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THE ZINC CONCENTRATION IN HARD AND SOFT TISSUES OF THE RAT

THE INFLUENCE OF ZINC DEFICIENT FEEDING

by

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INTRODUCTION

Zinc is present in most tissues and organs of man and animals. High concentrations have been found in mineralized tissues (for a detailed review see *Bergman*, 1970a). Several studies have shown that the zinc concentration in bones is markedly influenced by, among other factors, the zinc level in the diet. Zinc concentration in bone was elevated when the dietary level of the element was high and this effect, also observed in dentin and enamel, was far more drastic when a high zinc level was associated with low dietary calcium (*Huxley & Leaver*, 1966). Conversely, high levels of dietary calcium have been reported to depress the zinc concentration in bone (*Forbes*, 1964; *Heth & Hoekstra*, 1965; *Likuski & Forbes*, 1965). *Leaver* (1967) reported that the absence of vitamin D slowed down the uptake and release of zinc in bone of rats on a low calcium diet.

Several authors have reported that the bone zinc concentration was reduced in rats placed on a zinc deficient diet (see Table I). *Hove et al.* (1938) also reported a decrease of zinc concentration in teeth of zinc deficient rats. *Millar et al.* (1958) found a reduction of the zinc concentration in testes, epididymis and prostate of zinc deficient rats, and similar observations for testes have been reported by *Macapinlac et al.* (1966) and *Prasad et al.*

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Table I

A compilation of some previously reported studies on the zinc concentration in rat bone as influenced by zinc deficient feeding

Authors	Experimental period in weeks	Bone sample studied	Weight	Zinc concentration ppm mean values		
				Experimentals	*Ad-lib.*-controls	Paired-fed controls
Hove et al., 1938	10		dry	62	92	—
Day & McCollum, 1940	11	femur	ash	95	237	—
Millar et al., 1958	8	fibula	wet	79	134	—
Forbes, 1961	6	femur	ash	188	—	285
Macapinlac et al., 1966	7	femur	wet	35	141	109
Prasad et al., 1967	6		dry	69	—	168
Swenerton & Hurley, 1968	6	femur	ash	108	424	—

(1967) for rats, and *Boquist* and *Lernmark* (1969) for Chinese hamsters. *Prasad et al.* (1967) also reported reduced zinc concentrations in muscle, esophagus, and kidney.

In previous studies on zinc deficient rats, where bone has been analyzed, only femur or fibula have been included (see Table I). The whole bone seems to have been taken for analysis without distinction being made between various portions of the bone specimen. The experimental periods were comparatively long, varying between 6 and 11 weeks.

The present study is concerned with the early effects of zinc deficient feeding on the zinc concentration in some tissues of female albino rats. The effects on the concentrations in mandibular condyle, mandibular bone, tibia epiphysis, and tibia diaphysis were of primary interest. The incisors and some soft tissues were also included in the analysis for comparison.

MATERIALS AND METHODS

Weaned female rats of the Sprague-Dawley strain were used in this study. Twenty 21-day-old rats were used, four rats from each of five litters. Each animal was assigned to a group consisting of 5 rats, one from each of the five litters. Four groups were thus formed:

Group A: experimental rats, which were given a zinc deficient diet *ad libitum* plus a mineral supplement;

Group B: paired-fed controls, which were given a zinc deficient diet plus mineral and zinc supplements;

Group C: paired weight-fed controls, which were given a zinc deficient diet plus mineral and zinc supplements;

Group D: controls, which were given a conventional rat diet *ad libitum.* (Anticimex 210, Anticimex, Norrviken, Stockholm; zinc content 73 ppm.)

The paired-fed controls (group B) were given an amount of the experimental diet equal to the mean food intake of the experimental rats (group A) during the preceding day. For the paired weight-fed controls, group C, the food intake was restricted so that their weight gain would be the same as that for the experimental rats. The five animals in each group were housed in a single cage. The animals were weighed at least three times weekly. The experimental period was 18 days.

