Lactic and Malic Dehydrogenases in Testes of Zinc-Deficient Rats[1] (36759)

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Zinc deficiency in weanling rats causes growth retardation, dermal lesions, esophageal hyperplasia and, in males, testicular atrophy and spermatogonic arrest (1–3). In females, zinc deficiency impairs estrous cycles (2) and during pregnancy causes embryonic death and congenital malformations (4, 5). Because zinc is known to be an essential component in a number of enzyme systems (6–8), it was thought that the morphological lesions observed in zinc deficiency might be brought about by alterations in enzyme activity.

Prasad *et al.* (9) have reported histochemical observations suggesting that the activities of a number of enzymes were reduced in some tissues of zinc-deficient rats. In particular, lactic dehydrogenase (LDH) and malic dehydrogenase (MDH) activities were reported to be markedly decreased in the testis. However, preliminary spectrophotometric studies in our laboratory suggested that LDH activity was not lower in the testes of zinc-deficient rats than in controls.[2] An experiment was therefore designed in which both spectophotometric and histochemical methods were used to measure enzyme activities in the testes of zinc-deficient rats and their controls.

Methods. Weanling male Sprague-Dawley rats weighing 50 ± 5 g were purchased from a commercial source and were fed either a zinc-deficient ration or a zinc-supplemented control ration. The zinc-deficient ration contained 0.3 ppm of zinc[3] and had the following composition (%): isolated soybean protein,[4] 30.0; sucrose, 57.3; corn oil, 8.0; salt mix,[5] 4.0; and DL-methionine, 0.7. The soybean protein was extracted with a chelating agent[6] to lower its zinc content (2). The control ration was the same as the zinc-deficient diet except that zinc carbonate was added to the salt mix providing a total content in the diet of 100 ppm of zinc. Crystalline vitamins were given separately.[7] Extreme care was taken to eliminate sources of zinc contamination from the environment as well as from the diet. Details of the diets and procedures have been described previously (2).

Three groups of rats were used in this study: One group was fed the zinc-deficient diet *ad libitum*; a second group was fed the zinc-supplemented diet *ad libitum*; a third group, serving as inanition controls was fed the zinc-supplemented diet in restricted amounts.[8] After 28 days, rats were decapitated and both testes were rapidly removed and frozen in isopentane chilled in liquid nitrogen. One testis was stored at —15° for subsequent spectrophotometric analysis of

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[2] Swenerton, H., and Hurley, L. S., unpublished data.

[3] Determined by atomic absorption spectroscopy.

[4] Purina Assay Protein RP-100, Ralston Purina Company, St. Louis, MO.

[5] Composition of the salt mix (g): $CaCO_3$, 600; $Ca(H_2PO_4)_2 \cdot H_2O$, 220; K_2HPO_4, 650; NaCl, 336; $MgSO_4 \cdot 7H_2O$, 250; $FeSO_4 \cdot 7H_2O$, 50; $MnSO_4 \cdot H_2O$, 4.6; KI, 1.6; $CuSO_4 \cdot 5H_2O$, 0.6.

[6] The tetrasodium salt of ethylenediaminetetraacetic acid.

[7] A mixture of crystalline vitamins in glucose was given 3 times each week in amounts to provide the following intake (μ/day): Ca pantothenate, 500; *p*-aminobenzoic acid and riboflavin, each 100; thiamin·HCl, pyridoxine·HCl, and nicotinic acid, each 300; menadione, 250; folic acid, 6; biotin, 2.5; vitamin B_{12}, 0.3; and choline chloride, 10 mg; inositol, 5 mg; ascorbic acid, 1 mg; α-tocopheryl acetate, 1.2 IU; vitamin A palmitate, 150 IU, and vitamin D_3, 15 IU.

[8] Three grams per day.

