# Growth and zinc homeostasis in the severely Zn-deficient rat

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1. Male weanling rats were fed on diets either adequate (55 mg/kg), or severely deficient (0·4 mg/kg) in zinc, either *ad lib.* or in restricted amounts in four experiments. Measurements were made of growth rates and Zn contents of muscle and several individual tissues.

2. Zn-deficient rats exhibited the expected symptoms of deficiency including growth retardation, cyclic changes in food intake and body-weight.

3. Zn deficiency specifically reduced whole body and muscle growth rates as indicated by the fact that (*a*) growth rates were lower in *ad lib.*-fed Zn-deficient rats compared with rats pair-fed on the control diet in two experiments, (*b*) Zn supplementation increased body-weights of Zn-deficient rats given a restricted amount of diet at a level at which they maintained weight if unsupplemented, (*c*) Zn supplementation maintained body-weights of Zn-deficient rats fed a restricted amount of diet at a level at which they lost weight if unsupplemented (*d*) since the ratio, muscle mass:body-weight was lower in the Zn-deficient rats than in the pair-fed control groups, the reduction in muscle mass was greater than the reduction in body-weight.

4. Zn concentrations were maintained in muscle, spleen and thymus, reduced in comparison to some but not all control groups in liver, kidney, testis and intestine, and markedly reduced in plasma and bone. In plasma, Zn concentrations varied inversely with the rate of change of body-weight during the cyclic changes in body-weight.

5. Calculation of the total Zn in the tissues examined showed a marked increase in muscle Zn with a similar loss from bone, indicating that Zn can be redistributed from bone to allow the growth of other tissues.

6. The magnitude of the increase in muscle Zn in the severely Zn-deficient rat, together with the magnitude of the total losses of muscle tissue during the catabolic phases of the cycling, indicate that in the Zn-deficient rat Zn may be highly conserved in catabolic states.

Zinc is an important nutrient, and Zn deficiency in humans has been identified in several areas of the world (e.g. Hambidge & Walravens, 1976; Prasad, 1976; Shrimptom, 1980). The present paper is concerned with two aspects of the biological role of Zn. The first is the involvement of Zn in the regulation of tissue growth. Whereas Zn deficiency has repeatedly been shown to have marked effects on growth, investigations of the mechanism of these effects are complicated by the marked changes in appetite and food intake in animals fed on Zn-deficient diets (e.g. Chesters & Quarterman, 1970). The difficulty of separating effects of reduced food intake from Zn deficiency *per se* is highlighted by the study of O'Leary *et al.* (1979) who concluded from a study of the effects of Zn deficiency on muscle growth that conventional pair-feeding may be insufficient to allow such a distinction to be made. In the experiments reported here, in order to evaluate the effect of Zn deficiency on tissue growth, we have attempted to account for the effects of alterations in food intake in animals given a severely Zn-deficient diet by conducting pair-feeding experiments in both the depletion and repletion phases of Zn deficiency.

The second aspect of the present study concerns Zn homeostasis. The recommended dietary allowance of Zn is similar to that of iron ((US) Food and Nutrition Board, 1974) but, unlike Fe, it is generally considered that there are no specific substantial Zn stores. Thus none of the large number of Zn metallo-enzymes widely distributed throughout metabolism have been considered to serve as storage proteins. Even the role of the heavy metal-binding protein, metallothionein, is perceived by some as regulatory for Zn absorption (Cousins, 1979) rather than a storage protein *per se* (Olafson, 1983). In any case the amounts of Zn bound to this protein are not large enough to prevent the immediate development of the symptoms of Zn deficiency in experimental animals (Mills *et al.* 1969) and in children

Table 1. *Composition (g/kg) of zinc-deficient diet*

| | |
|---|---|
| Egg albumin | 180 |
| Sucrose | 657 |
| Maize oil | 100 |
| Salt mix* | 40 |
| B-vitamin mix† | 22 |
| Fat-soluble-vitamin mix‡ | 1 |

* Salt mix provided (mg/kg diet): aluminium (as sulphate) 0·48, calcium (as carbonate) 4958, cobalt (as chloride) 2·6, copper (as sulphate) 5·1, iron (as ferric sulphate) 189, magnesium (as sulphate) 200, manganese (as sulphate) 40·7, potassium (as iodide, dibasic phosphate and sulphate) 5314, sodium (as chloride, fluoride and borate) 2744.

† B-vitamin mix provided (g/kg diet): ascorbic acid 1·8, inositol 0·2, *p*-amino-benzoic acid 0·2, pyridoxine hydrochloride 0·04, aneurine hydrochloride 0·04, calcium pantothenate 0·12, biotin 0·008, folic acid 0·0036, cyanocobalamin 0·00008, riboflavin 0·08, choline chloride 3·0, nicotinic acid 4·5.

‡ Fat-soluble-vitamin mix provided (/kg diet): retinol 3·44 mg, ergocalciferol 12·5 $\mu$g, menaphthone 2 $\mu$g, mixed tocopherols 2 $\mu$g.

(Golden & Golden, 1981). Indeed, the rapidity of onset of symptoms, particularly growth retardation, led Golden & Golden (1981) to compare Zn with an essential amino acid as far as the response to Zn deficiency was concerned. However, the response of the rat to severe Zn deficiency is not an immediate total growth suppression. Rats can grow at near normal rates on Zn-deficient diets when tube fed (Faragi & Sweinseid, 1983). Even in animals given Zn-deficient diets *ad lib.*, some tissue growth occurs. For example, O'Leary *et al.* (1979) reported increases of between 31 and 90% in the weights of individual muscles in rats fed on a severely Zn-deficient diet and the Zn content of muscle was not reduced in these experiments. Clearly, the expansion of the Zn pool in any tissue in the Zn-deficient state is problematic if there are no Zn stores. We have investigated, therefore, the distribution of tissue Zn in muscle and several other tissues of rats fed on a severely Zn-deficient diet in order to understand better the regulation of the Zn content of the body.

## MATERIALS AND METHODS

### *Diet composition*

The two diets used in the experiments (Table 1) were based on egg albumin (at 180 g/kg) and contained either 0·4 $\mu$g Zn/g (Zn-deficient diet) or about 55 $\mu$g Zn/g (Zn-supplemented control diet) which was formulated by adding $ZnSO_4 . 7H_2O$ to the salt mix. The salt mix is that described by Hoppel & Tandler (1975) which was part of a synthetic rat diet from Nutritional Biochemicals Corp. Cleveland, Ohio. It is similar to that described by Bernhart & Tomarelli (1966) for the K and Na concentrations and to that described by Payne & Stewart (1972) for the other minerals. This latter diet provides amounts equal to or slightly above the recommended intakes for rats (Coates *et al.* 1969), so that intakes will be adequate when there is loss of appetite. The fat-soluble-vitamin mix was obtained from commercial sources (Special Diet Services Ltd, Stepfields, Witham, Essex) but all other main components of the diet were prepared in our laboratory in separate lots, i.e. salt mix (2 kg), B-vitamin mix (1 kg) and sucrose–egg albumin mix (3·65:1; 50 kg), in one large batch at the beginning of the experiment. The diet was then prepared in 1-kg quantities with 10 min mixing after the addition of each component and with maize oil added as the last component. Before every experiment at least three samples of diet were collected from the food bags and the Zn content analysed.