The experimental diet described by *Day & McCollum* (1940) and modified by *Millar et al.* (1958) was used with minor changes. A detailed description of the composition and the preparation of the diet is given by *Bergman et al.* (1970). The zinc content of the diet used in the present study was analyzed by neutron activation and gamma-ray spectrometry. No detectable amounts of zinc were found.

The experimental rats (group A) and the two groups of restricted-fed controls (groups B and C) received a supplement of mineral compounds suspended in purified water (*Millar et al.* 1958). These animals were given 0.1 ml of the mineral supplement twice weekly by oral administration (see *Bergman et al.*, 1970). The mineral supplement did not contain any detectable amounts of zinc as determined by neutron activation analysis. The restricted-fed controls (groups B and C) also received a daily oral supplement of zinc. Zn $SO_4 \cdot H_2O$, *p.a.*, was dissolved in purified water in a concentration corresponding to 1.0 mg Zn/ml purified water. Each rat in groups B and C was given 0.2 ml of the zinc supplement daily, or 200 μg zinc by oral administration.

Water used for drinking purposes as well as for the cleaning of cages and various utensils for preparation, storage, and feeding was purified according to method number 5 described by *Söremark & Johansson* (1963). The purified water was given *ad libitum* to all rats. During the experimental period, samples of water were continuously taken for zinc determination by neutron activation analysis. The highest value recorded was 3.5×10^{-4} ppm zinc.

In order to reduce the possibility of environmental zinc contamination, cages and feed containers of acrylic resin were used. To reduce coprophagy a perforated, 4 mm thick plate was inserted 70 mm above the bottom of the

cage. Drinking water bottles were of polyethylene and provided with tubes of quartz glass. Feed containers were cleaned every day and cages every second day. For further details concerning the cages and the cleaning method used for the various utensils see *Bergman et al.* (1970).

At the end of the experimental period, the rats were slightly anaesthetized with ether and sacrificed by decapitation. A blood sample was collected and the following specimens were taken: kidney, spleen, liver, heart, incisors, mandibular condyle, mandibular bone, tibia epiphysis and tibia diaphysis (only compact bone). Partial samples were taken from liver (median lobe), incisors (crowns), and mandibular bone (ramus and part of the corpus). Only polyethylene or polyethylene-covered instruments were used in order to avoid contamination of samples and standards before irradiation. For further details of the sampling and preparation of the various organs and tissues, see *Bergman* (1970a). The specimens were collected in polyethylene tubes and the wet weights of the samples were noted. The specimens were then dried for 24 hours at 70—80° in an electric oven.

A standard amount of zinc was inserted into a separate polyethylene tube and placed with the specimens in an aluminium can which was then irradiated by thermal neutrons for 7.5 days in the R2 reactor of the Swedish Atomic Energy Company in Studsvik, Nyköping. The flux was 3.17×10^{12} cm^{-2} sec^{-1}. When the period of irradiation was completed, standards and tissue samples were subjected to radiochemical separation using a method modified after *Samsahl et al.* (1963) (see *Bergman*, 1970a). The yield of this method was 97.3 % with a standard deviation of 3.5 %. The analysis was performed in a $3'' \times 3''$ well type NaI (Tl) scintillation detector connected to a transistorized 512-channel gamma-spectrometer. Quantitative data of the organ samples were obtained by comparing the gamma intensity, the photopeak area at 1.1156 MeV, of ^{65}Zn in each sample with that of the standard (*Marinelli et al.*, 1962).

RESULTS

Average weight curves for the four groups of female rats are shown in Figure 1. In Table II, the weight increase and the food consumption of the four groups are presented.

