The Impact of Experimental Zinc Deficiency on Growth, Morbidity and Ultrastructural Development of Intestinal Tissue

Swapan K. Roy[1] & *Andrew M. Tomkins*[2]

1. International Centre for Diarrhoeal Disease Research, Bangladesh.
2. London School of Hygiene and Tropical Medicine, Keppel Street, London, U.K.

Introduction

Among many other trace metals, zinc is particularly important for the growth, protein synthesis and maintenance of normal functions of the body. Zinc has been recongnized as an essential trace element for lower plants[1], animals[2] and human[3]. Zinc deficiency has been documented with retarded growth, hypogonadism and anorexia in subjects of Iran and Egypt[4]. Among human zinc deficiency syndrome acrodermatitis enteropathica (AE) presents with alarming clinical condition with persistent diarrhoea, vesicobulous skin lesions, infection, alopecia and growth faltering[5]. This dreadful disease responds to zinc therapy with return of normal growth and body functions[6]. School children from middle class families in the USA were found to have growth faltering[7] and similar abnormalities were reported in formula fed babies in the UK[8]. Although studies have shown effects of zinc on growth, food intake and enzymatic functions, no information is available on the growth of intestinal tissue on dietary zinc depletion and the response with zinc repletion in respect of diarrhoea observed during zinc deficiency.

Materials and Methods

Twenty three days old female Sprague-Dawley weanling rats were brought at about 70 g of body weight. They were divided into four groups and were individually housed in prewashed zinc free stainless steel cages in a room with 12 hours light and 12 hours dark cycle. They were provided with deionised distilled water and semisynthetic fresh diet every day and daily body weight and food intake were measured on an electronic balance with an accuracy of 0.1 g. The basic diet prepared with egg albumin has been described previously[15]. Zinc deficient (ZD) animals were provided with specially prepared semisynthetic powder diet with very low zinc content (0.8 ug Zn/g diet) for 16 days.

Zinc deficient repleted (ZDR) animals received the same treatment of diet for 16 days as ZD group and then received the same diet with added zinc (55 ug Zn/g diet) for 48 hours. Ad libitum fed control (ZAL) animals received zinc adequate diet (55 ug Zn/g diet) for a period of 16 days. Weight matched control (ZWM) animals received zinc adequate diet (55 ug Zn/g diet) until their body weight reached equal to that of the ZD group.

Clinical study: Apart from measuring body weight and food intake, daily observations were made on behavioural changes, skin lesions, alopecia, development of diarrhoea and other clinical changes.

Collection of specimen : At the end of their feeding schedules they were anaesthetized with ether inhalation and maintained with subcutaneous injection of pentobarbitone (4.5 mg/100 g). Abdomen was opened with mid line incision and about 15 cm long intestinal segment was obtained distal to the ligament of Treitz. The segment was dissected out with sharp scissors and weighed on electronic balance and length was measured with vertical scale with a

Bangladesh Journal of Nutrition Vol. 2, No. 2, 1- 7 , June, 1989, Printed in Bangladesh. Institute of Nutrition and Food Science, University of Dhaka.

weight load of 5g. The segment was placed on ice cooled glass surface and was slit longitudinally. Mucosa was scraped with the help of a microscopic slide and weight was measured on electronic balance (Sartorius M 1250). Tissue obtained were put into liquid nitrogen and then stored at-20°C for further analysis. Two samples of 1 mm thick mucosal sections were obtained while the blood supply was intact and were preserved in a solution containing 3% gluteraldehyde with 7.5% phosphate buffer and sodium cachodylate solution for electron microscopic examinations.

Analysis for protein DNA and RNA in the mucosal tissue : Protein was assayed from tissue homogeneate following the biuret folin reaction method[9]. RNA was measured by spectrophotometer. DNA was determined by Diphenylamine method described by Munro et al.[10]

Results

Growth retardation: Figure 1 shows daily body weight during the study period. From the 3rd day of feeding ZD group showed cyclical loss and gain of weight. Body weight increased steadily until the 3rd day and only a little from the 4th to 7th day, thereafter no net gain was noticed. The final mean body weight of the ZD group was 90 g compared to 180 g of ZAL group. The ZDR group gained 20 g over 48 hours from 90 to 110 g. Weight matched grop (ZWM) had the same weight as ZD animals.

