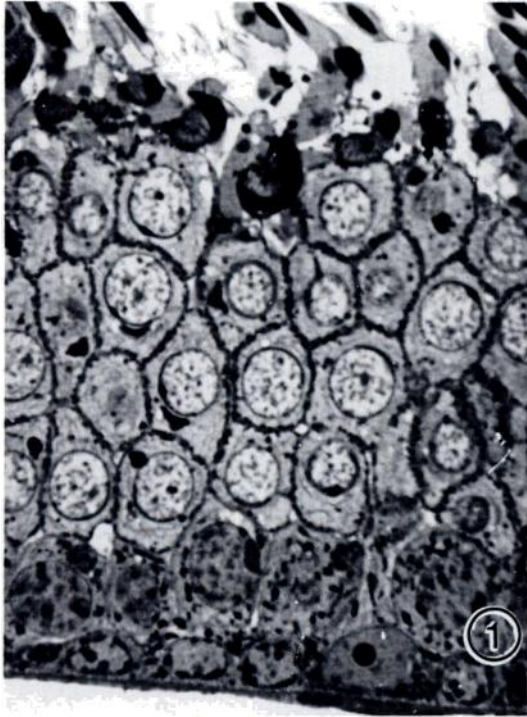


Fig. 1. Light micrograph of control specimen. Portion of a seminiferous tubule of a control rat, depicting cell association at stage VIII of the cycle. Note normal appearance of Sertoli cell and germ cells ($\times 800$).

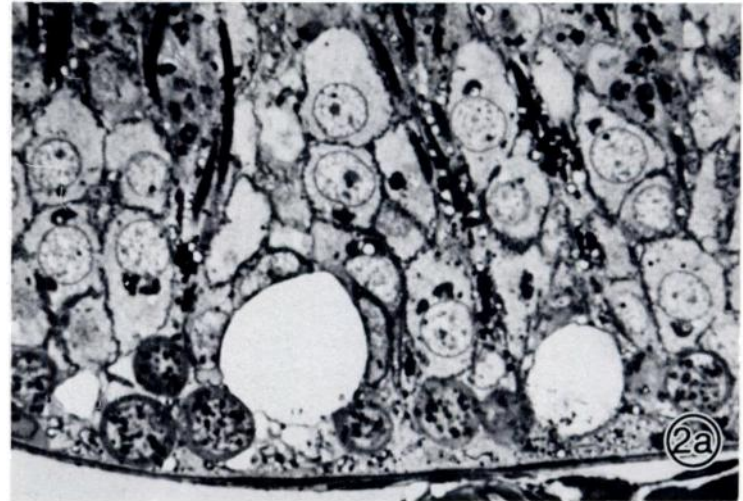


Fig. 2a. Light micrograph. Seminiferous tubule from an SC-12937-treated rat showing intraepithelial vacuoles ($\times 800$).

served. Although small intraepithelial vacuoles sometimes were observed in the seminiferous tubule of the control testis, a large round vacuole or multiple small vacuoles were characteristics of the SC-12937-treated rats and were never observed in control specimens. Degenerating germ cells were observed readily even in mild or moderately affected seminiferous tubules. Although many tubules were damaged, areas of normal-appearing seminiferous tubules still

