Increased Lactate Dehydrogenase Activity in Buccal Epithelium of Zinc-Deficient Rats

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ABSTRACT Previous studies by us and by others have shown that the body weight of rats fed a zinc-deficient diet is significantly lower than that of pair fed controls, but marked hyperplasia and parakeratosis are seen intra-orally in the buccal epithelium. No histologic changes occur in palatal epithelium. This investigation was undertaken to assay lactate dehydrogenase (LDH) activity in the buccal and palatal epithelium in rats fed a zinc-deficient diet. Weanling male Simonsen rats were fed a diet containing 1.2 ppm zinc for a 30-day period. Pair fed controls were fed the same diet containing 40 ppm zinc. LDH levels were determined by ultramicrochemical assay. In the buccal epithelium, a more than twofold increase over that of controls was observed in zinc-deficient rats, but there was no change in the palatal epithelium. These results support the conclusion of others of an organ-specific sensitivity of LDH to zinc deprivation. No increases in LDH activity in zinc-deficient rats, as observed in the hyperplastic buccal epithelium in this study, have previously been reported. J. Nutr. 107: 724-729, 1977.

INDEXING KEY WORDS lactate dehydrogenase · zinc deficiency · oral epithelium · hyperplasia · rats

A dietary deficiency of zinc has been shown to cause a slowing of growth resulting in smaller body weight and smaller size of most organs in young pigs (1-3), calves (4, 5), lambs (6), and other domestic species (7) as well as in laboratory animals (8-11). The retarded growth is accompanied in most organs by decreases in the synthesis of DNA (12-14) and RNA (12, 14) and in the activity of many zinc-dependent enzymes (3, 15-18). Lactate dehydrogenase (LDH; EC 1.1.1.27) is one of the enzymes that is often found to be reduced in activity (14, 18-20).

Although most organs respond to a dietary deficiency of zinc by retarded growth, the reverse of this response occurs in certain regions of epidermis and oral epithelium, which instead develop hypertrophy and hyperplasia, increased mitotic activity and parakeratosis (8, 10, 14, 91). The present study on oral epithelia of rats was carried out to examine the effect of a dietary deficiency of zinc on the activity of lactate dehydrogenase.

MATERIALS AND METHODS
Male rats of the Simonsen strain were used. At the start of the experiment, which lasted for 30 days, the rats were 21 days old and weighed an average of 53 ± 1.38 (SEM) g. They were fed a purified pelleted diet (table 1) in which the concentration of zinc was assayed at 1.2 ppm. Control rats of the same age and of matched body

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weight (53 ± 1.32 g) were pair fed with the same diet except that zinc carbonate was added to a final zinc concentration of 40 ppm. Except for ad libitum feeding on the first day, each rat in the control group received the amount of food consumed by its partner in the preceding 24-hour period. The rats were provided with double-distilled water from glass bottles with stainless steel nozzles and were housed singly in stainless steel cages.

At the end of the experiment, the rats were killed by a blow to the head. Specimens of buccal mucosa and the mucosa of the hard palate in the region of the molar teeth were quickly dissected free and frozen in liquid nitrogen in preparation for microdissection and ultramicrochemical analysis (22, 23). The advantage of this technique is that it permits the assay of isolated histologic layers. For the present study, cellular and keratin layers were isolated in buccal and palatal epithelium. Seven samples were assayed per layer in each region in each rat except for the cheek of zinc-deficient ones, where a minimum of 14 was used. The standard error of the mean LDH activity per layer did not exceed 10% in any rat. The individual samples weighed 25 to 150 ng.

Details of the LDH assay method may be found in Bonting et al. (24). Fluorescence of NAD was read in a fluorometer against quinine working standards. The amount of NAD formed was linear with time during the incubation periods used, and its net fluorescence was proportional to its concentration.

The significance of the differences in activity between groups was evaluated through the use of Student’s t-test (25).

RESULTS

The average weight of the zinc-deficient rats at killing was 83 ± 4.48 g, and that of the controls was 128 ± 7.55 g. The buccal epithelium of all zinc-deficient rats was thickened, and the increase in thickness was most marked in the stratum corneum. In addition, it had changed from orthokeratinization to parakeratinization (fig. 1).

This change was limited to, or more accentuated in, the stratum corneum overlying epithelial ridges. Samples for assay were taken only from these regions. The palatal epithelium in the zinc-deficient rats was often thinner than in their pair fed partners, but it was never thicker. Also, a change to parakeratosis was never observed (fig. 1).