Clinical observation: Figure 2 shows the clinical abnormalities observed in the ZD animals. Significant reduction in physical activities and loss of interest in food intake was observed as early as third day of feeding zinc deficient diet. They looked to be irritable at the end of the first week. Animals kept themselves away in the corner of their cases and spilled larger amount of diet as the study proceeded. From the fifty day on deficient diet, body weight remained unchanged and the body hair looked sparse. Hair fell from the abdomen and proximal part of the extremities. Animals had developed fissures and scales in paws by the first week. On about tenth day, bleeding from nose and paws was evident. None of the animals fed zinc adequate diet showed any of the above abnormalities. Diarrhoea appeared on the 3rd day of feeding zinc deficient diet and was present in 90 per cent of the animals on the 5th day and then gradually reduced.

Food intake: ZD animals exhibited cyclical variation in food intake expressed as intake permetabolic unit of body size ($g/w^{0.75}$) from the 3rd experimental day. Figure 3 shows this pattern from a single animal. A period of rise in food intake followed by a fall over 3.5 days was seen in all of the ZD animals. This type of cyclical pattern is only observed in experimental zinc deficiency when dietary concentration of zinc falls below 3 PPM[11]. The cycle was closely associated with the change in body weight. Food intake reduced after 5 days then continued at a lower level with a lower amplitude in the cycle. ZAL group maintained a fairly constant level of intake through out the experimental period with a steady growth. A comparison of food intake is shown in Table 1. The average food intake in the ZD and the ZAL groups were 0.26 and 0.32 $g/w^{.75}$ respectivelly. There was no correlation (r=-0.3) between body weight and amount of food intake in ZD group but it was significant (r-.9) in the ZAL group. Body weight increased steadily when ZD group was offered zinc containing diet (Figure 4).

Table 1. *Comparison of food intake per unit metabolicbody size between and libitumfed animals (ZAL) and zinc deficient (ZD) animals every five days.*

	Food intake ($g/g^{.75}$) Mean ± SEM		% of	Statistical Significance
	ZAL (n=12)	ZD (N=12)	ZD/ZAL	ZDVs ZAL
Day 1-5	0.33 ± 008	0.268 ± 008	81 %	NS
Day 6-10	0.33 ± 008	0.21 ± 008	63 5	P <0.05
Day 11-15	0.31 ± 002	0.21 ± 006	67 %	P <0.05

Change in mucosal tissue: Mucosal mass was expressed as mg of wet mucosa per cm of intestine and there was significant reduction in mucosal weight of ZD group compared to that of the ZAL animals (Table 2). Mucosal weight in the ZDR animals improved to 80 per cent of that of the ZAL group. Younger ZWM group had lower mucosal weight than that of ZD animals. Mucosal cell population was evaluated by expressing DNA per cm of intestine. Cell size was estimated by the protein/DNA ratio of mucosal tissue. Cell population was significantly reduced in the ZD group compared to that of the ZAL group. There was little effect in the ZDR group. ZWM group had fewer cells than ZD animals. Mucosal cell size was significantly reduced in the ZD group compared to that of the ZAL group. ZDR group showed a significant recovery of size of the cells.

Table 2. *Mucosal character in different groups ofrats (Mean + SD)*

	ZD (n=11)	ZAL (n=10)	ZDR (n=4)	ZWM (n=5)	Statistical Significance ZD vs ZAL	ZD vs ZDR
mg/cm Intestine	13.3±0.3	21.3±1.4	19.6±2.0	10.1±1.3	P<0.001	P<0.001
Mucosa: Serosa	0.5±0.1	0.8±0.03	0.7±0.02	0.4±0.02	P<0.01	P<0.05
No. of cell/cm (DNA ug/cm)	405±15	610±31	450±45	319±14	P<0.001	NS
Size of cells mg protein/ mg DNA	3.58±0.09	4.2±0.19	4.0±15	3.59±.12	P<0.05	P<0.01

Discussion

Growth retardation from zinc deficiency have been documented in children[7,4] and in experimental animals[13]. The present study confirmed the previous findings. The effect of growth retardation due to zinc deficiency can not be overcome by either increasing protein concentration in diet or by increasing energy intake[14]. Studies on protein synthesis and degradation during zinc deficiency have demonstrated cyclical change of muscle

mass with associated change in body weight. The synthesis and breakdown of protein are influenced by hormonal regulation among which insulin and corticosterone may be important[15]. The effect on tissue synthesis and protein metabolism might be related to the changes in food intake. Severe anorexia was marked during the deficiency period and the mechanism is not yet known but some suggestions have been put forward that high level of essential amino acids in blood may cause anorexia. The ability of recovery of body weight was striking as 48 hours zinc repletion increased 20 per cent body weight while that was only 6% at best during zinc deficiency.

