THE PROTRACTED EFFECT OF A SINGLE DOSE OF dl-ALPHA-TOCOPHEROL ACETATE UPON THE TESTES OF RATS ON VITAMIN E-DEFICIENT DIET *

HANS KAUNITZ, M.D., ALWIN M. PAPPENHEIMER, M.D., and CLAUDIA SCHOCOLEFF
(From the Department of Pathology, College of Physicians and Surgeons, Columbia University, New York, N. Y.)

In studies of the oxygen consumption of young male rats on a vitamin E-deficient diet, it has been found that a single dose of *dl*-alpha-tocopherol acetate given on the 15th day of life is followed by prolonged lowering of total oxygen consumption as compared with untreated litter mates. This effect is continued until the onset of sexual maturity.[1]

This paper is concerned with a similar protracted effect of a single dose of tocopherol, given to rats on the 15th day, upon postpubertal testicular degeneration.

METHODS

The mother rats were maintained from the time of weaning on a vitamin E-deficient diet which consisted of: casein (commercial), 320 gm.; cornstarch, 400 gm.; lard, 220 gm.; yeast (baker's dried), 100 gm.; salts (Hawke-Oser), 40 gm.; fish oil (Mead's blended), 20 gm. During lactation, 100 gm. of yeast were added to the diet.

When mating was positive, the litter was assured by protecting the mother with 50 drops of wheat germ oil, given within 5 days after mating. To the treated rats, *dl*-alpha-tocopherol acetate (Hoffmann-La Roche) was given by mouth. Dose and day of administration are stated in the tables. In order to make the tocopherol inaccessible to the untreated rats, they were separated from their litter mates for several hours after the administration. Controls on a Rockland pellet diet † supplied data for normal testicular weights. Comparison in individual experiments was always between treated and untreated litter mates; however, the data given in the tables represent mean values derived from different litters.

The right testicle was removed at various ages under ether narcosis; sperm from the vas deferens were examined in Locke's solution for motility or evidence of degeneration, and a histologic study was made of the testis. In grading the lesions, we have followed the stages de-

* Aided by a grant from the John and Mary R. Markle Foundation.
Received for publication, May 17, 1943.

† A commercial product containing ground yellow corn, ground hulled barley, ground hulled oats, ground whole wheat, soy bean meal, meat scraps, powdered whole milk, alfalfa meal, NaCl (not iodized), precipitated chalk ($CaCO_3$).

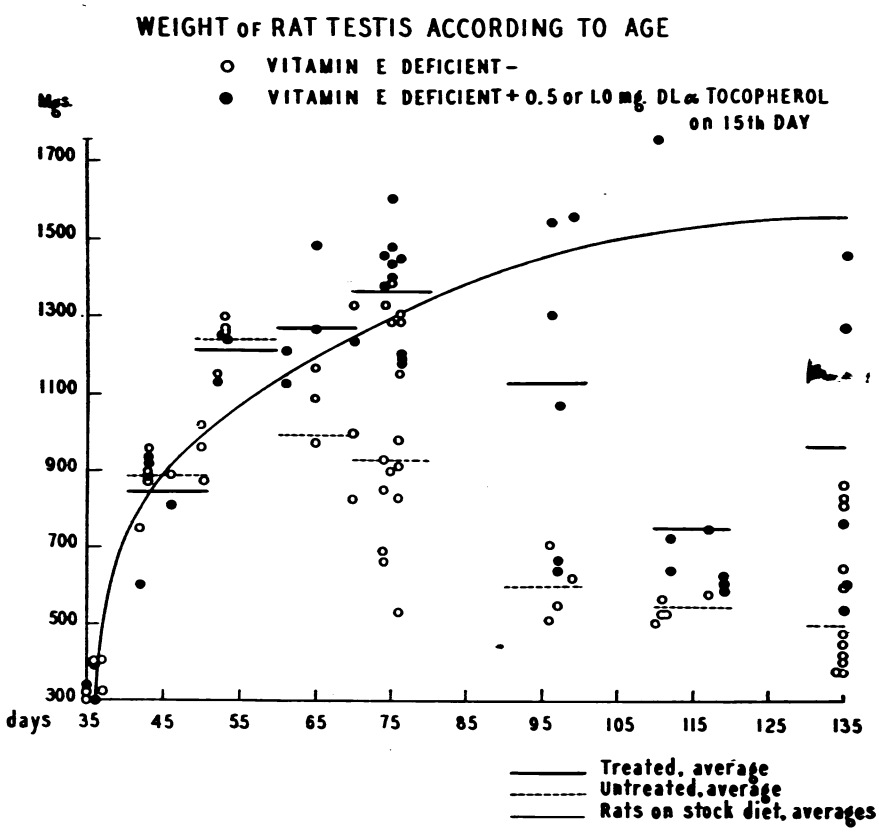
scribed by Mason.[2] After a further period of observation, the second testis was removed, also under ether anesthesia, and the animal killed.

