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CONCENTRATION OF ZINC IN SOME HARD AND SOFT TISSUES OF RAT DETERMINED BY NEUTRON ACTIVATION ANALYSIS

by

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Zinc is an essential element for the organism and is present in most organs and tissues in man and animals in varying quantities. High concentrations have been reported for hard tissues, such as bone (Lutz 1926, Taylor 1961, Alexander & Nusbaum 1962, Söremark & Bergman 1952) and teeth (Cruickshank 1936, Samsahl & Söremark 1961, Lundberg et coll. 1965, Babicky & Taylor 1966, Nixon et coll. 1967). Previously reported values of zinc concentration in skeletal tissues have most often been concerned with long bones. High concentrations of zinc have been localized by means of autoradiography in mineralizing areas in long bones (Haumont & Vincent 1961, Haumont 1963, Kinnamon 1963).

The present study was initiated in order to compare quantitatively the zinc concentration of the following skeletal tissues of young and adult rats: mandibular condyle, mandibular bone, tibia epiphysis and tibia diaphysis. To allow for a more complete comparison with previous studies, the teeth and some soft tissues were also included. The results obtained in the present investigation are intended

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Table 1

Sensitivity of neutron activation analysis for zinc: a compilation of values reported in the literature

Author(s)	Isotope measured	Neutron fluence cm^{-2} sec^{-1}	Irradiation time	Sensitivity
MEINKE 1955		5×10^{11} 10^{13}	Saturation	0.04 μg/ml 0.002 μg/ml
LEDDICOTTE et coll. 1958	^{65}Zn	5×10^{11}	Saturation	0.02 ppm
BOWEN 1959	^{69m}Zn	10^{12}	12 hours	0.05 ppm
PARR & TAYLOR 1964	^{65}Zn	10^{12}	7 days	0.01 ppm

to serve as a complement to a scintillation study on the distribution of radiozinc in rats (BERGMAN 1970).

Various methods have been utilized for the quantitative determination of microelements in biologic materials. For the determination of zinc in tissues and body fluids, colorimetric methods have been used extensively (VALLEE & GIBSON 1948, BERFENSTAM 1952, WOLFF 1956, MALMSTRÖM 1956). Spectrographic analysis has been employed by SCOULAR (1939), GRIFFITH et coll. (1954), MONACELLI et coll. (1956), BUTT et coll. (1964), PFEILSTICKER (1965), and STRAIN et coll. (1966) and radiometric titration by LANDGREBE et coll. (1968). Roentgen ray fluorescence has been used by ALEXANDER & NUSBAUM (1962) and ZEITZ & LEE (1966), and atomic absorption spectrophotometry by PRASAD et coll. (1965), MACAPINLAC et coll. (1966), PIHL & GUSTAFSON (1967), HACKLEY et coll. (1968), and BOQUIST & LERNMARK (1969).

In recent years neutron activation has come into use more and more for analysis of microelements in biologic tissues. The method has been reviewed and discussed in detail by e.g. BOWEN & GIBBONS (1963), SÖREMARK (1965) and BOWEN (1966).

Values reported in the literature on the sensitivity of neutron activation analysis for zinc are compiled in Table 1. The sensitivity will be further increased by radiochemical separation of the radioactive zinc present in the sample (SAMSAHL 1961).

In the present study neutron activation analysis was chosen for the following reasons (SÖREMARK 1965, BOWEN 1966): (1) there is a high degree of sensitivity and specificity, and (2) the risk of contamination and loss can be almost completely eliminated.

Material and Methods. The animals used in the present study were albino rats (Sprague—Dawley). All animals were normally lively, and no signs of infection,

deficiency, or any disease were observed. Only females were used; there were ten 3-week-old and ten 24-week-old rats. The young animals were housed together with their mothers in acrylic cages with steel covers until sacrifice. The mothers of the young rats and the 24-week-old rats had free access to tap water and a pellet rat diet, Anticimex 210 (Anticimex, Norrviken, Stockholm) with a zinc content of about 73 ppm.

