organ do indeed turnover. In our experiments so far we have observed that not only the cells of the taste organ turnover but that associate and sensory cells turnover at a different rate. The exact timing of the phenomenon for the 2 cells population will be furtherly elaborated in a subsequent report.

Conclusions. 1. Two morphologically-distinct types of cells exist in frog's taste organs, only one of which has direct functional involvement with the afferent nerve fibre.

2. The described sensory cells with their dendrite-like processes are typical of the frog and have not yet been described in any other animal. The authors believe that these processes are the receptive part of the sensory cell and that the tight junctional complexes observed between the distal parts of these dendrites should be furtherly investigated in regard to the possibility that it may subserve some form of interreceptor interaction.

3. The presence of typical synaptic contacts between nerve fibres and sensory cells shown by accumulation of dark core vesicles and mitochondria on the presynaptic membrane as well as membrane thickenings is taken as evidence for the existence of chemical transmission between the sensory cells and the sensory fibres. The data obtained through the outlined histochemical procedures, points to norepinephrine as the neurotransmitter involved in the sensory process of frog's taste organ[15].

Résumé. Dans les bourgeons gustatifs de *Rana pipiens* deux groupes de cellules ont été découverts. Le premier est directement en relation avec la réception de stimuli, tandis que le second ne comprend que des cellules d'association. Les cellules sensorielles offrent des contacts synaptiques avec les fibres sensorielles.

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Patterning of Resorption in the Cartilages from the Long Bones of Newts Treated with Parathyroid Extract[1]

Gross morphological studies of skeletal elements in *Rana pipiens* and *Triturus viridescens* treated with parathyroid extract have shown that there is an increase in the amount of matrix in the long bones which can be stained with methylene blue[2]. Widened and more densely staining matrix was observed at the ends of the tibiofibula, and the phalanges exhibited double bands of matrix between the articular cartilaginous cap and the ossified diaphyseal bone in the majority of animals after hormone treatment (Figure 1). When new bands of matrix appeared beneath the epiphyseal cap, they lay in a region topographically comparable to the zone of provisional calcification in mammalian long bone (= metaphysis). The types of cells which may be responsible for these changes in the bone matrix of hormone-treated amphibians are undetermined. Although SCHLUMBERGER and BERK[3] were unable to elicit a hormone-mediated increase of osteoclasts in bone of *R. pipiens*, other laboratories[4,5] have described elevated osteoclast numbers in several amphibian species as a direct or indirect (calcium-free lavage)[6] consequence of parathormone action. The exact nature of the 'metaphyseal' band of stainable matrix formed after hormone treatment is, however, unknown. The present study was, therefore, undertaken to investigate the origin of this tissue in the newt, *T. viridescens*, employing histochemical techniques.

Materials and methods. The majority of animals used in these studies were *T. viridescens viridescens*, but in a few cases, the closely related *T. viridescens dorsalis* were employed. All experiments were done with summer animals which were warm-acclimated in the laboratory at 18–22 °C for 1 week before use, in order to eliminate seasonal and temperature modifications of the response to parathormone. Newts were maintained in individual containers and environmental water was changed daily; calf liver was force fed once weekly. To avoid unfavorable changes due to conditions of captivity, no animals were used beyond 1 month after arrival in the laboratory.

The 104 *T. viridescens v.* used in this study weighed 1.0–4.5 g, and they were randomly placed into control and parathyroid extract-treated groups.

The experimental animals were injected i.p. with parathyroid extract (PTE) once daily for 7 days. Newts weighing over 3.0 g received 15 U; those with body

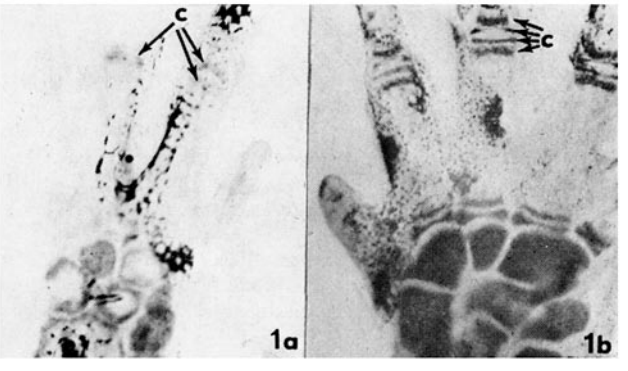

Fig. 1. Micrographs of appendages from control (1a) and PTE-treated newts (1b). Note the increased staining density and doubling of the carpal cartilages (c) in the PTE-treated animal.