Experimental Findings

Data upon the weights of one testis at various periods after the administration of 0.5 or 1.0 mg. of *dl*-alpha-tocopherol on the 15th day are given in Text-Figure 1, in which are recorded also the weights of one testis of the untreated litter mate controls.

It is obvious that during the prepubertal period treated and untreated rats have about the same testicular weights. A striking difference becomes manifest after the 60th day. The testicular weight of the untreated animals declines, at first rapidly, then more slowly, to reach a final average weight of about 500 mg.

The testicular weight of the treated rats continues to increase in completely normal fashion until about the 80th day. In 4 of 8 animals, maintenance of normal weight continued until the 110th day. Although



Text-Fig. 1.

Text-Figure 1 appears to show occasional overlapping in the later age groups, this was not the case when litter mates were compared. The average final weight of the testis in the treated rats at 135 to 160 days was nearly twice as great as that of the untreated controls, although there were wide individual variations.

It is seen that most of the testicular weights of treated rats fall above a curve based on the testicular weights of rats of the same colony on a stock diet.* This is explained by the better growth and heavier body weight of the litter on our experimental diet.

Plotting testicular weights against body weights has given comparable curves, although the treated animals tended to be somewhat heavier than the controls.

Motility of Sperm

The effect of the tocopherol in extending the period of sperm motility is clearly demonstrated. In 2 of the treated animals, mobile sperm were present on the 163rd day. In not a single animal were degenerative changes noted before the 100th day in the sperm obtained from the vas deferens, whereas in the nontreated rats, swelling and clumping of the sperm were observed in half of the cases during the period of 71 to 80 days, and in practically all cases thereafter.

Fertility

A limited number of fertility tests were made. In the age group from 61 to 76 days, 10 matings with normal females on stock diet† yielded 4 positive results. In 3 cases, the young, in all 27, were observed for 3 months, during which time they showed normal behavior and growth. Matings were attempted with 9 untreated litter mates of the same age group. No sperm were found in the vaginal plugs, and all matings were sterile.

Histologic Changes

The prolonged protective effect of the tocopherol was reflected in the maintenance of normal structure beyond the period in which degeneration of the testis becomes manifest with untreated vitamin E deficiency. This is brought out in Table II and in Figures 1 to 6. Not included are the histologic findings in 9 treated and 10 untreated animals examined between the 35th and 50th days, since there was no detectable difference in the testes at this time. Nor is there any evident

* We are indebted to Dr. Herbert Stoerk for these data.

† During the mating period, males and females were given only the vitamin E-deficient diet.

TABLE I

Motility or Degeneration of Sperm in Treated and Untreated Rats

Age (days)	35 to 50			51 to 60			61 to 70			71 to 81			90 to 100			110 to 120			120+		
	No.	Motile sperm	Degen. sperm	No.	Motile sperm	Degen. sperm	No.	Motile sperm	Degen. sperm	No.	Motile sperm	Degen. sperm	No.	Motile sperm	Degen. sperm	No.	Motile sperm	Degen. sperm	No.	Motile sperm	Degen. sperm
Treated with 0.5 or 1.0 mg. α-tocopherol on 15th day	9	Sperm absent	Sperm absent	3	3	0	6	5	0	13*	10	0	6	1	0	6	0	4	5	2	1
Untreated	10	Sperm absent	Sperm absent	4	3	0	5	0	0	12	0	6	4	0	3	5	0	5	7†	0	1

* The 3 rats with nonmotile sperm had received 0.5 mg. of α-tocopherol. All rats receiving 1.0 mg. had motile sperm.

† Four animals showed complete absence of sperm.