The rats were mildly anaesthetized with ether and killed by decapitation. About 0.8 ml of blood was collected from each rat immediately after decapitation. The various tissues to be analysed were then rapidly excised in the following order: kidney, pancreas, spleen, liver (median lobe), heart, incisors, mandibular condyle, mandibular bone, tibia epiphysis and tibia diaphysis. The tissues were not perfused. When possible, the whole organ was removed. The teeth examined were the four incisors, which were broken off at the gingival margin; thus, only the crowns were analysed. All visible pulp was removed. The mandibular bone sample consisted of the ramus and part of the corpus including both spongy and compact bone. The periosteum was scraped away. When removing the tibia diaphysis, great care was taken to avoid the metaphyseal part. The diaphyseal part was dissected free, sectioned along its long axis, and freed of its bone marrow and periosteum. In this way, attempts were made to collect only the compact bone of the diaphysis. During the removal and preparation of the various specimens, no metal instruments were used. In order to avoid contamination only polyethylene or polyethylene-covered instruments were in direct contact with the specimen. The specimens were collected in polyethylene tubes, and the wet weight was recorded immediately after removal of the tissue. The wet weights of the tissues varied between about 800 mg, e.g. blood and liver, and 15 mg, e.g. incisors and mandibular condyle. After weighing, the specimens were dried in an electric oven for about 24 hours at 70 to 80° C in order to prevent volatilization during irradiation. Care was taken to avoid contamination of samples and standards before irradiation. The water used for dissolution of the standards to be sent for irradiation as well as that used for rinsing instruments and glassware was carefully purified according to the method described by SÖREMARK & JOHANSSON (1963) and collected in polyethylene bottles.

A standard amount of zinc was inserted into a separate polyethylene tube and placed in the same aluminium can as the specimens. The aluminium can containing the standard and the specimens was irradiated by thermal neutrons for periods of 5 and 12 days in the R2 reactor of the Swedish Atomic Energy Company in Studsvik, Nyköping. The neutron fluence was approximately 1.4×10^{12} cm^{-2} sec^{-1}.

When the period of irradiation was completed, the samples were dissolved in hot H_2SO_4 p.a. followed by dropwise addition of 30 % H_2O_2 p.a. Thereafter,

Table 2

Concentration of zinc in some hard and soft tissues from 3-week-old female rats — The values are based on wet weights and expressed in ppm; $\bar{x}$ = mean of 10 rats; s = standard deviation

Tissue	$\bar{x}$	s	s % = 100 s/$\bar{x}$
Blood	6.3*	1.1	17 %
Kidney	18.6	2.9	16 %
Pancreas	30.0	5.7	19 %
Spleen	16.8	3.5	21 %
Liver	53.0	18.3	35 %
Heart	14.6	4.3	29 %
Incisors	128.0	40.7	32 %
Mandibular condyle	152.0*	57.7	38 %
Mandibular bone	121.0	25.0	21 %
Tibia epiphysis	45.0	9.5	21 %
Tibia diaphysis	140.0	22.8	16 %

* One sample spoiled during preparation, mean value based on nine samples.

Table 3

Concentration of zinc in some hard and soft tissues from 24-week-old female rats — The values are based on wet weights and expressed in ppm; $\bar{x}$= mean of 10 rats; s = standard deviation

Tissue	$\bar{x}$	s	s % = 100 s/$\bar{x}$
Blood	13.0	15.4	118 %
Kidney	18.4	4.2	23 %
Pancreas	22.6	19.8	88 %
Spleen	63.7	28.1	44 %
Liver	44.9	33.4	74 %
Heart	14.5	3.4	23 %
Incisors	111.0	33.0	30 %
Mandibular condyle	223.0	84.9	38 %
Mandibular bone	204.0	61.0	30 %
Tibia epiphysis	250.0	52.5	21 %
Tibia diaphysis	250.0	51.9	21 %

the solution was diluted with 0.7-n HCl p.a. The diluted solution flowed at a rate of 2 ml/min through an anion exchange column in chloride form, Dowex 2 × 10. The columns used were 10 cm high with a diameter of 1 cm. A faint suction was applied by means of a vacuum pump. After the dropping had ceased, the column was washed with 50 ml of 0.7-n HCl p.a. in order to remove traces

Table 4

Some previous results on zinc concentration in various rat tissues compiled together with the results obtained in the present study — Mean values are given and the concentrations are expressed in ppm