*Animals, tissue collection and Zn analysis*

All experiments involved male Sprague-Dawley outbred rats (Charles River UK Ltd, Margate, Kent), 25 d-old and weighing initially about 60 g. They were caged individually in stainless steel cages, in a room maintained at 24–25° with a 12 h light (08.00–20.00 hours)–12 h dark cycle. Before each experiment, cages were washed in water and detergent, and soaked in EDTA solution (10 g/l). Only acid-washed glass containers were used for food and water, and water bottles were fitted with melamine nozzles and filled every 2 d with fresh deionized distilled water. Animals were weighed and fed between 08.00 and 09.00 hours and measurements of food intake were made each 24 h.

All animals were killed between 08.00 and 11.00 hours by decapitation. Blood was collected from the neck for about 10 s through an acid-washed glass funnel into a Zn-free lithium heparin tube (LIP Equipment and Services Ltd, Shipley, West Yorkshire) kept on ice. Plasma was separated from whole blood by centrifugation and immediately transferred to 5- or 10-ml plastic tubes (Sterilin Ltd, Teddington, Middlesex). Plasma samples were stored at −20° before analysis.

Gastrocnemius, plantaris and soleus muscles, thymus and femur were dissected out in all experiments and; in addition, during the Zn-depletion experiment (Expt 1) and Zn-depletion–repletion experiment (Expt 3), liver, kidneys, spleen, testis and gut (small intestine from pylorus to caecum) were also removed and weighed. In the case of gut the contents were washed out with a syringe containing Zn-free saline (9 g sodium chloride/l) prior to weighing.

Water content was determined by drying at 110° in acid-washed Pyrex tubes or 10-ml beakers overnight or until the weight remained constant over two successive measurements at 4-h intervals. Tissues were then placed individually in small Zn-free strong plastic bags and pulverized between two aluminium blocks. Bone was dissected free from muscle and tendons, the heads cut off and the diaphysis opened longitudinally so that bone marrow could be carefully removed with a stainless steel needle before drying. All powdered tissues were kept in sealed 5-ml plastic tubes (Sterilin) at −20° for Zn analysis.

Samples of the diet were weighed into a 45 ml vitrified-porcelain crucible, dried at 100° and ashed at 450° in a muffle furnace for 24–36 h. Ash was dissolved in 20 ml HCl (100 ml/l) and made up to 100 ml in a volumetric flask with deionized, distilled water. For thymus, spleen, liver, kidney, testis, gut and muscle, between 5 and 15 mg dried powdered tissue were dissolved overnight at room temperature in 1 ml concentrated HCl in a 5 ml plastic container (Sterilin), thoroughly mixed and diluted to 5 ml with deionized, distilled water. Dried and powdered bone samples were dissolved in a mixture of concentrated hydrochloric and nitric acids (1:1). All other steps in bone sample preparation were as described previously. Plasma was diluted with 5 vol. distilled water.

The Zn analysis of these samples was performed on a Pye-Unicam SP2900 atomic absorption spectrometer (Pye-Unicam Ltd, Cambridge). Absorbance was measured at 213·9 nm with standard solutions prepared from zinc nitrate.

## EXPERIMENTAL DESIGN

In all, four primary experiments were performed, two involving feeding the Zn-deficient diet (Zn-depletion experiments (Expts 1 and 2), and two involving Zn-repletion (Zn-depletion–repletion experiments (Expts 3 and 4).

*Expt* 1, *Zn-depletion.* This was to verify the Zn-deficient state in terms of the animals' clinical and behavioural features, total body growth and food intake, and to determine the changes in weight, water and Zn contents of all the tissues and organs described previously.

Twenty-two rats were separated into four groups: baseline (BL), which were killed immediately; Zn deficient (ZD); pair-fed (PF), six rats each; and *ad lib*-fed (AL), four rats. The ZD group was fed on the Zn-deficient diet (ZD) *ad lib.* and daily intakes by the individual rats were measured. The pair-feeding was achieved by giving individual PF rats the Zn-supplemented control diet in an amount equal to the intakes of an individual ZD rat on the previous day. The AL group was fed on the Zn-supplemented control diet *ad lib.* In order to improve the interpretation of the results (see Tables 5 and 7 and Fig. 5) an additional group of six rats was fed on the Zn-deficient diet and treated as described previously.

*Expt* 2, *Zn-depletion.* In the second Zn-depletion experiment the pair-feeding was replaced by controlled intakes, based on the mean measured intakes in Expt 1, which were fed from the first day. Thirty-eight rats were separated into five groups, three groups of six animals (baseline (BL); Zn-deficient (ZD10), fed *ad lib.*; and controlled intakes (CI10)), with the controlled intake group (CI10) fed for 10 d on a fixed amount of the Zn-supplemented control diet equal to the measured intake of the Zn-deficient rats in the previous experiment (6 g/d), and two further groups (ZD17 (*n*13) and CI17, (*n*7)) fed on the respective diets for 17 d.

*Expt* 3, *Zn-depletion–repletion.* The objective of this experiment was to examine the response of whole body and muscle growth to Zn supplementation at a fixed dietary intake in order to determine the separate effects of Zn deficiency and food restriction.

Twelve male rats were fed on the Zn-deficient diet for 25 d and then separated into three groups of four animals, i.e. baseline (ZDBL, killed immediately), Zn-repleted either at a restricted intake (RCI, 5·5 g/rat per d, equal to approximately 0·19 g/g body-weight$^{0.75}$ which was the average intake observed during the previous 7 d on the Zn-deficient diet), or allowed to feed *ad lib.* (RAL) for 22 d.

*Expt* 4, *Zn-depletion–repletion.* The objective of this experiment was the same as in the previous one but the Zn repletion was achieved at a lower restricted intake (5 g/d equal to about 0·16 g/g body-weight$^{0.75}$). Eight rats were fed on the Zn-deficient diet for 24 d and were then divided into two groups: RIZD fed on the restricted amount of the Zn-deficient diet and RIZS fed on the same restricted amount of the Zn-supplemented diet for 8 d.

