PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 162, 58-64 (1979)

Persistence of Coitus during Induced Infertility in Male Rats (40618)

F. E. HARRINGTON, J. D. ROTHERMEL, R. S. HO, R. L. ROBISON, H. A. HARTMAN, AND G. E. VISSCHER

Sandoz, Inc., Route 10, East Hanover, New Jersey 07936

During routine safety evaluation of a maltase inhibitor, 2,2-dimethyl-1-(4-methylphenyl)-1-propanone, histological preparations of testes from treated rats showed suppression of spermatogenesis. Based on histological information the alterations in sperm cell development were found to be reversible since spermatogenesis returned upon drug withdrawal. The objective of this study was to determine if the histological observations were correlated with effects on coitus and/or fertility.

Materials and methods. Adult male and nonparous female rats of Wistar origin (Royal Hart Farms) were used in the breeding study. All animals were housed individually in open wire-bottom cages in a room with a 14:10 light/dark photoperiod. Midpoint of the light period was 12:00 noon.

The breeding study spanned a 27-week period as outlined in Table I. Nonparous females were exposed only to the pelleted ration. During cohabitation the medicated ration was withdrawn overnight from the male cage and returned the following morning when the female was returned to her cage. Cohabitation was allowed on Monday, Tuesday, or Wednesday of each week and males were exposed individually to proestrus females until a mating occurred. Copulation was verified by the presence of sperm cells in the vaginal smears and/or copulation plugs. If a male failed to copulate on all 3 days, he was not exposed to another female until the following week.

Male fertility was assessed by the presence or absence of fetuses in females autopsied 19–21 days postcoitus for the first 20 weeks of the study. The last 7 weeks it was based upon actual littering data.

The photographs of the histological preparations used in this paper were obtained in part from the safety evaluation study referred to in the introduction. Adult Sprague–Dawley males (Charles River Farms) were housed individually in open wire-bottom cages and fed the drug in the diet for 8 weeks as in the current study. The concentration of the drug in the diet (see below) was identical for the two studies. Tissue samples were obtained from Sprague–Dawley males fed the drug for 2, 4, or 8 weeks as well as from males autopsied 4 weeks following drug withdrawal. Wistar males in the current study were used to provide tissue samples from males autopsied 8 weeks following drug withdrawal. Tissues from both studies were fixed in Bouins solution and 5-μm sections stained with hematoxylin–eosin for microscopic evaluation.

The compound, 2,2-dimethyl-1-(4-methylphenyl)-1-propanone, was initially sprayed onto a stock of ground rat chow by dissolving it in acetone. Following evaporation of the acetone the stock was mixed using a Hobart feed mixer with the entire quantity of ration to be fed. The drug was incorporated at a concentration that would expose each male to an equivalent of 100 mg/kg (0.1% of the diet).

Results. A graphic representation of the results obtained for the parameters investigated is presented in Figs. 1A–D. Males in both groups were found to be fertile during the 8-week pretreatment period.

The various parameters remained unchanged among control males during the treatment period (Weeks 9 through 16). However, among treated males the number of females containing sperm cells in the vaginal smears fell throughout the treatment period (Fig. 1B). By the fourth week the number of sperm cells were greatly reduced and none were normal in appearance. Sperm cells were completely absent from the vaginal smears of females exposed to treated males during the last week (Week 16) of exposure to the drug. All treated males continued to copulate, since copulation plugs were observed under the male cages.

Litter sizes recorded for females exposed to

0037-9727/79/090058-07$01.00/0

TABLE I. EXPERIMENTAL DESIGN FOR BREEDING STUDY

Type of ration administered[a]	Week(s) of study	Number of Males	Number of Weeks fed	Observations
Pelleted	1–5	26	5	Sperm cells, vaginal plugs, number fetuses
Ground	6–8	26	3	Sperm cells, vaginal plugs, number fetuses
Ground	9–16	26	8	Sperm cells, vaginal plugs, number fetuses
(a) Nontreated		13		
(b) Treated[b]		13		
Pelleted	17–27	26	11	Sperm cells, vaginal plugs, litter size

[a] Purina Rat Chow.