A reduced rate of growth was noted in the experimental group and paired weight-fed controls (group C) compared to paired-fed controls (group B) after about 10—12 days. During the 18-day experimental period the weight increase of the experimental rats (group A) and the paired weight-fed con-

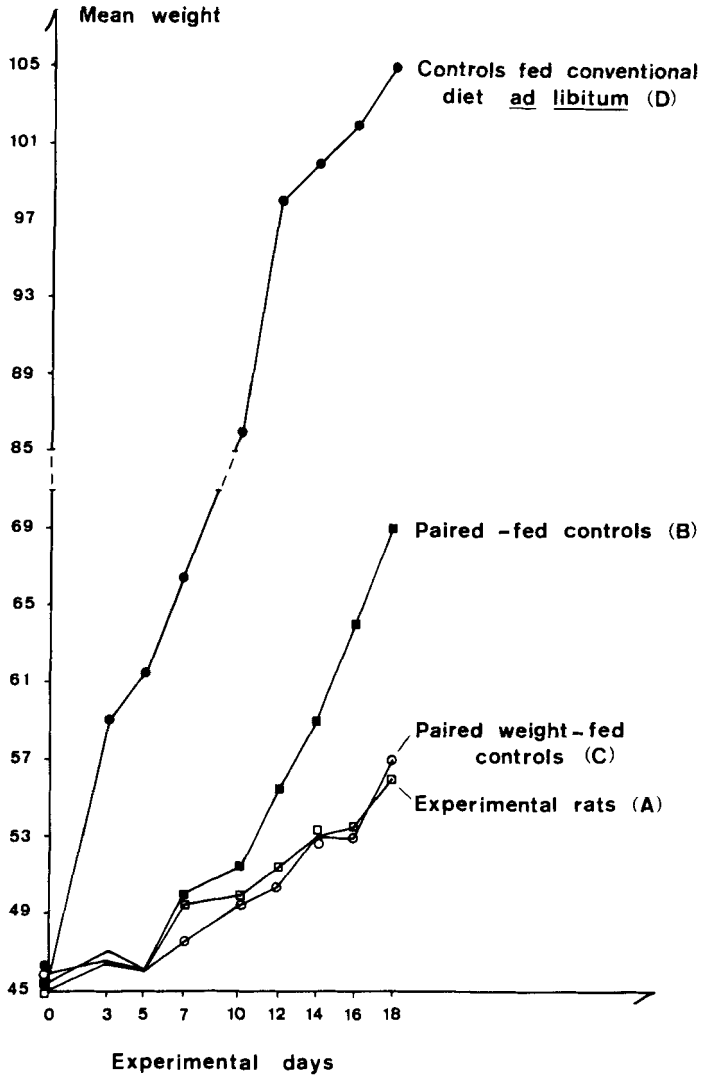

Fig. 1. Average weight curves for the four groups of female rats. Each group consisted of five rats.

trols (group C) was significantly lower than that of the paired-fed controls (group B), and each of these three groups differed significantly from the fourth group, the controls which were fed a conventional rat diet *ad libitum* (group D). After about 2 weeks, a loss of hair became noticeable in the

Table II

The weight increase and the food consumption in the four groups of animals; $\bar{x}$ = mean of 5 rats, s = standard deviation

Animals	Weight increase in g during the experimental period of 18 days		Food consumed per day in grams
	$\bar{x}$	s	$\bar{x}$
Experimental rats (group A)	11.0	1.6	8.2
Paired-fed controls (group B)	23.5	3.3	8.2
Paired weight-fed controls (group C)	11.5	2.2	6.3
Controls fed conventional rat diet *ad libitum* (group D)	59.0	0.9	not registered

experimental group. This loss progressed so that after 18 days, the experimental group exhibited almost completely denuded areas. All controls had normal fur.

The zinc concentrations obtained by means of neutron activation and gamma-ray spectrometric analysis are presented in Table III. The statistical analysis was performed in the following way: the differences between animals of the same litter from all possible pairs of groups were calculated, and the means of these within-litter differences were analyzed by means of the Student's t-test.

Of the skeletal samples containing spongy bone, mandibular bone and tibia epiphysis of the experimental rats (group A) showed almost significantly or significantly lower zinc concentrations than those from each of the three control groups (groups B, C and D). The zinc concentrations in the mandibular condyles of the experimentals (group A) were found to be significantly lower than those of the *ad libitum* controls (group D) and almost significantly lower than those of the paired-fed controls (group B). In addition, the concentrations in the mandibular condyles of the *ad libitum* controls (group D) were almost significantly higher than those of the two other control groups (groups B and C). In the tibia diaphysis, the zinc concentrations of those of the experimentals (group A) differed almost significantly only from those of the *ad libitum* controls (group D). No significant differences were found among the four groups in incisors, blood, kidney, spleen, liver or heart.

