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Decreased Citrate Synthesis: Possible Indication of Early Degenerative Changes in Testes of Vitamin E-Deficient Rats[1] (39052)

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Deficiency of Vitamin E (α-tocopherol), a derivative of 6-hydroxychroman, in animals causes a variety of pathological changes such as sterility in rats, skeletal muscle dystrophy in rabbits and encephalomalacia in chicks. At present the biochemical role of vitamin E is unknown although attempts have been made to explain its biological effects and the role it plays as an antioxidant, as a component of the respiratory chain, and also in the regulation of genetic expression (1). The role of vitamin E in carbohydrate metabolism has been the subject of several studies (2–5). Oral glucose tolerance tests conducted by Sinha *et al.* (6) indicated no changes resulting from vitamin E-deficiency in rats. However, a lower glycogen content was observed in the skeletal muscle, liver, and testes of E-deficient rats and guinea pigs. These authors suggest an indirect role for vitamin E in carbohydrate metabolism.

Alterations of the activity of enzymes involved with oxidative metabolism of glucose have been reported by many investigators. Increased oxygen consumption of skeletal muscle strips and slices from E-deficient animals has been reported by Weinstock *et al.* (7). These authors found that in ATP suplemented liver particulate fractions obtained from E-deficient rabbits, all of the citric acid (CA) cycle intermediates except citrate, when used as substrates showed increased oxygen consumption. Vitamin E-deficiency produced in rats fed a Torula yeast diet showed a respiratory decline in liver slices and homogenates (8).

Marked testicular degeneration occurs in vitamin E-deficient rats (9). A study of glucose metabolism in the E-deficient rat testes comparable to those reported for the E-deficient rat liver has not been done. The investigations reported here were undertaken to: a) Test the ability of the different tissues especially testes from E-deficient and control rats to incorporate 1-^{14}C-acetate into citrate. (b) Investigate the effect of administering an intravenous glucose load to vitamin E-deficient rats exhibiting manifest signs of testicular degeneration.

Materials and methods. Weanling Sprague–Dawley rats were fed at 28 days of age either a diet deficient in vitamin E or control (vitamin E supplemented) diet. The tocopherol deficient diet was prepared according to the formulation of Cawthorne *et al.* (10) with 0.2% of D,L-Methionine, the latter was added since casein in the diet might not provide adequate amounts of this amino acid for a growing rat (11). The control diets were prepared by adding 60 mg alpha tocopherol acetate per kilogram to the deficient diet.

The animals were pair fed and weight gains were checked at least three times weekly. The E-deficient state of the experimental animals were tested by at least two of the following criteria: (a) Erythrocyte hemolysis test (12). (b) Plasma tocopherol levels were determined by the method of Duggan (13) as modified by Hansen and Warwick (14). (c) Histological examinations of the E-deficient and control rat testes.

Glucose tolerance tests were performed by the intravenous (tail vein) injection of a 30% glucose solution in 0.9% saline. The dose was one ml. per 100 gram body weight. The animals were ether anesthetized and blood samples for glucose and insulin determinations were collected from the or-

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bital sinus at 15, 30, 45, 60, and 120 min after administering the glucose load. Blood glucose was determined by the glucose oxidase method. Plasma insulin assays were performed with the Phadebas insulin assay kit purchased from Pharmacia.

In vitro incorporation of 1-^{14}C acetate into citrate was carried out by a radiometric assay described recently by Dixit and Stern (15). The tissues from the rat were excised rapidly, placed in chilled beakers and minced with scissors. Fifty mg of minced tissue samples were weighed on coverslips and placed in flasks with side arm containing a 2 ml medium described by Dixon and Perkins (16). Oxaloacetate, monofluoroacetate (MFA) and 0.25 μCi of 1-^{14}C-acetate (purchased from New England Nuclear Corporation, Boston, specific activity 2 mCi/m*M*) were added as substrates to the vials. After incubation at 27° for 30 min the reaction was stopped by adding one ml of 9 *N* sulfuric acid containing 1 mg per ml citric acid. The labeled citrate formed was determined as described (15).

For histological examinations the testes

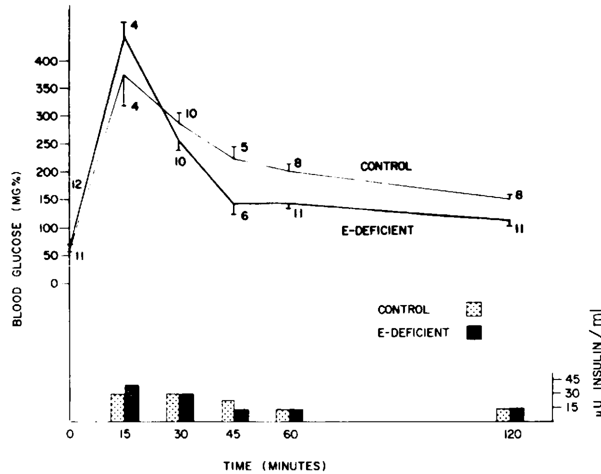

FIG. 1. Blood glucose and serum insulin levels in vitamin E-deficient and control rats following the intravenous administration of a glucose load (1 ml of 30% glucose solution per 100 g body weight). Vertical lines represent standard error of the mean. The numbers along the bars denote the number of rats. Insulin values are expressed as microunits (μU) per ml of serum.