[b] 2,2-Dimethyl-1-(4-methylphenyl)-1-propanone, concentration = 0.1% of diet.

treated males were similar in size to controls after 6-day exposure to the drug (Fig. 1D). Only one female cohabitated with treated males was found to be pregnant at autopsy during the second and third week of drug treatment. Both pregnancies were attributed to different males. Though all five fetuses were observed to be undergoing resorption at autopsy for the female sacrificed during the second week of drug exposure, all 16 fetuses were found to be normal in the female autopsied during the third week of drug exposure.

Microscopic examination of the testes at the second week of drug treatment revealed disruption of Sertoli cells and presence of syncytial giant cells in the lumen of some seminiferous tubules compared to the findings in controls (Figs. 2A and C). The presence of giant cells and cellular debris as well as a noticeable reduction in the number of sperm cells was noted in the epididymis of treated males (Figs. 2B and D). The seminiferous tubules of males autopsied on the fourth and eighth week of drug treatment were completely free of the latter stages of germ cell maturation and some contained giant cells (Figs. 3A and C). The tubules of the epididymis were free of spermatozoa and occasionally contained giant cells along with cellular debris during this time (Figs. 3B and D).

Sperm cells were noted in the vaginal smears of females exposed to control males throughout the study (Fig. 1B). Among females exposed to treated males the vaginal smears showed numerous sperm cells during the pretreatment period (Fig. 1B). However, during the treatment period the number of females with sperm cells in the vaginal smears as well as the number of sperm cells present declined throughout this period, even though treated males were copulating as evidenced by the presence of copulation plugs under each male's cage. Following withdrawal of the compound sperm cells did not reappear, with but one exception, until the fifth week and in only 2 females (neither pregnant) at this time (Figs. 1B and C). Six weeks after discontinuation of treatment 9 females were inseminated (Fig. 1B); 4 delivered litters slightly smaller in size than controls (Figs. 1C and D). Thereafter 9 to 10 females cohabitated with treated males were inseminated each week and the majority delivered litters of normal size (Figs. 1B, C, and D).

Microscopic examination of tissue taken 4 weeks following drug withdrawal revealed that the vast majority of seminiferous tubules had returned to normal (Fig. 4A). However, occasional seminiferous tubules not fully recovered were observed. Tubules of the epididymis were devoid of spermatozoa and contained cellular debris (Fig. 4B). Both seminiferous tubules and epididymides were found to be normal in all but one of the treated males 8 weeks following drug withdrawal from the diet (Figs. 4C and D).

Discussion. Abnormal levels of glucose in the testes of rats impairs spermatogenesis as a result of degenerative changes in the germinal epithelium (1). The importance of glucose in maintaining normal morphology of the testes is illustrated by the observations of Mancini *et al.* (2) who reported several types of alterations in the germinal epithelium of rats in which hypoglycemia had been induced experimentally. Zysk *et al.* (3) recently reported that 5-thio-D-glucose, an inhibitor of glucose active transport, fed in the diet of mice for 3 weeks inhibited spermatogenesis. Whether its effect on testicular function was by way of its inhibitory effect on glucose

