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# RECEPTOR CELL RENEWAL IN THE SENSORY EPITHELIA OF THE LIP OF THE SNAIL HELIX POMATIA L.

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## Abstract

$^3$H-thymidine incorporation was investigated in the sensory epithelia of the lips of *Helix pomatia* by light microscopic autoradiography. Labelled cells were observed among the epithelial cells as well as in the sensory lobules. After a short survival time following the $^3$H-timidine injection only heavily labelled cells could be detected. The heavily labelled cells in the receptor cell areas were always located at the periphery of the sensory lobules. After a long survival time the lightly labelled cells appeared and became dominant; they were always observed among the sensory cells in the sensory lobules. The number of labelled cells increased during short survival times (30 min, 4h) but later a continuous decrease was observed. This demonstrates a slow but continuous renewal and maturation of the sensory and epithelial cells in the sensory epithelia of the snail *Helix pomatia*.
Key words: *Helix pomatia*, lip, sensory epithelia, receptor cells

## Introduction

Gastropods receptor areas such as the lips and body wall seem to have the general ability of both chemo- and mechano-reception (SCHULTZ, 1938; KIECKEBUSH, 1953; KITTEL, 1956; STEPHENSON, 1979; CROLL and CHASE, 1980; CHASE and CROLL, 1981; CHASE, 1982; HERNÁDI et al., 1984; KEMENES et al., 1985.) According to the Golgi impregnation studies, the receptor areas of the body wall are densely innervated by primary sensory neurons (SCHULTZ, 1938; DEMAL, 1955; HERNÁDI, 1982). The ultrastructural studies have concentrated on sensory dendrites to establish a relationship between the sensory dendrites with different ultrastructural characteristics and the different receptor modalities. Numerous types of dendrites could be separated on the basis of their fine structural characteristics, and different receptor modalities have been correlated with them (ZYLSTRA, 1972; WRIGHT, 1974; WONDRAK, 1975; KATAOKA, 1976; BENEDECZKY, 1977, 1979; CROLL, 1983). The sensory dendrites in the sensory epithelia of the tentacles, the lips and the foot of *Helix pomatia* were classified into a series of transitional forms on the basis of their fine structural characteristics (the number of cilia and microvilli, the length of the roots of cilia, the width of the apical dendritic surface, the density of the dendritic cytoplasm). These transitional forms spread from the microvillous dendrites with centrioles through the dendrites possessing 1—2 cilia and microvilli with centrioles in their apical parts, to the

dendrites possessing numerous cilia on their apical surfaces (HERNÁDI and BENEDECZKY, 1978, 1983). It was supposed that this great variation in the structural appearance of the sensory dendrites could be explained by a renewal process of the primary sensory neurons (HERNÁDI and BENEDECZKY, 1978, 1983; HERNÁDI, 1981 a, b, TOTARO et al., 1984) similar to that in the vertebrate olfactory epithelia (GRAZIADEI and METCALF, 1971; MOULTON, 1974; HARDING et al., 1977; GRAZIADEI and MONTI GRAZIADEI, 1979). The aim of the present study was to demonstrate mitotic elements in the sensory lobules of the lips by applying light microscopical $^3$H-thymidine autoradiography and in this way to prove the sensory cell renewal in the sensory epithelia of *Helix pomatia*.

## Materials and Methods

Adult specimens of *Helix pomatia* were used for the experiments. 15 μCi $^3$H-thymidine/gr body weight was injected into the body cavity of each animal diluted in 250 μl ringer solution. The animals were sacrificed at 30 min, 4h, 1 day, 1 week, 2 weeks, and 1 month survival time following the injection. The lips were excised and fixed in 2.5% glutaraldehyde buffered with 0.1 M sodium cacodylate buffer (pH: 7.2) for 4h at 4 °C. After a short wash in the buffer the samples were postfixed in 1% $OsO_4$ buffered with 0.1 M s-collidine (pH: 7.4) for 2h at 4 °C. After fixation the samples were dehydrated through increasing concentration of ethanol and were embedded into Spurr media through propileneoxid. Serial sections consisting of 8—10 1μm thick cross sections containing the whole width of the sensory epithelia were cut and dried on slides and counterstained with toluidine blue. The sections were covered with Ilford L4 emulsion. After two weeks exposition the slides were developed with Kodak D—19 developer. The cells were considered to be labelled if there were at least 5 grain over their nucleus.

