Relation of Collagen Metabolism to Calcium Metabolism in the Bone

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According to the present view collagen molecules serve as nucleating agents in the mineralisation of bone.[1] Evidence also exists of a direct physical [2] and chemical [3] bonding between collagen fibres and mineral crystals in bone. Further, Firschein has shown that changes in the skeletal incorporation of radioactive calcium and hydroxyproline after injection of ^{45}Ca and proline-^{14}C are of the same order of magnitude during bone formation.[4] On the other hand, little has been known of the possible metabolic interactions of collagen and calcium in mature bone. Earlier reports from our laboratory have suggested that a definite correlation exists between these parameters in bone resorption, both under normal conditions [5] and in experimental endocrinological disorders.[6,7] The present communication provides evidence of the essential role of collagen in the overall metabolism of bone.

Materials and methods. Male albino Wistar rats, weighing 83–86 g, were used as test animals. The radioactive compounds, proline-^{14}C (8.2 mC/mmole) and ^{45}Ca (as calcium chloride, 2–5 C/g) were obtained from the Radiochemical Centre, Amersham. The rats received an intraperitoneal injection of 10 μC of proline-^{14}C and 10 μC of ^{45}Ca, and were subsequently killed at various periods after injection of the labels; two rats were used for each measurement. Measurement of the total radioactivity of hydroxyproline-^{14}C and ^{45}Ca in bone was made in the right femur of one rat and the left femur of the other killed at the same time. Femurs were removed, dissected free of cartilage and adhering soft tissues, and minced in distilled water, first with scissors, and then with an Ultra-Turrax homogenizer. The homogenates were hydrolysed with an equal volume of 12 N HCl for 8 h at 138°C, evaporated to dryness, and dissolved to 10 ml with distilled water. An aliquot was used for determination of the specific activity of hydroxyproline-^{14}C and of ^{45}Ca. Hydroxyproline-^{14}C was determined following the method of Peterkofsky and Prockop,[8] and the quantity of hydroxyproline was measured by the method of Kivirikko *et al.*[9] These values were employed for the calculation of the total radioactivity of hydroxyproline in bone collagen. For determination of the radioactivity of ^{45}Ca, 1 ml of the solution was diluted with 3 ml of distilled water, and precipitated with 1 ml of saturated ammonium oxalate at pH 5. The precipitate was washed three times with ammonium oxalate, dried on a steel planchet, and analysed for ^{45}Ca content with a gas-flow counter. The results were corrected for self-absorption and decay. The total calcium content was measured by EDTA-titration using Calcein [10] as indicator.

For determination of the radioactivity of hydroxyproline-^{14}C and ^{45}Ca in the neutral salt-soluble fraction of bone, the remaining

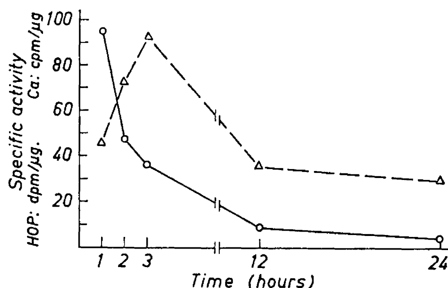

Fig. 1. Specific radioactivities of hydroxyproline-^{14}C (○) and ^{45}Ca (△) in the 0.45 M NaCl soluble fraction of bone homogenate after the injection of 10 μC of proline-^{14}C and 10 μC of ^{45}Ca.

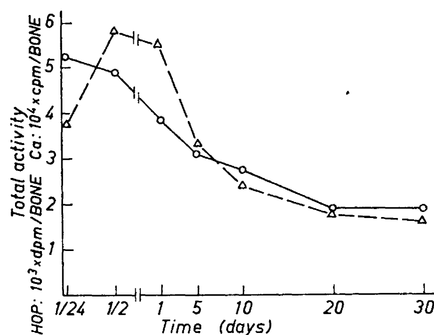

Fig. 2. Total radioactivities of hydroxyproline-^{14}C (○) and ^{45}Ca (△) in the bone, after the injection of 10 μC of proline-^{14}C and 10 μC ^{45}Ca.

femurs were prepared and homogenized as above. The homogenates were extracted with 0.45 M sodium chloride by vigorous shaking for 24 h at +4°C. The homogenates were centrifuged at 60 000 *g* for 120 min, and the supernate was hydrolysed with an equal volume of 12 N HCl. The hydrolysate was then used for determination of the amount and the radioactivity of hydroxyproline and calcium, as in the case of whole bone analyses.

Results and discussion. Fig. 1 indicates the very rapid incorporation of proline-^{14}C as hydroxyproline-^{14}C into the neutral salt soluble fraction of collagen which is known to represent newly formed collagen in bones (*cf.* Ref. 5). Calcium in this soluble fraction of the bone homogenate reaches its highest specific activity later than the peak specific activity of hydroxyproline-^{14}C, although the activities of hydroxyproline-^{14}C and ^{45}Ca decline simultaneously. Incorporation of both hydroxyproline-^{14}C and ^{45}Ca into the total bone is slower than that into the soluble fraction of bone, and again the incorporation of ^{45}Ca is delayed relative to that of hydroxyproline-^{14}C (Fig. 2). After the initial rise of the labels, the radioactivities of both hydroxyproline-^{14}C and ^{45}Ca decline at a rate characteristic of the slow turn-over of collagen, especially during days 5–30 of the experiment, when the labels are embedded in relatively old bone.[5]

The findings suggest that in the formation of bone, there is initially a rapid synthesis of collagen, which comprises the main fraction of the organic matrix. Thereafter, the mineralisation may be initiated by the newly formed collagen molecules as shown by the later incorporation of ^{45}Ca than that of hydroxyproline-^{14}C into bone. These findings are in agreement with the nucleation concept. The soluble collagen fraction, which represents newly synthesised forms of collagen, changes into insoluble, mature collagen. This is reflected in the rapid decline of radioactivity of hydroxyproline-^{14}C in the soluble fraction of bone homogenate, followed immediately by the corresponding change in calcium. After the neutral salt-soluble fraction of collagen has changed into the mature form, the metabolism of the mineral phase is tightly coupled to the metabolism of collagen; this is reflected in the similar decline of total radioactivities of both hydroxyproline-^{14}C and ^{45}Ca at a slow rate peculiar to collagen. Since, during this later phase, the specific activity of calcium in serum is lower than that in the bone,[11,12] an equilibrium between these two pools of calcium seems to be excluded. The present results can be interpreted as evidence of the primary role of collagen metabolism in the overall metabolism of bone; the changes in the mineral metabolism may largely be secondary reflections of changes in the collagen metabolism.

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