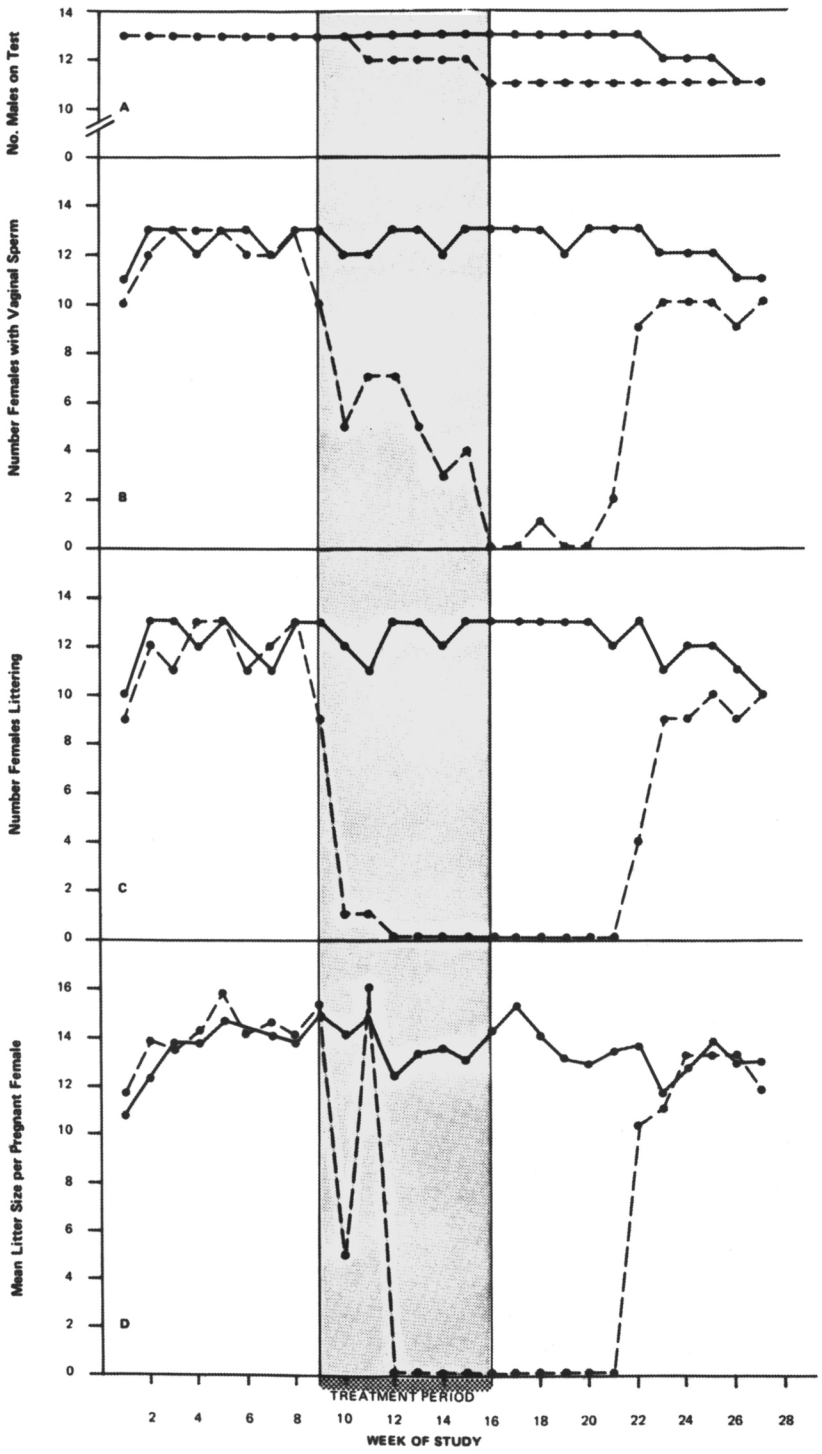

FIG. 1. The effect of 2,2-dimethyl-1-(4-methylphenyl)-1-propanone on fertility in male rats (●——● = controls, ·––· = treated).