TABLE I. Lactic and Malic Dehydrogenase Activities in Testis of Weanling Male Rats After 28 Days of Experimental Diet.[a]

Group	No. of rats	Body wt (g)	Testis[b] wt (g)	Lactic dehydrogenase activity[c]		Malic dehydrogenase activity[c]	
				Per g tissue	Sp act[d]	Per g tissue	Sp act[d]
Zinc-supplemented controls, *ad lib.*	12	246 ± 7	1.19 ± 0.03	44 ± 1	0.88 ± 0.03	55 ± 3	1.10 ± 0.05
Zinc-supplemented controls, restricted-intake	12	62 ± 2[e]	0.59 ± 0.04[e]	44 ± 2	0.85 ± 0.03	55 ± 2	1.06 ± 0.04
Zinc deficient, *ad lib.*	10	57 ± 2[e,f]	0.39 ± 0.04[e,f]	51 ± 3	1.04 ± 0.07	63 ± 5	1.29 ± 0.12

[a] Means ± standard errors.
[b] Weight of one testis.
[c] Units (μmoles DPNH converted/min).
[d] Expressed as units per milligram of supernatant protein.
[e] Significantly different from ad lib.-fed controls ($p < .001$).
[f] Significantly different from restricted-intake controls ($p < .01$).

LDH and MDH activity. Selected samples of the other testis were sectioned immediately in a cryostat and processed for histochemical demonstration of the same two enzymes.

Testes for the spectrophotometric assay were homogenized in a Potter-Elvehjem type of mechanical grinder in 10 vol of 0.14 *M* KCl for 1 min at 0°.[9] Homogenates were centrifuged at 30,000*g* for 30 min at 0°. Enzyme activities were measured in the supernatant fraction immediately after preparation, using a Gilford recording spectrophotometer (Model 2000), by measuring the change in absorbance at 340 mμ resulting from the oxidation of reduced nicotinamide adenine dinucleotide at 25°. LDH was assayed in a Tris·HCl buffer (pH 7.4) with sodium pyruvate as the substrate (10). MDH was assayed in the same buffer system with *cis*-oxalacetic acid as the substrate (10). Protein was determined by the Lowry and co-workers' method (11).

LDH and MDH were histochemically demonstrated by the cobalt-formazan method of Pearse (12) using nitro-blue tetrazolium as the final electron acceptor. Unfixed sections were incubated at 36° in air for 1 hr at pH 7.4 using DL-lactic acid as substrate for LDH and sodium malate as substrate for MDH. Reactive intensities were evaluated by microscopic examination.

Results and Discussion. After 28 days of the experimental regimen, growth was severely retarded in rats fed the zinc-deficient diet. In addition, these rats displayed the signs of zinc deficiency reported previously (2): immature hair coats, dermal lesions, emaciation, and abnormal posture. Animals fed the zinc-supplemented diet in restricted amounts had body weights similar to those of the zinc-deficient rats, but they displayed none of the signs of zinc deficiency.

Testis weight was significantly lower in both the zinc-deficient and restricted-intake groups than in *ad libitum*-fed controls; however, the testes of zinc-deficient rats appeared to be more severely affected than those of the inanition controls. These data concur with the results reported previously by Diamond, Swenerton and Hurley (3).

Spectrophotometric analysis (see Table I) showed no significant decrease in the activi-

[9] Preliminary studies indicated that frozen testis homogenized in either H_2O or 0.4 *M* KCl released the same amount of MDH into the supernatant fraction.