Table III

Zinc concentrations in different tissues in zinc deficient and control rats: ppm wet weight, $\bar{x}$ = mean of 5 rats, s = standard deviation, s % = $s \times 100/\bar{x}$. Differences were tested by means of Student's t-test

Tissue	Experimental rats (group A)			Paired-fed controls (group B)			Paired weight-fed controls (group C)			*Ad libitum*-fed controls, conventional rat diet. (group D)		
	$\bar{x}$	s	s %	$\bar{x}$	s	s %	$\bar{x}$	s	s %	$\bar{x}$	s	s %
Blood	19.7	12.8	65 %	16.8	3.2	19 %	26.3	24.5	93 %	16.9⊕	8.1	48 %
Kidney	29.3	8.9	30 %	27.9	2.3	8 %	25.2	12.9	51 %	25.3	3.7	15 %
Spleen	27.9	6.9	25 %	22.6	6.2	27 %	21.8	3.4	16 %	24.4⊕	7.5	31 %
Liver	41.3	8.8	21 %	34.5	5.3	15 %	34.5	6.1	18 %	34.7	14.0	40 %
Heart	22.0	10.8	49 %	23.5	8.8	37 %	19.9	1.4	7 %	19.8⊕	4.3	22 %
Incisors	139.2	42.5	31 %	154.6	12.9	8 %	148.7	10.7	7 %	188.2	100.6	53 %
Mandibular condyle	65.2⊕	4.4	7 %	93.3*	14.6	16 %	96.2	23.6	25 %	135.8‡**	21.4	16 %
Mandibular bone	92.1	22.8	25 %	149.8*	28.0	19 %	143.8*	24.1	17 %	176.5**	15.6	9 %
Tibia epiphysis	45.1	4.3	10 %	77.0**	15.1	20 %	85.6***	11.2	13 %	135.6*	75.6	56 %
Tibia diaphysis	148.3	24.6	17 %	169.5	25.6	15 %	177.4	27.0	15 %	187.4*	17.7	9 %

‡ Difference from paired-fed (B) and paired weight-fed (C) controls almost significant ($P < 0.05$)

* Difference from experimental group (A) almost significant ($P \leq 0.05$)

** Difference from experimental group significant ($0.001 < P \leq 0.01$)

*** Difference from experimental group highly significant ($P < 0.001$)

⊕ Mean of four rats; one sample spoiled during the preparation

DISCUSSION

The experimental period used in the present study — 18 days — is considerably shorter than in previous experiments of the same character (Table I). The aim of the present experiment was to study the early effects of zinc deficient feeding on the zinc concentrations in some rat tissues, where skeletal tissues were of primary interest. As *Bergman et al.* (1970) found that marked differences in growth rate were obtained between rats fed a zinc deficient diet and controls after about 2 weeks, it was considered adequate to interrupt the present study after 18 days.

It is of great importance that water used for drinking purposes as well as for the cleaning of the various experimental utensils is as zinc free as possible. The daily water consumption of a growing rat is about 15 g. In previous studies, the water used has been reported to be distilled, redistilled or deionized. However, no determination of the zinc concentration in water used has been reported. *Söremark* and *Johansson* (1963) found that their distilled or redistilled water contained about 9 and 33 ppm zinc respectively. The water used in the present study was purified according to a method recommended by these authors for use in activation analysis and the zinc concentration was found to be extremely low ($\leq 3.5 \times 10^{-4}$ ppm zinc). Hence, the daily zinc intake via the water was almost negligible in the present study.

