Plasma zinc, rate of weight gain, and the energy cost of tissue deposition in children recovering from severe malnutrition on a cow's milk or soya protein based diet[1,2]

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ABSTRACT Thirty-four malnourished children were rehabilitated on either a cow's milk or a soya protein based formula diet. The soya diet contained 25% less zinc than the cow's milk diet and 1.33 mmol/l phytic acid. When the marasmic children were given either of these diets they had a dramatic fall in their plasma zinc concentration. Children given the soya formula had an even more profound drop in plasma zinc, to levels associated with acrodermatitis enteropathica. Plasma zinc remained low throughout the phase of rapid weight gain, but rose when a mixed diet was given and the children ceased gaining weight. There was a negative correlation between the rate of weight gain and the plasma zinc concentration, the rate of new tissue synthesis being the predominant modulator of the plasma zinc concentration. The energy cost of tissue deposition was initially low on the cow's milk diet and relatively high on the soya diet. Later, the energy costs rose in the cow's milk fed children. The rate of weight gain was less on the soya diet; however, the appetite, as measured by the ad libitum dietary intakes, did not differ between the diets, or correlate with the plasma zinc concentration. This may be due to the relatively low protein content of the diets used. It is concluded that dietary zinc may limit the rate of weight gain during periods of recovery in body mass and may be a cause for the excessive deposition of adipose tissue. *Am. J. Clin. Nutr.* 34: 892–899, 1981.

KEY WORDS Malnutrition, protein-energy malnutrition, zinc, trace elements, body composition, cow's milk, soya

Introduction

Zinc is needed for tissue synthesis, both as a component of the new tissue and in the form of the zinc metalloenzymes essential for nucleic acid and protein synthesis. Animal studies indicate that the size of the body zinc store is extremely limited, so that there is a day-to-day requirement for dietary zinc (1, 2). In normal children, zinc is required to support growth as well as to replace losses. Hambidge's group has shown that diets marginal in zinc may be a cause of failure to thrive in North America (3, 4).

Children recovering from "protein-energy" malnutrition may gain weight at over 20 times the normal rate. In this situation, where the requirements for new tissue synthesis dominate the total dietary requirement, the amount of zinc that has to be sequestered in the new tissue may exceed the dietary supply. When this is the case, dietary zinc may both limit the absolute rate of weight gain and determine the composition of new tissue laid down.

Data from Jamaica (5) and Peru (6) show that children recovering from malnutrition tend to synthesise excessive amounts of adipose tissue, which has a low zinc content, at the expense of lean tissue, which is rich in

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zinc. Because lean tissue has a relatively low stored energy content, whereas the energy stored in fat tissue is high, an estimate of the fat to lean ratio of synthesised tissue can be obtained from the energy cost of tissue deposition.

Using plasma zinc concentration as the measure of zinc status, we have related zinc status to the rates of recovery and energy costs of tissue deposition in children recovering from malnutrition on two therapeutic diets, which differ in zinc concentration and availability.

Patients and methods

Thirty-four severely malnourished children aged 4 to 31 months (median 12, mean 12.5 ± 0.9 months) were studied throughout their recovery in a metabolic ward. There were 14 girls and 20 boys. On admission 16 children had marasmus, 12 marasmic-kwashiorkor, and six kwashiorkor (7). Their treatment was therapeutically divided into three stages. During the 1st stage the children were given a diet designed to maintain their edema-free body weight, while infections and specific nutrient imbalances were treated. Details of the diet are given in **Table 1**. No child received intravenous fluids or blood. On this regime, the marasmic children maintained their weight; the edematous children lost their edema.

When the children regained their appetites they entered the 2nd stage of treatment. Twenty-four children were given the cow's milk based formula diet, our routine recovery diet; 10 were randomly selected to have the diet based on soya protein (Table 1). During this stage, each child gained weight rapidly until his weight was 90 to 110% of the 50th percentile of the Boston Standard for a child of the same height as the patient. This stage took from 3 to 6 wk. The child was then described as clinically recovered and entered the 3rd stage where he was offered an ordinary mixed diet before discharge.