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Fig. 2. Photomicrograph of the epiphysis of the metatarsal from a normal untreated newt. PAS and Alcian blue. ×111.

weights less than 3.0 g received 10 U. Within 2 min after injection, jerking movements were observed in limbs, tail, and jaws. These uncoordinated responses, which subsided within 2 h, may be related to a transient hypocalcemia owing to the injected protein load rather than representing a physiological response to volume pressure; control newts injected with an equal volume of amphibian Ringer's solution failed to show such movements. Groups of 2–6 newts from both the control and PTE-treated series were sacrificed at 1, 2, 4, 8, 14 and 21 days by ether anaesthesia. Cumulative doses of PTE administered to each newt, then, ranged from 10–20 to 105–140 U. At autopsy, the hindlimbs were removed and fixed in 10% neutral formalin. The tissues were decalcified in 10% ethylenediaminetetraacetic acid (EDTA, pH 7.4), embedded in paraffin and sectioned at 5 µm along the long axis of the metatarsals and phalanges. Some sections were stained with hematoxylin and eosin and PAS + Alcian blue.

Results. Control newts. The normal appearance of the cartilaginous epiphyseal caps in the foot bones from control newts is shown in Figure 2. The cells are rounded and dispersed in 2 major zones of the matrix which are distinguished on the basis of their staining characteristics. The more basophilic region adjacent to the diaphysis houses the more mature hypertrophic chondrocytes and is presumably that region which is calcified. The diaphyseal surfaces of the cartilage are generally smooth, save for a few contiguous trabeculae formed by endochondral ossification, and in some instances their diaphyseal surfaces were overlain by a thin veneer of lamellar bone. Few cells lined the endosteal surfaces of the shaft,

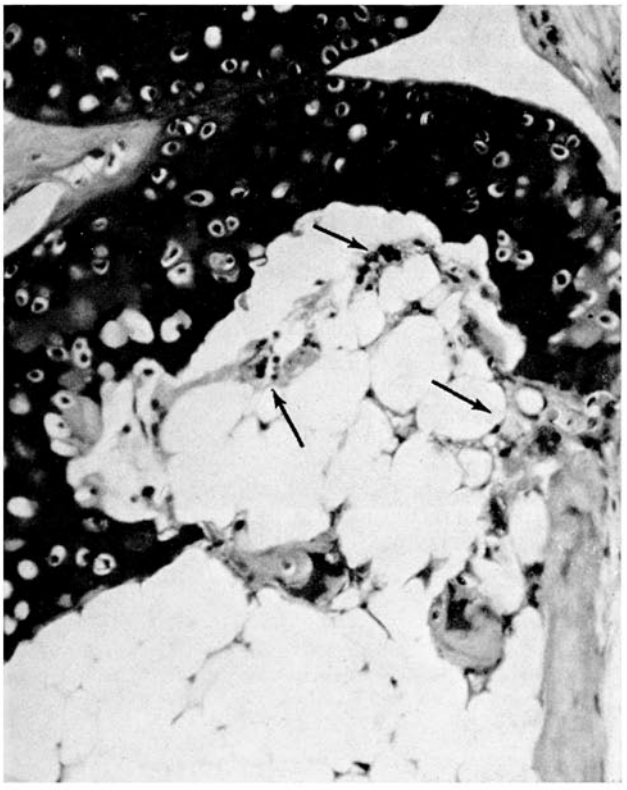

Fig. 3. Photomicrograph of the distal end of a tibiobibula from a newt sacrificed 14 days after PTE treatment (7 injections at 24 h intervals) showing large numbers of resorptive multinucleated chondroclasts (arrows) resorbing the cartilage. PAS and Alcian blue. ×111.

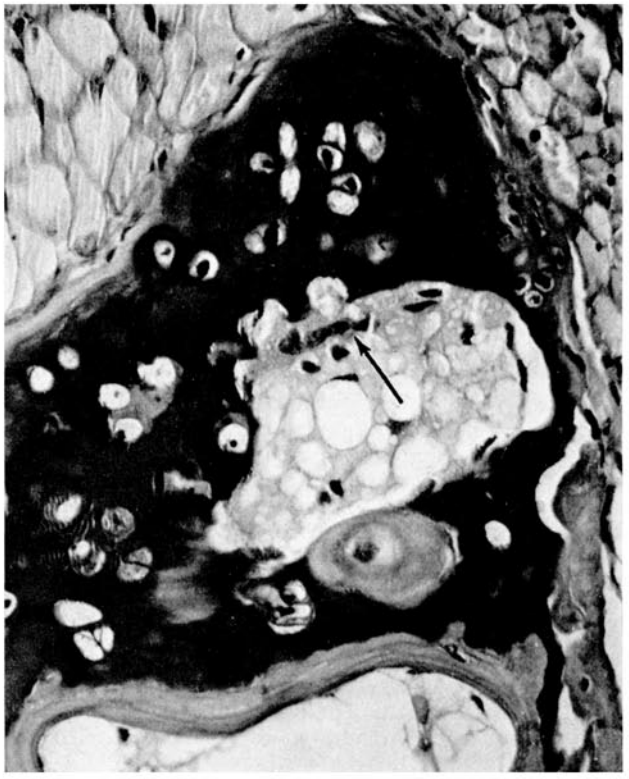

Fig. 4. Photomicrograph of the proximal cartilage in the metatarsal of a newt sacrificed 14 days after PTE treatment (7 injections at 24 h intervals). Note the presence of a resorptive cavity within the cartilage showing a large multinucleated chondroclast (arrow). PAS and Alcian blue. ×111.

and these could not be identified as histotypical osteoblasts or osteoclasts. This pattern was typical of femoral, tibiofibular, metatarsal, and phalangeal cartilages.

