THE TESTIS IN VITAMIN E-DEFICIENT GUINEA-PIGS *

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Loss of fertility associated with testicular degeneration is a familiar and well studied effect of vitamin E deficiency in the white rat. The very careful work of Mason[1-4] and others has made clear the underlying histopathology. The change is irreversible;[5, 6] once effected, the testicular structure and function cannot be restored by vitamin E administration. Daily doses of 0.75 mg. of alpha-tocopherol protect the testis against degeneration.[7]

This striking result of vitamin E deficiency has thus far been demonstrated only in the rat. Bryan and Mason[8] have shown that mice on vitamin E-deficient diets do not develop testicular degeneration even after 400 days, and this is confirmed by our own experience.[9, 10] Mackenzie and McCollum[11] have found that muscular dystrophy may be produced in rabbits in the absence of testicular degeneration. Only 3 of 11 rabbits used in their experiment received no tocopherol supplement. They were maintained for periods of 49 to 79 days on the vitamin E-deficient diet. While these authors do not draw from their experiments the conclusion that vitamin E is unessential for the integrity of the rabbit's testis, their observations do indicate that the skeletal muscle in this species is more sensitive than is the testis to lack of vitamin. Probably because of the difficulty in raising guinea-pigs beyond maturity on simplified vitamin E-deficient diets, there has been no previous study as to the rôle of vitamin E in preserving the integrity of the testis in this species. Since we have succeeded in maintaining guinea-pigs in good condition for several months after sexual maturity on a diet deficient in vitamin E, the opportunity has been offered to study the condition of the testis in such animals.

METHODS

Guinea-pigs were placed on vitamin E-deficient diets soon after weaning. Of 17 animals used in the experiments, 3 received the standard diet V used by Pappenheimer and Goettsch[12] in previous studies on muscular dystrophy, but it was supplemented by aqueous lettuce extract or linseed meal, or both.[13-15] Cod liver oil was incorporated in the diet. In spite of these additions, growth was poor and

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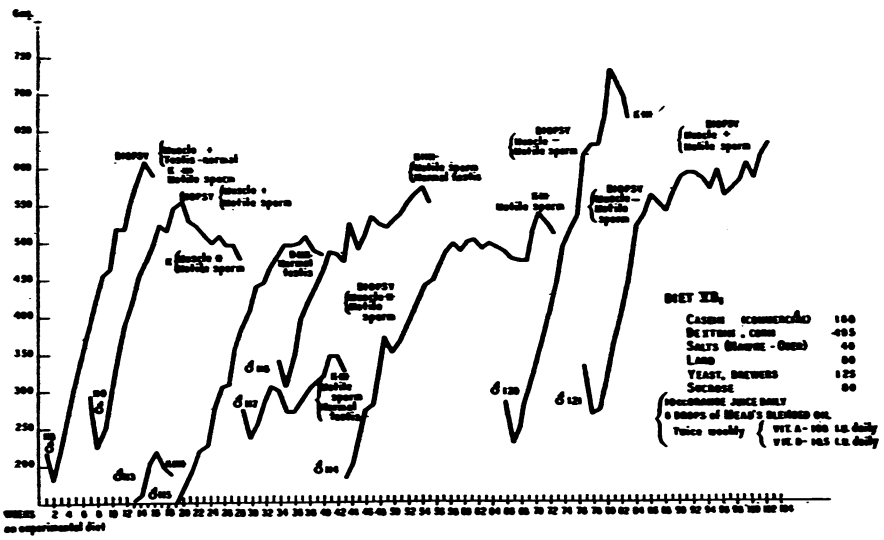
dystrophy developed between the 60th and 85th days. The testes were normal.

Five other guinea-pigs were given a diet in which dextrin was substituted for cornstarch. In some the diet was treated with ethereal ferric chloride before adding dextrin; in others, the complete diet was treated. All developed severe dystrophy, and the survival period did not exceed 70 days on the diet. The testes, in those animals which survived until after maturity, were normal. A series of 9 guinea-pigs was then given diet V B_1. In the hope of prolonging life, fish liver oil was not incorporated into the diet, but given separately twice weekly by pipette. The diet was mixed into a paste with water, dried with an electric fan, broken into small lumps and fed without additional roughage.

On this diet, the onset of muscular dystrophy was delayed, and the growth of 7 of the 9 animals was excellent, averaging 3.2 gm. daily weight gain for the first 70 days. Eventually all developed typical muscular lesions (Text-Fig. 1).



Text-Fig. 1. Guinea-pigs on a vitamin E-deficient diet.

The vitamin E content of diet V B_1 was tested on 3 pregnant rats, which were placed on the diet immediately after mating. All had resorptions.