Intakes and losses were recorded for each feed and the total daily dietary intake calculated. Body weight was recorded at the same time each morning. Height was measured weekly.

Two ml jugular venous blood were taken within 1 h of the 10 AM feed, once during the 1st stage, at weekly intervals during the 2nd stage, and once in the 3rd stage. Plasma zinc concentrations were measured as previously described (8). The values were compared with those of 16 control children whose weight and height were normal for their age and who had never suffered serious illness.

Informed consent for the study was obtained from the parents. It was approved by the Ethics Committee of the University Hospital of the West Indies.

Phytic acid was kindly assayed by Dr. B. Nävert using the method of Davies and Reid (9).

Calculations

The phase of rapid weight gain was divided into early and late recovery. Early recovery was defined as the first 3 wk of high energy feeding and later recovery was weight gain occurring after this period. This somewhat artificial division was made because most of the children commence recovery with a period of very rapid weight gain which tails off as the expected weight for height is approached. To assess the rates of weight gain, the weight charts were inspected and all periods showing a steady rate of weight gain delineated. The periods were of 12 ± 0.5 days (range 6 to 22 days). Thirty early periods and 33 late periods of steady weight change were identified. The rate of weight change was taken as the slope of the linear regression equation of body weight against days using time as the independent variable. Plasma zinc was taken as the mean of the individual measurements during the period of weight change. The range of these values was always less than 10% of the mean value. Dietary intake was calculated as the mean volume ingested/day/kg body weight for that day.

The energy cost of tissue deposition was assessed in

TABLE 1
Composition of diets

		Maintenance*	Cow's milk*†	Soya milk*†
Pelargon‡	g/l	27	190	
Sobee§	g/l			133
Sucrose	g/l	100		50
Arachis oil	g/l	20	60	59
Protein	g/l	4.4	31	31
Energy	MJ/l	2.95	5.67	5.67
Zinc	μmol/l	10	69	52
Phytic acid	μmol/l	0	0	1330
Protein energy/total/energy	%	2.5	9.2	9.2
Zinc/energy	μmol/MJ	3.4	12.2	9.2
Zinc/nitrogen	μmol/mol	210	199	150
Zinc/phytate	mol/mol	∞	∞	1:26
Volume given to patient	ml/kg/day	136	Ad libitum	Ad libitum

* All children received: KCl, 4 mmol/kg/day; $MgCl_2$, 1 mmol/kg/day; folic acid, 11.4 μmol/day; and a multivitamin preparation (Tropivite, Federated Pharmaceutical Co.), 0.5 ml/day.

† Children receiving the high energy formulae were also given ferrous sulphate, 270 μmol/day.

‡ Nestlé, Bahamas.

§ Mead-Johnson, Jamaica.

two ways. First, for the groups of children, it was taken as the slope of the regression of dietary intake against rate of weight change. As the errors in both variables were unknown, the regression equation described by Brace (10) was used. Second, the energy cost of tissue deposition was assessed for each individual child using the procedure of Jackson et al. (11).

Individual measurements were compared using Student's *t* test, paired or unpaired as appropriate. Differences between groups of paired data were assessed by an analysis of covariance. A p value of <0.05 was accepted as significant. Results are given as mean ± SEM.

Results

The plasma zinc concentration of the marasmic children (13.9 ± 0.8 μmol/l) was not different from thtof the control children (14.3 ± 0.6 μmol/l). The plasma zinc concentration of the kwashiorkor children (11.4 ± 1.7 μmol/l) was the same as the value of the children with marasmic-kwashiorkor (10.6 ± 0.9 μmol/l). These two groups of edematous malnutrition were combined. Their values were significantly lower than those of the control children ($p < 0.005$).

