Pigment formation in sensory cells of *Aplysia l.*[1]

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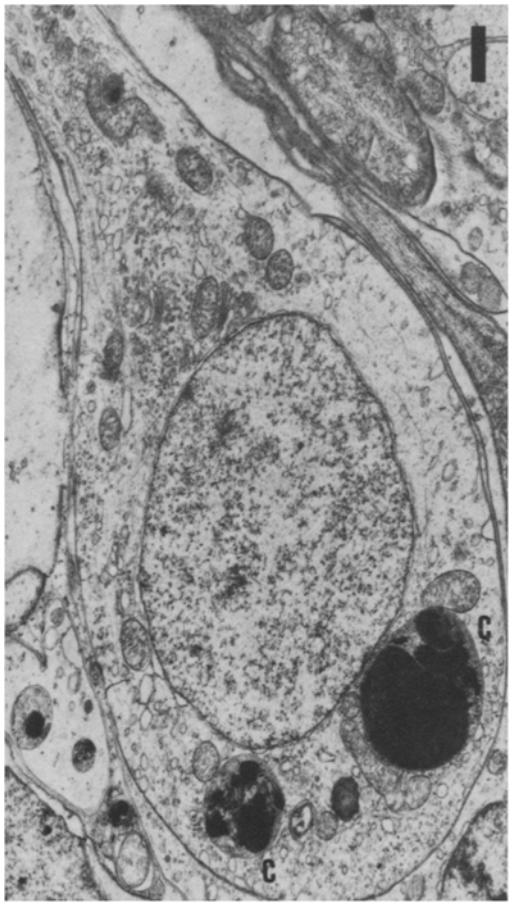
Summary. The yellow-brown pigment present in the sensory cells of *Aplysia limacina* was studied using light and electron microscopy. The ultrastructure, the high carotenoid content and the presence in neurons for which a turnover process has been hypothesized, indicate that these pigments are cytosomes, organelles involved in the production of energy in anaerobiosis.

Carotenoid-rich pigment granules have been observed in many molluscs[2]; they have been described as lysosomes[3], neurosecretory granules[4], and lipofuscin, accumulation material involved in cell aging[5]. Nolte[6] was the first to suggest that these granules were cytosomes, and later Zs.-Nagy[7] found a correlation between the degree of pigmentation and the marked ability of bivalve molluscs to survive anoxic conditions. It has been supposed[8] that in the cytosomes there is an 'anoxic endogenous oxidation mechanism' in which the function of terminal electron acceptor, normally performed by oxygen, is performed under anoxic conditions by an electron acceptor inside the pigment deposits. Zs.-Nagy[8] suggested that this acceptor could be the unsaturated fatty acids of the cytosomal lipids. This hypothesis was supported by the findings[9] that a dramatic reduction of the content of cytosomal fatty acids could be shown when tolerance to anoxia comes to an end.

The primary sensory nerve cells of vertebrates are peculiar in that they are the only nerve cells to undergo a continuous turnover[10–14]; this phenomenon has been hypothesized also in invertebrates[15], although support is still lacking. It has been demonstrated[14,16,17] that sensory cells contain pigment which is believed to be lipofuscin and is therefore considered as an index of cell aging. In the sensory cells of the foot of *Helix pomatia* yellow-brown granules have been described both as lipofuscin and as cytosomes[15]. Since the ganglion cells of *Aplysia limacina* contain both lipofuscin and cytosomes[8,18], this animal appears to be a suitable model for investigating whether the pigment granules found in sensory cells are involved in cell aging or whether they are metabolically active organelles.

Adult *Aplysia limacina* obtained from the Gulf of Naples were anesthesized with 2% ethanol in sea water. The upper tentacles were removed, cut into small pieces and fixed in 2% OsO_4 buffered with sodium cacodylate 0.1 M, pH 7.4, for 5 h at 4 °C. The samples were dehydrated and embedded in Spurr medium. Ultrathin sections were cut on an Ultracut Ultratome and stained with uranyl acetate and lead citrate. To observe the