Year	Author	Rat age in weeks	Method
1926	Lutz		Fluorescence
1927	Heller & Burke		Fluorescence
1938	Hove et coll.		Colorimetry
1940	Day & McCollum	15	Colorimetry
1951	Mawson & Fischer		Colorimetry
1956	Gilbert & Taylor	6—12	Colorimetry
1958	Millar et coll.	12—13	Colorimetry
1960	Forbes & Yohe		Colorimetry
1961	Forbes	9	Colorimetry
1961	Taylor	8—49	Colorimetry
1962	Alexander & Nusbaum	0—59	Roentgen ray fluorescence and emission spectrography
1966	Huxley & Leaver	14—15	Polarography
1966	Macapinlac et coll.	10	Atomic absorption spectrophotometry
1967	Reinhold et coll.		Colorimetry
1967	Prasad et coll.	3	Atomic absorption spectrophotometry
1968	Swenerton & Hurley		Atomic absorption spectrophotometry
Present study		3	Neutron activation analysis
		24	Neutron activation analysis

of ^{24}Na. The standards were treated in exactly the same way as the biologic samples. The radiochemical separation method used was a modification of the one described by Samsahl et coll. (1963).

After the radioactive zinc had been collected in the anion exchange column, the resin was transferred to stoppered polyethylene tubes for analysis in a $3'' \times 3''$ well type NaI (Tl) scintillation detector connected to a transistorized 512-channel gamma-spectrometer.

Quantitative data based on the wet weight of the organ samples were obtained by comparing the gamma intensity, the photopeak area at 1.1156 MeV of ^{65}Zn

Table 4 *(cont.)*

Weight	Whole blood	Kidney	Pancreas	Spleen	Liver	Heart	Teeth	Bone
Wet	6.7	14.4		36.3	20.7			178.4
Wet		15		31	20	14		
Dry					76		92 (dentin and enamel)	92
Ash								237
Wet			23.3		30.3			233 (tibia)
Wet	3.88	23.4		24	29.7			
Wet								133.9
Wet					43			
Ash								285 (femur)
Wet								77—191 (femur)
Ash								323 (femur shaft) 420 (femur end)
Dry							250 (dentin) 130 (enamel)	388 (femur)
Wet		41			34			162 (femur)
Wet		19.4	17.5	16.2	23.9			
Dry		91		105	101	73		168
Ash								424 (femur)
Wet	6.3	18.6	30	16.8	53	14.6	128 (dentin and enamel)	140 (tibia diaphysis)
Wet	13	18.4	22.6	63.7	44.9	14.5	111 (dentin and enamel)	250 (tibia diaphysis)

or at 0.439 MeV of ^{69m}Zn in the sample with that of the standard (MARINELLI et coll. 1962).

The method used has been discussed by SAMSAHL & SÖREMARK (1961), SÖREMARK & BERGMAN (1962), SAMSAHL et coll. (1963), BOWEN & GIBBONS (1963), OLEHY et coll. (1966) and HAHN et coll. (1968). BRUNE (1963) reported a yield for ^{65}Zn of 98 %, with a standard deviation of 8 %, by the chemical group separation method developed by SAMSAHL et coll. (1963). The loss of zinc due to the chemical procedures after the irradiation was also tested in the present study. To each of four hard and four soft tissue samples was added a known amount of ^{65}Zn. The samples then underwent the same chemical procedure as

the irradiated samples and were subjected to scintillation measurements. The yield was 97.3 % with a standard deviation of 3.5 %.

Various errors in the sampling method have been analysed elsewhere (BERGMAN 1970), and were found to be small. Therefore, it seems logical to assume that the large standard deviations obtained for some of the tissues in the present study — especially blood, pancreas and liver in the 24-week-old rats — can be ascribed mostly to biological variations.

Results

The zinc concentrations obtained by means of neutron activation and gamma ray spectrometric analysis of some hard and soft tissues from 3- and 24-week-old rats are presented in Tables 2 and 3.

Intra- and interindividual differences were tested by means of Student's t-test. Except for tibia epiphysis in the 3-week animals, all the bone samples ($p < 0.001$) and the incisors ($p < 0.01$), had significantly higher zinc concentrations than the soft tissue samples within both age groups. For incisors, blood, kidney, pancreas, liver, and heart, there were no significant differences between the two age groups. The zinc concentration was significantly higher in adult rats in spleen ($p < 0.001$), mandibular bone ($p < 0.01$), tibia epiphysis ($p < 0.001$), and tibia diaphysis ($p < 0.001$). For mandibular condyle the zinc concentration was almost significantly higher in adult rats ($p \sim 0.05$).