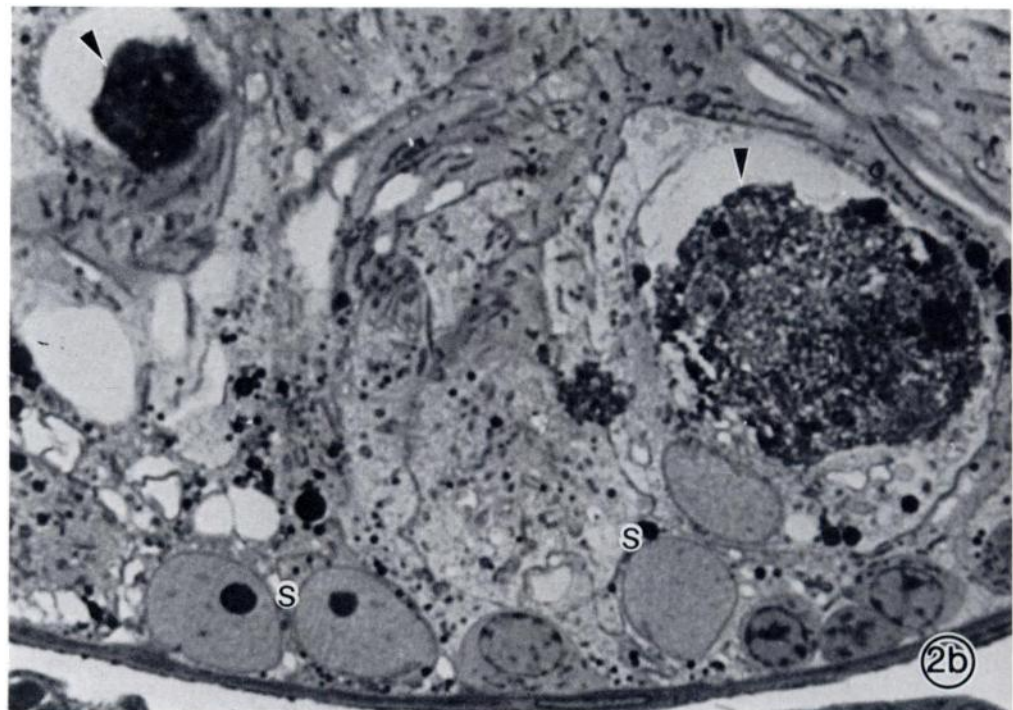


Fig. 2b. Light micrograph. Portion of a damaged seminiferous tubule from an SC-12937-treated rat showing a layer of Sertoli cells (S) with a few spermatogonia and degenerating germ cells (arrowheads) ($\times 1100$).

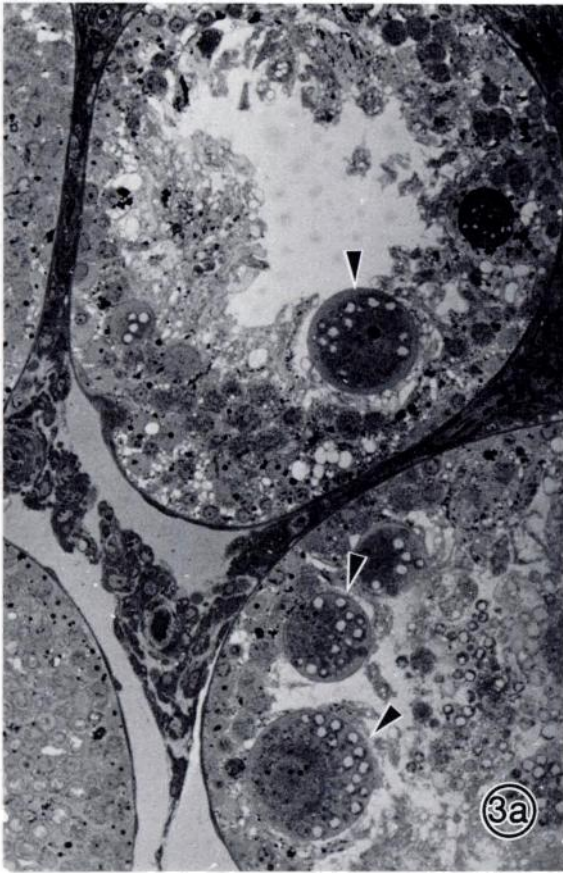


Fig. 3a. Light micrograph. Portions of two seminiferous tubules from SC-12937-treated rat containing multinucleated giant cells (arrowheads) ($\times 220$).



Fig. 3b. Light micrograph. At higher magnification, multinucleated giant cell is shown to better advantage ($\times 1100$).

were found in every block of tissue examined. These normal tubules either were intermingled with, or immediately adjacent to, groups of damaged seminiferous tubule profiles (Fig. 4). Tissues were obtained from different parts of the testis, but within an affected testis there was no pattern of regional distribution of the lesions. Complete cell depletion occurred only in a few tubules (Fig. 5).

