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Effects of Weightlessness on Amphibians
I. The Ultimobranchial Body

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This is the first in a series of articles describing our studies, extending over many years, into the effects of short-term space flights on various amphibian organs and systems, as exemplified by the newt *Pleurodele waltlii*.

During a space flight, anemia develops and signs of skeletal demineralization appear both in astronauts and in experimental animals [4,5]. These abnormalities may be caused by disorders of calcium and phosphorus metabolism at the cellular, tissue, organic, and/or systemic levels. The most important role in the regulation of mineral metabolism is played by the synthesis of calcitonin, a hormone that controls the mechanism of calcium binding in vertebrates. In mammals, including man, calcitonin-secreting cells (calcitoninocytes, or C cells) become incorporated into the thyroid gland during early stages of development [3] to be diffusely arranged among the thyroid follicles, which complicates their quantitative evaluation and makes impossible the analysis of nondifferentiated cells. Attempts at postflight analysis of C cells in mammals have failed to produce unequivocal results, although their secretory activity has been shown to deviate from normal [6]. To assess the impact of weightlessness on calcitonin secretion, amphibians were used as test animals [2]. The ultimobranchial body or gland (ULT) in amphibians is a distinct organ that is anatomically separated from the thyroid gland and is largely composed of C cells aggregated into follicles and surrounded by a dense network of capillaries arising from the arterial arch adjacent to the ULT. This gland contains both secretory and nonsecretory cells. Being an anatomically separate and asymmetrical organ that comprises cells in various phases of differentiation, the amphibian ULT appears to be an optimal model for studying changes in calcitonin secretion brought about by weightlessness.

The purpose of the present study was to examine morphological changes in the ULT of the newt *Pleurodeles waltlii* after a space flight and in particular during the readaptation period.

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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 to-

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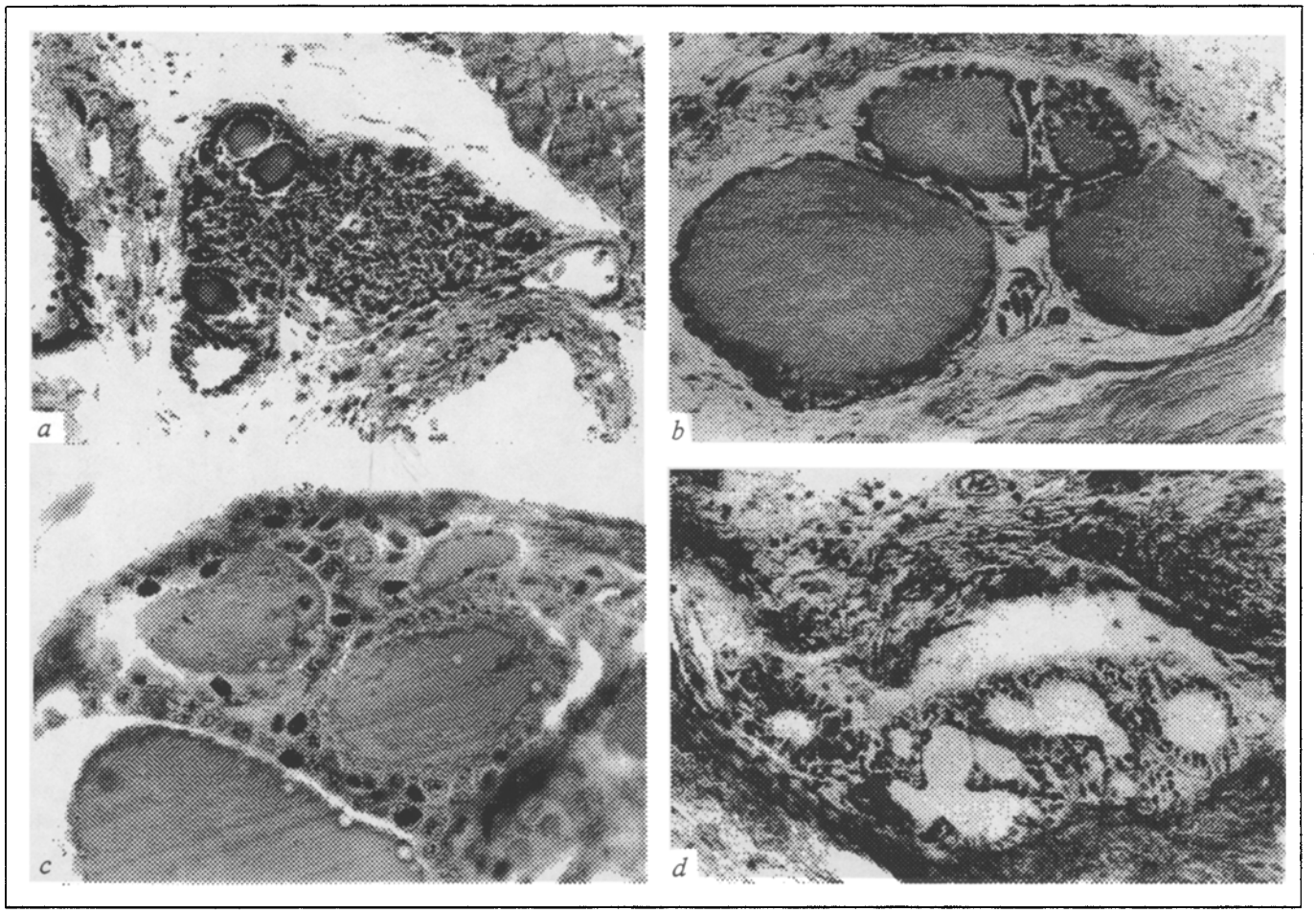