As no detactable amounts of zinc were found either in the diet or in the mineral supplement as analyzed by means of neutron activation and gamma-ray spectrometry, it is clear that the experimental rats in the present study were extremely zinc-deficient fed. The daily zinc intake per rat in the experimental group was calculated to be approximately 10^{-3} μg. In the two restricted-fed control groups, the corresponding value was 200 μg. *Millar et al.* (1958) observed no difference between control rats receiving 100 or 200 μg zinc daily, both control groups grew well and showed none of the symptoms of zinc deficiency. The specific compound of zinc used may have an influence on the availability of the metal (*Davis*, 1966). In the present study the two restricted-fed control groups were given zinc sulfate, which is one of the zinc compounds reported to be readily available from the intestines of chicks (*Edwards*, 1959).

A prenatal transfer of ^{65}Zn from the dam across the placenta to the fetus has been demonstrated in mice (*Bergman & Söremark*, 1968; *Gunn et al.*, 1963) rats (*Feaster et al.*, 1955; *Kinnamon*, 1963), rabbits (*Terry et al.*, 1960) and dogs (*Vallee et al.*, 1949). High concentrations of zinc have been observed in the prelactating mammary glands of mice (*Bergman & Söremark*, 1968), and postnatal transfer of zinc from the mother to their off-

springs takes place via the milk (*Feaster et al.*, 1955; *Gunn et al.*, 1963). It has been noted in man that colostrum contains a large amount of zinc and that the concentration falls significantly as lactation proceeds (*Berfenstam*, 1952). *Nishimura* (1953) reported that newborn rats deprived of colostrum displayed zinc deficiency symptoms. Up to weaned age, about 21 days, the rats consequently obtain practically all of their zinc via their mothers, but zinc intake due to environmental contamination cannot be excluded.

The rats in the present study were housed with their mothers until they were 21 days old when the experiment started. As the subsequent zinc intake via diet and water was extremely low, and environmental zinc contamination was minimized by the experimental set-up used, the rats of the experimental group from their twenty-first day of age throughout the experimental period were almost completely deprived of a zinc supply. However, it cannot be completely excluded that, in spite of the special cage used, zinc intake due to coprophagy might have taken place — the main route of excretion of zinc is by feces. Moreover, the loss of hair noted in the present study for the experimentals might have provided a possibility for an additional external contribution of zinc. It was namely reported by *Follis* (1966) that much ingested hair was found in the stomachs in zinc deficient rats, but it is unlikely that rats can avail themselves of zinc in ingested hair.

The results of the statistical analysis may be interpreted in more than one way. The five animals in each group were placed in the same cage. Thus it cannot be excluded that the observed differences may be due to unintentional diversities between the four groups, *e.g.* that the animals in one cage may have been subjected to an infection with retroactions on the results. However, no signs of a »cage effect» were observed. Growth is the most commonly used criterion to follow the development of experimental animals. In this respect it is of interest to compare the present study with two other (*Bergman et al.*, 1970, *Bergman et al.*, in preparation), as the design and execution of the three studies were similar. The weight increases of the experimentals and paired-fed controls are nearly the same in these three studies. This suggests that the differences in the weight increase observed in the present study were probably not due to a »cage effect». That retroactions on the zinc concentrations are not correlated to the weight is supported by the fact that the paired-fed and paired weight-fed controls did not differ concerning the zinc concentrations, despite the markedly different weight increases for these groups.

In previous experiments, *ad libitum*-fed and/or paired-fed controls have been used. With zinc deficiency, there is a reduced appetite (*Humphries & Quarterman*, 1968). Paired-fed controls are, therefore, of value in distin-