Electron Microscopic Observation

Germ cell degeneration in the affected tubules appeared to be a common occurrence. The Sertoli cell response to this drug was highly variable. Some Sertoli cells showed signs of complete necrosis, indicated by highly vacuolated cytoplasm, dense lobulated nuclei and almost completely degenerated mitochondria, while others still retained cellular integrity. The Sertoli cells that were not necrotic appeared to become highly phagocytic in nature, and many degenerative sperm tails were found in close associa-

tion with the lobulated nuclei (Fig. 6). Some of the Sertoli cells of these affected tubules contained a large number of lipid droplets, myeloid bodies and lysosomes (not shown).

Various changes also were seen in the extratubular areas. These included thickening of the basement membrane, invagination of the basal lamina, and presence of some extratubular cells in the invaginated pockets of the basal lamina (Fig. 7).

Quantitative Analysis

Light microscopic analyses showing the percent of affected tubules in SC-12937-treated rats are summarized in Table 2 and Fig. 8. The frequency of occurrence of the affected tubules increased with increasing SC-12937 dosage. A reduction of the mean seminiferous tubule diameter also was noted in the treated rats at both dose levels when compared

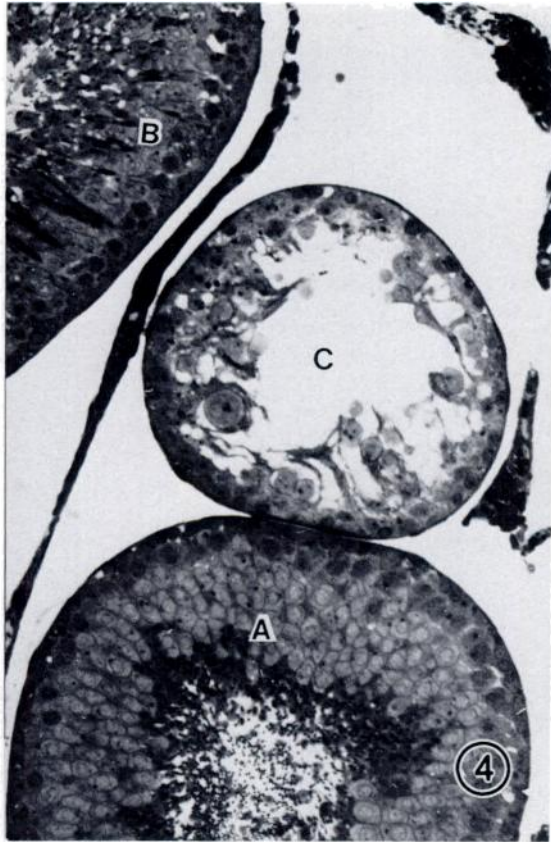


Fig. 4. Light micrograph. Testicular section from an SC-12937-treated rat showing the presence of two normal seminiferous tubules (A and B), containing normal germ cell layers adjacent to a damaged tubule (C) ($\times 200$).

with the controls (Table 3). It should be emphasized, however, that in the present study, only normal appearing tubules were considered for measuring the diameter of the seminiferous tubules in the affected testis. The intent of this screening was to eliminate the tubular profiles showing varying degrees of damage in SC-12937-treated rats.

Discussion

Information to date concerning the effects of SC-12937 on the mammalian testis is mainly based on light microscopic observations on a wild rodent pest, the bandicoot rat (Sinha Hikim, 1985). No attempt was previously made to explore its effects on the testis of any commonly used laboratory animals. Results of the present investigation demonstrate that SC-12937 also was effective in inducing spermatogenic disruption in laboratory rats, which confirmed findings on the bandicoot rat (Sinha Hikim, 1985). The present study possibly reports for the first time the fine structural changes in the testis of

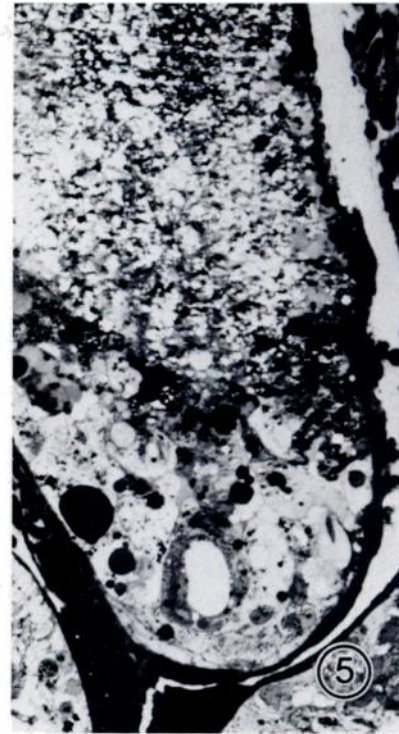


Fig. 5. Light micrograph. A highly degenerative tubule of an SC-12937-treated rat. The tubular lumen is filled with cellular debris ($\times 220$).

