

Presence and possible function of Zn in the hyaline cartilage

M.C. Pérez Castejón¹, M.J. Barral¹, A. Vera Gil¹, M.J. Pérez Castejón² and M. Lahoz¹

¹Department of Morphological Sciences, Faculty of Medicine, University of Zaragoza, Zaragoza and ²Department of Nuclear Medicine, Faculty of Medicine, University Complutense of Madrid, Madrid, Spain

Summary. Using histochemical and autoradiographical techniques the location of Zn in the hyaline cartilage of the Wistar rat was studied. A triple location was observed in the territorial matrix; in the chondrocyte; and in the perichondrio (cellular area). We believe that the molecules which were observed carrying Zn could be alkaline phosphatase, timidin-kinase and chondroitin-sulphuric acid.

Key words: Zn, Cartilage, Histochemistry, Autoradiography

Introduction

Recently, Greenberg (1989), in his revision of the role of Zn in histopathology, collected, among others, the data of the osteoblastic and chondrogenic diminution associated with the deficiency of this micronutrient.

Barral (1979) studied the presence of Zn in the cartilage and Vera Gil et al. (1981) made a histochemical and autoradiographical location of this heavy metal in the cartilage.

On the other hand, there exists evidence of the co-relation between the metabolic fraction of Zn and chondrogenic activity. This evidence justifies the present study of the localisation and function of the Zn in the cartilage.

Materials and methods

The Wistar rat was used as the experimental animal. 66 young adult rats of both sexes and weighing an average of 225 grs. were used. They were divided into 2 groups.

1.- The first group of 6 animals was used to realize the histochemical techniques for location of heavy metals

described by Timm (1958) and Phil (1968) for paraffin and semi-thin sections respectively.

They were killed without any previous manipulation and 2 types of samples of costal cartilage were taken from then, which were rapidly fixed, either in sulphidric alcohol (Timm, 1958) or in glutaraldehyde sulphidric (Phil, 1968). Later they were processed according to both of the above techniques mentioned in the references.

2.- The other 60 animals, forming a second group, were injected intraperitoneally with 0.5 ml of a saline solution of $\text{Cl}_2^{65}\text{Zn}$ called ZAS and made by Nuclear Iberica, with a calibrated activity of 355 μCi . Later they were killed in groups of 4 animals at intervals of 24 hours from the 1st post-injection day until the 15th day.

From each animal 2 types of samples of costal cartilage were extracted. Some pieces were studied by autoradiography in paraffin and semi-thin sections, which, after being fixed in Carnoy and later histologically treated, were processed autoradiographically by the Deeping method, using Ilford K5 photographic emulsion dissolved in double distilled water at a proportion of 50%. Later these pieces were exposed in a black plastic box with a drying system, and the box was put into the refrigerator at 4° C for 21 days. These pieces were developed using Kodak D-19 and fixed in Hypam.

We realized controls of the total autoradiographic blackening from the samples of the rats which had been injected and from those which had not been injected. No alteration of the expected behaviour was found, according to the origin of the samples, which would make the experiment invalid. Each of the other pieces was first weighed and then homogenized with 5 cc of nitric acid for 12 hours. Afterwards the radiation in each sample was measured with a CGR-Gammamatic 2 pit counter.

7 parameters for each animal were obtained and the average homogeneous values for each sample among the animals killed the same day were determined. Later a reconstruction of the profiles of uptake of ^{65}Zn in the cartilage every day was made.

Offprint requests to: Prof. M.C. Pérez Castejón, Departamento de Ciencias Morfológicas, Facultad de Medicina, C/. Domingo Miral s/n, 50009 Zaragoza, Spain



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Fig. 1. Histochemical technique of Timm. Conventional histology. $\times 78$

