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Characteristic Molar Ratios of Magnesium, Carbon Dioxide, Calcium and Phosphorus in the Mineralizing Fracture Callus and Predentine

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SUMMARY

From fracture callus in different stages of mineralization the contents of Mg, CO_2, Ca and P were determined and compared with those found in predentine. It was found that the Mg- and CO_2-contents are high in relation to Ca and P values during the prestages and early stages of mineralization. These relatively high Mg- and CO_2-values are connected with high Mg/Ca, Mg/P, CO_2/Ca and CO_2/P ratios which strongly decrease with the increasing degree of mineralization. These results seem to be part of a general controlling mechanism of the calcification.

INTRODUCTION

Knowledge of the components of the organic matrix and the changes which occur in the matrix is necessary for an understanding of the process of hard tissue formation. Since certain ions have a decisive influence on the process of mineralization, their initial accumulation in the prestages of mineralization must also be analyzed. A particular meaningful relationship concerns the relative proportions of carbonate and magnesium ions in addition to calcium and phosphate. Whereas it is recognised that carbonate is a component of a biological apatite (survey, 1), it is not certain that magnesium is also a component of the mineral portion. It is one of the most important ionic cofactors of alkaline phosphatase which splits phosphoesters to release phosphate near the mineralizing front (2). It has been shown in in vitro investigations of the synthesis of biological calcium phosphates that magnesium (3) hinders the transformation of amorphous calcium phosphates to hydroxyapatite and that carbonate inhibits the crystal growth (4) (survey, 5): together they show an additive effect (6).

The mineralizing turkey tibia tendon is a very convenient model for the study of hard tissue formation (2). Topological analyses of CO_2 and magnesium on this model have shown that there are relatively high magnesium/calcium and carbonate/calcium ratios in the stage before recognisable mineral nucleation (7). These ratios reduce rapidly during the phase of mineralization and become very low in the completely mineralized tissue. Similarly, high ratios of carbonate/ and magnesium/phosphorus are also found in the very earliest stages of mineralization of rat incisor enamel (8).

Key words: Mineralization — Collagen Rich Systems — Fracture Callus — Predentine.

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MATERIALS AND METHODS

Fracture Callus

Fracture callus was studied in the radius of "black headed sheep". These bones were completely transected using a saw. The bones were immobilised during healing with a metal plate. Portions of the fracture callus were frozen in liquid nitrogen, and freeze dried. As different regions of the fracture callus had a different overall degree of mineralization, fragments of tissues from different regions were analyzed as well as the whole callus sample after homogenization.

Predentine

Mandibular molar teeth from three-weeks old bull calves were freeze fractured after freezing in liquid nitrogen, and freeze dried at -65°C. After freeze drying the pulp tissue was dissected away and the remnants removed with a blast of dry dichlorodifluoromethane. The predentine surfaces were scraped with sharp dental excavators and the resulting powder collected for analysis.

For the chemical analyses the samples were treated with 1 N HCl and the liberated CO_2 gas absorbed by barium hydroxide. The contents of CO_2, Mg, Ca and P were determined according to (9).(The samples of callus weighed approximately 1 mg and the predentine samples approximately o.1 mg)

RESULTS AND DISCUSSION

The Contents of Ca, Mg, P, and CO_2

The calcium content in fracture callus rose after two-three weeks after operation to about 6% the phosphorus content remained slightly less than 3%. At eight weeks post operation, the calcium rose to about 15% and the phosphorus to 7%. At the end of the period of investigation, at 16 weeks, the calcium had risen to 21.54 (± o.14% S.D., n=11) and the phosphorus value was 1o.36 (± o.14% S.D.).

The total calcium content was taken as an indicator of the degree of mineralization. With a calcium content less than 1% (average Ca: o.48%; P: o.4o%) at the commencement of mineralization, the mean magnesium value was o.o75%. In the partly mineralized condition (average Ca: 5.1%; P: 2.45%) the magnesium content lay about o.19%. In the mineralized condition (average Ca: 18.28%; P: 8.35%) the magnesium content lay in the region of o.4-o.8%, with the mean value of o.56%. The CO_2 content increased in a corresponding fashion, with o.36% at the earliest stage, o.8o% at the partly mineralized stage, and reaching 1.59% when the calcium content had exceeded 1o%. At the end of the investigative period the CO_2 content of the mineralized tissue was 2.51 (± o.33 S.D.). Two predentine samples gave mean values of 2.5% Ca, o.26% Mg, 1.2% P, o.53% CO_2.

Corresponding values were received for the mineralizing turkey leg tendon (7). At the earliest phase investigated when the mean Ca value was o.22% and the mean P o.3o%, the Mg value was o.o4% and the CO_2 o.87%. At the stage when recognisable mineral deposition was present (average Ca: o.7o% and P: o.52%) the magnesium value was o.o6% and the CO_2 1.2%. In the partly mineralized condition (mean values Ca: 5.45%, P: 2.69%) Mg was o.15% and CO_2 1.33%. In the mineralized tissue with 15% (and 6.9% P, the Mg was o.36% and the CO_2 was 1.93%.

In the interpretation of the present results it is important to note that identical CO_2 analyses were obtained where o.1 N HCl was used to release the gas.