TABLE II

Comparison of Testicular Lesions of Treated and Untreated Rats on a Vitamin E-Deficient Diet

Age (days)	51 to 60				61 to 70				71 to 81				90 to 100				110 to 120				120+			
Testicular lesions*	No.	I to 0	III to II	V to IV	No.	I to 0	III to II	V to IV	No.	I to 0	III to II	V to IV	No.	I to 0	III to II	V to IV	No.	I to 0	III to II	V to IV	No.	I to 0	III to II	V to IV
Treated with 0.5 or 1.0 mg. α-tocopherol on 15th day	3	3	—	—	6	6	—	—	13	12	1	—	6	3	1	2	6	1	—	5	5	2	—	3
Untreated	4	4	—	—	5	2	3	—	12	2	6	4	4	4	—	—	5	—	1	4	7	—	1	6

* Mason's[8] grading of testicular degeneration:

0 Normal testis.

I Degeneration of spermatozoa.

II Degeneration of spermatozoa and "bead-like" degeneration of spermatids.

III Karyorrhexis of spermatids. Giant cells.

IV Disappearance of giant cells. Degeneration of spermatocytes and spermatogonia.

V Tubules lined with Sertoli cells. Disappearance of all spermatogenic elements.

difference between the 51st and 60th day, corresponding to the onset of spermatogenesis. From this time on, as the table shows, degenerative changes are consistently more advanced in the untreated rats. Even after 121 days, one occasionally finds histologically normal testes in the treated rats.

The lesions were graded without previous knowledge as to whether the individual rat had or had not received tocopherol. When comparisons were made between rats of the same litter, the differences were always consistent.

Effect of Increased Dosage

No systematic study has been made of the comparative effect of varying doses. Through inadvertence, however, 4 rats from three litters received 5 mg. on the 15th day, instead of the usual dose of 0.5 or 1.0 mg. This exercised a more delayed protective effect. In the age period of 90 to 101 days, the average weight of one testis from this group was 1.450 gm. as compared with an average weight of 1.134 gm. in rats which had received the smaller dose. All of the animals had motile sperm, and the testes were histologically normal. At 110 to 125 days, the testicular weight in 4 animals still averaged 1.271, as contrasted with a weight of 0.500 gm. in the untreated controls. Motile sperm were still present in 2, and microscopic examination showed only very early degeneration (Figs. 7 and 8).

Influence of Age at Which Tocopherol Was Administered

It has been shown that tocopherol given during the early period of lactation is relatively ineffective in preventing the onset of muscular dystrophy.[3] This raised the possibility that it might be equally ineffective in delaying the postpubertal testicular degeneration. Experiments bearing on this point are summarized in Table III, from which it is obvious that there is no protective effect whatever when the tocopherol is given in the early lactational period.

Even more interesting is the fact that administration of 1.0 mg. of alpha-tocopherol on the 29th or 30th day—that is to say, immediately after weaning—is less efficacious than when it is given on the 15th day. This is clear from the observations presented in Table IV. Although there is a definite protective effect, it is distinctly less than when the tocopherol is given on the 15th day; and in some litters, the weight of the testis of individual untreated rats exceeds that of the controls.

Discussion

These observations indicate that a single dose of 0.5 or 1.0 mg. of *dl*-alpha-tocopherol acetate given on the 15th day of life produces a

TABLE III

Influence of 1.0 mg. dl-Alpha-Tocopherol Acetate Administered on the 6th to 8th Day of Life on Postpubertal Testicular Degeneration

	Age (days)	No.	Average wt. of 1 testis	Motility of sperm	Degeneration of sperm	Histologic changes (Mason[3])		
						0 to I	I to III	IV to V
			(gm.)					
Treated	71–80	12	1.106	2	5	3	8	1
Untreated	71–80	3	1.177	0	0	—	3	—
Treated	90–100	12	0.608	0	8	—	—	12
Untreated	90–100	3	0.643	0	3	—	—	3

TABLE IV

Difference in Effect Between a Single Dose of dl-Alpha-Tocopherol Acetate Administered on the 15th or on the 29th to 30th Day of Life