Fig. 1. Morphological organization of the ultimobranchial body (ULT) in the newt *Pleurodeles waltlii*. *a*) ULT of a control newt; *b*) hypertrophic ULT of an intact test newt after landing; *c*) death of C cells in follicular wall of ULT from an intact newt after landing; *d*) ULT with empty follicles taken after landing from a newt injured before the flight, Mallory's triple stain. *a*, *b*, and *d*) ×140; *c*) ×280.

tal of seven space flights) were used as test animals; another 79 newts of the same species and age served as controls. The newts were killed immediately after landing or after days 10-14, 23-28, 49, and 72. In some animals, limbs were removed and the retina was damaged before the flight in order to impose additional loads on mineral metabolism.

The ULT of newts does not readily lend itself to dissection because of its small size (500-1300 μ) and its position between the pericardial and peritoneal cavities. For this reason, the entire central portion of the pharynx was dissected out. It was then fixed in Bouin's fluid, 10% formaldehyde, or in Bouin's fluid modified for immunohistochemical analysis. The fixed material was dehydrated through 1,4-dioxane and embedded in paraffin, and serial sections 10 μ in thickness were prepared and subjected to various treatments including staining by Mallory's triple stain, impregnation with silver after Grimelius, Brachet's reactions, or an indirect immunofluorescence assay using an anti-calcitonin monoclonal antibody [3]. The preparations thus treated were examined and photographed in Ortholux 2POL BK (Leitz) and Lyumam I-3 microscopes, and follicles, dying cells, and calcitonin-secreting cells were counted.

RESULTS

In the ULT from control animals, not more than 5 to 10% of cells were secreting calcitonin. The ULT of the *Pleurodeles waltlii* newt usually consists of 15 to 18 follicles each containing 10 to 70 cells. Secretory cells occur in only 1 or 2 follicles that contain a colloid marking calcitonin secretion (Fig. 1, *a*). ULT in such a state were encountered in newts that had not been injured before the flight. After a 14-day flight, the intact newts were fixed at the site of landing, and the histological study of ULT from these animals showed that virtually all cells of the follicular walls had been secreting calcitonin (Fig. 1, *b*).