Lactate dehydrogenase activity in the cellular layers and stratum corneum of buccal epithelium was in every instance considerably higher in the zinc-deficient rat than in its pair fed partner. In palatal epithelium the differences between the pairs of rats were small and inconsistent. A summary of the results is shown in table 2. In the buccal epithelium there was, on the average, a more than twofold increase in activity in the cellular layers and a more than fivefold increase in the stratum corneum. In the palatal epithelium, there was no significant change in activity in either layer. The addition of zinc acetate to the reaction mixture at a final zinc concentration

<p>| TABLE 1 |</p>
<table>
<thead>
<tr>
<th>Composition of diet</th>
<th>%</th>
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<tr>
<td>Egg white solids¹</td>
<td>20</td>
</tr>
<tr>
<td>Glucose</td>
<td>50</td>
</tr>
<tr>
<td>Cellulose</td>
<td>5</td>
</tr>
<tr>
<td>Lard</td>
<td>11</td>
</tr>
<tr>
<td>Vitamin mixture²</td>
<td>1</td>
</tr>
<tr>
<td>Salt mixture³</td>
<td>4</td>
</tr>
</tbody>
</table>

¹ Egg-white solids treated to denature avidin.
² Composition of vitamin mixture in mg or units/100 g of final diet: p-aminobenzoic acid 11.013; ascorbic acid 99.12; biotin 0.044; vitamin B₁₂ 0.003; calcium pantothenate 6.608; choline 143.37; folic acid 0.198; inositol 11.013; menadione (vitamin K₃) 4.956; niacin 9.912; pyridoxine HCl 2.203; riboflavin 2.203; thiamin HCl 2.203; retinyl palmitate (500,000 U/g) 1982 units; ergocalciferol (500,000 U/g) 220.55 units; tocopherol acetate (500 U/g) 12.115 units; corn starch 406.7.
³ Composition of salt mixture in g/100 g of final diet: CaCO₃ 2.2; KH₂PO₄ 0.83; KCl 0.44; NaCl 0.27; MgSO₄ 0.16; FeSO₄ 0.08; MnSO₄·H₂O 0.08; MgSO₄ 0.06; NaF 0.004; CuSO₄·5H₂O 0.0034; AIK(SO₄)·12H₂O 0.0003; KI 0.0066. Diet formulated by manufacturer so that vitamin and mineral content exceed twice the daily requirement of the rat.
tion of $1 \times 10^{-8}$ M caused no change in activity in either region in both controls and zinc-deficient rats.

DISCUSSION

The effect of the zinc-deficient diet on body weight in this series of rats (a 57% gain as compared to 141% in control rats) was similar to that noted by other authors and very much like that noted in those of our previous series which were carried out under nearly identical conditions (26, 27).

Fig. 1 Buccal and palatal epithelium, 16 μm frozen sections, hematoxylin and eosin, ×100. a. Buccal epithelium from zinc-deficient rat. b. Buccal epithelium from pair fed control. In zinc-deficient rat, note increased thickness of epithelium, especially in stratum corneum, and parakeratosis over epithelial ridges. c. Palatal epithelium from zinc-deficient rat. d. Palatal epithelium from pair fed control. The palatal epithelium in zinc-deficiency is often moderately atrophic, as in this example, and it shows no signs of parakeratosis.

The increase in LDH activity that we are reporting is in sharp contrast to all previous studies on this enzyme in zinc-deficient rats, which have shown either a decrease or no change. Huber and Gershoff (19) found reduced activity in skeletal muscle and heart. Kidney activity is also reduced according to them, but Prasad and Oberleas (18) found no change. Im et al. (20), who also used the ultramicrochemical technique, reported a decrease in atrophic dorsal epidermis. Huber and
LACTATE DEHYDROGENASE IN ZINC-DEFICIENT RAT

TABLE 2

Lactate dehydrogenase activity* in buccal and palatal epithelium of rats fed zinc-deficient and control (zinc-supplemented) diets

<table>
<thead>
<tr>
<th>Layer</th>
<th>Buccal</th>
<th>Palatal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Zinc-deficient</td>
<td>Control</td>
</tr>
<tr>
<td>Cellular</td>
<td>1344.6±90.0</td>
<td>610.9±45.3</td>
</tr>
<tr>
<td>Stratum</td>
<td>1208.7±71.3</td>
<td>224.7±41.3</td>
</tr>
</tbody>
</table>

*Activity units are μmoles of substrate converted/minute/g of dried tissue at 37°C. Values are means of 6 rats for buccal and 5 rats for palatal epithelium±se. 2Significantly different from control value (P < 0.002).