PTE-treated newts. After PTE-treatment, there was a marked increase in the numbers of osteoclasts throughout the skeletons of the newts. Yet the pattern and timing of resorption in the different bone was variable and restricted almost entirely to the cartilaginous ends. The earliest changes were observed in the carpals. Serial sections revealed numerous foci of resorption after 24 to 48 h, but histotypical multinucleated osteoclasts were not invariably present within the spaces. Changes in the long bones of the foot were first evident at 14 days. Resorptive cells eroded the cartilages centrifugally, leaving a narrow zone of tissue adhering to the endosteum as the bones grew in length (Figure 3). In the metatarsals and phalanges, however, chondroclasts frequently dissected away a part of the calcified zone of cartilage, and the spaces were filled with loose connective tissue (Figure 4). In the bones most severely affected, the epiphysis was effectively amputated. Fibrotic changes in the marrow were never observed during PTE treatment, and they were rare even after 14–21 days. At no time did PTE induce cortical bone remodelling.

Discussion. The results of this study appear to explain the curious staining properties with methylene blue observed in cleared whole mounts of bone from PTE-treated anurans and urodeles described by one of us[2]. They are all consequences of hormone-induced resorptive activity. While this process in the tibiofibula primarily thins the cartilages, it leaves a shell of matrix laterally at the epiphyseal-diaphyseal junction, and under conditions of optimum cartilage growth, gross specimens stained for cartilage would appear to have thicker epiphyses than normal. The peculiar mode of intracartilaginous matrix resorption mediated by chondroclasts and subsequent connective tissue formation in the epiphyses of the metatarsals and phalanges could produce double bands of stainable matrix. All of these findings are consistent with the observation of increased numbers of resorptive cells in amphibian bone following the administration of parathyroid extract[4,5]. Although resorptive osteoclasts were apparent within 48 h after initiating PTE therapy, their origin is in question. In parallel studies with a pulse label of ^{3}HTdr (unpublished observations), labeling of marrow elements did not occur until the 3rd day after injection, and osteoclasts were not labeled until the 7th day[7].

Résumé. L'extrait parathyroïdien a augmenté le nombre des ostéoclastes dans tous les os des jambes des salamandres *Triturus sp.* Cependant, dans ces éléments, les modèles résorptifs et le moment de leur apparition sont variables. Ces modèles de résorption expliquent leur apparition charactéristique dans les préparations teintées graduellement, en particulier lors du redoublement des cartilages terminaux des phalanges.

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Kynurenine Localization in the Egg of *Drosophila melanogaster*

Studies on the genetics of eye color in *Drosophila* have demonstrated that the pathway of xanthommatin synthesis involves tryptophan, kynurenine and hydroxykynurenine (see[1] for a review). The mutation vermilion is known to block the formation of kynurenine from tryptophan via formylkynurenine and the mutant cinnabar the formation of hydroxykynurenine from kynurenine[1]. These two mutants therefore can provide convenient tools for the study of kynurenine metabolism during the life cycle and in different organs of the fruit fly. The accumulation of kynurenine in the larval fat body of *Drosophila*[2–5] and its presence in the Malpighian tubules has been clearly documented[6–9]. Earlier reports on the presence of kynurenine in the ovary[10] and eggs of *Drosophila*[11] suggested that a study of the ovaries combining chromatography and fluorescence microscopy should reveal the location of kynurenine in the developing and mature egg. This study provides such a demonstration.

Materials and methods. Descending paper chromatograms of ovaries were prepared by squashing the ovaries from a 2-day-old fly on Whatman No. 1 filter paper. Solvent systems used consisted of *n*-butanol:acetic acid:water (20:3:7) or *n*-propanol:1% ammonia (2:1).

For studies with the fluorescence microscope ovaries were prepared in one of two ways: 1. They were dissected out of 2-day-old flies in *Drosophila* Ringer's solution and smeared on a slide with a drop of fluid so that individual ovarioles and eggs could be viewed. Preparations were then air dried and viewed uncovered or mounted in Zeiss mounting medium ($L25 - n_D = 1.525$) or paraffin oil. 2. Some ovaries were dissected out and the egg chambers separated in paraffin oil on a slide.

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