Figure 1 shows the effect of introduction of

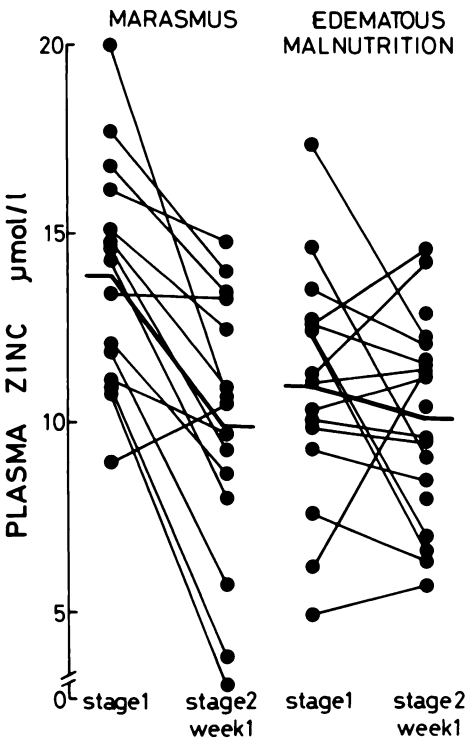

FIG. 1. The effect on plasma zinc concentration of changing from a maintenance diet to a high energy recovery diet in children with marasmus or edematous-malnutrition.

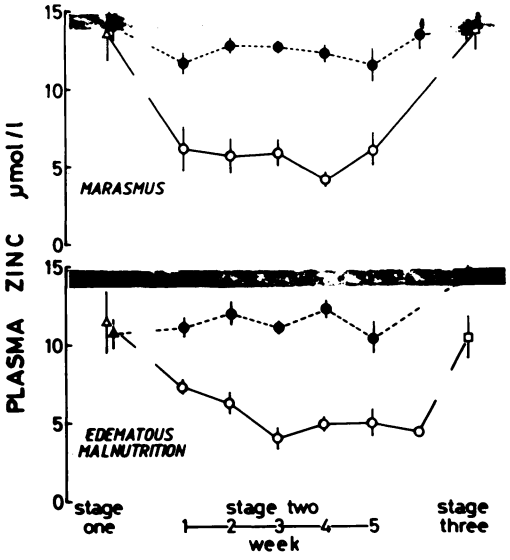

FIG. 2. Plasma zinc concentration during recovery from severe malnutrition. The *upper graphs* are for children with an admission diagnosis of marasmus and the *lower graphs* for children with edematous malnutrition. △ and ▲, maintenance diet; ●, cow's milk based formula; ○, soya protein based formula; □ and ■, mixed diet. The *open symbols* are for children given the soya diet and the *closed symbols* for those given the cow's milk diet. The *shaded bar* represents the mean ± SEM for the control children. Errors shown are SEM.

the high energy diet. The plasma zinc concentration of the marasmic children fell immediately and dramatically ($p < 0.005$) to a mean of 9.9 ± 0.9 μmol/l. The initially edematous children, all of whom had lost their edema before starting the high energy diet, had a slight fall in the plasma zinc concentration (to 10.1 ± 0.6 μmol/l). This fall was not significant, largely because of one child's plasma zinc which rose from a very low value.

Throughout the stage of high energy feeding, the plasma zinc concentration of all children remained low (**Fig. 2**). For the marasmic children the fall in plasma zinc was much more profound in those that were given the soya based formula (to 5.8 ± 0.5 μmol/l) than in those given the cow's milk based diet (to 12.4 ± 0.3 μmol/l). Similarly, the mean of all values for the initially edematous children fed the cow's milk diet (11.6 ± 0.3 μmol/l) was higher than those fed the soya based diet (5.7 ± 0.4 μmol/l). Although the initially edematous children given the cow's milk had slightly lower plasma zinc concentrations than the initially marasmic children, the predominant factor affecting their plasma zinc

concentration from the beginning of high energy feeding was the diet and not the initial diagnosis or clinical state. There was no difference in the two groups of children who received the soya based diet either clinically or anthropometrically. In the soya fed children, there was a tendency for the plasma zinc to continue to fall as the children remained on the diet.

