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DEVELOPMENTAL CHANGES IN BONE MINERAL STRUCTURE DEMONSTRATED BY EXTENDED X-RAY ABSORPTION FINE STRUCTURE (EXAFS) SPECTROSCOPY

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Summary EXAFS spectra have been recorded above the calcium K edge from bones of mice aged 3 days, 1 week, 1 month, 2 months and 7 months. Spectra indicated that the calcium ion environment in bone mineral changes during development. Results were compared with those obtained from amorphous calcium phosphate and a poorly crystalline hydroxyapatite matured from this amorphous calcium phosphate in the presence of water. Spectra from the older mice closely resembled those of the matured product but those from the younger mice were more like those from the freshly prepared amorphous calcium phosphate.

Introduction We have used extended X-ray absorption fine structure (EXAFS) spectroscopy to obtain information on changes in the calcium ion environment during the development of bone mineral. It is often supposed that bone mineral development involves the transformation of amorphous calcium phosphate into a poorly crystalline hydroxyapatite (1,2) although this view has been questioned (3) as the nature of the initial bone mineral is obscure (4). Here we compare EXAFS spectra of femurs from mice aged from 3 days to 7 months with the spectra of synthetic amorphous calcium phosphate and hydroxyapatite obtained from it by maturation in the presence of water.

EXAFS spectra, recorded above the calcium K edge, have previously provided information on the calcium ion environment in mature bone (5,6). A plot of X-ray absorption against photon energy shows damped oscillations on the high energy side of the edge. These oscillations are the EXAFS spectrum which arises from interference of emitted photoelectrons with those scattered back by neighbouring atoms; the spectrum is thus very sensitive to the immediate chemical environment of the absorbing atom (7). We have now succeeded in recording spectra, using the newly commissioned Synchrotron Radiation Source at Daresbury Laboratory, which provide information on structural changes accompanying the development of bone mineral.

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Materials and Methods Femurs were dissected from freshly killed mice aged 3 days, 1 week, 1 month, 2 months and 7 months. Attached muscle, as well as the proximal and distal extremities, were discarded to leave the femoral shaft. Shafts were split, quickly dried with filter paper and then thoroughly dried for at least 1 hour over silica gel in a vacuum dessicator. This dried material was then ground with acetone to a fine powder in an agate mortar.

EXAFS spectra were recorded on the same day as the animal was killed. Each age group was represented by two samples. For the two youngest age groups each sample consisted of material from three individuals; for the remainder each sample was from a single mouse. A thin layer of each powder was mounted between strips of Sellotape. Spectra above the calcium K edge were recorded in the transmission mode at the Daresbury Synchrotron Radiation Source (8). (The storage ring was operating at 1.9 GeV with an average current of 90 mA or at 1.8 GeV and 120 mA when these experiments were performed). Harmonic contamination of the incident beam was minimised by using a Si 111 vertically dispersing, channel cut monochromator and by choosing a sample thickness, t, such that $\mu t < 1$ where μ was the attenuation coefficient of the sample. Furthermore the ionisation chambers used to measure the incident (I_o) and transmitted (I_t) intensities were optimised to have attenuations of 20% and 60% respectively. Absorbance ($\log[I_o/I_t]$) was then calculated as a function of photon energy and the EXAFS spectrum extracted from the smoothly varying background (9). In fig.1 spectral peak heights are presented as a fraction of the height of the calcium K edge.

Results EXAFS spectra of the mouse bones are compared with those of model compounds in fig.1. Fig.1(a) shows the spectrum of amorphous calcium phosphate which has been published previously (5). When moist this material gradually matures into poorly crystalline hydroxyapatite (10). The EXAFS spectrum of the matured material (maturation time a few weeks) resembled that of adult rat bone more closely than the spectrum of any other plausible model compound, including highly crystalline apatites (5). Comparison of figs.1(f) and (g) shows that the spectrum of 7 month-old mouse bone also closely resembles that of matured amorphous calcium phosphate.