### *Statistics*

All results are given as means with 1 SEM. The significance levels for the differences between means were assessed by the Student's *t* test as described by Bailey (1981). However, tests for significance were generally only performed between Zn-deficient and the appropriate pair-fed control groups. Correlation between two variables was assessed by calculation of the correlation coeficient, *r*, the appropriate *P* value for that coefficient being obtained from Table VII of Fisher & Yates (1963) with $n-2$ df where $n$ is the sample number. In all significance tests *P* values of 0·05 or less were considered to be signficant.

## RESULTS

The severe Zn deficiency resulted in reduced food intake (Table 2), growth retardation after 3 d (Fig. 1), losses of hair after 10 d, and hyperkeratosis of the paws and dermatological changes of other areas after 3 weeks, which are all characteristic symptoms (see Swenerton & Hurley, 1968). Also the characteristic cyclic changes in body-weight in response to cyclic changes in food intake (Williams & Mills, 1970) were observed after 5 d. The results for a typical rat are shown in Fig. 2. The periodicity was about 3 d, with the lowest intakes resulting in losses of body-weight of up to 6%/d and with the highest intakes, which were similar to intakes in the AL group (see Table 2), inducing growth of up to 7%/d.

Of these various responses, only the growth retardation was observed in the PF rats

Table 2. *Expt 1. Zinc depletion. Food intakes (g/g body-weight$^{0.75}$ per d) of Zn-deficient (ZD), pair-fed (PF) and* ad lib.*-fed control (AL) rats expressed as weekly average intakes for individual rats*

(Mean values with their standard errors)

| Group... | ZD | | PF | | AL | | ZD:PF | ZD:AL |
|---|---|---|---|---|---|---|---|---|
| *n*... | 6 | | 6 | | 4 | | | |
| Week no. | Mean | SE | Mean | SE | Mean | SE | (×100) | (×100) |
| 1 | 0·28 | 0·012 | 0·29 | 0·009 | 0·37 | 0·024 | 96·6 | 75·7 |
| 2 | 0·22 | 0·018 | 0·20 | 0·008 | 0·38 | 0·023 | 110·0 | 57·9 |
| 3 | 0·20 | 0·014 | 0·17 | 0·009 | 0·38 | 0·028 | 117·6 | 52·6 |

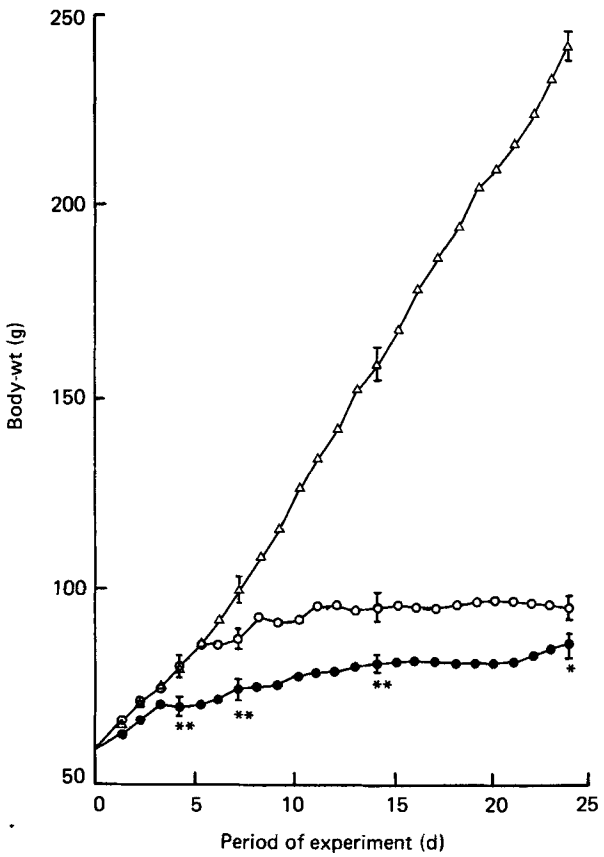


Fig. 1. Expt 1. Zinc depletion. Mean body-weight of Zn-deficient (●, ZD), pair-fed (○, PF) and *ad lib.*-fed (△, AL) rats, during 24 d on the Zn-deficient diet. Significance of the difference between groups ZD and PF: * $P < 0·05$, ** $P < 0·01$.

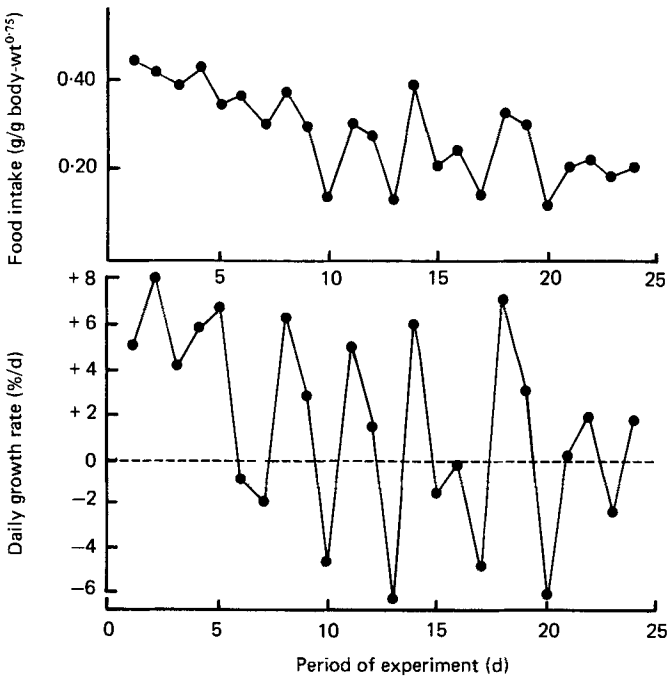


Fig. 2. Expt 1. Zinc depletion. Cyclic pattern of food intake and growth in a Zn-deficient rat during 24 d on the diet.

(Fig. 1). Although the overall growth of the ZD rats was less than that of the PF rats the interpretation of this response was complicated by the pair-feeding technique in this experiment. This involved feeding to the PF rats the same absolute amount of food as consumed the previous day by the ZD rats, so that there was a 1 d lag which resulted in an initial gain in body-weight of the PF rats (Fig. 1). Thus food intake as a function of body-weight became progressively lower compared with that of the ZD rats after the first week. This can be seen in Table 2 (showing food intakes calculated as a function of body-weight$^{0.75}$ because of the divergance of body-weights and because the intakes of the *ad lib.*-fed groups varied in proportion to body-weight$^{0.75}$). Thus while the intake of the ZD rats fell from 75% of that of the AL group in the first week to 52% in the third week, in the PF group the intake fell to only 45% of that of the AL group. A smaller ultimate body-weight on a slightly higher intake suggests that Zn-deficiency does have a specific growth-retarding effect. This is also suggested by the subsequent experiments. In the second Zn-depletion experiment the CI group was fed the same average intake as the ZD group from the first day (6 g/d) and, although body-weights (g) were similar after 10 d (ZD10, 88·3 (SE 1·0); CI10, 90·5 (SE 1·6)), after 17 d CI rats were heavier (114 (SE 2)) than the ZD rats (98 (SE 2)).