The postflight immunohistochemical study indicated the presence of calcitonin in the follicular

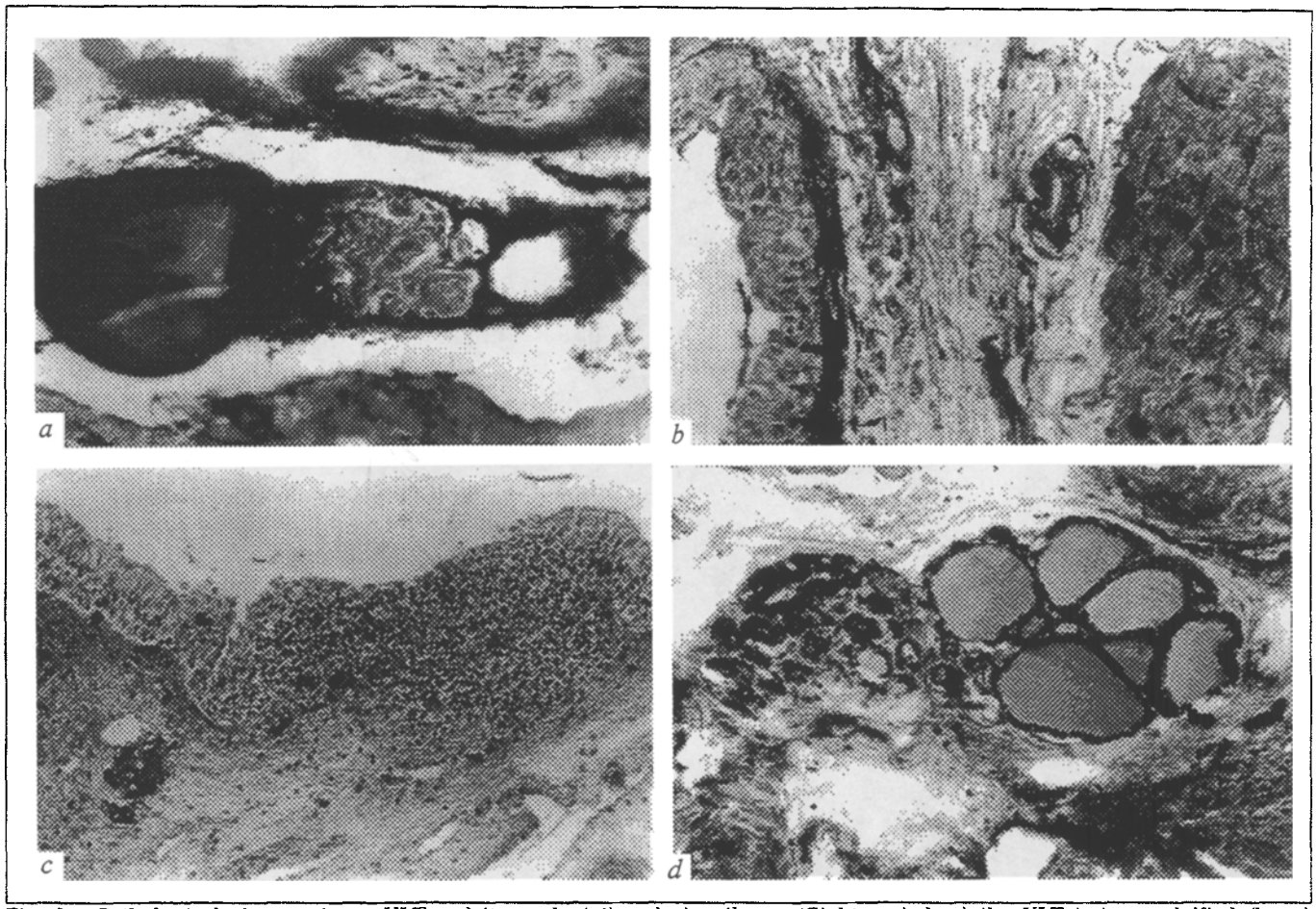

Fig. 2. Pathological changes in an ULT and its readaptation during the postflight period. *a*) the ULT is in a calcified (bony) capsule after landing; *b*) secondary follicle laid down after landing; *c*) impaired basement membrane and migration of pharyngeal epithelial cells in the area of the ULT; *d*) ULT with hypertrophic and nondifferentiated follicles at the end of readaptation (day 72 after landing). Mallory's triple stain. ×140.

colloid and the cytoplasm of secretory cells in intact animals. This finding is important, since C cells may secrete a calcitonin-bound peptide that does not influence calcium metabolism. Active calcitonin secretion was accompanied by massive death of C cells in the follicular walls (Fig. 1, *c*). In intact newts that had been in flight for 10 to 14 days, 40 to 60% of all C cells died, although the ULT had not undergone complete degeneration. The follicles that remained were hypertrophic, and the local cell death led to the establishment of direct contacts between the colloid and the capillary wall. Calcitonin was retained in most of the hypertrophic ULT, where it accumulated in follicles and interfollicular spaces without being transported to its target organs.