Decarboxylation of amino acids that commonly occur in proteins can be excluded under these circumstances. Degradation of γ-carboxy glutamic acid as a possible donor of CO_2 in the as yet unmineralized tendon can also be excluded since Glimcher et al. (1o) have shown that it can only be found in the mineralized regions. A rough calculation on the basis of the results of these authors would indicate that γ-carboxy glutamic acid would only account for o.1% of the CO_2 content in the mineralized area. Since we have found high concentrations of Na and K in the prestages and early stages of mineral formation with both the fracture callus and the tendon, we assume that the high CO_2 content is mostly present in the form of fixed HCO_3^- and possibly also as carbamino-CO_2 at the place where mineral formation is present.

Mg/Ca Molar Ratios

Figure 1 shows the mean Mg/Ca ratios at the different stages of mineralization of the sheep fracture callus and turkey leg tendon. At the beginning of mineralization with calcium values below 1% these ratios lie in the range of o.3 in both tissues, that means fairly in the range of blood serum. It is noteworthy that in vitro experiments (4) have shown that the transformation of amorphous calcium phosphate to hydroxyapatite is prevented when the Mg/Ca ratio is greater than o.2. Comparing these results we also came to the conclusion that a great similarity excists between the initial phase in vivo and the formation of hydroxyapatite in vitro. The ratios for the mineralizing tissues fell with increasing mineralization to o.o5, with an increase in total magnesium content. The largest deviations are found at the starting phase of mineralization, probably reflecting a pushlike influx of these elements at this stage. We believe that the CO_2 also

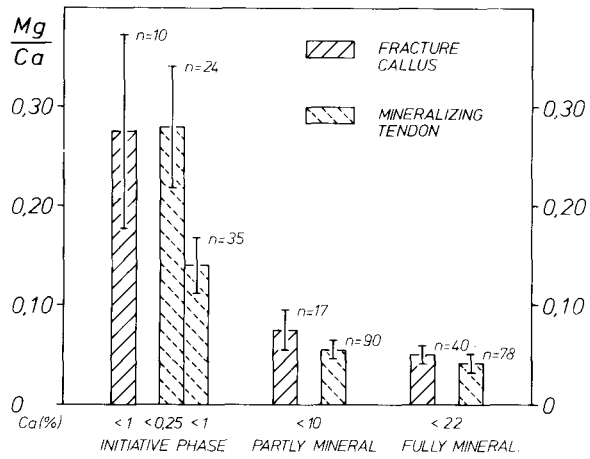

Fig. 1. The molar ratios of Mg/Ca in the prestages of mineralization, in partially and fully mineralized fracture callus and in mineralizing turkey leg tendon (MINERAL.: mineralized)

increases in a similar wavelike manner. A corresponding fall in the Mg/Ca ratios is found in the transition from predentine to dentine (Fig. 2). Furthermore, Wuthier's (11) results also show a similar tendency in the zones of transition from the proliferating to the calcified cartilage of the epiphyseal growth plate (7). A similar fall in Mg/P ratios also occurs in all the hard tissues which we have discussed, as well as in dental enamel (8). A high concentration of Mg^{2+} ions in the early stages of the calcification was also noticed in experimental cutaneous calcinosis (12).

CO_2/Ca Molar Ratios

The carbonate content in the mineralizing turkey leg tendon varies in a wavelike fashion in the zone of transition from completely unmineralized to the mineralized condition, as long as mineralization is not yet complete (7). If one takes the mean CO_2 content half-way between the peaks and the troughs of the distribution it is then found that the CO_2/Ca and CO_2/P ratios both decrease with increasing mineralization. The CO_2/Ca ratio falls from about 2

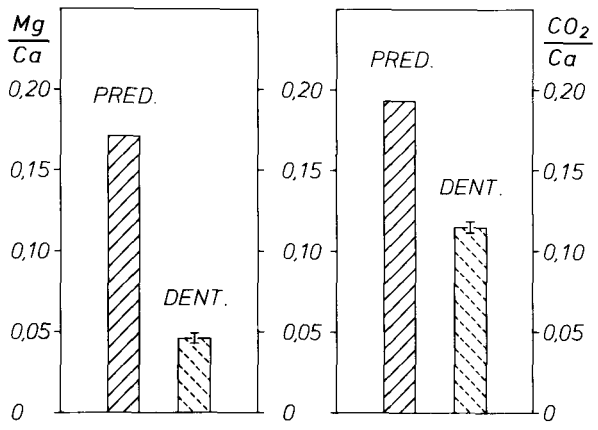

Fig. 2. The molar ratios of Mg/Ca and CO_2/Ca of predentine and dentine (PRED.: predentine; Ca: 2.5%, DENT.: dentine; Ca: 27.2%)

at the earliest phase of mineral deposition (the Ca content: between o.25% and 1%) to o.o8 in the completely mineralized tissue. For partly mineralized regions with calcium between 1% and 1o% the CO_2/Ca ratio was about o.3. The same trend was shown in the transition from predentine to dentine (Fig. 2, right). In the same way the CO_2/Ca ratio fell with an increasing mineralization and increasing CO_2 content in the healing fracture callus. It was o.88 ($\pm$ o.36 S.D., n=1o) at the starting phase, o.14 ($\pm$ o.12 S.D., n=17) in the partly mineralized callus and o.o8 ($\pm$ o.o3 S.D., n=4o) in the mineralized callus with a mineral content similar to bone. The CO_2/P ratios alter in a similar fashion.

Agreeing with the in vitro experiments being discussed above, the high Mg and CO_2 values in relation to Ca and P (which were found in the prestages and early stages of mineralization of 4 different collagen rich hard tissues and of developing enamel) may represent an indicator of a general regulating activity for mineral formation.

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