	Age (days)	No.	Average wt. of 1 testis	Motility of sperm	Degeneration of sperm	Histologic Changes (Mason[3])		
						0 to I	I to III	IV to V
			(gm.)					
Treated with 1.0 mg. α-tocopherol, 29th or 30th day	71–80	11	1.240	4	0	6	3	2
Treated with 0.5 or 1.0 mg. α-tocopherol, 15th day	71–80	13	1.362	10	0	12	1	0
Treated with 1.0 mg. α-tocopherol, 29th or 30th day	90–100	10	0.774	0	8	1	2	7
Treated with 0.5 or 1.0 mg. α-tocopherol, 15th day	90–100	6	1.134	1	0	3	1	2

significant retardation of postpubertal testicular degeneration in rats maintained on a vitamin E-deficient diet. In agreement with the findings of Mason,[4] we can detect no effect during the developmental period. The onset of spermatogenesis is not delayed in the vitamin E-deficient rats, and the spermatozoa for a short time exhibit normal motility. Beginning at the 60th day, however, there is sharp divergence in the behavior of the testis in treated and untreated rats. The continuing effect of the single early dose of tocopherol is reflected in the greater weight of the testes, in the conservation of sperm motility, in the percentage of fertile matings, and in the histologic structure of the organ. This is consistently true, in spite of wide individual variation in the degree of degenerative change at any given period. A still more evident protection is obtained when the dose is raised to 5 mg.

In contrast to this striking protective effect of tocopherol when given on the 15th day is the complete lack of it when the vitamin is administered on the 6th to the 8th day. This is perhaps less surprising in view of the fact that the early administration fails also to protect against the occurrence of muscular dystrophy.

In the experiments of Mason,[4] the mother and infant rats were transferred on the 14th day from a stock diet containing three times the required amount of vitamin E to a vitamin E-deficient diet. Under these conditions, histologic signs of testicular degeneration began between the 65th and 70th days. This corresponds closely to what has been observed by us in untreated animals. Since Mason's rats presumably received a certain amount of vitamin E during the first 2 weeks of the nursing period, it would seem that a deficiency during the third week is of critical import, and that, as in our experiments, the provision of vitamin E during the early lactational period will not arrest or delay subsequent testicular degeneration. That the third week of lactation is a critical one is further borne out by the fact that when tocopherol is given after weaning it is less effective than when given on the 15th day.

The difference in the average weights of testes between treated and untreated litter mates at the age of 71 to 80 days is thus greatest in those receiving tocopherol on the 15th day, less in those receiving it on the 29th or 30th day, and entirely absent when it is given on the 6th to 8th day. The figures are 0.435 gm., 0.329 gm. and minus 0.061 gm., respectively.

Since over half of the rats treated on the 29th or 30th day had shown previous clinical evidence of muscular dystrophy, it is interesting to inquire whether this may have had a deleterious influence upon the

testicular changes. This is definitely not the case. There is no difference in absolute or relative testicular weights at the age of 71 to 80 days between the animals with and without clinical signs of muscular disease. The lack of correlation between symptoms of muscular disease and testicular degeneration is true also of the untreated animals.

Conclusions

1. Administration of a single dose of 0.5 or 1.0 mg. of *dl*-alpha-tocopherol to the offspring of vitamin-depleted mother rats delays the onset and retards the course of postpubertal testicular degeneration.

2. Administration of 5 mg. on the 15th day produces a still greater protective effect.

3. Administration of 1 mg. on the 6th to 8th day is without effect.

4. Administration of 1 mg. on the 29th to 30th day affords less protection than when given on the 15th day.

We are greatly indebted to Dr. R. D. Shaner of Hoffmann-La Roche, Inc., Nutley, N. J., for the tocopherol used in these experiments.

References

1. Kaunitz, H., and Pappenheimer, A. M. Oxygen consumption in vitamin E-deficiency. *Am. J. Physiol.*, 1943, 138, 328–340.
2. Mason, K. E. Testicular degeneration in albino rats fed a purified food ration. *J. Exper. Zoöl.*, 1926, 45, 159–229.
3. Goettsch, M., and Pappenheimer, A. M. Alpha-tocopherol requirement of the rat for reproduction in the female and prevention of muscular dystrophy in the young. *J. Nutrition*, 1941, 22, 463–476.
4. Mason, K. E. Minimal requirements of male and female rats for vitamin E. *Am. J. Physiol.*, 1940–41, 131, 268–280.