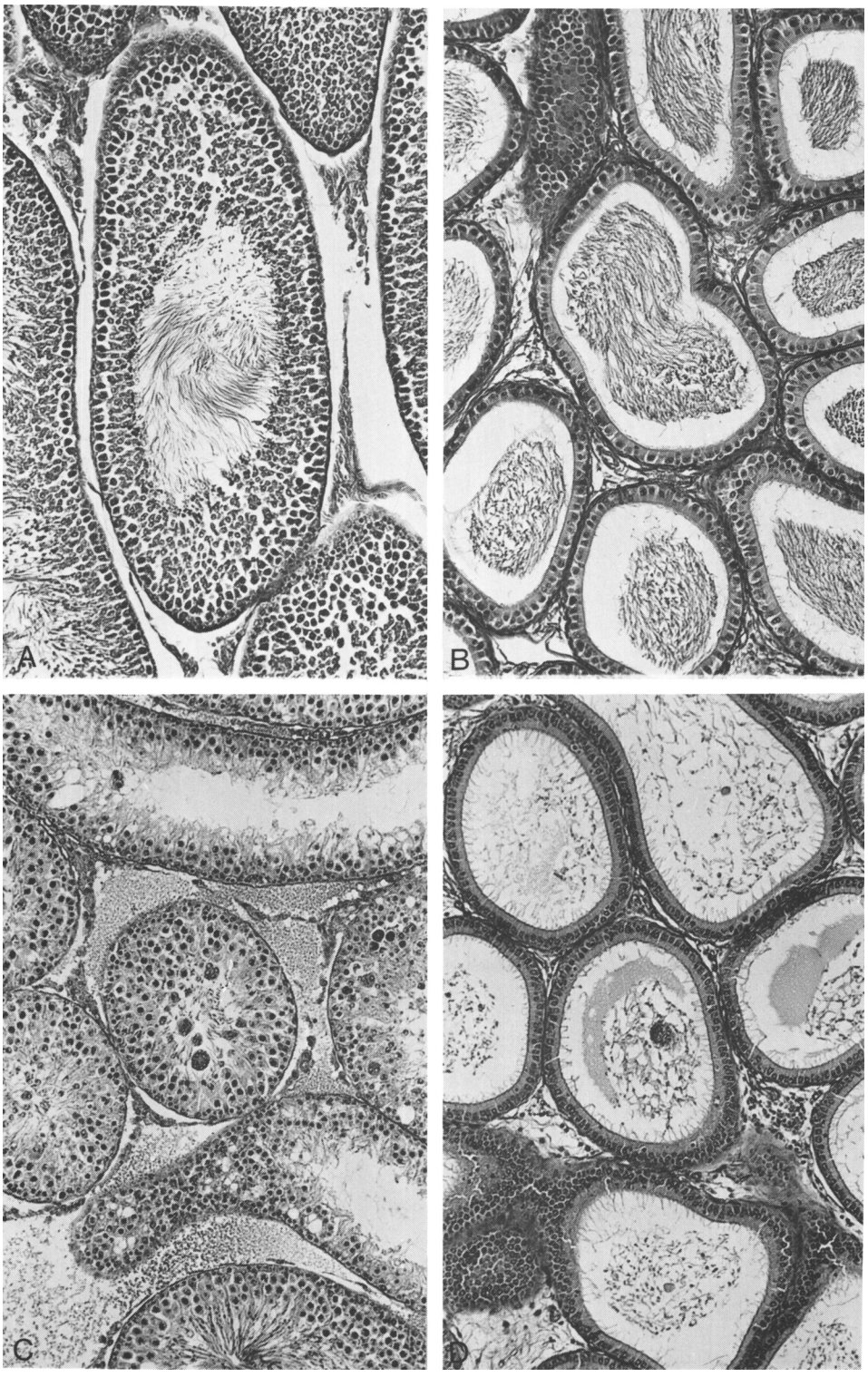

FIG. 2. Seminiferous tubules (A) and epididymis (B) obtained from a control male. Interruption of normal germ cell maturation and presence of syncitial giant cells are seen (C) in the seminiferous tubules of a rat treated for 2 weeks. The epididymis (D) of the same rat contained both giant cells and cellular debris. Note in particular the lack of spermatozoa (× 332.5).