## Results

In the 1 μm thick toluidine blue stained cross sections the lobular organization of the receptor cells as well as the typical structure of the lips are clearly visible (Fig. 1.). The different cellular elements can be separated on the basis of their typical morphological characteristics (e. g. diameter and the heterochromatin pattern of the nucleus). Labelled cells can be observed in the sensory lobules and among the epithelial cells. Nonsensory neuronal elements do not have grains over their nuclei. After a short survival time (30 min) only heavily labelled cells can be detected with numerous silver grains over their nuclei. These cells can be observed usually as pairs both among the epithelial cells located on the basal lamina (Fig. 2) and in the region of the sensory lobulus (Fig. 3). The heavily labelled cells are usually located at the periphery of the lobules (Fig. 3). Of the 6—8 lobules in the investigated section only 1—2 contained heavily labelled cell pairs. After a longer survival time (4h) the number of heavily labelled cells increases and lightly labelled cells begin to appear among the sensory cells in the lobulus. These lightly labelled cells have only 5—10 silver grains over their nucleus (Fig. 4). At short survival times (30 min to 4h) the heavily labelled cells are dominant, but after a longer survival time (1 day) the number of lightly labelled cells inreases, and these become dominant. Heavily

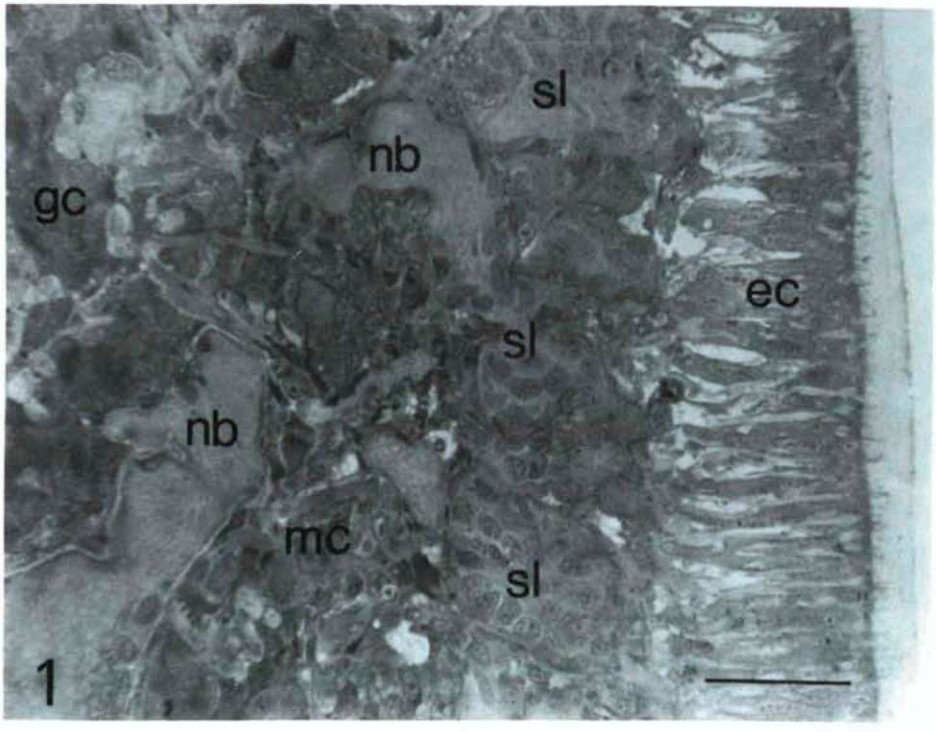
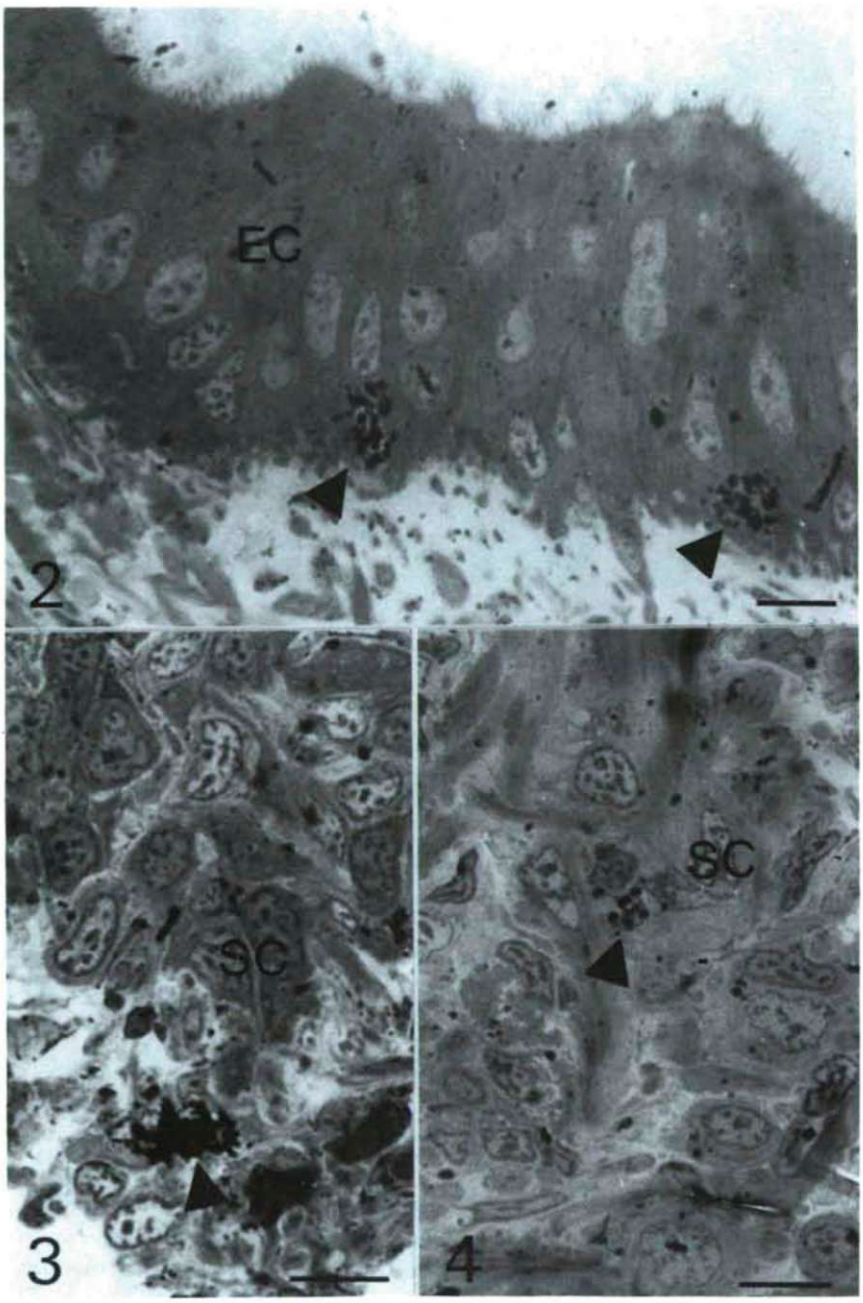