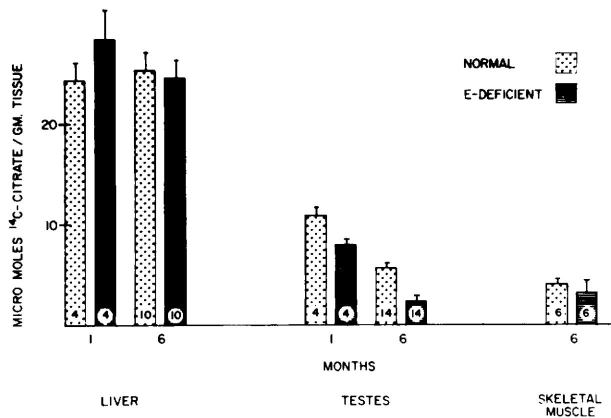

FIG. 2. *In vitro* incorporation of acetate into citrate by tissues obtained from normal and vitamin E-deficient rats. The bars represent the mean values, while the vertical lines on the bars denote standard error of the mean (SEM). The rats were fed the E-deficient and α-tocopherol supplemented diets at age 28 days.

from experimental and control animals were excised rapidly and placed in Bouins fixative for periods of up to 12–24 hr. They were then dehydrated, embedded in paraffin, sectioned routinely and stained with haematoxylin and eosin.

Results. Glucose tolerance. The blood glucose levels from E-deficient rats were not significantly different from the control group between 0 and 30 min after the glucose load was administered. Blood glucose levels between the two groups, however, differed significantly after 30 min in that the levels from E-deficient rats approximated normal levels at 45 min ($P < 0.02$), whereas those of the control group did not approach normal levels until 120 min after the administration of the initial glucose load (Fig. 1).

Plasma insulin levels while higher in the E-deficient rats at 15 minutes, dropped to low levels after 30 min, whereas, in the control animals the plasma insulin levels decreased to these low levels 45 min after the initial glucose load (Fig. 1).

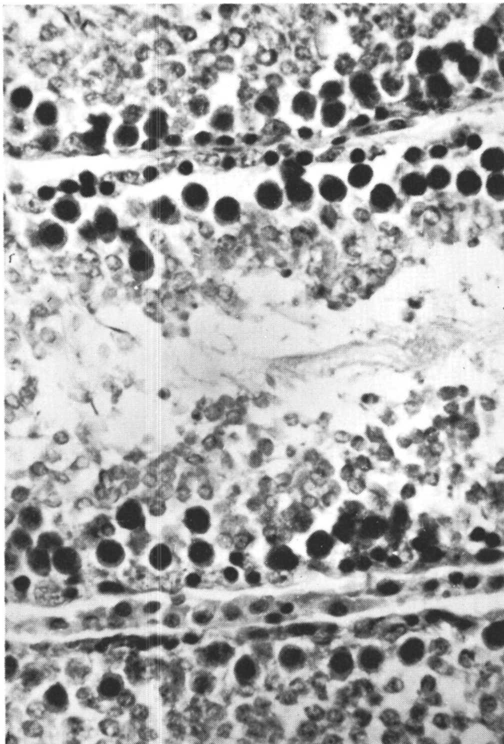

FIG. 3. Photomicrograph of seminiferous tubules from a rat fed an E-deficient diet for 1 month. Normal germinal epithelium and the presence of spermatozoa is indicative of active spermio and spermatogenesis. Hematoxylin–Eosin (H and E). Magnification ×750.

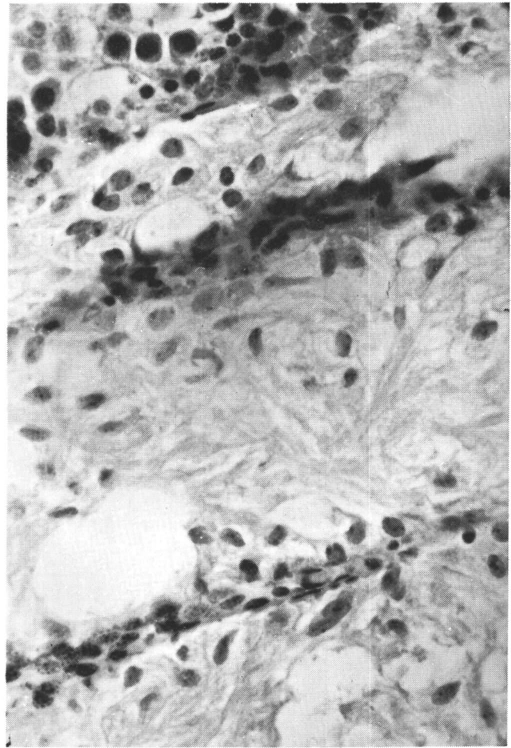

FIG. 4. Photomicrograph of seminiferous tubules from a rat fed an E-deficient diet for 6 months. Classical degenerative changes are evident in the tubules as described by Mason (9). H and E, magnification × 750.