Diarrhoea observed during severe dietary zinc deficiency was comparable with that during acrodermatitis enteropathica (AE) in human. Skin lesion, alopecia and growth retardation occur in AE patients which are reversed to normal by oral zinc therapy[5]. Diarrhoea was associated with growth retardation and there might be excess loss of zinc with stool but it was not measured in this study. Increased loss of body zinc in chronic diarrhoeal state has been documented previously[16]. The mechanism of diarrhoea is not yet known but could be due to increased permeability of intestinal mucosa reduced absorption of water and electrolytes, and mucosal abnormalities which has been demonstrated in this study. The ultrastructural abnormalities might have been responsible for increased intestinal permeability and decreased transport function. Degenerative changes, loss of intercellular tight junction and appearance of large lateral space could be causal to diarrhoeal sysmptom in zinc deficiency and improvement of these abnormalities with short zinc supplementation may support the hypothesis.

Alopecia in experimental zinc deficiency and in AE patients has been related to inadequacy of zinc. Dermatities observed in this study could be related to reduced availability of zinc for synthesis of cell membrane, keratin and collagen tissue[17]. Total parenteral nutrition (TPN) with low level of zinc in parenteral fluid can cause skin leasion with low plasma zinc level[18]. Application of topical zinc resulted in improvement of would healing demonstrating that epithelial tissue was essentially zinc dependent[19].

Number of epithelial cells did not increase significantly within 48 hours which probably required longer time for multiplication. Growth retardation could be due to reduction of protein synthesis and multiplication of cells during zinc deficiency.

It was evident that zinc had profound effects on mucosal tissue which is directly related to water and electrolyte transport. The study hypothesizes that zinc supplementation may improve mucosal functions related to diarrhoea in human subjects.

Summary

Animal studies were undertaken to demonstrate the exact nature of clinical abnormalities and changes in intestinal mucosa related to diarrhoea. Four groups of weanling Sprague-Dawley rats were given zinc deficient or zinc adequate diets. Anorexia, severe growth retardation, alopecia, cyclical weight change and diarrhoea were observed during feeding zinc deficient diet. Intestinal mucosal weight reduced significantly in zinc deficient animals with reduction of mucosal cell population and cell size. Forty eight hours of zinc supplementation reversed the mucosal abnormalities. The study hypothesizes that zinc supplementation may improve mucosal structure and function related to diarrhoea.