laboratory rats following treatment with this compound. The major deleterious effects of SC-12937 on the rat testis included occurrence of intraepithelial vacuoles of varying sizes, multinucleated giant cell formation, exfoliation of germ cells, and tubular atrophy. These changes appear to be dose-dependent. For example, a greater number of seminiferous tubules (34.6%) were damaged in rats receiving 30 mg/kg of SC-12937 compared with the group of animals receiving 10 mg/kg (19.6%). Moreover, the percentage of severely affected tubules also was higher in rats treated with higher doses of this compound when compared with the low-dose group (Fig. 8). SC-12937 is a hypocholesterolemic agent (Elder, 1964). Interestingly, testicular lesions of a similar nature have been observed in rats following administration of another hypocholesterolemic agent, chlorcyclizine (Wong and Hruban, 1972), and other anti-spermatogenic agents such as PMHI, DL-6-(N- α -pipercolino-methyl)-5-hydroxy-indane maleate (Fang and Anderson, 1976), and AF1312/TS; 1-p-chlorobenzyl-1H-indazol-3-carboxylic acid (DeMartino et al, 1975).

The basis for the extreme focal nature of the seminiferous tubular damage in the SC-12937-treated

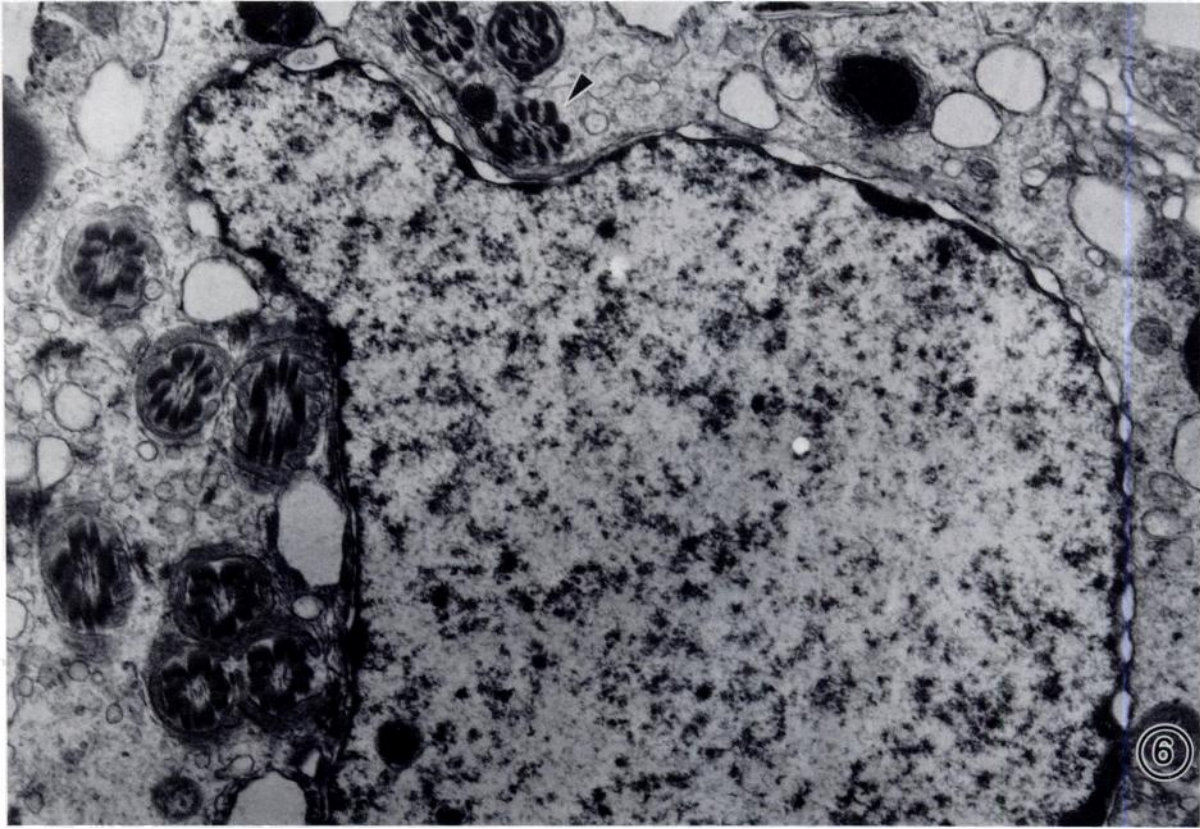


Fig. 6. Electron micrograph. Portion of a normal-looking Sertoli cell of an affected seminiferous tubule containing many degenerative sperm tails, as indicated by the loss of the plasma membrane and fibrous sheath (arrowhead) in close association with the Sertoli cell nucleus. ($\times 15,400$).

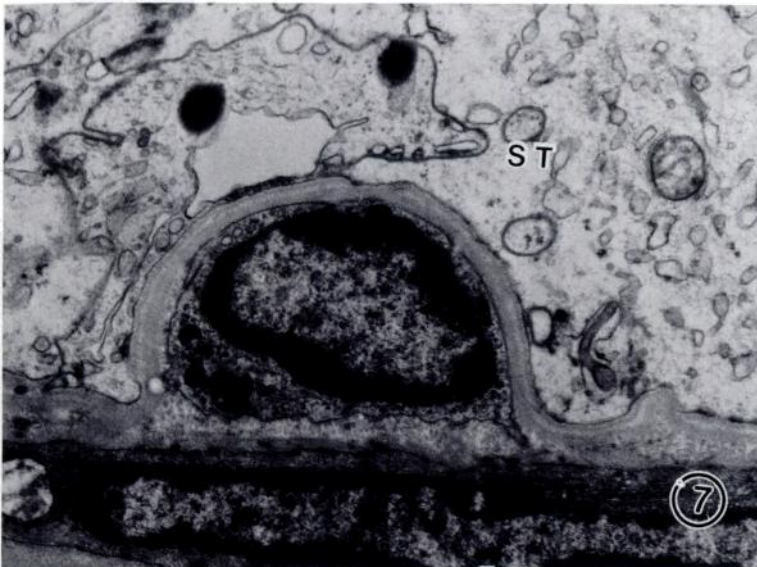


