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Short Communication

Crystalline Changes in Avian Bone Related to the Reproductive Cycle

II. Percent Crystallinity Changes

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This is an X-ray diffraction study of the changes in the proportional amounts of the amorphous and apatitic calcium phosphate phases in pigeon femur during the egg-laying cycle. The medullary portion of pigeon femur is completely resorbed in the reproductive cycle. In this process the portion of bone which is higher in amorphous calcium phosphate is resorbed before the crystal-rich portion. Thus, bone with a higher amorphous content is more metabolically active than more crystalline bone. Finally, medullary bone which can be produced in male pigeons by estrogen treatment was found by X-ray diffraction to resemble female medullary bone at the beginning of the reproductive cycle.

Key words: Bone — Reproductive cycle — Pigeons — Amorphous — Crystalline.

Une étude de diffraction aux rayons X, au cours du cycle de ponte d'oeufs de pigeon, a été entreprise pour évaluer les modifications de la proportion entre les phases de phosphate de calcium amorphe et apatitique. La partie médullaire du fémur de pigeon est totalement résorbée au cours du cycle de reproduction. Au cours de ce processus, l'os contenant plus de phosphate de calcium amorphe est résorbé avant celui qui est riche en cristaux. Ainsi l'os à contenu amorphe plus élevé est métaboliquement plus actif que l'os riche en cristaux. L'os médullaire qui peut être induit chez le pigeon mâle par traitement aux oestrogènes, ressemble, au point de vue diffraction aux rayons X, à l'os médullaire des femelles au début du cycle de reproduction.

Mittels Röntgen-Diffraktion wurden die Veränderungen in den prozentualen Mengen der amorphen und der kristallinen Calciumphosphat-Phasen im Femur der Taube während des Eierlegens untersucht. Der medulläre Teil des Taubenfemur wird während des Fortpflanzungscyclus vollständig resorbiert. Dabei wird der Teil des Knochens, der mehr amorphes Calciumphosphat enthält, vor dem kristallreichen Teil resorbiert. Daraus folgt, daß der Stoffwechsel des Knochens mit höherem amorphem Gehalt aktiver ist als derjenige des Knochens mit höherem kristallinem Gehalt. Weiter konnte mittels Röntgendiffraktion festgestellt werden, daß der medulläre Knochen, welcher in männlichen Tauben durch Oestrogenbehandlung erzeugt werden kann, dem weiblichen medullären Knochen am Anfang des Fortpflanzungscyclus gleicht.

Introduction

It has been suggested [3] that bone mineral consists of two phases (a) a finely-divided bone apatite and (b) a second calcium phosphate phase which is amorphous to X-ray diffraction. It is assumed by analogy to synthetic systems that the amorphous calcium phosphate is a precursor phase which transforms

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autocatalytically to bone apatite unless stabilized in some way [7]. In line with this is the observation that young bone is higher in amorphous than in crystalline content, while the reverse is true for mature bone [11].

The question arises as to whether the amorphous and crystalline phases are contained in different metabolic bone pools. Preliminary work by Lopez *et al.* (1970) suggests that in parathyroid hormone induced resorption of eel spine, the amorphous pool is removed preferentially as compared to the crystalline mineral pool. The resorption of medullary bone in the pigeon during the reproductive (i.e., egg-laying) cycle [4] is a normal physiological process in which we can study the proportional resorption of these two mineral phases. This resorptive process provides calcium for the formation of calcium carbonate in the egg shells. Medullary bone, which yields a typical bone mineral pattern by electron diffraction [1] occurs only in females during the egg-laying cycle and is formed under the influence of sex hormones stimulated by mating. The medullary portion is more highly developed in certain bones than in others with the femur providing one of the best sources [10]. This new-formed bone is completely resorbed about one week after the last egg is deposited in a given reproductive period [2]. Cortical bone does not participate significantly in this resorptive process [6, 9].

A previous crystallographic study carried out on this system [8] dealt with changes in the apatite pool alone. This paper reports on a study of the changes in the proportional amounts of amorphous and apatitic calcium phosphate phases in pigeon femur during the egg-laying cycle.