A specific effect of Zn is also indicated by the Zn-supplementation experiments. Zn supplementation resulted in an immediate restoration of food intake and a consequent restoration of growth (Fig. 3). Even when the ZD rats were given a restricted intake of the Zn-supplemented diet (5·5 g), they increased their body-weights by about 20% (see Fig. 3). In the second repletion experiment, in response to a slightly lower intake (5 g), the rats fed on the Zn-supplemented diet maintained weight, whereas those fed on the Zn-deficient diet at this level lost weight (Fig. 4).

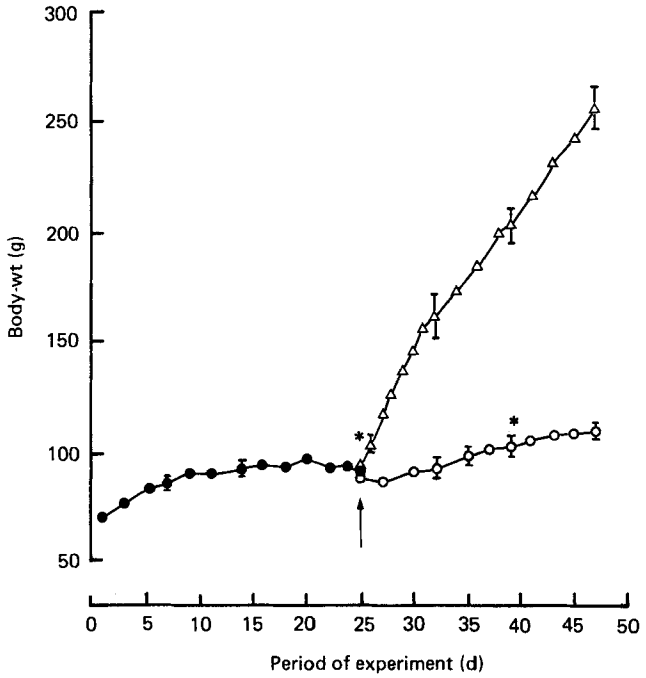


Fig. 3. Expt 3. Zinc depletion–repletion. Mean body-weight of rats which were baseline Zn-deficient (●, ZDBL) and then Zn-supplemented (↑) at a restricted intake (○, RCI), or *ad lib.*-fed (△, RAL) before and after 22 d supplementation. Significance of the difference between groups RAL and ZDBL, RCI and ZDBL: $*P < 0{\cdot}05$.

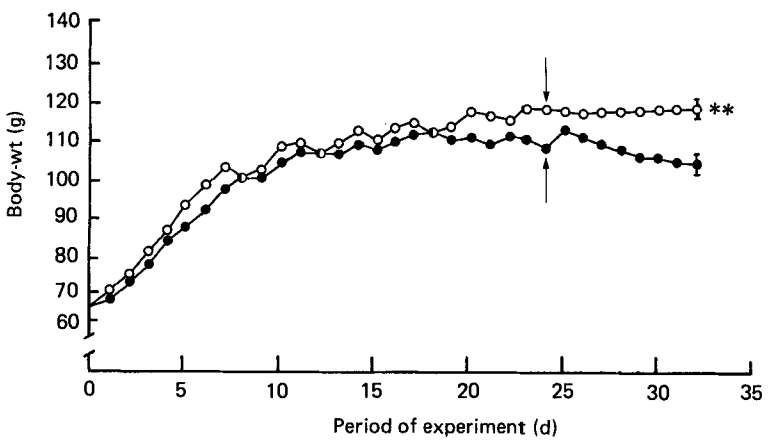


Fig. 4. Expt 4. Zinc depletion–repletion. Mean body-weights of *ad lib.*-fed Zn-deficient rats which were fed at 25 d (↑) restricted amounts of the diet either with Zn-supplementation (○, RIZS) or unsupplemented (●, RIZD). Significance of the difference between RIZS and RIZD: $** P < 0{\cdot}01$.

Table 3 shows the effects of the Zn deficiency on body composition with organ weights shown in relation to body-weight. These organs were chosen as representatives of systems and processes known to be affected by Zn deficiency and include spleen and thymus (defence), general metabolism (liver, kidney and gut) and muscle as the largest component of the lean body mass. Since Zn concentration in red oxidative muscle has been reported

Table 3. *Expt 1. Zinc depletion. Organ weight (g/kg total body-weight) of baseline control (BL), Zn-deficient (ZD), pair-fed (PF) and* ad-lib.*-fed control (AL) rats after* 24 *d on the diets*
(Mean values with their standard errors)

| | BL (*n*6) | | ZD (*n*6) | | PF (*n*6) | | AL (*n*4) | |
|---|---|---|---|---|---|---|---|---|
| | Mean | SE | Mean | SE | Mean | SE | Mean | SE |
| Body-weight (g) | 59·0 | 1·1 | 87·7 | 1·9 | 97·3 | 3·1 | 240 | 5·1 |
| Spleen | 4·9 | 0·4 | 2·7 | 0·2 | 2·6 | 0·1 | 3·2 | 0·1 |
| Thymus | 5·1 | 0·3 | 2·3 | 0·2 | 2·8 | 0·3 | 3·8 | 0·3 |
| Liver | 56·1 | 3·1 | 47·7** | 3·3 | 36·9 | 0·5 | 59·7 | 3·4 |
| Kidney (two) | 14·3 | 0·3 | 13·7** | 0·2 | 12·0 | 0·1 | 10·6 | 0·3 |
| Testis (two) | 8·9 | 0·2 | 17·9 | 0·9 | 18·0 | 1·6 | 9·8 | 0·2 |
| Gut | 93·3 | 5·2 | 57·5 | 4·0 | 48·8 | 3·6 | 47·7 | 2·5 |
| Plantaris | 0·78 | 0·05 | 0·90** | 0·02 | 1·05 | 0·02 | 0·96 | 0·03 |
| Gastrocnemius | 4·3 | 0·02 | 4·8** | 0·1 | 5·8 | 0·1 | 4·9 | 0·2 |
| Soleus | 0·42 | 0·02 | 0·43** | 0·01 | 0·52 | 0·03 | 0·42 | 0·01 |

Statistical significance of the difference between mean values was tested between ZD and PF groups: ** $P < 0·01$.