Discussion

Comparisons of different studies concerning microelements such as zinc in biologic tissues are complicated for many reasons. The materials and methods vary and results are expressed in different ways, e. g. based on wet weight, dry weight, or ash weight. Furthermore, age is an important variable for the zinc concentration in skeletal samples.

For all the tissues analysed in the present study, except mandibular condyle and mandibular bone, data on zinc concentrations in rats are available from earlier studies. In Table 4, some previously reported results for rat tissues are compiled together with the results obtained in the present study.

Where comparisons can be made, the results obtained in the present study agree comparatively well with those previously published. Spleen was the only soft tissue tested where a significant difference could be shown between the two age groups in the present study. At present no explanation can be given for this difference. It is also noteworthy that the zinc concentration in the spleen of the 24-week-old rats differs considerably from previously published values (Table 4).

It was found in the present study that the skeletal samples of the adult rats

Table 5

Concentration of zinc in some hard tissues of female rats — The values in Tables 2 and 3 have been used and are here recalculated on ash weight and expressed in ppm — Numbers within parentheses indicate the mean ratios ash weight/wet weight each obtained from 6 rats; $\bar{x}$ = mean of 10 rats; s = standard deviation

Tissue	3-week rats		24-week rats	
	$\bar{x}$	s	$\bar{x}$	s
Incisors	197 (65 %)	63	154 (72 %)	46
Mandibular condyle	755 (20 %)	242	572 (39 %)	218
Mandibular bone	404 (30 %)	83	371 (55 %)	111
Tibia epiphysis	566 (8 %)	188	735 (34 %)	154
Tibia diaphysis	350 (40 %)	46	391 (64 %)	81

had a significantly higher zinc concentration per gram wet weight than those of the young rats; for mandibular condyle the difference was almost significant. The zinc concentrations found for skeletal tissues containing spongy bone (mandibular condyle, mandibular bone and tibia epiphysis) will also include zinc in the hemopoietic bone marrow. Zinc concentrations in bones from various ages have previously been analysed by TAYLOR (1961) and ALEXANDER & NUSBAUM (1962). TAYLOR (1961) reported that the mean zinc content of femur, humerus and pelvis in rats continued to rise from 56 days to 679 days from 77 up to 200 ppm wet weight. The zinc content of the ribs did not increase as much as that of the other bones. ALEXANDER & NUSBAUM (1962), contrary to TAYLOR (1961), were not able to show any elevation of zinc in rat bone with increasing age from newborn up to 414 days. These authors were also unable to show any correlation between age and zinc content in human ribs. They found it likely that the level of zinc in bone was directly related to the zinc to calcium ratio of the diet. The results of TAYLOR (1961) and those of the present study are based on wet weight, while those of ALEXANDER & NUSBAUM (1962) are based on ash weight. This fact complicates direct comparisons between the studies. The bone of the adult animals give more ash per unit volume than that of young animals due to a higher degree of mineralization.

The following additional experiment was carried out in order to determine whether or not comparison is possible between results based on wet weight and

those based on ash weight. Six 3-week-old and six 24-week-old rats were injected intraperitoneally with 0.2 μCi ^{65}Zn per gram body weight and killed after 24 hours. The tibia diaphysis of the hind legs of each rat was dissected free and freed from bone marrow and periosteum, the wet weight was recorded, and the total concentration of ^{65}Zn in the bone samples determined. The ash weight was recorded after 16 hours at 600° C (ad modum ROBINSON & ELLIOT 1957, ALEXANDER & NUSBAUM 1962), and the total concentration of ^{65}Zn was determined in the ashed samples. By using metabolically incorporated ^{65}Zn in this way, it could be verified that no loss of zinc took place during the conditions of ashing. The ratio ash weight/wet weight was further recorded for the incisors, mandibular condyle, mandibular bone and tibia epiphysis. Using these figures, the zinc concentrations for the hard tissues (Tables 2 and 3) were recalculated on the basis of ash weights (Table 5). When the differences between 3- and 24-week-old rats were tested statistically, only the mean ratios ash weight/wet weight were considered, as the standard deviations of these mean ratios were of negligible magnitude ($< 3\ \%$).