Figure 1

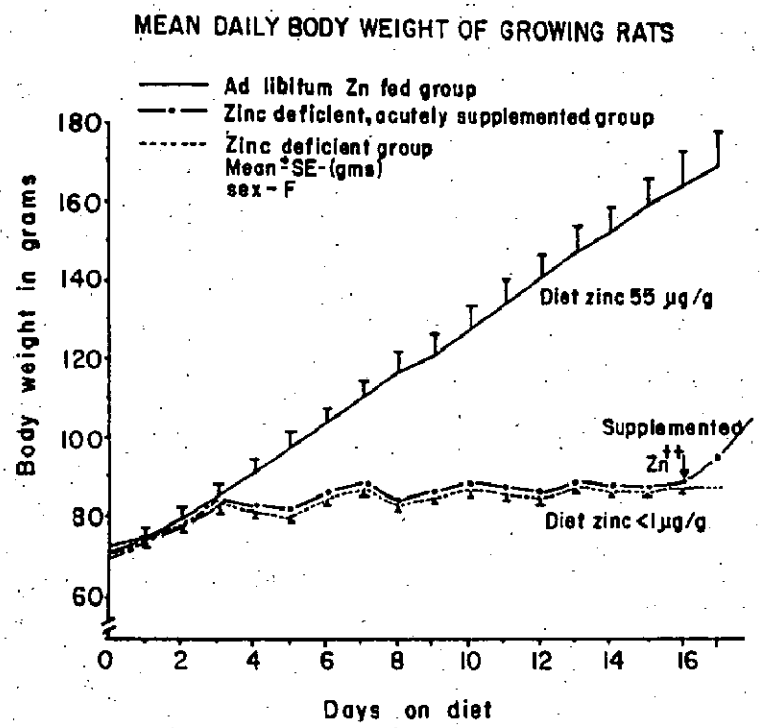


Figure 2

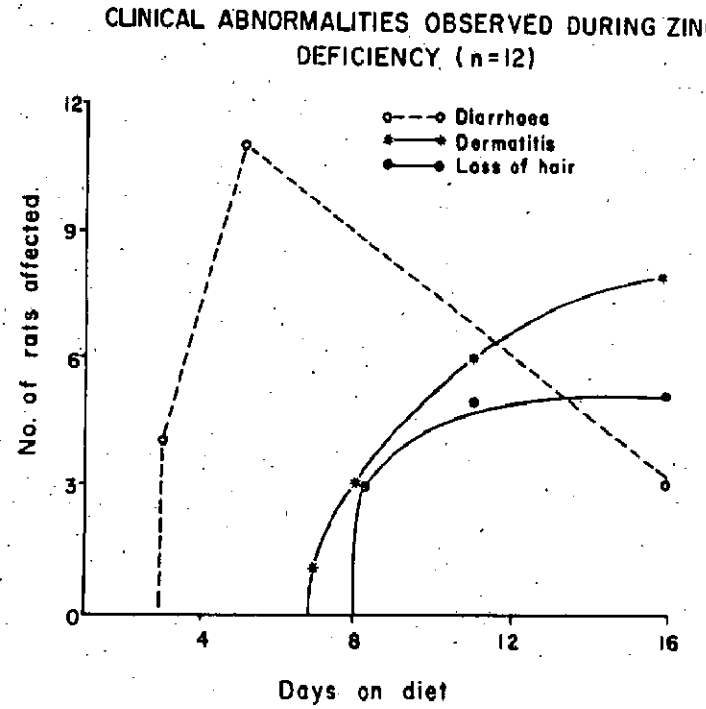


Figure 3

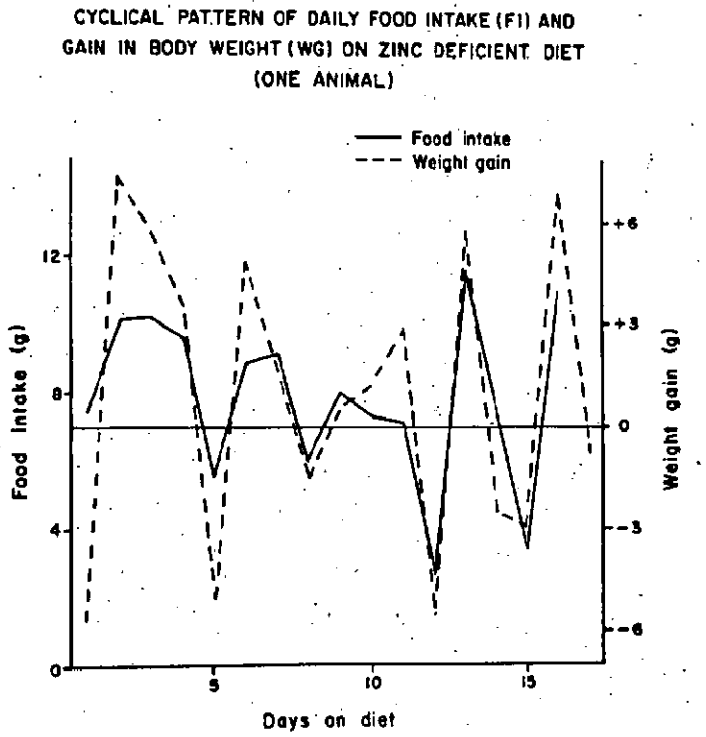
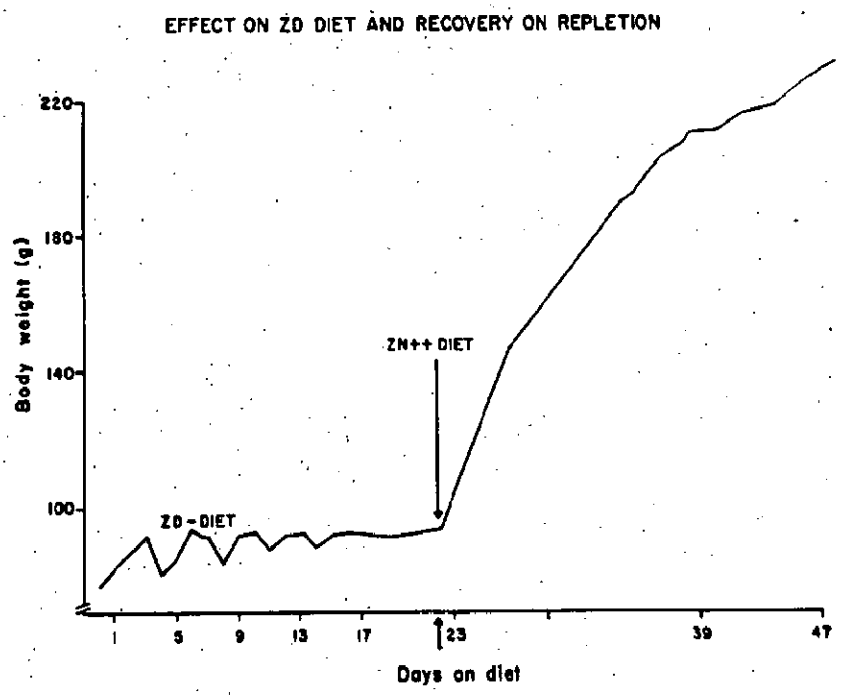