to be higher than in pale glycolytic muscle (Cassens *et al.* 1967; O'Leary *et al.* 1979; Jackson *et al.* 1982), soleus (red oxidative), and gastrocnemius and plantaris muscles (mixed fibre but mainly glycolytic) were examined. The effects of the Zn deficiency have to be judged against the trend of the changes in body composition which occur with age in well-fed rats (Miller, 1969), as well as the changes occurring in the PF rats. Thus in the spleen, thymus and intestine the decline of relative organ size with age was accelerated by Zn deficiency to the extent that these organs actually lost weight. All of the other organs gained weight although in the case of liver, which exhibited a fall in the percentage of body-weight, weight gain was slower than body-weight growth. In the case of muscle, however, there was no indication of any selective growth retardation, since gastrocnemius and plantaris weight increased as a percentage of body-weight at least as fast as the age-dependent increase, and the soleus muscle maintained its percentage of body-weight. However, judging by the changes in the PF group, reduced food intake played a role in initiating these changes in organ size. In spleen, thymus, testis and intestine there were no differences between the ZD and PF groups. However, liver and kidney were somewhat surprisingly larger in the ZD group. In contrast, muscle in the PF group was a significantly increased percentage of body mass compared with both BL and AL groups as reported by O'Leary *et al.* (1979), indicating that these food-restricted rats were much leaner than the well-fed groups, and that the voluntary reduction in intake of the ZD diet did not allow a similar increase in leanness.

The effect of the Zn deficiency on muscle growth can be further examined with the results in Table 4 showing muscle weights and muscle weight:body-weight values in all the experimental groups. At the end of both Zn-depletion experiments (Table 4), because gastrocnemius and plantaris muscles together constitute a smaller percentage of body-weight than in the food-restricted rats, differences in muscle mass were even greater than differences in body-weight. Zn repletion at a restricted intake of diet selectively increased muscle mass (RCI, Table 4) while Zn repletion at an intake of diet which induced weight loss when unsupplemented (Fig. 4) induced the highest muscle mass:body-weight value observed in these experiments (RIZS, Table 4), resulting in a 25% larger muscle than in the Zn-deficient rats. Although the differences were not so marked, the changes in the soleus muscle:body-weight values were similar.

Table 4. *Expts 1–4. Zinc depletion and repletion. Muscle weight (mg from both legs and g/kg body-weight) in well-fed (BL, AL), food-restricted (PF, CI10, CI17), Zn-deficient (ZD, ZDBL, RIZD) and Zn-repleted (RCI, RAL, RIZS) rats*

(Mean values with their standard errors)

| Expt no. | Expt | Group | *n* | Gastrocnemius + plantaris: Weight Mean | SE | Gastrocnemius + plantaris: g/kg body-weight Mean | SE | Soleus: Weight Mean | SE | Soleus: g/kg body-weight Mean | SE |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. | Zn depletion 1 (24 d) | BL | 6 | 600 | 22 | 10·3 | 0·3 | 50 | 2 | 0·85 | 0·04 |
| | | ZD | 6 | 992** | 54 | 11·3** | 0·2 | 74** | 4 | 0·84** | 0·03 |
| | | PF | 6 | 1316 | 74 | 13·5 | 0·2 | 102 | 6 | 1·03 | 0·05 |
| | | AL | 4 | 2838 | 79 | 11·5 | 0·4 | 204 | 6 | 0·83 | 0·03 |
| 2. | Zn depletion 2 (10 and 17 d) | BL | 6 | 658 | 21 | 10·6 | 0·4 | 59 | 2 | 0·94 | 0·04 |
| | | ZD10 | 6 | 1153 | 24 | 13·1 | 0·3 | 94 | 4 | 1·06 | 0·05 |
| | | CI10 | 6 | 1175 | 27 | 13·0 | 0·3 | 103 | 6 | 1·14 | 0·04 |
| | | ZD17 | 13 | 1273** | 39 | 12·8** | 0·2 | 94** | 8 | 0·95 | 0·02 |
| | | CI17 | 7 | 1618 | 76 | 14·2 | 0·4 | 118 | 10 | 1·03 | 0·04 |
| 3. | Zn depletion–repletion 1 (25 and 22 d) | ZDBL | 4 | 1058 | 40 | 11·5 | 0·4 | 80 | 3 | 0·87 | 0·03 |
| | | RCI | 4 | 1485** | 40 | 13·5** | 0·4 | 108** | 5 | 0·98* | 0·04 |
| | | RAL | 4 | 2914 | 60 | 11·4 | 0·6 | 203 | 12 | 0·78 | 0·04 |
| 4. | Zn depletion–repletion 2 (24 and 8 d) | RIZD | 4 | 1468** | 62 | 15·4 | 0·5 | 126** | 8 | 1·32 | 0·06 |
| | | RIZS | 4 | 1845 | 63 | 16·8 | 0·5 | 145 | 3 | 1·32 | 0·04 |

Statistical significance of the differences between mean values was tested between groups ZD and PF, ZD17 and CI17, ZDBL and RCI, and RIZD and RIZS: * $P < 0.05$, ** $< 0.01$.

Table 5. *Expts 1 and 2. Zn depletion. Skeletal muscle weight (g/kg total body-weight) in Zn-deficient rats in the anabolic (+) or catabolic (−) phase of their body-weight cycle*

(Mean values with their standard errors)

| Body-wt change† | *n* | Gastrocnemius + plantaris Mean | SE | Soleus Mean | SE |
|---|---|---|---|---|---|
| + | 15 | 12·6 | 0·4 | 0·96 | 0·03 |
| − | 16 | 12·3 | 0·4 | 0·97 | 0·03 |

† Based on body-wt change over previous 24 h.

These observations of reduced muscle mass:body-weight values and smaller muscles in the ZD rats compared with food-restricted control rats, do suggest a specific response of muscle growth to the Zn-deficiency independent of the reduction in food intake. However, because of the cycling in body-weight in the ZD rats, and the possibility that body composition may vary according to the phase of the cycle influencing the measured values, measurements of muscle mass:body-weight values were made in positive and negative

Table 6. *Expts 1 and 3. Zn depletion and repletion. Mean Zn concentration (μg/g dry weight) in individual organs and plasma from control (BL, AL), Zn-deficient (ZD, ZDBL) and Zn-repleted (RCI, RAL) rats*