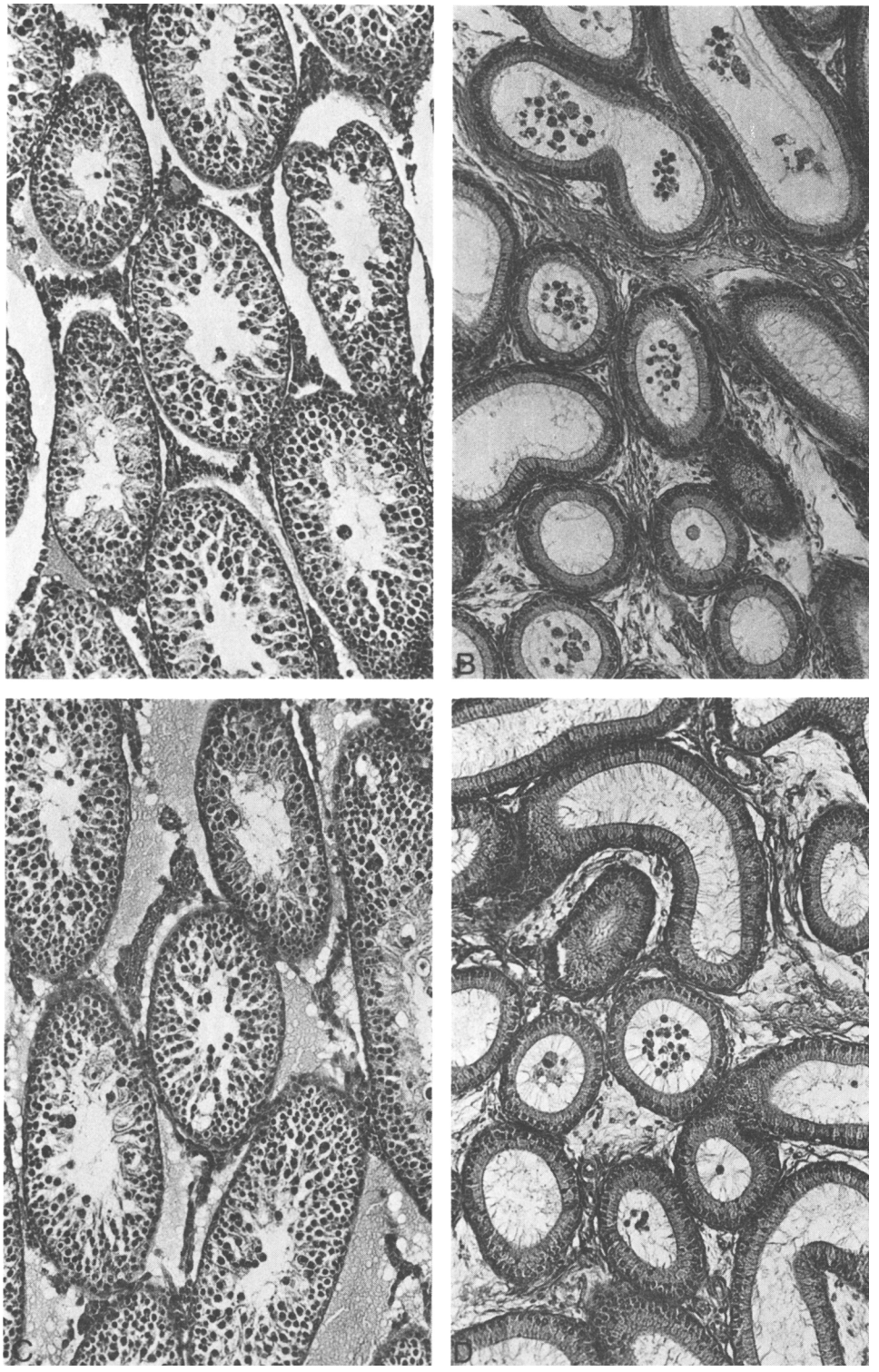

FIG. 3. Seminiferous tubules (A) of a male after 4 weeks of treatment contained reduced numbers of spermatogonia and primary spermatocytes and are completely devoid of spermatids. The epididymis (B) contained only scanty cellular debris. The seminiferous tubules (C) and epididymis (D) of males treated for 8 weeks were similar to those treated for 4 weeks (× 332.5).