Most ULT from newts operated upon (injured) before the space flight were in a state of degeneration and had empty follicles; hypertrophic follicles were seen in only 40% of ULT from such animals. C-cell mortality was of the apoptoid type in most instances, although chromatolysis was also often observed, as were nonspecific types of death in which cases the cytoplasmic membrane became disintegrated and the cellular contents were discharged into the follicular lumen. The nuclear membrane was preserved in the colloidal contents of the follicular lumen so that nuclei freely lying within the ULT secretion could be seen. It should be stressed that although the ULT was degenerated or hypertrophic, the number of capillaries filled with blood cells was normal in both intact and operated newts. In 12% of the operated newts, a bony capsule investing the ULT was noted (Fig. 2, *a*). The bony capsule formed as a result of the connective tissue metaplasia and calcification under the action of calcitonin present in abnormally high concentrations around the ULT.

The recovery period after landing proceeded in two stages. In the first stage, the morphological structure of the ULT underwent restitution. The ULT of tailed amphibia is devoid of its own proliferation zone and is restored through the laying down of new follicles by cells of the pharyngeal

epithelium (Fig. 2, *b* and *d*). The walls of follicles that had not yet differentiated contained lipids which were undetectable in the ULT from control animals or in those undergoing normal development. In addition to lipids, the walls of young follicles were found to contain multinucleate calcitoninocytes, which may be regarded as an abnormality resulting from exposure to weightlessness. It should be mentioned that the basement membrane of the pharyngeal epithelium was altered in most newts after landing and that active migration of cells occurred through the altered membrane toward the ULT (Fig. 2, *c*), but signs of its restoration were not yet in evidence. It was only during the readaptation period that nondifferentiated cell accumulations were observed in the ULT (Fig. 2, *d*). The number of cells contained in ULT amounted to 20-60% of their normal number in intact newts, but only to 30% at most in operated ones. The first stage of ULT regeneration lasted for 14 to 18 days in both intact and operated animals. It was also found that in newts with a largely or completely degenerated ULT, regenerative processes could proceed, with an equal probability, on the opposite side of the pharyngeal epithelium. In four cases, for example, remnants of the primary ULT were present together with a new group of follicles on the other side of this epithelium.

The first stage of morphological regeneration was succeeded by one of functional recovery lasting 50 to 60 days. In ULT where active calcitonin secretion started when new follicles had been laid down, the number of colloid-containing follicles decreased after 1 to 1.5 months; 72 days after landing, the undifferentiated cells were not secreting calcitonin any longer (Fig. 2, *d*).

The results of these experiments suggest that ULT responses to weightlessness may be of two types, differing in the magnitude of pathological changes. The first type is exhibited by intact animals, in which nonsecretory C cells also begin secreting calcitonin under weightless conditions. Most such cells degenerated during the 14-day flight. However, since no more than 10% of the cells are involved in calcitonin secretion in terrestrial environments, readaptation occurred through the regeneration of ULT cells (Fig. 1). The second type of response to weightlessness was displayed by injured newts. In such animals, massive involvement of C cells in calcitonin secretion occurred even before the space flight. During it, their ULT became hypertrophic, as did those of intact animals, but the additional stimulation of the ULT by regenerative processes led to degeneration of its follicles, calcification of the gland, and to the initiation of abnormal regenerative processes. During the postflight period, follicles were regenerating by processes similar to those proceeding during ULT development in embryogeny.

In summary, the newt ULT becomes hypertrophic and abundant calcitonin is secreted by its cells under weightless conditions; however, retention of the secreted calcitonin may result in calcification of the ULT and prevent this hormone from being transported to its target organs, which is likely to be a specific effect of weightlessness. During the readaptation period after landing, the ULT undergoes regeneration that resembles its development during embryogeny.

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