(Mean values with their standard errors)

| Group... | BL (*n*6) | | ZD (*n*6) | | PF (*n*6) | | AL (*n*6) | | ZDBL (*n*4) | | RCI (*n*4) | | RAL (*n*4) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Tissue | Mean | SE | Mean | SE | Mean | SE | Mean | SE | Mean | SE | Mean | SE | Mean | SE |
| Spleen | 84·9 | 2·0 | 87·7 | 2·4 | 87·5 | 1·5 | 89·0 | 1·6 | 88·7 | 1·9 | 96·6 | 1·5 | 94·8 | 1·9 |
| Thymus | 92·5 | 1·2 | 93·4 | 2·9 | 89·7 | 1·7 | 87·3 | 1·3 | 87·4 | 2·7 | 91·2 | 1·0 | 87·0 | 1·3 |
| Liver | 92·9 | 3·7 | 85·8** | 3·3 | 123·0 | 3·8 | 88·2 | 4·9 | 75·0** | 3·7 | 112·0 | 1·9 | 56·9 | 1·3 |
| Kidney | 81·5 | 2·6 | 80·9** | 2·1 | 102·4 | 2·4 | 103·5 | 1·7 | 82·2** | 1·5 | 105·4 | 1·0 | 108·2 | 1·1 |
| Testis | 129·6 | 4·1 | 123·0** | 1·9 | 176·9 | 5·6 | 187·8 | 2·1 | 129·7** | 5·4 | 164·2 | 6·0 | 167·7 | 7·5 |
| Gut† | 99·8 | 1·8 | 99·1** | 3·4 | 169·5 | 6·0 | 155·8 | 8·3 | 87·8** | 3·4 | 129·4 | 4·1 | 136·3 | 4·7 |
| Plantaris | 63·9 | 1·9 | 61·9 | 2·8 | 62·8 | 2·9 | 67·7 | 2·1 | 66·5 | 4·4 | 58·0 | 4·3 | 65·8 | 4·7 |
| Gastrocnemius | 63·2 | 3·9 | 60·6 | 4·2 | 62·8 | 4·5 | 58·3 | 5·8 | 64·7 | 3·7 | 65·2 | 5·7 | 58·5 | 2·7 |
| Soleus | 207·2 | 8·3 | 222·3 | 6·4 | 227·6 | 7·5 | 210·2 | 6·6 | 212·9** | 7·3 | 245·8 | 3·4 | 251·4 | 3·0 |
| Bone‡ | 177·8 | 4·9 | 62·9** | 3·6 | 235·0 | 6·7 | 242·8 | 7·3 | 80·8** | 2·1 | 135·6 | 5·7 | 166·3 | 14·0 |
| Plasma | 127·4 | 12·2 | 68·1** | 4·6 | 202·5 | 10·2 | 219·1 | 4·8 | 50·8** | 3·3 | 120·4 | 5·8 | 145·0 | 13·7 |

† Gut refers to small intestine.
‡ Bone refers to femur.
Statistical significance of the differences between mean values was tested between ZD and PF, and ZDBL and RCI groups: ** $P < 0.01$.

growth balance. As shown in Table 5 there were no differences, indicating that the cyclic changes in body-weight involved similar changes in muscle mass.

Table 6 shows the Zn concentration in plasma and several tissues and organs from animals in the first Zn-depletion experiment (BL, ZD, PF and AL), and in Zn depletion–repletion Expt 3 (Zn deficient baseline, ZDBL; Zn-repleted restricted, RCI; and Zn-repleted *ad lib.*, RAL). No differences between any of the groups were observed in the Zn contents of spleen, thymus, gastrocnemius and plantaris muscles, while the Zn content of the soleus muscle only changed in the Zn-repleted groups where it increased slightly. The maintainance of muscle Zn confirms the findings of Halsted *et al.* 1974, O'Leary *et al.* (1979) and Jackson *et al.* (1982) who all reported that Zn concentration was maintained in muscle in Zn deficiency. However, O'Leary *et al.* (1979) reported that in the soleus muscle from Zn-depleted rats, Zn concentration did not increase as it did in weight-restricted animals.

In liver, kidney, testis and gut there were changes in the Zn concentrations but the interpretation of these changes is somewhat complicated by the fact that there were higher levels in both the *ad lib.* control (AL kidney, testis and spleen), and the pair-fed group (PF liver, kidney, testis and gut) compared with the initial baseline group (BL). Why this occurred is not known but may reflect the relatively high Zn concentration and availability in the control diet, which was free of fibre and phytate, coupled with the fact that the Zn content of some tissues does increase with age (Georgievskii, 1982). Thus in gut, testis and kidney of the ZD rats, although the concentration did not fall below that of the BL group, it was lower than in both PF and AL groups. Furthermore, in the Zn-repletion experiments the Zn concentrations increased in all three tissues. Liver was somewhat unusual in that its Zn concentration was actually increased in the PF rats compared with all other groups, and for this reason there was a lower Zn concentration in the ZD groups compared with the PF group. Zn supplementation increased liver Zn but only when food was restricted, since in the supplemented *ad lib.*-fed rats Zn concentration fell. However, in plasma and

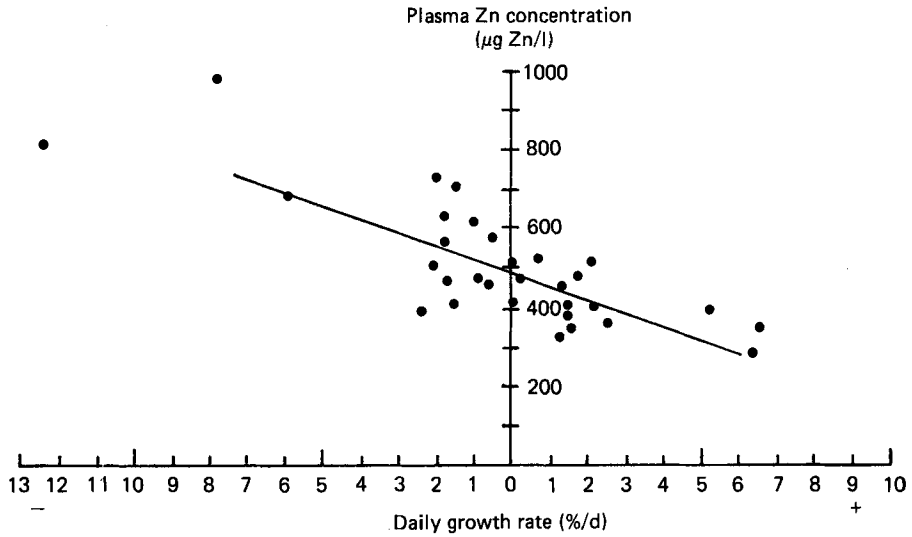


Fig. 5. Expts 1 and 4. Zinc depletion. Relationship between plasma Zn concentration and body-weight change of Zn-deficient rats during previous 24 h. The line is the regression of plasma Zn values on body-weight change which are significantly correlated ($r-0{\cdot}78$, $P < 0{\cdot}005$, $n31$). The intercept value (i.e. plasma Zn at zero balance) is 487 μg/l, while the slope is $-33$ μg/l per 1% per d increase in growth rate.

bone the Zn concentration was much lower in the ZD and ZDBL rats compared with all the other groups.

