Sheep bone collagen, rat tail tendon collagen and apatite seeds were used in a model system to study nucleation catalysis and mineral deposition in a metastable calcification buffer.

Sheep bone collagen was shown to be a good nucleation catalyst, while earlier experiments have shown that rat tail tendon collagen was a very poor catalyst.

The rapid phase of apatite deposition in the collagen was terminated as soon as a mineral content of not more than 50–60 per cent was reached, although the buffer was still highly supersaturated.

The results suggest that the amount of mineral deposited in such a model system is regulated by factors similar to those operating in biological calcification.

Key words: Nucleation — Mineralization — Bone — Collagen — Apatite.

Nous avons utilisé de la collagène d’os de mouton et de tendons de queues de rats et des cristaux d’apatite pour étudier dans un système modélisé la catalyse de la nucléation et la déposition de minéraux dans un tampon métastable.

La collagène d’os de mouton se trouvait être un bon catalyseur, tandis que des expériences antérieures ont démontré que la collagène de tendons de queues de rats était un catalyseur très faible.

Le phase rapide de la déposition de l’apatite dans la collagène se termine aussitôt que le contenu du minéral a atteint au plus 50 à 60 pour cent, bien que la supersaturation du tampon est encore bien élevée.

Les résultats montrent que dans un tel système modélisé la quantité du dépôt minéral est régulée par des facteurs semblables à ceux qui opèrent pendant la calcification biologique.

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Kollagen aus Schafsknochen war ein guter Katalysator für die Nukleation, während in früheren Versuchen sich herausstellte, daß Rattenschwanzkollagen ein ganz schlechter Katalysator ist.

Die schnelle Phase der Apatitfällung im Kollagen war beendet, wenn der Mineralgehalt bis zu 50–60% angestiegen war, obwohl der Puffer noch stark übersättigt war.

Die Resultate weisen daraufhin, daß die Menge des gefallenen Minerals in einem solchen Modell-System von ähnlichen Faktoren reguliert wird wie die biologische Verkalkung.

Introduction

Model systems for the study of apatite deposition in vitro have come into wide use. Systems can be designed that are able to catalyze the nucleation of apatite in metastable solutions. In spite of the oversimplification, necessarily involved in designing a model system for a complex physiological phenomenon, such systems have yielded useful information. Some of the advantages and limitations of such systems...
systems for gaining an understanding of the biochemical and biophysical aspects of the process of biological calcification have been discussed recently by one of the present authors (Bachra, 1967).

In earlier experiments (Bachra et al., 1959) it was noted that fibers reconstituted from acetic acid solutions of rat tail tendon collagen can act as a catalyst for the nucleation of apatite. Such fibers required a higher degree of metastability of the calcification buffer than rachitic epiphysial rat bone cartilage. The fibers calcified only after renewal of the buffer solution (Bachra, 1967). In later experiments it was found that bone collagen will also mineralize in vitro and that it requires a similarly high degree of metastability. This material, however, does not require renewal of the buffer solution. The reasons for the mandatory renewal of the buffer solution in the case of the fibers have been investigated in greater detail (Bachra and Fischer, 1968).

The present paper consists of a study of some quantitative aspects of the recalcification of bone collagen in vitro and of the mineral deposition which can be obtained in fibers of reconstituted rat tail tendon collagen after renewal of the calcification buffer. Some of the biological implications of this work are discussed.

**Materials and Methods**

**Rat Tail Tendon Collagen**

Tendons were obtained from rat tails stored in the frozen state at $-5^\circ$. Freshly excised tendons were carefully cleaned from adhering tissues, washed with distilled water, cut finely with scissors and extracted for 2 or 3 days with ice cold 0.04% acetic acid (250 ml per g of wet tendon weight). The mixture was then centrifuged for 1.5 h in a Spinco rotor No. 30 at 30,000 r.p.m. and filtered under suction in the cold through Whatman No. 541 filter paper. The clear collagen solutions were stored in the refrigerator until further used.

Fibers of rat tail tendon collagen were reconstituted by adding to the collagen solutions 0.5 volume of 2.5 M NaCl, amounting to a final NaCl concentration of 0.83 M or 1.16%. The precipitated fibers were washed twice with 0.83 M NaCl and then triplicate samples (3–20 mg dry weight for the combined triplicates) were immersed in the calcification buffer.

**Sheep Bone Collagen**

The diaphyseal portions of sheep long bones were carefully cleaned from adhering marrow and other tissues, washed with acetone to remove fat and stored at $-20^\circ$. The bone was powdered in a steel mortar. Bone particles less than about 1 mm in size were collected by sieving the powdered bone through a clean wire-gauze. About 10 g of bone powder per 1 of EDTA were used. The EDTA solution was renewed after 2 weeks and demineralization was continued with the fresh solution for an additional week. The demineralized bone powder was centrifuged off, washed three times with about 200 ml of cold distilled water and then stirred in the cold with 11 of distilled water for 3 days, the water being renewed each day. The demineralized bone powder was homogenized further in ice cold distilled water in a 100 ml stainless steel cup of a Servall Omnimix at top speed. Only particles which were small enough to remain in suspension for not less than about 10 sec were collected for use in the seeding studies. The resulting fine particles of demineralized bone largely consisted of collagen fibers. These showed the familiar native 640 A banding pattern, but no apatite crystals were visible in the electron microscope and no calcium and phosphate were detected. The bone collagen was stored at $-20^\circ$.

Triplicate samples of decalcified bone collagen (5–60 mg dry weight for the combined triplicates) were immersed in the calcification buffer.