Effects of Weightlessness on Amphibians II. The Skeleton and Mineral Metabolism

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UDC 577.462+591.83-84

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 116, № 7, pp. 90-92, July, 1993
Original article submitted March 4, 1993

Key Words: *amphibians; skeleton; weightlessness*

In weightlessness, the skeletal system undergoes substantial alterations in both humans and animals. In particular, the resorption of osseous tissue is intensified, which is manifested in a loss of calcium by the bones and in their reduced strength [1,2,5]. Many hypotheses have been advanced purporting to explain how the mineral balance is upset under conditions of weightlessness [3,4,6]. The most plausible appears to be the one according to which the balance between the secretion of calcitonin and that of parathyroid hormone is disrupted and the piezoelectric characteristics of mechanically unloaded skeletal elements are altered. However, no comprehensive studies examining the secretory activity of C cells (which are responsible for the regulation of calcium binding in the body) and skeletal status in weightlessness have been reported.

In the preceding communication we considered the impact of weightlessness on the hormonal component of calcium metabolism in the newt *Pleurodeles waltlii*. The study described here is concerned with the impact of a two-week space flight on the morphological organization and mineral balance of skeletal elements in this amphibian species.

MATERIALS AND METHODS

In this study, 32 young adult *Pleurodeles waltlii* newts that had been on board a biosatellite (Kosmos 1887, Kosmos 2044, Bion, or Foton - a total of seven flights) were used as test animals, while 79 other newts of the same species and age served as controls (synchronous, transport, and laboratory control groups). In some test newts, limbs were removed and the retina was damaged before the space flight or immediately after it in order to impose additional loads on mineral metabolism.

In all newts, both visceral and somatic parts of the skeleton were examined, special attention being paid to the following bones or bone elements: hypobranchiale, ceratobranchiale, basibranchiale, humerus, radius, ulna, coracoideum, procoracoideum, scapula, sternum, ceratohyale, and hypohyale. After landing, the skeletal system was examined on days 0, 10-14, 23-29, 49, or 72. On these days, newts were killed and fixed in Bouin's fluid or 10% formaldehyde. The fixed material was dehydrated through 1,4-dioxane and embedded in paraffin, and serial 10 μ sections of various skeletal elements were prepared. The sections were stained with Mallory's triple stain or with hematoxylin and eosin or else were examined unstained in polarized light, utilizing the interference effect, under a Leitz Ortholux 2 Pol BK microscope.

Some newts were not fixed. Their skeletal elements were freeze-dried and dusted with carbon in order to determine skeletal composition, for which purpose a KEVEX-S100 x-ray microspectrometer (USA), installed on a Hitachi S-500 scanning electron microscope (Japan), was used. In these determinations, 30 to 40 point measurements were made after precalibrating the signal. The pro-

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0007-4888/93/0007-0877 $12.50