These results indentify liver, kidney, gut, testis, bone and plasma as tissues where the Zn concentration was responsive to Zn deficiency. In contrast, in spleen, thymus and muscle, dietary Zn had little or no effect on Zn concentration. Reduced plasma Zn has been observed by several previous authors (e.g. Wilkins *et al.* 1972; Fleming *et al.* 1976; Hess *et al.* 1977). Similarly, reductions in bone Zn have been frequently observed (see Prasad & Oberleas, 1971; Halsted *et al.* 1974; Jackson *et al.* 1982). However, as far as the liver, kidney and testis are concerned there are mixed findings in the literature but this may reflect the fact that tissue responsiveness to Zn deficiency may be manifest not only as a loss of Zn but also as a failure to increase Zn in response to energy deficiency, as shown here in the liver (see Table 6). It may also reflect the fact that Zn levels can vary in some tissues between apparently well-fed animals, as shown here in gut, testis and kidney (Table 6).

Closer examination of the plasma Zn concentrations indicated a variability which was related to the phase of the body-weight cycle, and this is shown in Fig. 5. Thus the highest Zn concentrations were observed in rats in a catabolic state while the lowest levels occurred in rats in an anabolic state. Since this suggests that Zn is lost from tissues in rats losing weight, an attempt was made to determine if any tissues selectively lost Zn in the catabolic phase of the cycle. The results of this analysis are shown in Table 7. It is apparent that in no tissue, with the exception of plasma, were any differences apparent in Zn concentrations according to the phase of the cycle. Of course the loss of tissue Zn with tissue protein in the catabolic phase would liberate Zn without changing Zn concentration and, in any case, the total quantity of Zn necessary to increase plasma Zn by the observed amount (equal to about 3 μg) would be a very small proportion of the Zn pool in most of the examined tissues and unlikely to be measureable. For example, 3 μg Zn would be liberated during

Table 7. *Expts 1 and 3. Zn depletion. Zn concentration (μg/g dry weight) in tissues and plasma from Zn-deficient rats after 24 or 25 d of Zn deficiency in either the anabolic (+) or catabolic (−) phase of their body-weight cycle*

(Mean values with their standard errors)

| Body-weight change ... | + | | | − | | |
|---|---|---|---|---|---|---|
| Tissue | Mean | SE | *n* | Mean | SE | *n* |
| Bone | 77·7 | 3·8 | 8 | 76·2 | 4·0 | 9 |
| Gastrocnemius | 62·8 | 1·9 | 8 | 65·5 | 2·4 | 9 |
| Soleus | 232·2 | 11·1 | 8 | 233·5 | 8·7 | 9 |
| Liver | 82·5 | 2·7 | 8 | 79·6 | 3·0 | 9 |
| Kidney | 82·4 | 1·4 | 8 | 82·4 | 1·4 | 9 |
| Testis | 126·9 | 1·9 | 8 | 130·9 | 3·7 | 9 |
| Gut† | 101·3 | 5·0 | 8 | 98·7 | 5·9 | 9 |
| Spleen | 90·7 | 1·9 | 8 | 87·1 | 2·2 | 9 |
| Thymus | 92·6 | 2·2 | 8 | 91·1 | 2·5 | 9 |
| Plasma‡ | 409 | 17 | 15 | 585*** | 42 | 16 |

Mean values of plasma Zn concentrations in the catabolic phase were significantly higher than in the anabolic phase: *** $P < 0{\cdot}001$. None of the other values differed between the two phases of the cycle.

† Small intestine.

‡ μg/l; values include measurements made in 17 d Zn-deficient rats.

the catabolism of only 0·4% of the total musculature. In fact in muscle, although there were no significant differences in Zn concentrations in the two phases of the cycle, the mean value was actually higher in the catabolic phase. The implication of this will be discussed.

## DISCUSSION

There are several important implications of these results. First, the present results confirm that Zn deficiency *per se* does have a restrictive effect on growth which is independent of the reduction in food intake in accord with most previous studies (e.g. Chesters & Quarterman, 1970; O'Leary *et al.* 1979). In all four experiments body-weight and particularly muscle weight, were less in the ZD rats compared with the Zn-supplemented rats on the same intake. Although several authors have discussed the possible mechanism of the reduced food conversion efficiency (Chesters & Quarterman, 1970; Chesters & Will, 1973; Underwood, 1977; Prasad, 1978; Mills, 1981) the mechanism is not understood. It would be expected that the cyclic changes in body-weight would reduce food utilization efficiency. This is because the energy cost of the excess tissue deposited in the anabolic phase (i.e. tissue subsequently lost in the catabolic phase of the cycle), would result in increased overall energy expenditure and consequently increased energy requirements compared with animals with the same net tissue gain which were not experiencing cyclic changes. However, in the second Zn-repletion experiment there were differences in body-weight and muscle weight between the ZD rats and the Zn-supplemented rats on the same intake, even though the ZD rats were given fixed restricted amounts of food and were therefore unable to cycle their body-weights as in the *ad lib.*-fed state. Thus the cyclic changes in body-weight cannot be the only explanation for the reduced food conversion efficiency. A reduced rate of tissue protein deposition must ultimately involve an influence of Zn deficiency on the rates of protein synthesis and degradation. This is investigated in a subsequent paper (R. Giugliano and D. J. Millward, unpublished results).

Table 8. *Expt 1. Zinc depletion. Estimated Zn pool sizes (μg total tissue) in baseline (BL) and Zn-deficient (ZD) rats after 24 d of Zn deficiency*

| | BL | ZD | Zinc gain or loss |
|---|---|---|---|
| Skeletal muscle (white) | 1710 | 2791 | +1082 |
| Skeletal muscle (red) | 930 | 1450 | +520 |
| Liver | 505 | 581 | +76 |
| Kidney | 86 | 124 | +38 |
| Testis | 96 | 265 | +169 |
| Intestine (small) | 731 | 662 | −69 |
| Spleen | 37 | 32 | −5 |
| Thymus | 37 | 21 | −16 |
| Bone | 2756 | 1417 | −1339 |
| Plasma | 37 | 28 | −9 |
| Total | 6925 | 7371 | +446 |
| Diet | | | 313 |

Calculations based on six rats per group. Total body-weight (g): BL 358·9, ZD 521·3. Zn concentrations from Table 6 corrected for water content (g/g): muscle water 0·75, bone water 0·28. Organ weights (g/kg body-wt): white muscle 300 (BL), 350 (ZD), red muscle 50, bone 60, plasma 80. Total diet consumed 870 g (Zn absorption 90%).