No significant differences could be found between the young and adult rats using the ash weights for the zinc concentrations in the incisors, mandibular condyle, mandibular bone or tibia diaphysis. For tibia epiphysis, the adult rats showed an almost significantly higher zinc concentration than the young rats ($p < 0.05$). The mean values for zinc concentration in ashed tibia diaphysis in the present study (350 and 391 ppm) agree well with those reported by MAWSON & FISCHER (1951) for ashed tibia (390 ppm = 233 ppm wet weight) and ALEXANDER & NUSBAUM (1962) for ashed femur shaft (323 ppm). Thus, it appears that zinc concentration in skeletal tissues and incisors of rats increases as mineralization proceeds, corresponding to the situation in the human enamel. Surface enamel in man has been reported to contain a higher concentration of mineral than the deeper layers (THEWLIS 1940, SONI & BRUDEVOLD 1959, ANGMAR et coll. 1963), and BRUDEVOLD et coll. (1963) found zinc to be more concentrated in surface layers than in deeper layers of the enamel in man. The most striking difference obtained in the present study between young and adult rats was found for tibia epiphysis, 45 and 250 ppm zinc based on wet weights. This difference may reflect the late start in mineralization, as indicated by the low ash weight at three weeks, and the continuing replacement of existing epiphyseal cartilage with bone with increasing age.

No significant difference could be found in the present study between the incisors in the two age groups. This can probably be explained by the fact that the rat incisors are almost fully mineralized at 3 weeks of age, as indicated by the ash weight/wet weight ratios, and, thereafter, are subject to continuous growth and abrasion.

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SUMMARY

The concentration of zinc (ppm/wet weight) was determined in the mandibular condyle and in some selected hard and soft tissues of 3- and 24-week-old rats by means of neutron activation and gamma ray spectrometry. Except for tibia epiphysis in the 3-week-old animals, the skeletal samples and the incisors had significantly higher zinc concentration than the soft tissue samples in both age groups. Excepting the spleen, which had a significantly increased zinc concentration in adult rats, none of the soft tissues sampled showed significant differences in zinc concentrations between young and old rats. The skeletal samples of the adult rats had a significantly higher zinc concentration than those of the young rats. These increases were shown to result most probably from increasing mineralization with increasing age. No significant difference was found between the zinc concentrations of the incisors of the two age groups.

ZUSAMMENFASSUNG

Die Konzentration von Zink (ppm/Feuchtgewicht) wurde in der Mandibularcondyle und verschiedenen ausgewählten harten und weichen Geweben von 3 und 24 Wochen alten Ratten mit Hilfe von Neutronenaktivierung und Gammastrahlen-Spektrometrie bestimmt. Mit Ausnahme der Epiphyse der Tibia von 3 Wochen alten Tieren war die Zink-Konzentration der Skelettproben und der Inzisoren in beiden Altersgruppen signifikant höher als in den Proben der weichen Gewebe. Mit Ausnahme der Milz, deren Zink-Konzentration bei erwachsenen Ratten signifikant angestiegen war, bestanden keine signifikanten Unterschiede der Zink-Konzentration der Proben weicher Gewebe zwischen jungen und alten Ratten. Die Skelettproben erwachsener Ratten hatten eine signifikant höhere Zink-Konzentration als die der jungen Ratten. Dieser Anstieg ist am wahrscheinlichsten das Resultat der steigenden Mineralisation mit steigendem Alter. Es wurde keine signifikante Differenz zwischen der Zink-Konzentration der Inzisoren der beiden Altersgruppen gefunden.

RÉSUMÉ

L'auteur a déterminé par activation neutronique et par spectrométrie de rayons gamma la teneur en zinc (ppm/poids humide) dans le condyle mandibulaire et dans certains tissus durs et mous de rats âgés de 3 et de 24 semaines. À l'exception de l'épiphyse tibiale des rats âgés de 3 semaines, les échantillons osseux et les incisives avaient une teneur en zinc significativement plus élevée que celle des échantillons de tissu mou dans les deux groupes d'âge. À l'exception de la rate dont la teneur en zinc était significativement augmentée chez les rats adultes, aucun des échantillons de tissu mou ne présentait de différence significative de leur teneur en zinc entre les jeunes rats et les vieux rats. Les échantillons osseux de rats adultes avaient une teneur en zinc significativement plus élevée que celle de jeunes rats.

L'auteur a montré que cette élévation de la teneur en zinc résulte très probablement de l'augmentation de la minéralisation osseuse en fonction de l'âge. Il n'a pas constaté de différence significative de la teneur en zinc des incisives dans les deux groupes d'âge.

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