Fig. 7. Electron micrograph. Portion of the basement membrane of an affected seminiferous tubule, showing the invagination of the basal lamina and the presence of a peritubular cell within an invaginated pocket of the basal lamina inside the seminiferous tubule (ST) ($\times 11,800$).

TABLE 2. Light Microscope Analysis of the Percent of Affected Tubular Profiles in Testes of SC-12937-Treated Rats

Type of Effect	Treatment		
	Group 1 (10 mg/kg of SC-12937)	Group 2 (30 mg/kg of SC-12937)	Group 3 (Vehicle Only)
Tubules exhibiting intra-epithelial vacuolization	13.4*	16.5	7.4
Presence of multi-nucleated giant cell in the tubular wall	0.9	3.7	—
Tubules lined with Sertoli cells with few or no germ cells	4.8	8.1	—
Atrophied tubules with no cellularity	0.5	6.3	—

*Percent of affected tubular profiles.

rat, however, remains unclear. Even at the highest dose level (30 mg/kg), only 34.6% of the tubules exhibited morphologic alterations. Such a nonuniform response of seminiferous tubules to SC-12937 may be attributed to focal alterations in the microenvironment of the spermatogenic compartment. Perhaps due to the retraction of the cytoplasmic processes of some Sertoli cells, the immature spermatids were denuded prematurely and became degenerative (Lee and Gillies, 1984). Conversely, the possibility of increased susceptibility of the germ cells to this compound at one or a few stages of the spermatogenic cycle should not be ruled out. Further systematic investigation is needed to establish the cause and effect relationship of this drug in inducing testicular damage.