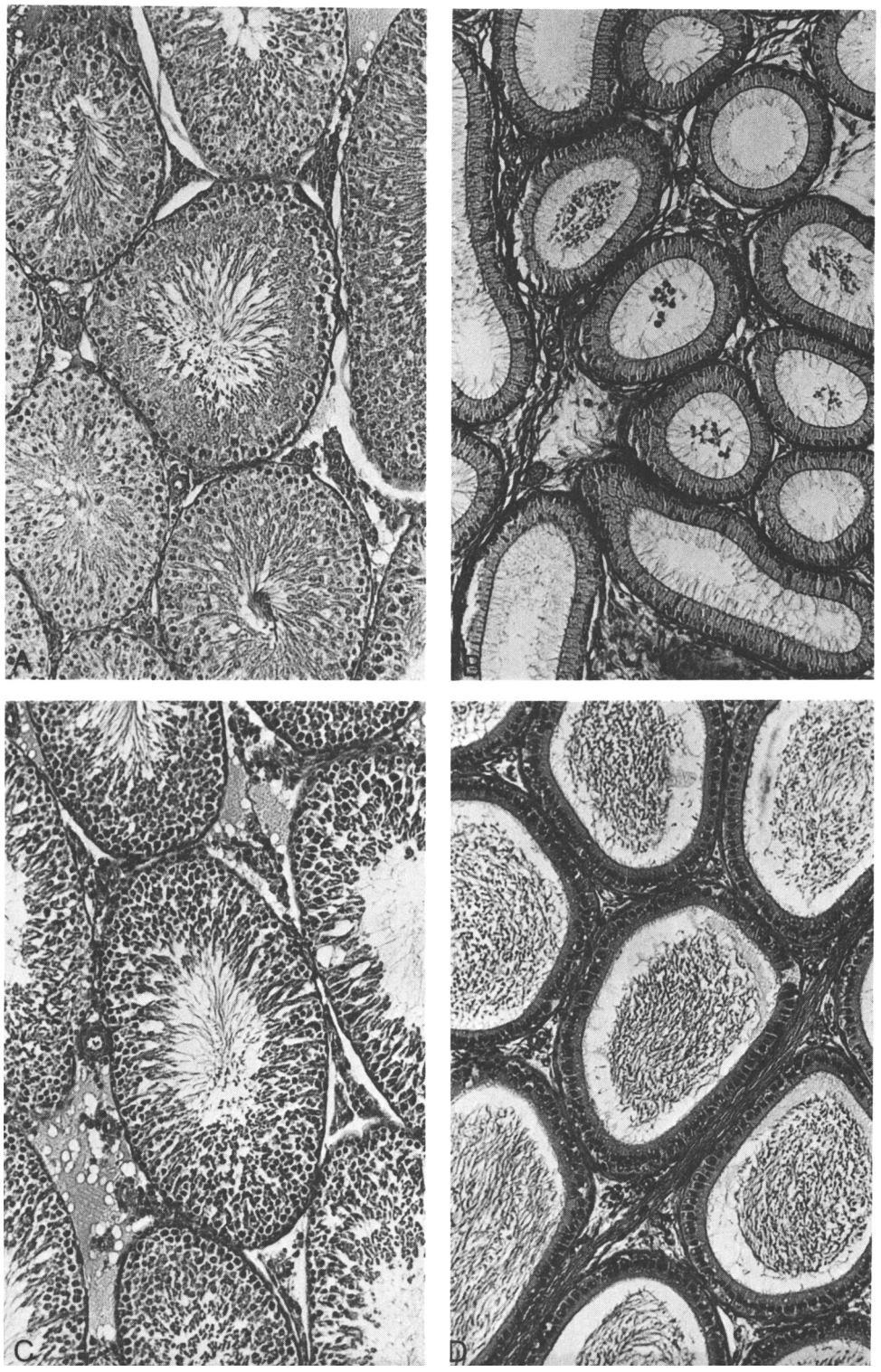

FIG. 4. Four weeks following drug withdrawal the seminiferous tubules (A) showed normal germ cell maturation and presence of spermatids. The epididymis (B) was devoid of spermatozoa and contained only cellular debris. The seminiferous tubules (C) and epididymis (D) were found to be completely normal 8 weeks following drug withdrawal (× 332.5).

active transport across cell membranes or by some unknown mechanism of action was not determined.