Gershoff (19) found no change in lung, pancreas, duodenum or stomach, and Prasad and Oberleas (18) found no change in testis, bone and thymus. Activity in the liver was found unchanged in several studies (14, 28, 29). These previously reported findings led to the conclusion that in the rat there is an organ-specific sensitivity to zinc deprivation, and our finding of unaltered (palate) and increased (cheek) levels of activity support the conclusion. Whether unaltered or increased activity of LDH in some tissues is due to the fact that it is zinc-independent or to selective sequestration of zinc as, e.g., reported in mast cells (30), remains an open question.

In the present control rats at 7 weeks of age, lactate dehydrogenase activity levels in the cellular layers of the epithelium were 610.9 μmoles/g/minute in the cheek and 719.8 in the palate (table 2). In a previous study, values for adult male rats of the same strain were 559 for the cheek and 548 for the palate (31). The lower activities in the adult rats may be due to the age difference, but the values are close enough to support the validity of the technique.

The responses of buccal and palatal epithelium noted in the present study have been as similar to those of previous series in this laboratory as were the effects on body weight described above. A change from ortho- to parakeratinization, thickening of the cellular layer and greater thickening of the stratum corneum occur without fail in the buccal mucosa, while the palatal mucosa remains unaltered or atrophies somewhat (28, 32-35). Hyperplastic and hypertrophic changes also occur in all other regions of oral epithelium which differentiate according to the pattern seen in buccal mucosa, i.e., in epithelium with sparse, large keratohyalin granules, haphazardly dispersed tonofilaments, and a loose stratum corneum. Such changes were in fact noted in the esophagus and circumscribed regions of oral mucosa in one of the earliest studies of zinc deficiency in the rat (8). These changes in rat buccal epithelium are associated with increased mitotic rates and increased size and dry weight of the cells estimated to represent a sixfold acceleration of synthetic activity (26, 34). Similar changes, i.e., parakeratosis, thickening and increased mitotic rate, have been noted in "scaling lesions" in the skin of pigs (2, 14).

These instances of increased epithelial activity in zinc deficiency occur in the face of marked slowing of body growth including a smaller size of most organs, decreased synthesis of DNA and RNA, and reduced activities of many enzymes. It has not been appreciated that the contrary responses to dietary zinc deficiency in some regions of oral epithelium and skin take the opposite direction from that in the bulk of the animal, i.e., that they represent increased anabolic activity in contrast to the predominant effect of zinc deficiency, which is manifested by the retardation of growth. While the inhibitory effect has been studied extensively, no metabolic studies on the stimulatory effect (other than ours) have been made.

In an effort to understand the change from ortho- to parakeratinization, we had previously investigated acid phosphatase activity in the hypertrophic buccal epithelium of zinc-deficient rats and found a
70% increase in spite of evidence for decreased lysis in vivo (see below and 27). The increase in acid phosphatase activity and our current finding of a 100% increase in LDH activity show that the increase in anabolic activity is associated with increased activity of enzymes. Assuming that the increase in dry weight per volume reported by Meyer and Alvare (26) is due chiefly to proteins, the increase in anaerobic energy conversion, as measured by LDH activity, could well have provided the necessary energy.

Residual enzyme activities in the stratum corneum obviously depend on how complete has been the action of lytic enzymes by the time the cells leave the stratum granulosum. In controls, this was more complete in the palate than in the cheek (residual activity in palate was 19% of activity in cellular layer; in cheek, 36%). This agrees with previous observations on normal rats comparing LDH in the two regions (17% vs. 34%; 31). Whereas, in the palate of the zinc-deficient rats, residual activity was virtually the same as in the controls (20%), in the cheek, activity in the stratum corneum was 97% of that in the cellular layer. Light microscopy of the cheek of zinc-deficient rats shows sharp histologic differences at the boundary between the cellular layer and the stratum corneum (fig. 1); electron microscopy shows the same internal thickening of the plasma membrane in the deepest cells of the stratum corneum which characterizes this boundary in control rats (33). Chen et al. (35), however, have shown that together with the preservation of pyknotic nuclei there is preservation and only gradual and incomplete lysis of cell organelles. The present findings suggest a virtual failure of the proteolytic enzyme activity, if LDH is assumed to be representative of cellular proteins.

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LITERATURE CITED