Description of Plates

Plate 46

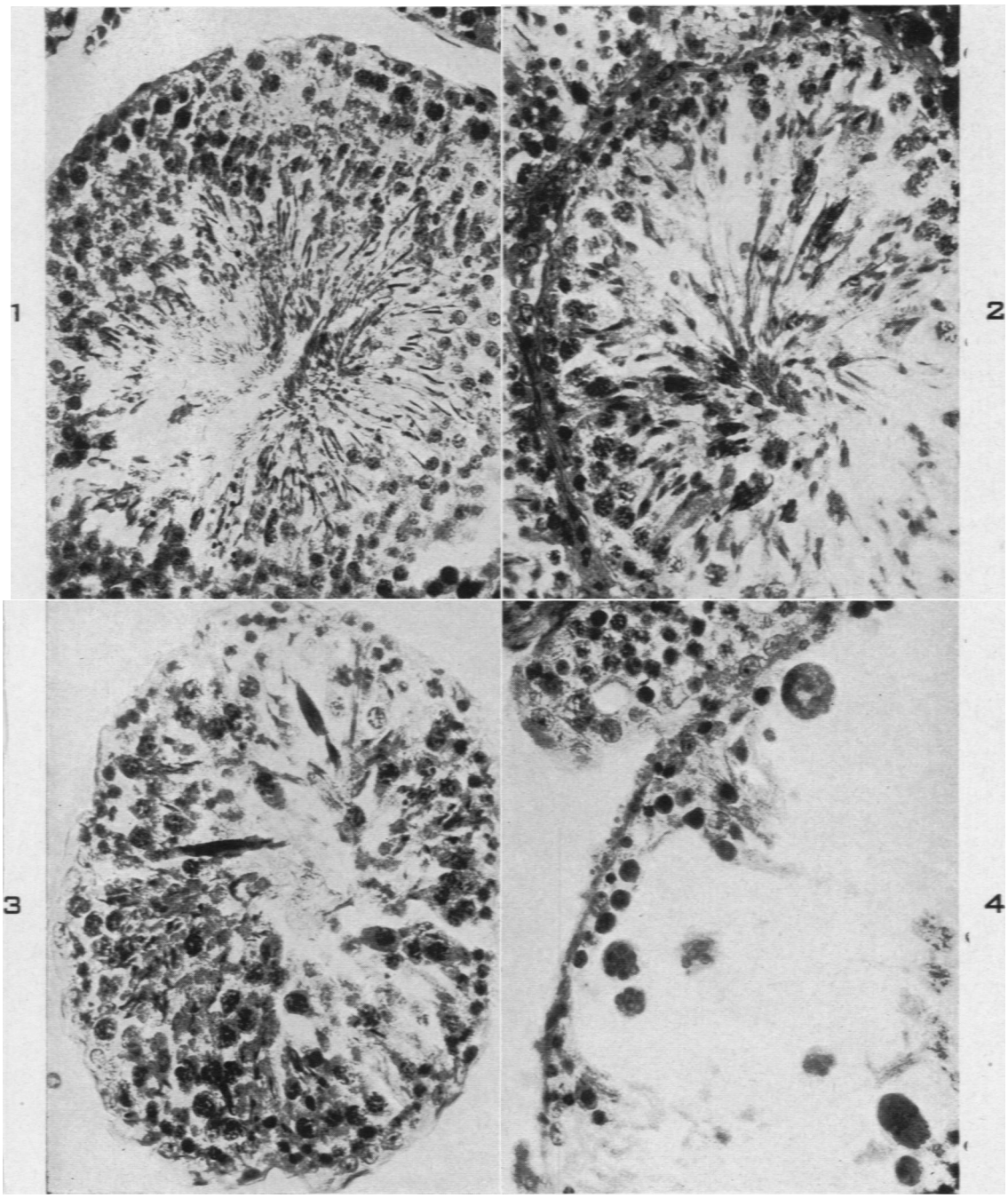
Each pair of figures, beginning with Figures 1 and 2, represents testes from litter mates. Sections are stained with Weigert's iron hematoxylin and eosin stain. × 490. See Table II for Mason grading.

Fig. 1. 65 days. 1 mg. *dl*-alpha-tocopherol on 15th day. Testicular weight, 1.482 gm. Mason stage 0.

Fig. 2. 65 days. Untreated control. Testicular weight, 1.086 gm. Mason stage I.

Fig. 3. 75 days. 1 mg. *dl*-alpha-tocopherol on 15th day. Testicular weight, 1.378 gm. Mason stage I.

Fig. 4. 75 days. Untreated control. Testicular weight, 0.936 gm. Mason stage III.