It is clear from this study that the testicular changes observed with the administration of a maltase inhibitor were reversible upon withdrawal of the compound from the diet. Evidence for this was based not only upon microscopic examination of the testes of treated males, but also upon resumption of fertility in breeding studies.

Equally important was the observation that treated males continued to copulate during the period of time males were found to be infertile. Sperm cells or fragments thereof were noted in the vaginal smears of females throughout 7 of the 8 weeks of drug treatment. During the eighth week of exposure to the drug, copulation plugs were recorded under the cage of each treated male even though sperm cells were completely absent from the vaginal smears at this time. In addition copulation plugs continued to be observed under each male's cage during the period of time following drug withdrawal that the males remained infertile.

The testicular changes obtained in this study bear a striking resemblance to those reported in rats in which hypoglycemic comas were induced with insulin (2) as well as mice fed 5-thio-D-glucose (3). Since both studies (2, 3) reported similar types of alterations to those found in this study, it may be the changes were associated with a testicular deficiency in available glucose.

Mancini *et al.* (2) concluded that in their study the testicular changes were not readily reversible since they observed incomplete recovery of testicular damage 5 weeks following induction of the last hypoglycemic coma. In this study the alterations were detectable in some seminiferous tubules of a few males 4 weeks following drug withdrawal but germ cell development was proceeding normally in the vast majority of seminiferous tubules, and by the eighth week testes were found to be completely normal histologically. Thus, one may conclude that the differences between these two studies may reflect in part the type of agent used to inhibit spermatogenesis as well as time of autopsy.

Zysk *et al.* (3) found that administration of 5-thio-D-glucose in the diet of male mice inhibited spermatogenesis within 3 weeks and remained so for 7 weeks without impairment of libido. Resumption of normal fertility was obtained within 5 to 8 weeks following removal of the compound from the diet. A similar pattern was observed in this study insofar as fertility was completely inhibited by the fourth week of drug treatment and remained so for 5 weeks following drug withdrawal from the diet without preventing coitus.

Clinically a compound which would inhibit spermatogenesis during administration without compromising libido and reverse its effect on spermatogenesis upon withdrawal within a reasonable length of time would be highly desirable as a male contraceptive. In this regard a nonsteroidal agent would be more desirable than a steroidal agent with its attendant hazard of disruption of the normal hormonal milieu. The findings obtained in this study as well as those reported by Zysk *et al.* (3) demonstrate in rodents that such a concept is certainly valid. Indeed, 2,2-dimethyl-1-(4-methylphenyl)-1-propanone may represent a chemical lead in the development of a clinically effective male contraceptive.

Summary. Incorporation of 2,2-dimethyl-1-(4-methylphenyl)-1-propanone into the diet of male rats inhibited fertility within 4 weeks. Examination of the testes showed that spermatogenesis was inhibited and they remained infertile without preventing coitus throughout the treatment period. Six weeks following withdrawal of the compound from the diet, litters of normal size were sired by treated males. The results obtained in this study demonstrate that male fertility can be inhibited without preventing coitus and that normal fertility can be realized upon drug withdrawal.

The authors wish to express their gratitude to Dr. Richard Elton for reviewing the manuscript and to Miss Cherle Knott for her secretarial assistance.

1. Davis, J. R., and Langford, G. A., *in* "The Testis. II Biochemistry," Chap. 5. Academic Press, New York (1970).
2. Mancini, R. E., Penkos, J. C., Izquierdo, I. A., and Heinrich, J. J., Proc. Soc. Exp. Biol. Med. **104,** 699 (1960).
3. Zysk, J. R., Buskway, A. A., Whistler, R. L., and Carlton, W. W., J. Reprod. Fert. **45,** 69 (1975).

Received February 27, 1979. P.S.E.B.M. 1979, Vol. 162.