The incorporation of labeled acetate into citrate in the liver and skeletal muscle of E-deficient rats did not differ from their respective controls. However, in the testis tissue of the E-deficient rat the ability to synthesize citrate was significantly lower than that of the controls (Fig. 2). This difference was noted quite early e.g., only one month after placing the animals on an E-deficient diet. It is noteworthy that at this early stage degenerative histological changes were not observed in the testes (Fig. 3), although the serum tocopherol levels were low. Marked degenerative changes in the seminiferous tubules noted in our rats fed the E-deficient diet for 6 months compared to their controls are shown in Figs. 4 and 5 respectively.

Discussion. The results of our glucose

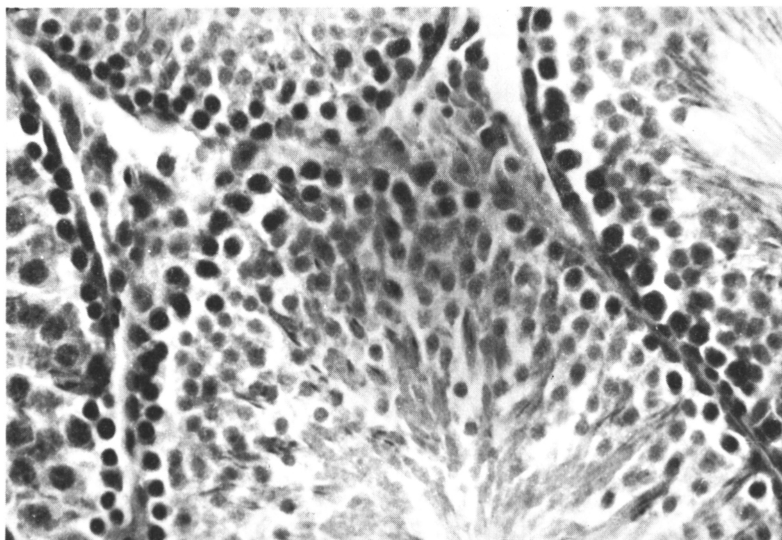

FIG. 5. Photomicrograph of seminiferous tubules from a rat fed a diet supplemented with adequate amounts of vitamin E (α-tocopherol) for 6 months. Normal germinal epithelium with active spermiogenesis is evident. H and E, magnification $\times$ 750.

tolerance studies are different from those reported by Sinha *et al.* (6) who found no significant difference between the glucose tolerance tests of E-deficient and control rats. It should be pointed out, however, that in the study conducted by Sinha *et al.* the rats were fed an E-deficient diet for 12 weeks, whereas in the present study the animals were fed the diet for 18–20 weeks. Furthermore, Sinha *et al.* used an oral glucose tolerance test, and in this investigation we used the intravenous route for glucose administration.

The blood glucose levels of our E-deficient rats were significantly lower than that of the controls 45 min after the glucose load was administered and remained lower than the control blood glucose values during the remainder of the time intervals. The marked drop in blood glucose levels of E-deficient rats 30 min after the glucose load could not be attributed to an effect of insulin release, since the plasma insulin levels were low at that point (Fig. 1). Since the intravenously administered glucose disappears from the blood at faster rates in vitamin E-deficient rats compared with the control rats, it is possible that changes in tissue membrane permeability may account for the observations reported. In this connection it may be pointed out that vitamin E-deficiency results in altered membrane structure (17).

Of the various tissues studied, the testes of our E-deficient rats showed a significant drop in their ability to synthesize citrate. This drop was noted as early as one month after the rats were placed on the E-deficient diet. It is important to note that histologically no degenerative changes were apparent in the testes of these rats.

Marked testicular degenerative changes, gross and histological, in addition to a marked decrease in the ability of the testes to synthesize citrate was noted in rats fed the E-deficient diet for 24 weeks. This decrease in their ability to synthesize citrate could be attributed to a decrease in the total mass of testicular tissue. However, the dry weights of whole or minced testicular tissue from these rats were not significantly different from their respective controls. Furthermore, as pointed out earlier, the decreased ability of testes tissue to synthesize citrate was also noted at a time when no histologically degenerative changes were evident.

We suggest, therefore, that the reduced citrate synthesis reported may be an early manifestation of the effect of E-deficiency in the testis. Any further interpretation of our

citrate synthesis experiments is difficult because the effect of E-deficiency on other metabolites or substrates of the citric acid cycle in E-deficient testis tissue has not been reported in the literature.

Summary. We have shown that intravenously administered glucose disappears from the blood of E-deficient rats at different rates compared to that of the control rats and that this difference could possibly be explained by membrane permeability changes in E-deficiency. We have also shown that the ability of E-deficient rat testis tissue to synthesize citrate is decreased, and that this decrease is probably an early manifestation of testicular degeneration.

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