guishing between primary effects of zinc deficiency and secondary effects of reduced food intake. In the present study significant differences were found in the weight increases between experimentals and paired-fed controls, thus confirming the results of previous studies (*Forbes*, 1961; *Macapinlac et al.*, 1966; *Prasad et al.*, 1967; *Bergman et al.*, 1970). The great difference in growth rate between the *ad libitum* controls and the two restricted-fed control groups can be explained by differences between the conventional rat diet and the experimental diet. A similar difference in growth rate was also observed by *Macapinlac et al.* (1966). The *ad libitum* rats fed conventional diet, therefore, may not be considered as a control group in the strict sense, but it was considered to be of interest to include them in the present study for comparison. Paired-fed controls normally grow more than deficient animals because they are better able to utilize their restricted food intake (*Follis*, 1958). In order to avoid the possibility that differences in zinc concentrations might be ascribed to this fact, paired weight-fed controls were also included as a control group in the present study. It was of special interest to compare the experimental rats with the paired weight-fed controls. The low zinc concentration of the mandibular bone and the tibia epiphysis of the experimentals is probably not a consequence of primary or secondary inanition, since the zinc concentrations in these tissues from not only the *ad libitum* and paired-fed controls but also the paired weight-fed controls differed significantly or almost significantly from the experimentals.

In general, when animals are restricted to a diet deficient in an essential mineral element, homeostatic mechanisms are brought into play, which tend to conserve the element and make more effective use of the limited amounts that are available. This is particularly true in young animals in which active growth increases basic requirements (*Copp & Suiker*, 1962). According to *Cotzias et al.* (1962) and *Cotzias & Papavasiliou* (1964), zinc metabolism is controlled by at least two homeostatic mechanisms which act at the sites of absorption and excretion when these mechanisms are functioning together. Some studies in rats (*Feaster et al.*, 1955; *Rubini et al.*, 1961) and man (*McCance & Widdowson*, 1942) indicate that only some 5 to 10 per cent of dietary zinc is absorbed from the intestine at a normal intake level. However, higher values of intestinal zinc absorption have been reported by *Hoekstra* (1964) in rats (40 %) and *Spencer et al.* (1966) in man (50 %).

Normally, zinc is mainly excreted by the feces, primarily via the pancreatic secretions (*Sheline et al.*, 1943; *Birnstingl et al.*, 1956; *Wakeley et al.*, 1960; *Molina et al.*, 1961; *Cotzias et al.*, 1962; *Robertson & Burns*, 1963;

Andrási & Fehér, 1967). *McCance* and *Widdowson* (1942) found a direct relation between dietary zinc level and fecal excretion in human adults. *Prasad et al.* (1963) reported that excretion of ^{65}Zn in stool and urine was less in zinc deficient human subjects than in controls. *Forbes* and *Yohe* (1960) demonstrated that fecal zinc excretion in rats was decreased as the dietary zinc intake was lowered. In the present study, in which the experimental rats were deprived of zinc, the excretion probably was slowed down as a result of homeostatic regulations. However, despite this attempt at conservation there will be a reduced total zinc concentration in the experimental rats and, if not all tissues are to lose zinc, a translocation within the rat body must take place.

The turnover of ^{65}Zn in soft tissues is normally very rapid (*Bergman*, 1970b). No significant differences were found in the present study between the soft tissue concentrations of zinc in the various groups. Several authors have reported that the bone zinc concentration was reduced in rats placed on a zinc deficient diet (see Table I).

For compact bone, such as tibia diaphysis, zinc is presumably released mainly as a result of remodelling processes. It is difficult to estimate the degree of remodelling which has taken place during the 18-day experimental period. However, previous experiments using radiozinc have shown that turnover of zinc in well-mineralized tissues of rats is normally very slow (*Gilbert & Taylor*, 1956; *Ballou & Thompson*, 1961; *Taylor*, 1961; *Bergman*, 1970b). The experimental rats in the present short-term study were, therefore, probably not able to any significant degree to avail themselves of the zinc present in compact bone. However, in longer zinc deficient periods, zinc in compact bone could also be made available through remodelling.

It was shown in a previous study that the turnover of radiozinc was slow in the incisors; the incisors were the only tissue studied which showed a steady increase in ^{65}Zn concentration in both young and adult rats during the experimental periods (*Bergman*, 1970b). Several studies have shown that strontium and calcium in the incisors are less accessible to exchange than that in bone (*Bauer et al.*, 1961; *Bauer & Shtacher*, 1968). A decrease in zinc concentration in teeth of zinc deficient rats has been reported by *Hove et al.* (1938). However, this result was based on only one composite sample from two rats. The crowns of the rat incisors are almost fully mineralized at 3 weeks of age and are subject to a continuous growth and abrasion. In the incisors, zinc is lost mainly due to abrasion of the incisal edges. Therefore, the zinc present in incisors is probably not available in zinc deficient conditions, unless abraded particles are ingested and zinc absorbed from this source.

