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# Effect of Zinc Deficiency on Folate Absorption in Rats*

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*ABSTRACT*

*A zinc-deficient diet fed to rats resulted in a 23% reduction in plasma zinc levels and 22% reduction in folate levels. The reduction in folate appears to be related to the relative lack of pancreatic conjugase (54·8% reduction) necessary for folate absorption.*

## INTRODUCTION

Folate deficiency, caused by inadequate dietary intake, is considered the most common hypovitaminosis of man. Dietary folate consists mainly of reduced pteroylpolyglutamates in which the poly-$\gamma$-glutamyl side chain usually contains three to four residues. Conjugated folate is quantitatively deconjugated by intestinal pteroyl-$\gamma$-polyglutamyl carboxypeptidase (conjugase) before release into the mesentric circulation (Butterworth *et al.* 1969; Rosenberg, 1976). The conjugase has been shown to be a zinc metalloenzyme (Silink *et al.*, 1975). This fact suggests that a deficiency of

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dietary zinc may adversely affect intestinal pteroylpolyglutamate deconjugation by conjugase and thereby folic acid status.

Tamura *et al.* (1978) observed a decreased folate absorption from pteroylpolyglutamate but not from pteroylmonoglutamate in zinc-deficient human volunteers. Zinc deficiency caused lower plasma folate levels and decreased 5-$CH_3$—$H_4$ folate in liver (Tamura *et al.*, 1987).

The relationship between dietary zinc deficiency and the folic acid status of rats was studied by Williams *et al.* (1973) who found that zinc-deficient rats had lower liver folate concentrations than pair-fed zinc-supplement rats, but significant differences attributable to zinc were not detected in plasma folate concentrations. Keating *et al.* (1987) reported that there was no inhibition of zinc utilization by folic acid in humans and rats.

The current study was undertaken in order to confirm the short term effect of dietary zinc deficiency on folate absorption and to elucidate the biochemical lesion responsible for the observed reduction of folate in zinc-deficient rats.

## MATERIALS AND METHODS

### Animals and diet

Fifty male 21-day old weanling Wistar rats weighing 52–56 g were purchased from a commercial source. All animals were housed individually in suspended plastic cages fitted with wire floors, tops and food hoppers made of stainless steel, in a room controlled for temperature, light and humidity (22°C, 12-h light–dark cycle, 50% humidity). Cages, fittings and water bottles were prewashed in 5% $HNO_3$ and rinsed with distilled water before use. Rats were allowed free access to distilled water immediately after arrival, and were housed in the animal house and acclimatized for 7 days during which time they were fed a complete diet containing 100 ppm zinc. At 28 days of age rats were randomized into three groups of approximately equal average weight. The composition of the control diet is shown in Table 1 and contains 100 mg zinc/kg diet. The zinc-deficient diet had the same composition as the control diet except that zinc was deleted. Zinc-deficient diet containing 1 ppm zinc was offered *ad libitum* to one group of rats; a second group was fed the zinc-supplemented control diet and each rat was pair-fed with an animal of similar weight in the zinc deficient group. The third group of rats was fed the zinc-supplemented control diet *ad libitum*. The duration of the experiment was 3 weeks and the daily food consumption and 3 day weight gain were accurately recorded for each rat. Food was removed from the cages 24 h before the animals were killed.

**TABLE 1**
The Composition of the Basal Diet

| *Ingredient* | *Composition (%)* |
|---|---|
| Egg albumin | 25 |
| Dextrose | 17 |
| Sucrose | 16·3 |
| Dextrin | 17 |
| Maize oil | 10 |
| Lard | 5 |
| Methionine | 0·7 |
| Vitamin mixture | 5 |
| Mineral mixture | 4 |

## Procedure and analysis

On the 21st day of the experiment, rats were lightly anaesthetized with chloroform and about 2 ml blood was removed by puncture of the retro-orbital venous plexus using heparinized capillary tubes. Blood was collected in heparinized plastic tubes and centrifuged at 3000 rpm for 20 min. Plasma was removed by a micropipette fitted with plastic tips, transferred to plastic vials and stored frozen at −30°C until analyzed for zinc and folate. Plasma samples showing signs of hemolysis were discarded. Capillary tubes, heparinized tubes and micropipette tips were pre-checked and shown to contain no detectable signs of zinc contamination. After blood collection, the animals were immediately killed by chloroform anesthesia, the abdominal cavity cut and the pancreas and liver excised, weighed and frozen by clamping in liquid nitrogen. The duodenum was excised, everted and cut longitudinally; the mucosal cells were removed by scraping with a sharp scalpel blade, weighed and frozen in liquid nitrogen.

## Plasma zinc determination

Each plasma sample was diluted 1:1 with deionized water and analyzed in duplicate for total zinc concentration using an atomic absorption spectrophotometer (Perkin Elmer, Model 103). Samples were read against zinc standard in 10% glycerol. Glass and plastic were decontaminated of mineral residues by soaking overnight in 5% $HNO_3$; subsequently they were rinsed at least three times with deionized water.

## Plasma folate determination

Folate activity was determined by aseptic condition microbiological assay using *Lactobacillus casei*, ATCC 7469, as the test organism, folic acid as the

reference standard and Difco basal medium as previously described by Narasimha and Noronha (1977). All plasma samples were assayed in duplicate and referred for quantitation to a calibration curve which was run in triplicate with the unknown. Plasma folate concentration was determined by aseptic addition of 20 $\mu$l of plasma diluted (1:10) with deionized water.