The Sertoli cells were consistently affected by SC-12937. There was a marked increase in the phagocytic activity and lipid droplet accumulation in these altered Sertoli cells. Similar stimulated phagocytic activity of the Sertoli cells has been reported in the testis damaged by various antispermatogenic agents (Reddy and Svoboda, 1967; DeMartino et al, 1975; Lee and Gillies, 1984). Although an increase in the lipid droplets in Sertoli cells may be caused by spermatogenic inhibition and increased phospholipids and triacylglycerol synthesis (Gillies and Lee, 1983), massive accumulation of myeloid bodies may be indicative of cholesterol deficiency. Massive accumulation of myeloid bodies has been observed in Sertoli cells and in the epididymal epithelium of the rat following exposure to another hypocholesterolemic agent, chlorcyclizine (Wong and Hruban, 1972; Wong

TABLE 3. Seminiferous Tubule Diameters of Control and SC-12937-Treated Rats

Treatment	Number of Rats	Tubule Diameter (μm)*
Group 1 (10 mg/kg of SC-12937)	5	309.8 \pm 7.7 \dagger
Group 2 (30 mg/kg of SC-12937)	5	302.8 \pm 8.3 \ddagger
Group 3 (vehicle only)	5	334.7 \pm 4.9

*Mean \pm SEM, $\dagger P < 0.05$; $\ddagger P < 0.02$. (*P* values refer to comparison with control; Student's *t* test).

et al, 1972). It should be pointed out that the myeloid bodies may be derived from autophagic vacuoles and represent lysosomes in which the membraneous elements have been inadequately digested (Wong et al., 1972). Based on the above studies, it seems likely that structural changes in the Sertoli cell may be significant participating events responsible for the spermatogenic disruption noted in SC-12937-treated rats. Although at present we are unable to speculate regarding the exact mechanism(s) of action by which this drug causes focal spermatogenic disruption in

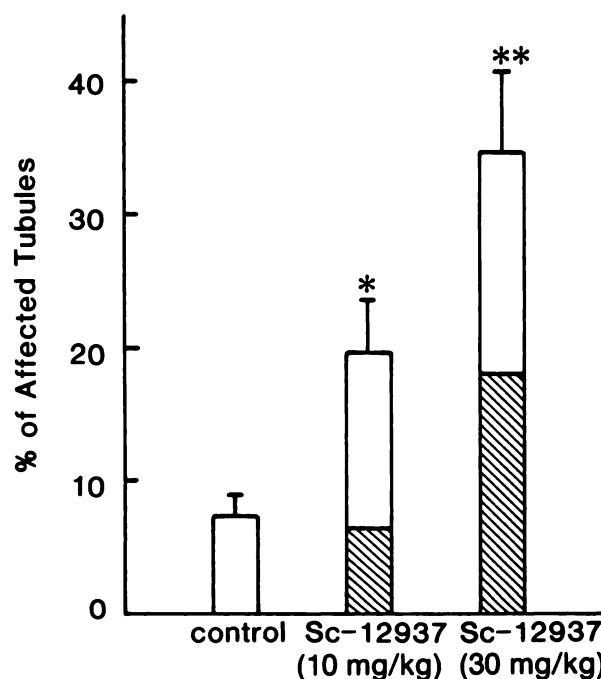


Fig. 8. A histogram showing the percentage of the affected seminiferous tubules in control and SC-12937-treated rats at two different dose levels. The clear area of each bar represents the percentage of total affected tubules, while the hatched portions indicate the percentage of severely altered seminiferous tubules. * $P < 0.05$; ** $P < 0.02$ (*P* values refer to comparison with control; Student's *t* test).

rats, it is clear that this drug is not only useful for its antispermatogenic property, but can also be effectively used to study the delicate response and intricate details of the spermatogenic process of laboratory rodents.

Acknowledgments

The generous gift of SC-12937 from G.D. Searle (Skokie, IL) is gratefully acknowledged.