The less mineralized structures are in a state of very rapid exchange with body fluids, and mineralized tissue in the body may act as an ion-exchanger. The skeleton may, therefore, play a great physiological role in normal and diseased conditions. It is well-known that in long bones the turnover of constituents of the epiphyseal and metaphyseal regions containing spongy bone is more rapid than that of the diaphysis, mainly composed of compact bone. In the present short-term study tibia epiphysis, mandibular bone, and mandibular condyle of the zinc deficient rats showed almost significantly or significantly lower zinc concentrations compared to those of the controls with one exception. It is possible that skeletal tissues containing spongy bone was the main source of the zinc supplied to meet the needs of the growing soft tissues. However, there may be other explanations for the results. For example it may be thought that the turnover of zinc in soft tissues was slowed down in the zinc deficient rats due to homeostatic mechanisms tending to reduce those portions of zinc in the soft tissues which are normally exchangeable. Even if at the same time the exchangeable fraction of zinc in spongy bone tissue was unaltered, it cannot be established from the present data to what extent this available zinc was needed by the soft tissues.

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SUMMARY

In the present short-term study, spongy bone samples (mandibular condyle, mandibular bone, and tibia epiphysis) from young rats on a zincdeficient diet showed almost significantly or significantly lower zinc concentrations compared to paired-fed controls, paired weight-fed controls and *ad libitum* controls with some few exceptions. No significant differences were found for compact bone, incisors or soft tissues, except for the zinc concentrations of the tibia diaphysis from the experimentals which were almost significantly lower than those from the *ad libitum* controls. The results are discussed from the point of view that bone may be a zinc reservoir for the organism.

RÉSUMÉ

TENEUR EN ZINC DES TISSUS MOUS ET DURS DU RAT
INFLUENCE D'UN RÉGIME DÉFICIENT EN ZINC

Dans la présente étude à court terme, les teneurs en zinc d'échantillons d'os spongieux (condyle mandibulaire, os mandibulaire et épiphyse du tibia) provenant de jeunes rats soumis à un régime déficient en zinc étaient presque significativement ou significativement plus basses que chez les animaux témoins, couplés pour le régime ou pour l'alimentation au poids, et chez des témoins nourris *ad libitum*, à quelques exceptions près. Il n'a pas été constaté de différences significatives en ce qui concerne l'os compact, les incisives ni les tissus mous, à l'exception de la teneur en zinc dans la diaphyse du tibia des animaux de l'expérience, qui était à peu près significativement plus basse que pour les animaux témoins nourris *ad libitum*. Partant du point de vue que l'os pourrait constituer une réserve de zinc pour l'organisme, l'auteur discute les résultats trouvés.

ZUSAMMENFASSUNG

DIE ZINKKONZENTRATION IN HARTEN UND WEICHEN GEWEBEN DER RATTE.
DIE EINWIRKUNG VON MANGELHAFTER ZINKDIÄT

Während dieser kurzen Studienperiode zeigten schwammartige poröse Knochenproben von jungen Ratten, die man mit einer mangelhaften Zinkdiät gezuchtet hatte, fast signifikant oder signifikant niedriger Zinkkonzentratione im Vergleich mit Kontrolltieren mit einiger wenigen Ausnahmen. Keine signifikanten Unterschiede konnten aufgezeigt werden im Hinblick auf übrige harten und weichen Geweben mit Ausnahme von dem Kompakta des Schienbeins von den Experimenttieren, die fast signifikant niedriger Zinkkonzentratione als die von den ad libitum Kontrolltieren zeigte. Man hat die Resultate diskutiert, ausgehend davon, dass Knochen ein Zinkreservoir für den Organismus sein könnten.

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