When the children were given a mixed diet they ceased to grow rapidly: indeed, most children initially lost some weight with the change in diet. Their plasma zinc concentrations rose to normal values within 1 wk in all groups except the edematous children who had recovered on the soya based diet. These latter children, nevertheless, had a substantial rise in plasma zinc concentration. **Figure 3** shows the rates of weight gain in the children given the two diets. The children taking the cow's milk diet gained weight significantly more rapidly than those given the soya diet. When the early periods of recovery are separated from the later periods, it becomes apparent that the rate of weight gain diminishes on both diets in the later periods of recovery. The differential between the two diets is much more pronounced during the early periods ($p < 0.02$) than during the later periods ($p < 0.05$).

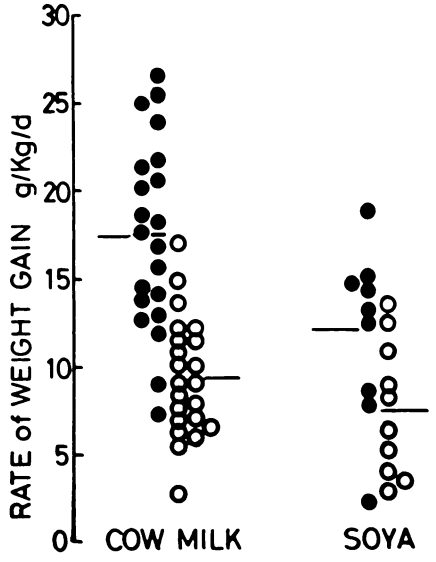

FIG. 3. Rate of weight gain in children recovering on cow's milk or soya based diets. ●, early recovery periods; ○, late recovery periods.

There was no difference in the ad libitum dietary intakes between the cow's milk group (774 ± 25 KJ/kg/day) and the soya group (740 ± 50 KJ/kg/day).

In both groups of children, a significant negative linear relationship existed between the rate of weight gain and the plasma zinc concentration (**Fig. 4**). The faster the children gained weight the lower the plasma zinc concentration. The soya fed children formed a completely distinct group from the cow's milk fed children; however, the slopes of the two regression lines were similar. At the same rates of weight gain, the children fed the soya formula had a plasma zinc concentration 7.2 μmol/l lower than those fed the cow's milk formula. The intercept at zero weight gain was subnormal for the soya group, at 8.8 μmol/l, whereas it was normal for the cow's milk fed children, 16.4 μmol/l.

There were positive correlations between the rates of weight gain and the energy intake in all groups of children (**Fig. 5**). The intercepts of these lines at zero weight gain give a measure for the maintenance energy requirements on the two diets at the different stages of recovery. These were 364 and 431 KJ/kg/day on the soya diet and 410 and 412 KJ/kg/day on the cow's milk based diet. The relatively small number of points in these regressions accounts for the minor differences; it is reasonable to assume that the maintenance energy requirements did not differ between the groups.

The mean energy intake per gram of weight gained, or the energy cost of tissue deposition, for the groups was estimated from the slopes of the lines. Children fed the cow's milk diet needed 21 KJ/g during the early part of recovery; this rose to 35 KJ/g later in recovery. There was no significant change between the phases of recovery in the children receiving the soya based diet (28 and 32 KJ/g). Only in the earlier period was the energy cost of tissue deposition significantly lower in the children given cow's milk than in those given the soya diet.

The values obtained from computing the energy cost of tissue deposition for each individual child gave similar results to those obtained from the regression equations.

The children did not experience a catch up in linear growth during the period of high energy feeding.