Fig. 1. The semithin section of the lip demonstrates the typical organization of the sensory epithelium. Under the epithelial cell layer (ec) sensory lobules (sl) are located. The nerve branches (nb) of the medial lip nerve reach the sensory lobules. Under the sensory lobules muscle cells (mc) and gland cells (gc) can be seen. scale bar: 50 μm

labelled cells, however can be observed even after 30 day survival but only scarcely among both the sensory and the epithelial cells. The number of labelled cells decreases in time. By the 30th day their number is about 60% of that observed at 4h survival. At this time only one lightly labelled cell can be detected in the cross section.

## Discussion

According to the $^{3}$H-thymidine autoradiography numerous mitotic elements can be observed both among the epithelial cells and the sensory neurons in the sensory epithelia of the lips. Our findings show that the sensory neurons originate from stem cells that undergo a slow mitotic process producing heavily labelled cell pairs that undergo a second mitosis which produces the lightly labelled cells. Following the $^{3}$H-thymidine injection, the number of heavily labelled cells


EC
2
SC
3
SC
4

increased; in time, however, all of the labelled cells decreased in number. Therefore, the mass of primary sensory neurons in snails undergoes a spontaneous and continuous process of renewal and maturation, which last from mitosis to neuronal death. Similar findings were demonstrated recently in the tentacles of *Achatina fulica* (CHASE and RIELING, 1986). According to these observations, the primary sensory neurons in the snail sensory epithelia behave similarly to the primary olfactory sensory neurons in vertebrate olfactory epithelia (GRAZIADEI and METCALF, 1971; MOULTON, 1974; GRAZIADEI and MONTI GRAZIADEI, 1979). This maturation process can explain the previously described great structural variety of the primary sensory neurons in the sensory epithelia of the *Helix* tentacles and lips (HERNÁDI and BENEDECZKY, 1978, 1983, HERNÁDI 1981).

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Fig. 2. At short survival time (30 min) heavily labelled cell pairs (triangles) can be seen among the epithelial cells (EC). scale bar: 10 μm

Fig. 3. At the periphery of the sensory lobules heavily labelled cell pair (triangle) can be detected after a short survival time (30 min) following the $^3$H-timidine injection. SC: sensory cell, scale bar: 10 μm

Fig. 4. At long survival time (1 day) lightly labelled cell (triangle) can be seen among the sensory cells (SC). scale bar: 10 μm

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