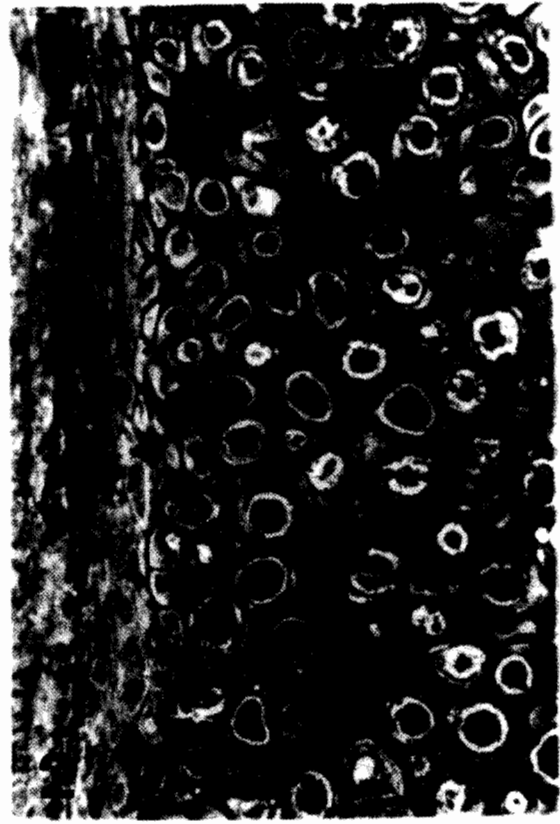
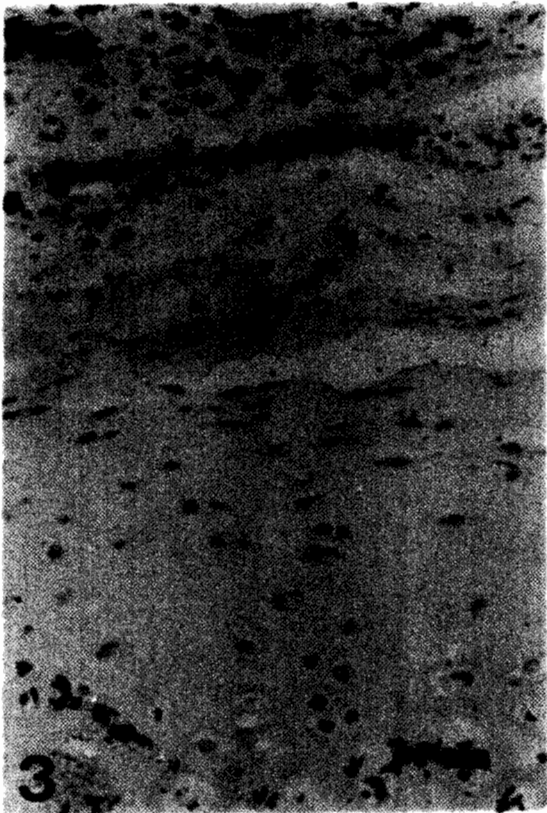
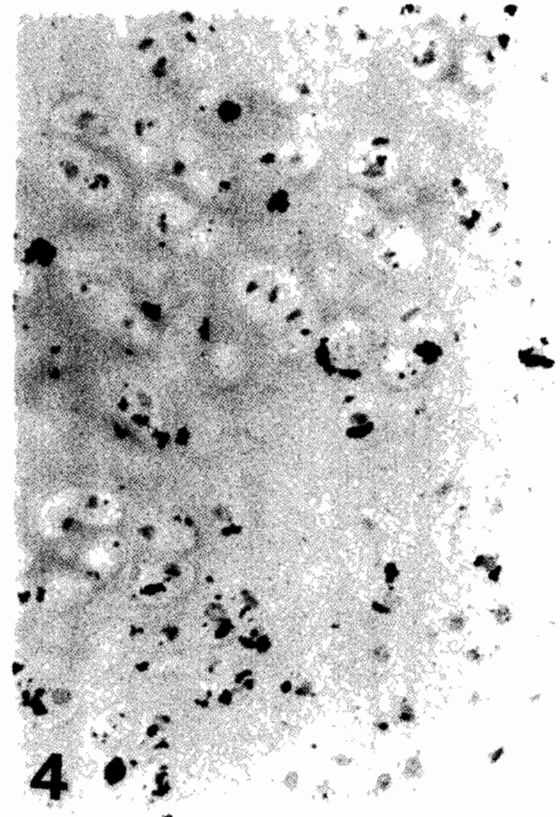


Fig. 2. Histochemical technique of Phil. Semi-thin sections. $\times 500$



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Fig. 3. Autoradiography with ^{65}Zn . Conventional histology. $\times 200$



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Fig. 4. Autoradiography with ^{65}Zn . Conventional histology. $\times 500$