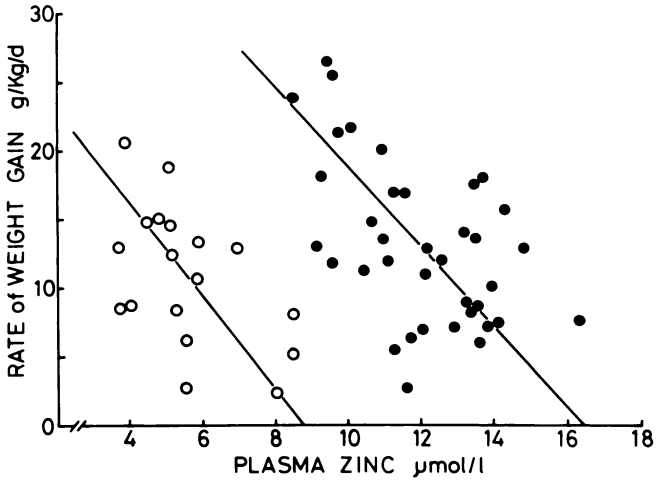

FIG. 4. Correlations between the rate of weight gain (RWG) and the plasma zinc concentration (PZn) in children recovering on two diets. ○, soya based diet, PZn = −0.30 RWG + 8.8, r = 0.50, $p < 0.05$. ●, cow's milk based diet, PZn = −0.34 RWG + 16.4, r = 0.60, $p < 0.01$.

Discussion

The Recommended Dietary Allowance of zinc for a normal 1-yr-old child is 77 µmol/day (5 mg zinc/day) (12). For a child of normal weight (9.4 kg) this gives a recommended intake of 8.2 µmol zinc/kg/day. Yet, in confirmation of our previous results (8), the marasmic children had normal plasma zinc concentrations on our "maintenance" diet, which supplied only 1:5 µmol zinc/kg/day. The children were not gaining weight on this diet so that there was no growth component to the requirement; it was simply required to replace losses. The requirement for zinc may be low in a child who has adapted to an habitually inadequate intake, in a similar way to the reduction in requirement for energy and nitrogen (13). If any of the children were receiving inadequate energy to preserve their tissues, they may have maintained their plasma zinc concentration through tissue catabolism. Fasting may lead to a rise in plasma zinc concentration (14).

When the children were given an energy dense diet, and started to synthesise new tissue, they had a precipitous fall in their plasma zinc levels, despite the much higher zinc concentration in these diets than in the maintenance diet. Indeed, nearly all the children taking the cow's milk formula were receiving more than the Recommended Dietary Allowance for zinc. While the children were gaining weight and laying down new tissue, their plasma zinc concentrations remained depressed. The metabolic state of the children with regard to anabolism or catabolism seems to be a major determinant of the plasma zinc concentration and presumably the dietary requirement for zinc. As these states are determined, to a large extent, by the dietary energy intake, it would seem more appropriate to specify the dietary zinc requirement in relation to the energy intake. A normal 1-yr-old child should ingest about 452 KJ/kg/day (12), which gives a desired zinc to energy ratio of 18µmol/MJ: on this basis our high energy diets could be considered to be zinc deficient (see Table 1).

The changes in plasma zinc concentration were much more pronounced in the children receiving the soya based formula than in those on the cow's milk formula. It is unlikely that the 25% lower zinc content of the soya diet entirely explains this difference, for at the same quantities of zinc ingested from the two diets, the soya fed children had a very much lower plasma zinc concentration. The soya powder used is a commercial infant feeding formula, and contained 10 mmol phytic acid/kg powder; this gives a concentration of 1.33 mmols of phytic acid per litre of made up feed with a molar ratio of zinc to phytate of 1:26. Phytic acid is known to reduce zinc availability (9) and we presume that this makes a substantial contribution to