Kaunitz, Pappenheimer and Schogoleff

Effect of *dl*-Alpha-Tocopherol Acetate upon Testes

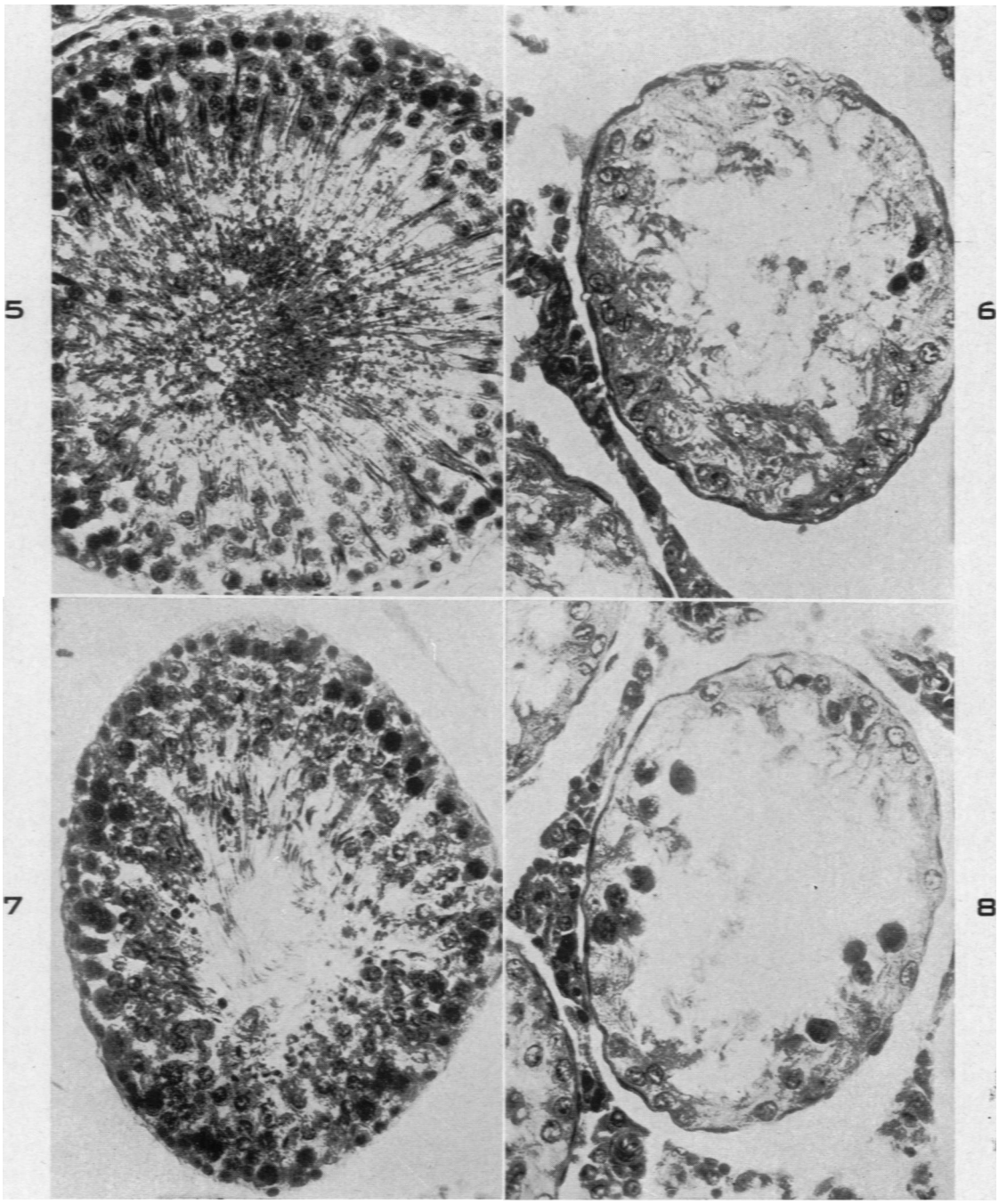
PLATE 47

FIG. 5. 97 days. 0.5 mg. *dl*-alpha-tocopherol on 15th day. Testicular weight, 1.071 gm. Mason stage 0.

FIG. 6. 97 days. Untreated control. Testicular weight, 0.549 gm. Mason stage V.

FIG. 7. 121 days. 1 mg. *dl*-alpha-tocopherol on 15th day. Testicular weight, 1.491 gm. Mason stage I.

FIG. 8. 121 days. Untreated control. Testicular weight, 0.594 gm. Mason stage V.



Kaunitz, Pappenheimer and Schogoleff

Effect of *dl*-Alpha-Tocopherol Acetate upon Testes