References

- Chakraborty J, Jhunjhunwala J. Experimental unilateral torsion of the spermatic cord in guinea pigs. Effects on the contralateral testis. *J Androl* 1982; 3:117-123.
- Chakraborty J, Sinha Hikim AP, Jhunjhunwala JS. Quantitative evaluation of testicular biopsies from men with unilateral torsion. *Urology* 1985; 25:145-150.
- Davies AG, Meanock SJ. Potential of 5-thio-D-glucose as an agent for controlling male fertility. *Arch Androl* 1981; 7:153-158.
- DeMartino C, Stefanini M, Agrestini A, Cocchia D, Morelli M, Scorza Barcellona P. Antispermogenic activity of 1-p-chlorobenzyl-1H-indazol-3-carboxylic acid (AF 1312/TS) in rats: III. A light and electron microscopic study after single oral doses. *Exp Mol Pathol* 1975; 23:321-356.
- Elder WH. Chemical inhibitors of ovulation in the pigeon. *J Wildl Manag* 1964; 28:556-575.
- Fang VS, Anderson WA. Studies on the antitesticular action of DL-6-(N-2-pipecolino-methyl)-5-hydroxy-indane (PMHI) in the rat. *Endocrinology* 1976; 99:358-370.
- Gillies PJ, Lee KP. Effects of hexafluoroacetone on testicular morphology and lipid metabolism in the rat. *Toxicol Appl Pharmacol* 1983; 68:188-197.
- Gomes WR. Pharmacological agents and male fertility. In: Johnson AD, Gomes WR, eds. *The testis*; Vol. IV. New York: Academic Press, 1977; 605-628.
- Gould SF, Powell D, Nett T, Glode LM. A rat model for chemotherapy-induced male infertility. *Arch Androl* 1983; 11:141-150.
- Jhunjhunwala JS, Sinha Hikim AP, Budd CA, Chakraborty J. Germ cell degeneration in the contralateral testis of the guinea pig with unilateral torsion of the spermatic cord: quantitative and ultrastructural studies. *J Androl* 1986; 7:16-22.
- Lee KP, Gillies PJ. Ultrastructural alterations in hexafluoroacetone-induced testicular atrophy in the rat. *Exp Mol Pathol* 1984; 40:29-37.
- Lofts B, Murton RK, Thearle RJP. The effects of 22,25-diazacholesterol dihydrochloride on the pigeon testis and on reproductive behavior. *J Reprod Fertil* 1968; 15:145-148.
- Patanelli DJ. Suppression of fertility in the male. In: Hamilton DW, Greep RO, eds. *Handbook of physiology*; Vol. 5. Male reproductive system. Washington, D.C.: American Physiological Society, 1975; 245-258.
- Reddy KJ, Svoboda DJ. Lysosomal activity in Sertoli cells of normal and degenerating seminiferous epithelium of rat testis. An ultrastructural and biochemical study. *Am J Pathol* 1967; 51:1-17.
- Sinha Hikim AP. Effect of 22,25-diazacholesterol dihydrochloride on the spermatogenesis of a wild rat, *Bandicota bengalensis*. *Int J Fertil* 1985; (In press).
- Wong TW, Hruban Z. Testicular degeneration and necrosis induced by chlorcyclizine. *Lab Invest* 1972; 26:278-289.
- Wong TW, Hruban Z, Slesers A. Chlorcyclizine-induced changes in the epididymides and the prostatic complex of rats. *Biol Reprod* 1972; 7:398-413.

Fellowships in Reproductive Biology Vanderbilt University

Predoctoral and postdoctoral fellowships are available at Vanderbilt University in reproductive biology, biochemistry, and endocrinology. Training includes testicular biochemistry and physiology, epididymal, endometrial, and luteal function, fertilization, peptide and steroid hormone receptors, steroid chemistry, and male reproductive behavior. Participating faculty including: Frank Chytil (Mechanism of vitamin A in reproductive tissues), Benjamin J. Danzo (Hormonal regulation of male reproductive function), David L. Garbers (Physiology of sperm), Loren H. Hoffman (Structural and functional aspects of mammalian implantation), Lee E. Limbird (Adrenergic receptors), Gary E. Olson (Post-testicular sperm development), Marie-Claire Orgebin-Crist (Male reproductive physiology), J. Bradley Powers (Neuroendocrine regulation of male reproductive behavior), Howard E. Smith (Affinity labeling of steroid receptors and binding proteins). Only U.S. citizens and permanent residents are eligible for appointment. For further information, please write:

Dr. Frank Chytil
Program Director
Department of Biochemistry
Vanderbilt University
Nashville, Tennessee, 37232.

Vanderbilt University is an equal opportunity employer.