The guinea-pigs were sacrificed after they had shown evident symptoms of muscle weakness. The motility of the sperm from vas deferens or epididymis was examined at the time of autopsy, and the testis fixed

in Zenker's fluid for histologic study. In several animals, previous specimens of one testis and muscle were obtained for biopsy, and the motility of the sperm tested.

Experimental Findings

Our observations on diet V B_1 are summarized in Table I. Because of their better growth and longer survival period, the animals in this group have yielded the most informative data.

The testes of 3 animals, killed after 105 days on the diet, presented a normal gross and histologic structure. Motile sperm were obtained from all.

After 130 to 139 days on the vitamin E-deficient diet, 2 of 4 animals showed early degenerative changes in some tubules, from swelling and clumping of the spermatozoa to complete disappearance of spermatozoa and spermatids (Fig. 1). The other 2 animals had normal testes. Motile sperm were present in the epididymides of all.

Guinea-pig 114, from which a normal testis was obtained by hemicastration after 131 days, was killed on the 165th day. The testis then contained a moderate number of degenerated tubules, but motile sperm were still present in the epididymis. Guinea pig 121, having a normal testis on the 84th day, had developed a very advanced atrophy of the remaining testis when killed on the 175th day (Fig. 2). The organ was shrunken, flabby and distinctly brown. Spermatogenesis was absent; the tubules were lined with Sertoli cells, and contained occasional larger elements filled with brownish pigment. The appearance corresponded to Mason's [1] stage V in rats. In spite of the extreme degeneration, motile sperm could still be obtained from the epididymis.

Attempts to prove the fertility of these animals by matings with normal females on stock diet have thus far not succeeded. Seven matings after the males had been from 62 to 112 days on the vitamin E-deficient diet have all been sterile.

The skeletal muscles of all guinea-pigs on diet V B_1 have shown characteristic dystrophic lesions (Figs. 3 and 4).

Discussion

These experiments, like those of Mackenzie and McCollum[11] with rabbits, demonstrate that muscular dystrophy in guinea-pigs may be produced while the testis is still histologically normal and elaborating actively motile sperm. They do not bring proof that vitamin E is unessential for spermatogenesis. Testicular degeneration was advanced in the single animal that survived for 175 days, while early degenera-

TABLE I

Muscular Dystrophy and Testicular Findings in Guinea-Pigs on a Vitamin E-Deficient Diet

No.	Days on diet V B_1*	Initial weight	Maximum weight	Final weight	Muscular dystrophy	Mobile sperm	Testicular structure	Incidental lesions
		(gm.)	(gm.)	(gm.)				
117	K105	280	342	322	+++	+105 days	Normal	—
118	K105	216	605	535	+++	+ 96 days +105 days	Normal	—
120	K105	286	730	650	+++	+105 days	Normal	—
115	D130	150	500	365	++++	?	Normal	—
116	K132	340	560	430	++++	+132 days	Swelling and agglutination of sperm (Mason stage I)	Lipoid pneumonia
114	Biopsy 131				++	+131 days	Normal	—
119	K139	296	552	470	++	+139 days	Few tubules degenerated	Hemorrhages over thorax; necrosis of liver
114	K165	180	525	498	+++	+165 days	Moderate number of degenerated tubules	—
121	K175	335	675	635	+	+ 84 days +163 days +175 days	Normal Complete degeneration	—

* Diet VB_1: Commercial casein, 180 gm.; dextrin (corn), Merck N.F.V. 495 gm.; salts (Hawke-Oser), 40 gm.; lard, 80 gm.; brewer's yeast, 125 gm.; sucrose, 80 gm.; 10 cc. of orange juice daily; 8 drops of Mead's blended fish oil twice weekly, stated to be equivalent to 108 I.U. of vitamin A and 10.5 I.U. of vitamin D.

tive changes in some tubules were observed in guinea-pigs sacrificed at periods from 130 to 165 days.

In the rat, degeneration of the testis is usually present after 40 to 50 days on a vitamin E-deficient diet begun immediately after weaning. From experiments reported in the following article,[16] we have learned that a single small dose of tocopherol given to young rats on vitamin E-deficient diet during the late nursing period will significantly delay the onset of testicular degeneration. Since our guinea-pigs were suckled by mothers on a stock diet containing vitamin E, the late appearance of the testicular degeneration may perhaps be ascribed to this fact. Attempts to gain further information on the influence of this milk factor are being made.

Conclusion

Muscular dystrophy developed in guinea-pigs on a vitamin E-deficient diet before the appearance of testicular degeneration. Early degenerative changes were first noted in the testicles after 130 days on the diet, and advanced degeneration was present after 175 days.