### Determination of pancreatic folyl conjugase activity

A 10% (w/v) homogenate of rat pancreas was prepared in 0·25M sucrose, centrifuged in an ultracentrifuge at 10 500 rpm for 15 min at 0–4°C and the protein content of the supernatant was determined by the method of Lowry *et al.* (1951). Folyl conjugase activity was measured in the supernatant by the procedure outlined by Narasimha and Noronha (1977) using pteroylpolyglutamates extracted from Torula yeast as substrate.

### Statistics

All data were analyzed for significance by student's *t*-test.

## RESULTS AND DISCUSSION

Table 2 shows the growth rate of the experimental and pair-fed control rats. As shown, a lower growth rate (2·7 g/day) was obtained when the rats were fed a zinc-deficient diet compared with (3·32 g/day) the pair-fed control group. These results illustrate that zinc deficiency significantly influences

**TABLE 2**

Effect of Zinc Deficiency on Body Weight Gain, Food Consumption and Liver Weight. (Mean values with their standard errors)

| | *Pair-fed* | | *Percentage change* | P-*value* |
|---|---|---|---|---|
| | *Control* | *Experimental* | | |
| Initial weight (g) | 78·7 ± 5·1 | 78·9 ± 5·3 | — | NS |
| Final weight (g) | 149·1 ± 12·6 | 135·7 ± 13·5 | 9·0 | 0·004 3 |
| Weight gain (g) | 70·4 ± 12·1 | 56·8 ± 12·3 | 11·3 | 0·002 2 |
| Weight gain (g)/day | 3·34 ± 0·29 | 2·70 ± 0·30 | 19·2 | |
| Food consumption (g) | 205·4 ± 16·6 | 205·4 ± 16·6 | — | NS |
| Food efficiency | 0·338 ± 0·038 | 0·272 ± 0·043 | 19·5 | 0·000 02 |
| Liver weight (g) | 5·5 ± 0·8 | 4·6 ± 0·56 | 16·4 | 0·024 |
| Liver weight (mg)/ g body weight | 36·0 ± 3·4 | 34·3 ± 1·7 | 4·70 | NS |

NS, not significant.

the growth rate. The data in Table 2 show that food efficiency of the experimental group was 0·276 g body weight/g diet, while the pair-fed control group which received the same amount of food consumed by the experimental group showed a higher food efficiency (0·34 g body weight/g diet). Plasma zinc level was significantly affected by zinc content in the diet. Plasma zinc level was lower in the experimental group than the control by 23% (Table 3). The decrease of plasma zinc in the group fed a zinc-deficient diet was accompanied by a decrease in plasma folate; a similar finding has been reported by Keating *et al.* (1987).

Pancreatic conjugase activity of the rats fed a zinc-deficient diet was lower than the pancreatic conjugase activity of the control rats by 55% (Table 3). Similarly, pancreas weight of the rats fed a zinc-deficient diet was 0·79 ± 0·12 g while the pancreas weight of the pair-fed control group was 1·02 ± 0·06 g. The reduction of conjugase activity could retard the hydrolysis of dietary folates and hence result in decreased absorption. As the data show, the decrease of serum folate level in the experimental group confirms that a decrease in pancreatic conjugase could be the main cause of folate malabsorption in the experimental group.

It has been reported that most of the folate in the tissues of plants and animals occurs in the form of polygammaglutamyl peptides attached in peptide linkage to the glutamyl moiety of 5-methyl derivatives of folic acid (Stokstad *et al.*, 1977). This is hydrolyzed to the monoglutamate by a zinc-containing conjugase enzyme [Pteroylpolygammaglutamyl hydrolase EC 3.4.22-12] at the brush border immediately before absorption (Butterworth *et al.*, 1969). This has been confirmed by Silink *et al.* (1975) who reported that bovine hepatic conjugase is a zinc metalloprotein and this was based on the observation that zinc was essential for enzyme stability and zinc atoms were detected in purified enzyme after 8 days dialysis against distilled water.

**TABLE 3**

Effect of Zinc Deficiency on Plasma Zinc, Plasma Folate, Pancreatic Conjugase Activity and Pancreas Weight. (Mean values with their standard errors)

| | *Pair-fed* | | *Percentage change* | P-*value* |
|---|---|---|---|---|
| | *Control* | *Experimental* | | |
| Plasma zinc μg/100 ml | 150·8 ± 8·8 | 116·3 ± 9·0 | 22·9 | 0·000 1 |
| Plasma folate ng/ml | 107·4 ± 14·8 | 83·6 ± 13·5 | 22·2 | 0·000 02 |
| Pancreatic conjugase activity[a] | 4·34 ± 0·84 | 1·96 ± 0·41 | 54·8 | 0·000 01 |
| Pancreas weight (g) | 1·02 ± 0·06 | 0·79 ± 0·12 | 22·5 | 0·000 3 |
| Pancreas weight mg/ body weight | 6·8 ± 0·64 | 5·9 ± 0·43 | 13·2 | 0·006 3 |

[a] Conjugase activity expressed as ng folate/mg protein.

The results of this research indicate that zinc plays an important role in folate absorption, since zinc deficiency was followed by lower pancreatic conjugase activity. It has been reported that the pancreas is the major source of folyl conjugase and that the enzyme activity in human pancreatic fistula twice at pH 5·0 was three times that found in intestinal mucosal biopsy specimens (Jagerstad *et al.*, 1972). It is unlikely that the conjugase is of hepatic origin because very little conjugase activity has been observed in the tube bile after folate ingestion (Pratt & Cooper, 1972).

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