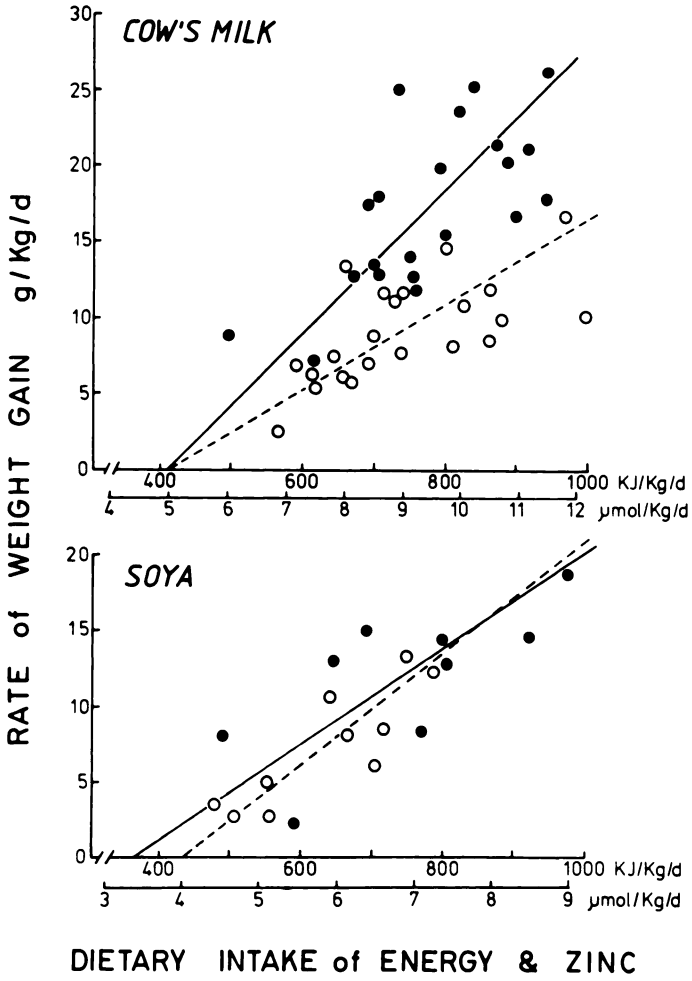

FIG. 5. The relationship between the rate of weight gain (RWG) and the dietary intakes of energy (E) and zinc (Zn) in children given cow's milk based diet (*upper figure*) or soya protein based diet (*lower figure*). ●, early recovery; ○, late recovery periods. The equations for the lines are as follows: *Cow's milk.* (*solid lines* early recovery, *dashed lines* late recovery). Early recovery: E = 21 RWG + 410; Zn = 0.26 RWG + 5.0; r = 0.69, $p < 0.001$. Late recovery: E = 35 RWG + 412; Zn = 0.43 RWG + 5.0; r = 0.64, $p < 0.001$. *Soya.* Early recovery: E = 32 RWG + 362; Zn = 0.29 RWG + 3.3; r = 0.72, $p < 0.05$. Late recovery: E = 28 RWG + 432; Zn = 0.25 RWG + 4.0; r = 0.86, $p < 0.01$.

the much lower plasma zinc values in the soya fed children.

That new tissue synthesis is a cause for a low plasma zinc concentration is demonstrated by the significant correlations between the rate of weight gain and the plasma zinc concentration. Indeed, the fall in plasma zinc in relation to the rate of weight gain was almost the same on the two diets (soya, 0.30 µmol/g/kg/day; cow's milk, 0.34 µmol/g/kg/day). The regression lines also show that children growing at a normal rate of about 1 g/kg/day will have only a minor drop in plasma zinc concentration; the growth effect is only significant during periods of accelerated weight gain.