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DESCRIPTION OF PLATE

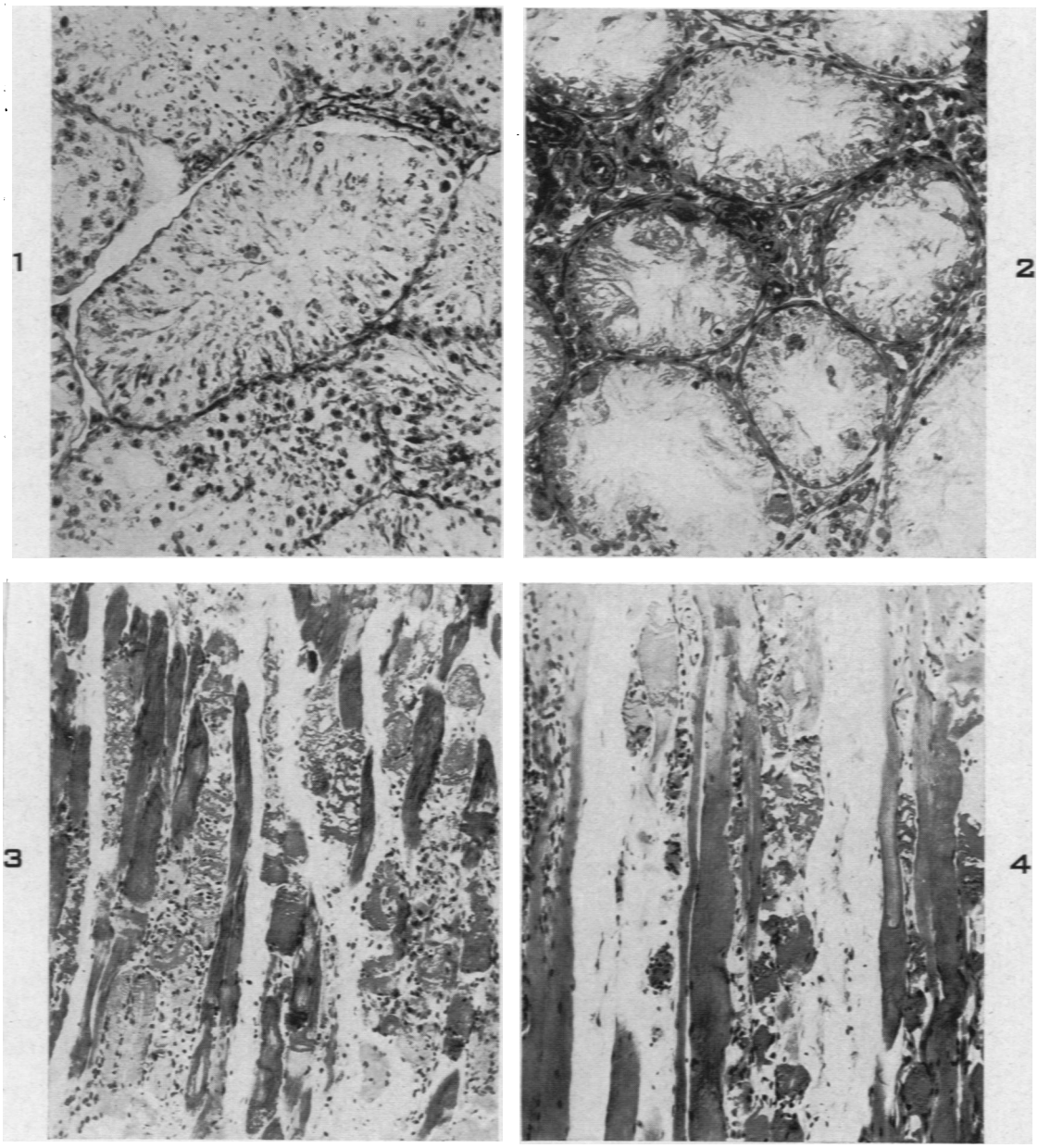
PLATE 45

FIG. 1. Guinea-pig 116. Killed after 132 days on diet V B_1. Early degeneration of testis. Hematoxylin and eosin stain. × 190.

FIG. 2. Guinea-pig 121. Killed after 175 days on diet V B_1. Advanced degeneration of testis. Large pigment cells. Hematoxylin and eosin stain. × 190.

FIG. 3. Guinea-pig 116. Skeletal muscles. Hematoxylin and eosin stain. × 90.

FIG. 4. Guinea-pig 121. Skeletal muscles. Hematoxylin and eosin stain. × 90.



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