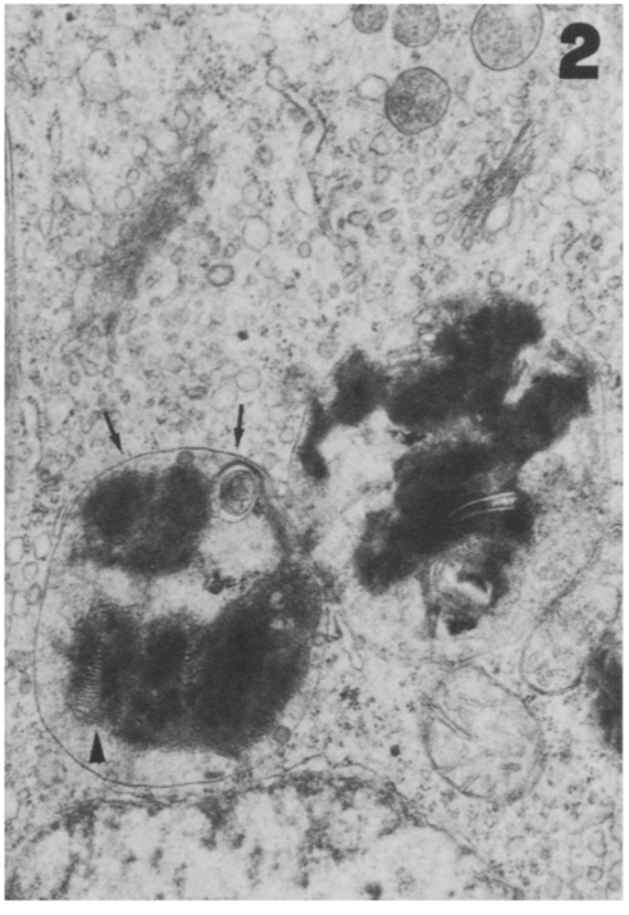


Figure 1. Sensory cells of *Aplysia limacina*. Two cytosomes (c) are visible in the cytoplasm of the pear-shaped sensory neuron. × 10,500.

Figure 2. High magnification of cytosomes in the sensory cell. Note the monolayer limiting membrane (arrow) and the structural heterogeneity of the cytosomes. The internal lamellar structures (arrow-head) are clearly visible. × 39,000.

carotenoid content of the sensory cells at light microscopic level, the cryostat sections were treated with concentrated sulphuric acid[19].

The sensory cells are gathered in small clumps immediately under the surface epithelium. Under the electron microscope, the sensory cells have a characteristic pear-shaped body. Figure 1 shows cytoplasmic zones delimited by membranes in which grossly granular lipochromic material stands out from a finely granular matrix. At a higher magnification, the structural heterogeneity with some granules of varying electron density and some lamellary formations can be observed (fig. 2). The monolayer structure of the pigment-limiting membrane is also evident. These pigment masses, which on the basis of number, size and cytoplasmic position correspond to those observed with the electron microscope, are sulphuric acid positive, and thus have a high carotenoid content.

The theory that the sensory cells are neurons subject to continuous renewal, the positive histochemical reaction for carotenoids and the morphology of the pigment-formations studied suggest that these are cytosomes, which play a metabolically active role, rather than they are accumulation material deposited during the aging of sensory cells, although the histospectrofluorimetric analysis[20] failed to reveal any marked spectral difference between these organelles and the typical lipofuscin masses present in ganglion cells of *Aplysia* and other marine animals.

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0014-4754/84/040382-02$1.50 + 0.20/0

Inhibitory effect of direct current on cell division and cell proliferation

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Summary. Direct current (0.1 to 0.2 mA) fully inhibited cell division and cell proliferation at the site of the non-polarizing anode, probably due to electrolysis and electro-osmotic processes affecting the chromatin of the cell nucleus. It resulted in cessation of the growth of the root-germs of the onion. Plant germs lost their ability to germinate 18–20 h after the application of the weak direct current.

In the twenties it became generally known, mainly from the investigations of Ebbecke[1] and Rein[2] that weak direct current, when applied to the skin by means of non-polarizing electrodes, evokes both subjective and objective symptoms. According to the authors mentioned above the subjective symtoms are caused by excitation of the nerves and nerve endings of the skin. These subjective symptoms are easily distinguished from the gross alterations of the skin which are displaced considerably in time and are not associated with subjective symptoms. The objective signs, developed in the skin as a result of the direct current, are consequences of a general tissue irritation[2]. They depend on several factors such as the chemical effect of the electrolyte, the electrolysis and electro-osmosis of the electrolyte solution due to the current and the ions migrating into the skin from the electrolyte solution.

Ebbecke[1] recognized that after application of an acidic electrolyte solution the changes appearing at the anode were most evident and those at the cathode were hardly visible. In contrast, application of an alkaline electrolyte solution resulted in increased irritation at the site of the cathode. The changes appearing at the sites of the electrodes were ascribed to H^+ and OH^- ions, respectively, migrating into the skin when the current was applied[1].

The physiological effect of direct current in human and various species of animals was studied by Holzer[3] in detail. Of special interest are their data taken from the literature concerning the effect of direct current of Ehrlich's mouse cancer and on various trypanosomes.

According to the experiences of Rein[2] weak direct current applied to the corium leads to the development at the cathode of a picture similar to that of an acute inflammation with the presence of a lot of leukocytes. In contrast, the cells at the anode undergo shrinking, no leukocytes are observed and the signs of cellular infiltration are absent.

In the present study, small-sized onions (Allium cepa) were kept in physiological saline solution (0.15 Mol/l NaCl) at room temperature until 2–3 mm root-germs developed. Then the non-polarizing cathode was placed in the body of the onion and the anode in the solution close to the root-germs, and 0.1–0.2 mA direct current was applied for 30 to 120 min.

The application of this direct current (0.1 to 0.2 mA in intensity) to the vegetal eukaryotic cells showed that this weak current, which is also used in human therapy, fully inhibited cell-division and, thus, cell proliferation at the site of the non-polarizing anode. This action is probably brought about by