Figure 4



Acknowledgement

This research was supported by the United Nations' University and International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B) and was conducted in the Department of Human Nutrition, London School of Hygiene and Tropical Medicine, London. The authors acknowledge with gratitude the excellent help and cooperation received from P.R. Payne, J.P. Rivers, D.J. Millward and E.F. Wheeler. The authors are grateful to Dr. Ellis for his help with electron microscopy and would like to thank Mrs. Mahbooba Shamsuddin for her secretarial work.

References

1. Raulin, J. Etudes clinigues sural vegetation. Ann. Sci. Natur. Bot. Biol. Veg. 11:93-s109, 1969.
2. Todd, W. R., Elvehyem, C. A. and HART, E. B. Zinc in the nutrition of the rat. Am. J. Physiol. 107: 146-156, 1934.
3. Prasad, A. S., Halstead, J. A., and NADIMI, M. Syndrome of iron deficiency anaemia, anaemia, hepatosplenomegaly, hypogonadism, dwarfism and geophagia. Am. J. Med. 31: 532, 1961.
4. Prasad, A. S. Miale, A., FARID, Z. C., SCHULERT, H. H., and SANDSTEAD, H. H. Zinc metabolism in patients with the syndrome of iron deficiency anaemia, hypogonadism and dwarfism. J. Lab. Clin. Med. 61: 537-549, 1963.
5. Moynehan, E. J. Acrodermatities enteropathica: a lethal inherited human zinc deficiency disorder. Letter. Lancet 2: 399-400, 1974.
6. Kelly, R. Davidson, G. P., Townly, R. R. W. and Campbell p. e.. Reversible intestinal mucosal abnormality in acrodermatitis enteropathica. Arch. Dis. Child. 51: 219-22, 1976.
7. Hambridge, K. M. Hambridge, C;, Jacob, S. M. and Baun, J. D. Low levels of zinc in hair, anorexia, poor growth and hypogeusia in children. Pediatr. Res. 6: 868-874, 1972.
8. Aggett, P. S. Atherton, D. J., More, J. . Symptomaticzinc deficiency in a breast fed premature infant. Arch. Dis. Child. 55:547, 1980.
9. Lowry, O. H. Rosebrough, N. J. Lewise Farr, A and Randall, R. J.. Protein measurement with the folin phenol reagent. J. Biol. CHem. 193: 265-275, 1951.
10. Munro, H. N. and Fleck, A. Analysis of tissues and body fluids for nitrogen constituents. In: Munro H. N. ed. Mammalian protein metabolism. Vol. 3, pp 423-525. Academic Press, London and New York, 1969.
11. Chesters, J. K., Quaterman, J. Effect of zinc deficiency on food intake and feeding patterns of rats. Br. J. Nutr. 24: 1061-1069, 1970.
12. Elmes, M. E. and Jones, J. G. Ultrastructural changes in the small intestine of zinc deficient rats. J. Pathol. 130-37-43, 1980.
13. Williams, R. B., Milis, C. F. The experimental production of zinc deficiency in the rats. Br. J. Nutr. 24: 989-1003, 1970.
14. Chesters, J. K., Will, M. Some factors controlling food intake by zinc deficient rats. Br. J. Nutr. 30: 555-566, 1973.
15. Giugliano, R. Growth and protein turnover in zinc deficient rats. Ph. D. Thesis, University of London, U. K. 1983.
16. Solomons, N. W., Kactu K. V., Sandstead, H. H. Rosenberg,, I. H. Zinc nutrition in regional enteritis. Am. J. Clin. Nutr, 27: P. 438 (abs.) 1974.
17. Horrobin, D. F. and Cunnane, S. C. Interactions between zinc, essential fatty acids and prostaglandin, relevance to acrodermatitis enteropathica, total parenteral nutrition, the glucogonoma syndrome, diabetes, anorexia nervosa and sickle cell anaemia. Med, Hypothesis. 6: 277-296. 1980.
18. Kay, R. G. and Tasman, Jones C. Zinc deficiency and intravenous feeding (letter0> Lancet 1: 605-606, 1975.
19. Greaves, M. W. Zinc and copper in psoriasis. Br. J. Dermatol. 86: 439-440, 1972.