The rate of weight gain is a relatively insensitive measure of the metabolic changes in a child recovering from malnutrition. The energy cost of tissue deposition gives information on the efficiency of recovery and also the type of tissue which is being synthesised. If adipose tissue is being synthesised it incurs a high energy cost, the sequestered energy being about 33 KJ/g. Lean tissue, on the other hand, has a stored energy of about 5

KJ/g (11). Early in recovery the children fed cow's milk had a lower energy cost of tissue deposition relative to late recovery and to the children fed the soya formula. Nevertheless, the energy costs were still high compared to the theoretical costs of lean and fat tissue deposition and indicate that the children were predominantly synthesising adipose tissue—with the soya fed children synthesising an even greater proportion of fat tissue than the cow's milk fed children. This is supported by skinfold measurements in recovering children (5) and also by metabolic balance which shows a minute nitrogen retention to weight gain (6). Muscle biopsies from children who have made good their weight deficit are still grossly abnormal, with thin atrophic fibers (15).

Mixed muscle tissue from normal children contains about 1.7 μmol zinc/g wet tissue (16). Figure 5 shows that the additional zinc ingested for each gram of weight gained ranged from 0.25 to 0.43 μmol zinc/g. If we consider a child commencing to gain weight, at 15 g/kg/day on the cow's milk formula, his total intake of zinc is 8.8 μmol/kg/day which gives a total dietary intake of 0.6 μmol zinc ingested/gram new tissue formed: a similar calculation for the soya formula gives a figure of 0.5 μmol zinc/g. Thus, there is insufficient zinc to make more than a maximum of one-third of new tissue as normal muscle tissue. The actual amount of zinc available to the new tissue will be much less than 0.6 μmol zinc/g, because body losses of zinc and a factor for availability have not been considered. A conservative estimate of body losses of 2 μmol/kg/day and a 50% zinc availability would give a zinc retention of about 0.2 μmol/g of new tissue. Obviously, there is insufficient zinc in the diet to support accelerated rates of lean tissue synthesis. As the demand for zinc during new tissue synthesis outstrips the supply there will be inefficient and limited weight gain, with the excess of ingested energy being deposited as adipose tissue. It is of interest that childhood zinc nutriture is often inadequate in the United States (3, 4) where childhood obesity is prevalent.

The children on the soya diet had plasma zinc concentrations that were as low as those of patients with untreated acrodermatitis enteropathica (17). Five of these children developed perineal candidiasis, which, at the time, was not associated with zinc deficiency. However, we now know that immunosuppression is an early feature of zinc deficiency (18). The children also showed a therapeutic response to zinc supplementation with a regrowth of their thymus glands (19), activation of their white cell sodium pump (20), and an acceleration of weight gain (see companion paper).

If these children were zinc deficient, it is important to ask why they did not develop anorexia. Anorexia is one of the most consistent features of experimental zinc deficiency. It was because of this lack of anorexia that we initially failed to attribute the infections in the soya fed children to zinc deficiency. It seems that the children induced zinc deficiency in themselves by attempting to grow rapidly in the face of an inadequate zinc supply. The answer may lie in the elegant experiments of Chesters and coworkers (21, 22). They showed that the zinc-deficient rat was sensitive to dietary protein, particularly to methionine, phenylalanine, threonine, and tryptophan, but not to dietary energy supply. When the protein content was reduced in the zinc deficient diet, there was an increased energy consumption and a disappearance of the cyclical anorexia. The anorexia may therefore be specifically caused by an inability to metabolise nitrogen in the face of a zinc deficiency. All our diets had similar, and relatively low, nitrogen to zinc ratios (Table 1), which could account for the lack of anorexia in the children.

Normal children do not grow continuously and steadily: they have infections and set backs which require periods of rapid "catch up growth" during convalescence. Recovery from all serious illnesses requires new tissue synthesis. Childhood malnutrition results from both frequent infections and from a failure to achieve catch up growth between infections. Our results show that zinc supply may be a limiting factor in the ability to resynthesize lost tissue, particularly from diets high in phytic acid. A relatively low protein diet in conjunction with zinc deficiency may lead to excess adiposity in man.

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