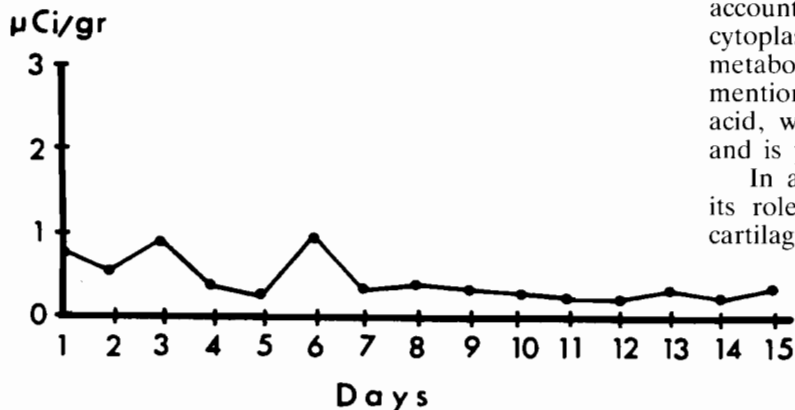


Fig. 5. Autoradiography with ^{65}Zn . Semi-thin sections. $\times 1,250$

Results

The histochemical technique offered a spectacular image of positivity in the territorial matrix of the cartilage (Figs. 1, 2) fundamentally, in the capsular matrix. A certain density of reaction was also observed in the chondrocyte nucleus (Fig. 2) in contrast to the slight reaction in the chondrocyte cytoplasm.

Autoradiography in paraffin (Figs. 3, 4) and semi-thin sections (Fig. 5) showed an uptake of radioisotope which was apparently greater in the territorial matrix, with which a parallel with the histochemical



Graph 1. Metabolic pathway of ^{65}Zn in cartilage.

answer was established. Positivity and uptake in the perichondric area (Figs. 2, 3) were also observed.

The metabolic pathway of the radio-isotope measured by a pit counter gave a graph with two maximum peaks on the 3rd and 6th post-injection day with values close to $1 \mu\text{Ci}$ per gram of tissue. This graph shows later until the 15th day after injection a continuous value close to the $0.3 \mu\text{Ci}$ per gram of tissue (Graph 1).

The combination of the results obtained using different techniques indicated that the process found autoradiographically on the 6th post-injection day (biological half-life of the radio-isotope according to Khristov, 1965), is the moment of metabolical incorporation of the ^{65}Zn in the cartilage. So we observe a preferential location of the grains in the extracellular matrix.

Discussion

The histochemical results suggest that the metabolism of Zn in the cartilage must pass through a large concentration of this metal in the extracellular matrix (fundamentally capsular matrix) and in this atmosphere the functional use of Zn by the chondrocyte is produced. This functional use justifies the level of activity maintained by the radio-isotope on the days after the 6th post-injection day.

The problem is: in which molecules is the detected Zn joined?

It is evident that the micronutrient found in the cartilage was metabolised and incorporated into the liver in molecules of greater complexity, during the 1st post-injection day. Proofs of this were found, by Pérez Catejón (1987) in the dynamic pathway of the ^{65}Zn in the liver.

These autoradiographical and histochemical locations of Zn suggest that the possible molecules to which the Zn is bound are, on one hand, alkaline phosphatase (in great quantities). This alkaline phosphatase is present in the extracellular matrix of the cartilage. On the other hand they also suggest timidin-kinase which is present in the chondrocyte nucleus. A third element which we must take into account is the location of Zn in the chondrocyte cytoplasm. This location could be joined to the metabolic process of either of the two molecules mentioned above, or, joined to chondroitin-sulphuric acid, which is an essential component of the matrix and is produced mainly by the chondrocyte.

In any of the three functional associations of Zn, its role in the maintenance and metabolism of the cartilage is evident. This role is also evident in the interstitial and appositional growth of the cartilage. It is observed as much in the maintenance of the vital conditions of the cartilage as in its growth. The studies of status of Zn in man (Hambidge, 1988; Vallee, 1988) and the facts known about the deficit in the skeletal development in

situations lacking in Zn, either chronic (Prasad, 1988) or congenital (enteropathic acrodermatitis), are explained by this role.

In support of this interpretation we would add the recent papers, Yamaguchi et al. (1986, 1987, 1988), Yamaguchi and Uchiyama (1987) and Kaji et al. (1988) which show the protective role of Zn in bone formation and growth. These experiments were realized «in vitro», using tissue cultures. Moreover the studies about the influence of Zn in the glycosaminoglycan metabolism in rats (Westmorland, 1971; Lohmander et al., 1972; Bolze et al., 1987), also support this paper.

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