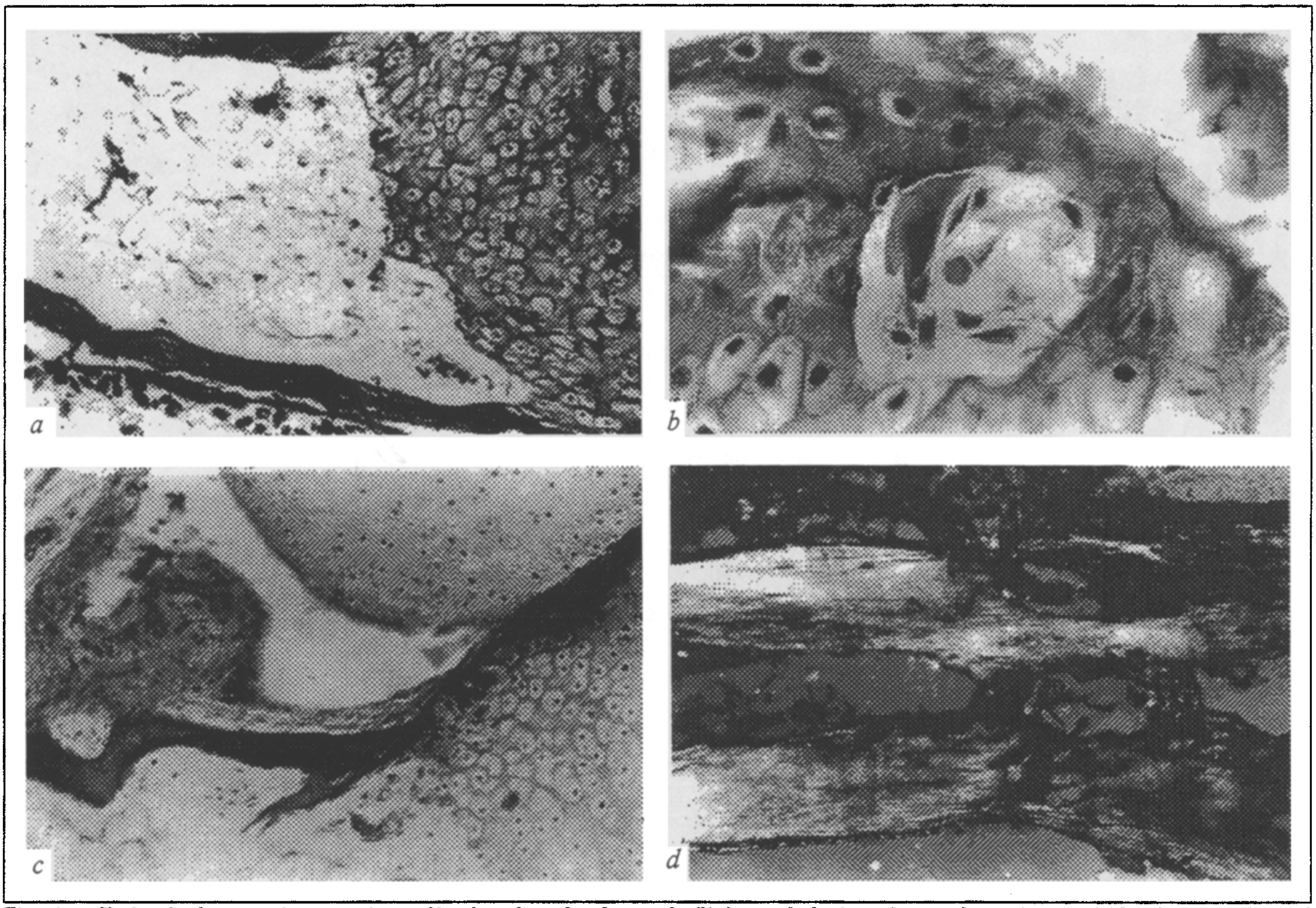

Fig. 1. Skeletal changes in newt immediately after the 2–week flight and during the readaptation period. *a*) resorption of cartilage and endochondral trabeculae in the humerus of a newt fixed just after landing; *b*) osteoclast within the diaphysis of the radius of a newt fixed just after landing; *c*) ossification centers have appeared in the articulating ends of the humerus during the readaptation period; *d*) interference of the humerus in polarized light, showing matrix fibers atypically arranged around the areas regenerating during the readaptation period. Mallory's triple stain. *a* and *c*) ×70; *b* and *d*) ×140.

portions of various chemical elements in the bones of both test and control newts were estimated.

RESULTS

The greatest changes under weightless conditions were found to be undergone by skeletal elements of the limbs and by the visceral skeleton.

In intact newts of the synchronous and laboratory control groups, endochondral bone formation occurred at the site of the cartilaginous humeral bone. In these animals, active penetration of vessels into cartilage resorption areas was observed together with the formation of endochondral trabeculae and perichondrial bone within the diaphysis, and osteoclasts were seen around the cartilaginous portions of the humeral bone. The axial skeleton was completely osseous and no signs of osteoporosis were in evidence. The visceral skeleton remained cartilaginous in most newts; initial endochondral ossification was detectable only occasionally.

In intact test newts after the 2-week flight, resorption of both osseous and cartilaginous skeletal elements had occurred, ossification of the limb skeleton was impaired, and the mineral composition of bones was altered. In the humeral, ulnar, and radial bones, cartilage and trabeculae of endochondral bone were in a process of active resorption after landing (Fig. 1, *a*). The humerus was represented only by a thin bony perichondrial band and small areas of cartilage within the epiphyses. As a result of osteoporosis, considerable portions of perichondrial bone and of the articulating ends of cartilaginous areas were destroyed in the limb skeleton in 35% of the newts. In the terrestrial control groups, none of these phenomena were detectable. Intact animals exposed to weightlessness also had large numbers of osteoclasts with a conspicuous brush border, but, unlike in the controls, such cells were present both around resorbing cartilages and around trabecular remnants of endochondral bone (Fig. 1, *b*). Under weightless conditions, chondrocyte proliferation became impaired and the appositional growth of cartilage ceased, as was indicated by the presence of binucleate chondrocytes in cartilage lacunae and the accumulation of young flattened chondrocytes. In the visceral skeleton, osteoporosis extended to large areas of the hyoid portion of the visceral skeleton, so that the diaphyseal zone of cartilages was completely destroyed in some newts and the cartilages could only be identified by noting traces of perichondrium and densely packed mesenchymal cells. The axial skeleton was changed to a much lesser degree than the visceral or the skeleton of the limbs. The small areas of resorption that were seen did not compromise bone integrity, although their presence indicated that osteoporosis had started to develop.

Examination of the axial skeleton in the x-ray microspectrometer revealed that, on average, 11% of its minerals were lost during the 2-week space flight, but the loss of different minerals was disproportionate. For example, the amounts of calcium and sulfur decreased by 7%, while that of phosphorus only by 3% as compared to the controls. On the other hand, the content of potassium increased by an average of 6%. Similar changes occurred in the mineral composition of what remained of the visceral skeleton and limb skeleton.

The postflight readaptation period was characterized by a relatively slow skeletal restoration. In the course of the first two postflight weeks, osteoclasts were not encountered around perichondrial portions of the limb bones or of the axial skeleton, but were present in cartilaginous skeletal areas. Two weeks later, newly formed trabeculae were in evidence and ossification started. Full skeletal regeneration never occurred, however. On day 72 postflight, the articulating ends of the limb bones showed ossification centers (Fig. 1, *c*), which are usually not observed in lower vertebrates. In the regenerated zones, atypical layers of osseous tissue had formed. Examination of matrix organization in polarized light demonstrated atypically arranged collagen fibers in the regenerated areas (Fig. 1, *d*). It should be noted that in newts injured before the flight, the bones were demineralized by as much as 21% compared with 11% in intact test animals. In newts injured immediately after the flight, osteoporosis continued for 2 subsequent weeks.

To summarize, osteoporosis and demineralization of the skeleton started to develop in newts during their 2-week space flight. The greatest pathological changes were undergone by cartilages of the visceral skeleton and by limb bones. Chondrocyte proliferation was impaired, and osteoclasts were activated, resulting in resorption of trabeculae in the limb bones. The mineral balance in the bones was upset, leading to losses of calcium, phosphorus, and sulfur and to accumulation of potassium in cartilage and bone matrices. The skeleton failed to regenerate completely during the readaptation period; the regenerated bones had an atypical lamellar structure.

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