Short Communication

Haemocyanin Production in Pore Cells of the Freshwater Snail Lymnaea stagnalis

T. Sminia and H. H. Boer

Department of Biology, Free University, Amsterdam-Buitenveldert, The Netherlands

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Summary. Electron micrographs of pore cells of Lymnaea stagnalis suggest that these cells produce and store haemocyanin.

Key words: Haemocyanin — Pore cells — Lymnaea stagnalis — Electron microscopy.

One of the most conspicuous cell types in the connective tissue of gastropods and bivalves is the pore cell. To these cells, which have been described under various names, several different functions have been attributed (e.g., Wolburg-Buchholz and Nolte, 1973), one of them being the production and secretion into the haemolymph of blood pigments (Sminia, 1972). In a previous study (Sminia et al., 1972) this latter hypothesis was tested with histochemical and electron microscopical techniques in two gastropod species having haemoglobin as blood pigment (Biomphalaria glabrata, Planorbarius corneus) and in one having haemocyanin (Lymnaea stagnalis). The pore cells of the planorbids appeared to react strongly to the histochemical test for haemoglobin. However, since there is no histochemical reaction available to demonstrate the presence of haemocyanin, the conclusion that the pore cells of L. stagnalis produce this pigment, had to be drawn mainly on the basis of the examination of electron micrographs of these cells. The micrographs showed that the cells obviously are protein producing cells, as they possess a very extensive granular endoplasmic reticulum (GER). The contents of the cisterns of this reticulum usually have a crystalline pattern. Since in the characteristic plasma membrane invaginations of the cells granules are present, which can be identified as haemocyanin molecules, it has been supposed that the cells produce this haemocyanin and that the crystalline material inside the GER consists of this pigment.

During further studies on the pore cells of L. stagnalis a number of electron micrographs was obtained supporting this assumption (Figs. 1 and 2). Inside the cisterns of the GER large numbers of granules are present, which have the characteristic shape and dimensions of haemocyanin molecules (cf. Mellema and Klug, 1972). In some places the particles are arranged in a highly ordered pattern. In other parts of the GER they can not be distinguished individually. Apparently the haemocyanin is at these places aggregated into large crystals.

The observations strongly suggest that the GER of the pore cells of L. stagnalis synthesizes and stores haemocyanin. Since this material is only found inside the GER and not in the Golgi apparatus or in some kind of secretion granule, it may be assumed that the Golgi apparatus is by-passed in the secretion of this material.



Figs. 1 and 2

Figs. 1 and 2. Electron micrographs of parts of pore cells of *L. stagnalis*. Inside the strongly dilated cisterns of the granular endoplasmic reticulum (*GER*) haemocyanin is present as individual particles (arrows) as highly ordered rows of particles (*RH*) or in a crystalline form (*CR*). *M* mitochondrion; *P* pores of the invaginated plasma membrane; *R* ribosomes. Fixation: Glutaraldehyde/OsO₄; Embedding: Epon; Electron microscope: Philips EM 300; $\times 70000$

References

- Mellema, J.E., Klug, A.: Quaternary structure of gastropod haemocyanin. Nature (Lond.) 239, 146-150 (1972)
- Sminia, T.: Structure and function of blood and connective tissue cells of the fresh water pulmonate Lymnaea stagnalis studied by electron microscopy and enzyme histochemistry. Z. Zellforsch. 130, 497–526 (1972)
- Sminia, T., Boer, H.H., Niemantsverdriet, A.: Haemoglobin producing cells in freshwater snails. Z. Zellforsch. 135, 563–568 (1972)
- Wolburg-Buchholz, K., Nolte, A.: Vergleichende Untersuchungen an Amoebozyten und Blasenzellen von Cepaea nemoralis L. (Gastropoda, Stylommatophora). Unterschiedliche Endozytosefähigkeit der Zellen. Z. Zellforsch. 137, 281–292 (1973)

Note Added in Proof. After completion of this manuscript an article was published by J. F. Reger [J. Ultrastr. Res. 43, 377–387 (1973)] reporting the occurrence of haemocyanin producing cells in the connective tissue of the slug *Limax* spec. It is not clear whether or not these cells have to be considered as pore cells.

T. Sminia Department of Biology Free University de Boelelaan 1087 Amsterdam-Buitenveldert The Netherlands