However the spectrum of 3 day-old mouse bone, fig.1(b), is clearly different and more nearly resembles that of amorphous calcium phosphate in fig.1(a). Furthermore a gradual change occurs in the spectra with increased age from 3 days to 7 months as shown in figs.1(b) to (f). All the spectra show three strong peaks at about 60, 120 and 180 eV above the calcium K edge; at higher photon energies these oscillations are strongly damped, presumably because of high structural disorder in the samples. The compound nature of the peak at 120 eV is clearly apparent. It is formed by the superposition of two or more oscillations and its shape is therefore very sensitive to small changes in their relative displacements and hence to the calcium ion environment in the various samples. For the older mice the resultant peak shape, shown in fig.1(f), is almost identical to that in fig.1(g) which is the spectrum of matured amorphous calcium phosphate. In contrast the shape of the

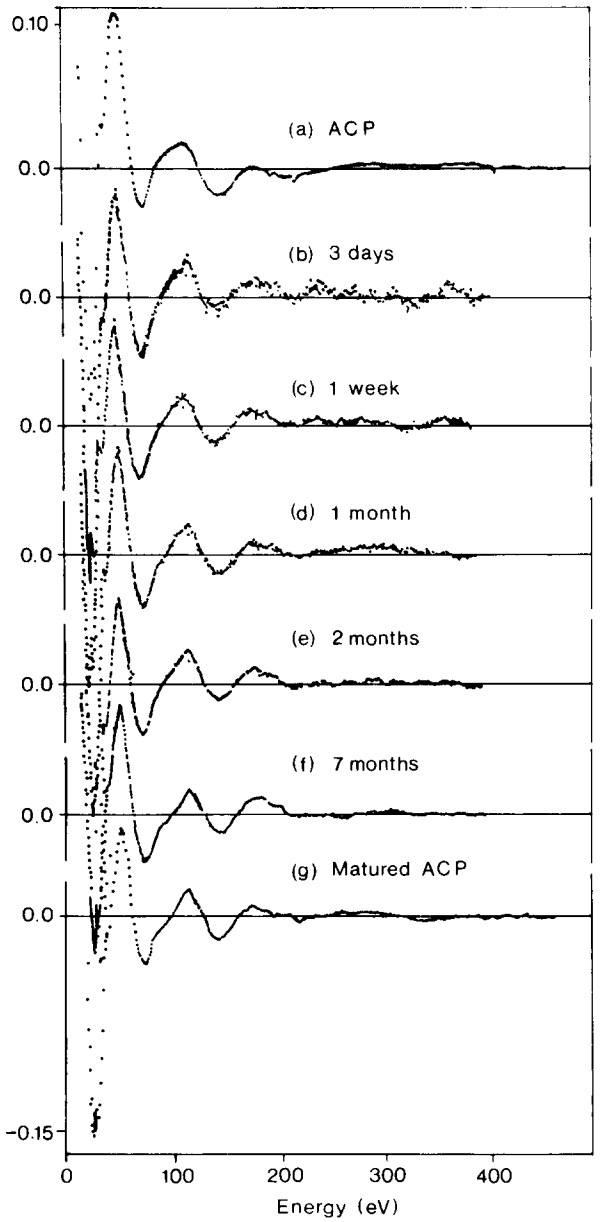

Fig.1 EXAFS spectra of (a) amorphous calcium phosphate, (b) 3 day-old mouse bone, (c) 1 week-old mouse bone, (d) 1 month-old mouse bone, (e) 2 month-old mouse bone, (f) 7 month-old mouse bone and (g) poorly crystalline hydroxyapatite obtained by maturation of amorphous calcium phosphate. All spectra are plotted against photon energy expressed as the energy above the calcium K absorption edge.

corresponding peak in the spectrum from the 3 day-old mouse bone, shown in fig.1(b), more nearly resembles that from the freshly prepared amorphous calcium phosphate in fig.1 (a). Spectral changes accompanying increased age of bone mineral closely resemble those occuring during maturation of amorphous calcium phosphate.

Discussion We have shown previously that EXAFS spectra are very sensitive to changes in the calcium ion environment in model compounds resembling bone mineral (5). For example the spectrum of hydroxyapatite obtained by maturation of amorphous calcium phosphate is clearly different from that of the highly crystalline material. These earlier results (5) were consistent with the view that bone mineral closely resembles poorly crystalline hydroxyapatite (4).

Our present results show that the calcium ion environment changes during the maturation of bone mineral and that the changes are very similar to those which occur when amorphous calcium phosphate matures into poorly crystalline hydroxyapatite. Thus it appears that the post natal developmental changes which occur in the structure of bone mineral closely resemble those accompanying the maturation of amorphous calcium phosphate. However we cannot rule out the strong possibility that other calcium phosphates such as brushite occur as minor phases in embryonic bone (3,4).

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