Material and Methods

Female pigeons in different phases of the egg-laying cycle were killed by decapitation. Males were similarly killed 8 days after a single administration of 10 mg of estradiol valerate (Squibb). The femora were removed, cleaned of adhering tissue and frozen in acetone cooled with dry ice; they were then lyophilized for 2 days and subsequently defatted with ether for 8 h. The bones were again lyophilized overnight and stored in a desiccator.

The proximal and distal ends of the femurs were then removed, the shafts split and the medullary bone scraped out. A Wiley mill with the hopper packed with dry ice (to avoid heating the bone) was used to grind the medullary bone to pass through a 60 mesh sieve. The samples were then ground in a mortar to 100 mesh for X-ray analysis.

X-ray diffraction was performed using a Siemens scintillation counter diffractometer with a high resolution slit system (copper K-α radiation). Percent crystallinity was determined from the X-ray data by the method of Harper and Posner (1966) which measures the percent of bone mineral which is in the apatite crystal form as opposed to the amorphous calcium phosphate form.

Results and Discussion

Table 1 lists the experimental animals giving a description of the phase of the egg-laying cycle at time of sacrifice. Samples 14 and 15 are the estrogen treated males.

Fig. 1 shows that prior to egg shell calcification there is more amorphous bone mineral in medullary bone than during shell calcification. It appears that during the egg-laying cycle the portion of medullary bone which contains the amorphous mineral is used preferentially. The figure also shows that the crystalline properties of the medullary bone in estrogen treated males resembles the female medullary bone at the beginning of the reproductive cycle.

Table 1. Listing of pigeon medullary bone samples according to phase of egg-laying cycle. Samples 1–7: before 1st egg calcified (1–6, before 1st egg ovulated); samples 8–13: during 2nd egg calcification

Pigeon No.	Description of bird at time of death
1.	ovum 0.5 cm diameter
2.	ovum 0.6 cm diameter
3.	ovum 1.1 cm diameter
4.	ovum 1.1 cm diameter
5.	ovum 1.8 cm diameter
6.	ovum 2.3 cm diameter
7.	1st soft shelled egg in shell gland
8.	2nd egg in shell gland, soft shell
9.	2nd egg in shell gland, hard shell
10.	2nd egg in shell gland, hard shell
11.	2nd egg deposited
12.	2nd egg deposited
13.	2nd egg deposited
14.	male, estradiol valerate 8 days
15.	male, estradiol valerate 8 days

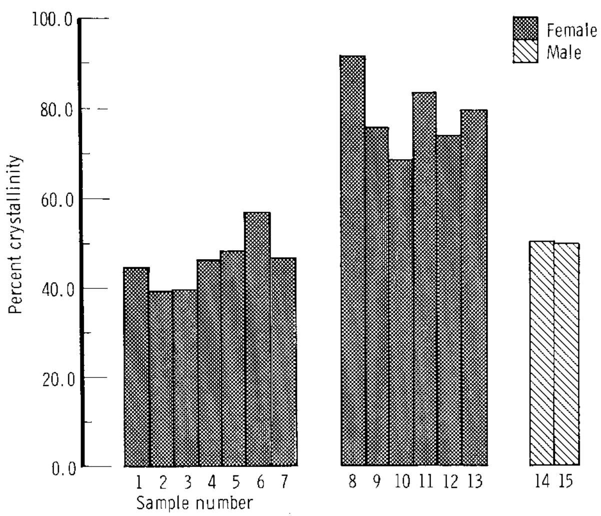

Fig. 1. Percent crystallinity of a series of medullary bone samples from the femur of pigeons taken during the egg-laying cycle (*1–13*) and from estrogen treated males (*14, 15*). The sample numbers are identified in Table 1. The average percent crystallinity for samples *1–7* (before egg is formed or calcified) is 45.8% ± S.D. 6.1; for samples *8–13* (after shell is formed is 78.7% ± S.D. 8.0. A student "t" test shows these groups to be significantly different ($P < 0.05$)

As mentioned above, younger, more recently formed bone is richer in the amorphous phase than a more mature or older bone [11]. In addition, it is well established that newly formed bone is more metabolically active than a more mature bone [12]. Thus, it seems logical in the experiment described above that the portion of bone which is richer in the amorphous phase is resorbed before the crystal-rich portion of bone. These observations provide a physical basis for explaining the high degree of lability required by medullary bone for its role in egg shell formation.

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