The results of the present study have important implications for the regulation of tissue Zn content in the severely Zn-deficient state. It has previously been shown that some tissues, including muscle, do not appear selectively to lose Zn on a Zn-deficient diet and never exhibit reductions in Zn concentrations during growth in the Zn-deficient state (e.g. O'Leary *et al.* 1979; Jackson *et al.* 1982). This is clearly shown in the current studies (Table 6). Furthermore, in comparison with the initial baseline animals most other tissues examined maintained their Zn concentrations in the Zn-deficient state (although as previously indicated some of these other tissues did increase their Zn in the *ad lib.* controls and with Zn supplementation). In the case of muscle and, to a lesser extent, in these other tissues, the analogy of Golden & Golden (1981) between Zn and an essential amino acid is supported. However, there is no doubt that although growth is severely retarded in Zn deficiency, Zn-deficient rats do expand their muscle mass to some extent and therefore increase their total muscle Zn in excess of the dietary supply. The extent of this can be seen in Table 8 which shows the estimated changes in the Zn pool sizes in the tissues examined during 24 d of Zn deficiency. On the assumption that the changes in the relative sizes of the muscles examined reflects the changes in the whole musculature it would appear that muscle increased its total Zn content, which is a large proportion of total body Zn, by about 60% and increases occurred in several other tissues. Since these increases amounted to more Zn than could have come from the diet, other tissues must have lost Zn and it is clear that amongst the tissues examined bone was the main source. In fact the sum of the Zn lost from the bone together with the dietary Zn is, notwithstanding the approximate nature of these calculations, remarkably similar to the amount of Zn deposited in muscle. Thus the analogy of Zn with an essential amino acid is less appropriate when the losses of Zn from bone (which result in an actual fall in Zn concentration) are taken into account. Indeed, the fact that in tissues like kidney, testis and gut, Zn levels in presumably well-fed rats can be increased by feeding high-Zn diets (Table 6, BL cf. PF and AL) also raises the possibility that in some tissues Zn levels are more variable than was thought previously.

The role of bone Zn as a potential store has been discussed with varying conclusions.

Thus the observation that no losses of bone Zn occurred in Zn-deficient pregnant rats led Hurley & Swenerton (1971) to reject the possibility that bone Zn could be redistributed when needed elsewhere. Furthermore, Murray & Messer (1981) argued that since Zn deficiency did not affect the reabsorption of bone in weanling rats, bone Zn could not serve as a store. However, Harland *et al.* (1975) showed that Zn supplementation before Zn restriction in birds resulted in improved growth on the restricted-Zn diet. Also, Brown *et al.* (1978) argued that the 30% reduction in total femur Zn in the Zn-deficient rat must be partly available for growth in other tissues. Our results are in line with the latter study and lead us to suggest that the idea that there are no substantial Zn stores can be challenged and replaced with the concept of variable priorities with which tissues retain Zn, bone being a low priority tissue and hence capable of releasing Zn for the growth of the high priority tissues such as muscle.

There is another important implication of the results shown in Table 8. Although this is not a whole body Zn balance, the results do suggest that Zn has been well conserved during the period on the Zn-deficient diet. Jackson *et al.* (1982) showed that rats on a Zn-deficient diet increased their body-weights from 50 to 200 g but only exhibited a 30% fall in total body Zn concentration, implying that total body Zn content increased during the period, no doubt reflecting the higher dietary Zn levels used in those studies. Although it might be expected that Zn is well conserved when deficient in the diet the cyclic changes in body-weight should make conservation difficult. Thus in the catabolic phase of the cycle, Zn losses should occur as the Zn associated with the tissue mobilized is released into the circulation and excreted. Certainly Zn losses are a well-recognized feature of tissue catabolism under normal circumstances (Cuthbertson *et al.* 1972; Fell *et al.* 1973; Jackson & Edwards, 1982). In the absence of dietary Zn, any Zn lost in the catabolic phase cannot be replaced in the anabolic phase. Thus the cycling should result in Zn being progressively lost from the body. It is possible to calculate the extent of these losses roughly from the values in Fig. 2. The sum of the catabolism calculated as the product of the daily percentage weight loss and time, amounts to some 30% of the body-weight over the 24 d. These losses of body-weight do indicate losses of lean tissue including muscle according to the constancy of muscle mass:body-weight values throughout the cycle (Table 5). However, there is no evidence that such losses of Zn occur since Zn appears to be well conserved in the tissues examined which account for more than 75% of the total body Zn as reported by Jackson *et al.* (1982). Furthermore, the main tissue Zn pool not examined in our study was skin which has also been shown not to exhibit a fall in Zn concentration in Zn deficiency (Jackson *et al.* 1982). Indeed, it would even appear that very little leaves the tissues during the catabolic phases since although plasma Zn is higher at this time (Fig. 5), the increase is very small compared with that expected if Zn was liberated as the tissues were catabolized. All this suggests that Zn is conserved in the tissues during tissue wasting, or is very avidly taken up by some other tissue after it is released into the plasma. If tissue Zn was conserved *in situ* this would mean that the Zn turnover would be very slow and this has been reported by Jackson (1980) who was unable to observe any Zn turnover in muscle of Zn-deficient rats. The retention of Zn *in situ* necessitates that tissue Zn concentrations should increase as animals lose weight by the same proportion as the extent of the tissue catabolism, which was up to 6% in the present experiments (Fig. 2) and this is likely to be very difficult to measure. It is nevertheless interesting that in muscle from rats in the catabolic phase of the cycle the mean Zn concentration was 4% higher than in muscle from rats in the anabolic phase, and although this difference is not statistically significant it is consistent with such a mechanism. How Zn could be retained in tissues which are wasting to the extent that the Zn concentration would increase is difficult to envisage however, and implies either that the numerous Zn metalloenzymes are in some way resistant to degradation in catabolic

states or that there is some (unknown) mechanism by which Zn is retained in the tissues subsequent to release from such enzymes during tissue wasting. The alternative explanation, that Zn is retained by being taken up into some other 'sink' tissue during body catabolism, is not ruled out by these results, especially since measurements have not been made in all the tissues of the body (e.g. not in skin). However, unless this 'sink' tissue Zn pool is equal to or larger than the muscle Zn pool, the Zn concentration would have to increase in that pool to an extent that would be disproportionately greater than the 6% loss of tissue during the catabolism. Of the tissues which we have not examined skin is a possibility, although unlikely in our opinion. Of the tissues which we have examined bone is the only possibility since in all the other tissues we would have observed such increases in Zn. Even in bone, since the Zn pool was only one-third of the muscle pool in the depleted rats, the retention of Zn lost by the catabolism of 6% of muscle would result in an 18% increase in bone Zn concentration and it is clear that this did not occur. It seems to us therefore most likely that the mechanism by which Zn is conserved is most likely to involve a specific retention in muscle, by some mechanism which remains to be characterized.

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