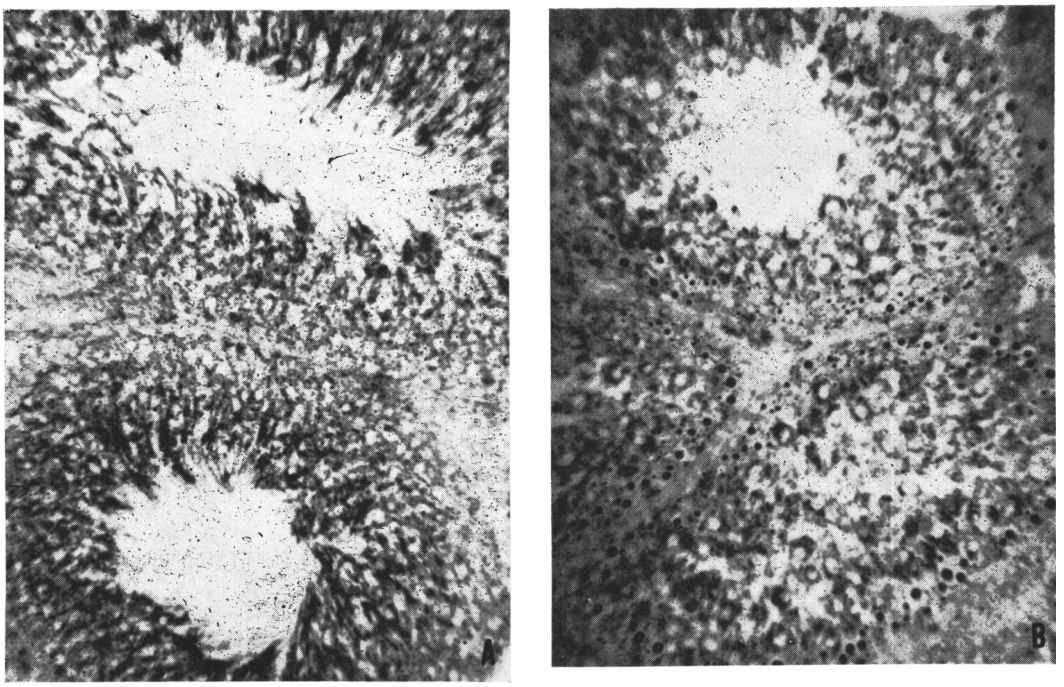

FIG. 1. LDH in seminiferous tubules of (A) control and (B) zinc-deficient rat testis. The lack of spermatogenesis in the testis of the zinc-deficient rat and the consequent alteration in the cellular composition of the tubular layers has not resulted in detectable changes in the intensity of reactivity of the enzyme. The dark globules near the basement membrane in the testis of the zinc-deficient rat are fat droplets.

ties of LDH and MDH in the supernatant fraction of testis homogenates from zinc-deficient rats as compared to *ad libitum*-fed or restricted-intake controls.

Histological changes were observed in the testes of zinc-deficient rats. These consisted of decreased tubule size, spermatogonic arrest, nuclear chromatolysis in spermatids and secondary spermatocytes, and pyknosis of epithelial cells. In some tubules, cellular debris filled the lumen. The testes of both *ad libitum*-fed and restricted-intake controls were morphologically normal.

The intensity of reactivity for both MDH and LDH in the cells of the seminiferous tubules of the zinc-deficient rats was not different from the reactivity present in the testes of controls. However, all of the cells appeared to be equally reactive in the tubules of zinc-deficient testes, while the tubules of both control groups showed localized zones with slightly more intense activity (Fig. 1).

It is apparent that the testes of control rats carrying on normal spermatogenesis were composed of a different population of cells than were those of zinc-deficient rats in which spermatogenesis had been arrested. The cellular localization of enzyme-reactive sites in the normal testes is related to the stage of spermatogenesis (13). The lack of concurrence of the present results with those of Prasad *et al.* (9) may be due to differences in the severity and duration of the zinc-deficient regimens used by the two laboratories.

If the morphological lesions of zinc deficiency are caused by alterations in enzyme activities, changes in enzyme activity should precede or at least occur concomitantly with overt signs of deficiency. The results of the present study indicate that severe symptoms of zinc deficiency may occur without a quantitative decrease in the activities of LDH and MDH, and thus suggest that there is no causal relationship between these enzymes and the lesions of zinc deficiency.

Summary. The activities of LDH and MDH were evaluated in the testes of zinc-deficient and control rats by both spectrophotometric and histochemical methods. There was no significant decrease in the activities of these enzymes by either method in the testes of rats fed the zinc-deficient ration for 28 days as compared with rats fed the zinc-supplemented diet *ad libitum* or in restricted amounts. Histological changes were observed in the testes of zinc-deficient rats consisting of decreased tubule size, spermatogonic arrest, nuclear chromatolysis in spermatids and secondary spermatocytes, and pyknotic epithelial cells. The testes of restricted or *ad libitum*-fed controls appeared normal. The evidence presented does not support the hypothesis that reduced activity of these enzymes is a primary cause of the physiological and morphological changes observed in zinc deficiency.

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