Quinone tanning in Agnatha

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Summary. The buccal teeth and notochordal sheath of the marine hagfish, *Petromyzon marinus* (L.) are phenolically tanned like the cuticle of insects and other arthropods.

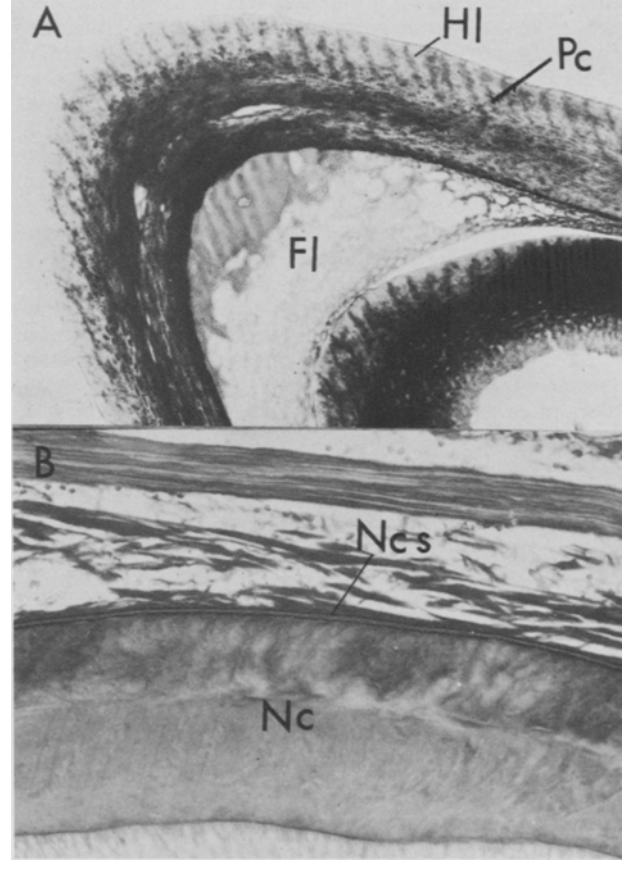
The presence of protein tanned by an orthoquinone has been established in the cuticle of arthropods[3], cysts of nematodes[4], shells of helminths[5], chetae of earthworms[6], the byssus of the bivalve mollusc, *Mytilus edulis*[7] and the central capsule of the radiolarian, *Thalassicola* (Protozoa)[8]. Among the Chordata, the hardening of the gill bars of *Amphioxus* (Cephalochordata)[9,10] and the egg cases of the selachian, *Chiloscyllium griseum*[11], seems to involve a process very like sclerotization. In the course of our studies on the skeletal tissue of the marine hagfish, *Petromyzon marinus* (L.) (Agnatha), we found that the buccal teeth and notochordal sheath are phenolically tanned like the cuticle of insects and other arthropods.

The required amount of buccal teeth and notochordal sheath were dissected from specimens preserved in 5% formaldehyde. Routine paraffin sections were made 5 µm thick, and stained in Mallory's triple stain. Histochemical tests employed included Millon's, the potassium iodide, potassium bichromate, argentaffin, xanthoproteic, Nadi, Sudan black-B and Libermann-Burchardt tests[12]. Unidimensional descending chromatography of the acid hydrolysate of the materials on Whatman No. 1 filter paper was also used.

The buccal teeth and notochordal sheath are faintly amber in colour with a bluish tinge and are mechanically rigid. Transverse sections through teeth stained with Mallory's triple stain show that they are solid structures. They consist of an outer 'hyaline' region and inner fuchsinophil region. The 'hyaline' region is similar to the exocuticle of the ant, *Formica fusca*[13] and the epicuticle of the scorpion, *Palamneus swammerdami*[14], whereas the fuchsinophil region resembles the mesocuticle of insects and other arthropods[15]. Spirally coiled vertical striations analogous with the pore canals of arthropod cuticle are present in the hyaline region (figure, A). Histological preparations of the notochordal sheath show that it consists of a tough layer with affinity for acid fuchsin; in this it resembles the mesocuticle of arthropods (figure, B). The bluish tinge seems to be due to the presence of bluish pigments in the epidermis, the chemical nature of which is unknown at present.

Maceration of the buccal teeth sections with mineral acids demonstrates that the outer hyaline region is more resistant the the inner fuchsinophil layer. The entire width of the notochordal sheath is markedly resistant to the acid treatment. The Millon's, xanthoproteic, potassium iodide and potassium bichromate tests give positive results in the outer and inner regions indicating the presence of tyrosine, tryptophane and other phenolic compounds. Evidence that the hyaline region is tanned is given by the fact that, even after boiling, it induces a rapid oxidation of the Nadi reagent which has been used to indicate orthoquinones. The argentaffin reaction for polyphenols and polyamines is most marked in the outermost region of the hyaline zone. However, the Sudan black-B and Libermann-Burchardt tests give negative reactions indicating the absence of simple as well as of steroid types of lipid. Besides, after detanning the teeth and notochordal sheath by treating them in diaphanol, they become soft and white in colour. Histological inspection of such diaphanol-treated materials show that the region corresponding to the nyaline and fuchsinophil regions are coloured red and blue respectively when stained with Mallory's triple stain. In view of the above observations it may be assumed that the teeth and notochordal sheath are quinone tanned.

The foregoing observations denote that the buccal teeth and notochordal sheath of *P. marinus* are hardened by phenolic tanning, a process comparable to that found in the cuticle of insects and other arthropods. The outer mechanically resistant hyaline region is homologous with the outer amber and/or hyaline exocuticle, while the inner fuchsinophil portion is analogous with the mesocuticle of other insects. However, certain points of difference are significant: 1. unlike that in the insect cuticle, the substrate involved in tanning seems to be a protein rich in phenolic groups without a lipid component; 2. chitin is absent; and 3. there is no differentiation into epi- and procuticle. In these respects, they resemble the ootheca of the cockroach, *Blatella germanica* [15], and spore walls of the fungus, *Aspergillus* sp.[16].



A and *B*. Transverse section through the buccal teeth and notochordal sheath of the marine hagfish, *Petromyzon marinus* (L.) after staining in Mallory's triple stain. Fl, fuchsinophil layer; Hl, hyaline layer; Nc, notochord; Ncs, notochordal sheath; Pc, pore canals. ×300.

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Spore germination in *Hebeloma* stimulated by living plant roots[1]

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Summary. Basidiospores from 14 strains of *Hebeloma* (Agaricales) representing 5 groups of mycorrhiza-forming species were tested for germination on a nutrient agar medium. Germination occurred in 13 strains but never exceeded 0.1%. A 10-fold increase or more in germination percentage was obtained in 4 out of 7 tested spore collections only by placing the growing root of a pine seedling among the spores on the agar medium.

Basidiospores of mycorrhiza-forming Homobasidiomycetes generally do not germinate on ordinary agar media but require special conditions for germination[4]. However, in the genus *Hebeloma* (Cortinariaceae, Agaricales) where most species form ectomycorrhiza with trees, spore germination occurs readily within the section Denudata, subsection C, as was demonstrated by Bruchet[5]. In other sections of *Hebeloma* he observed no, or merely a very sparse and slow, germination.

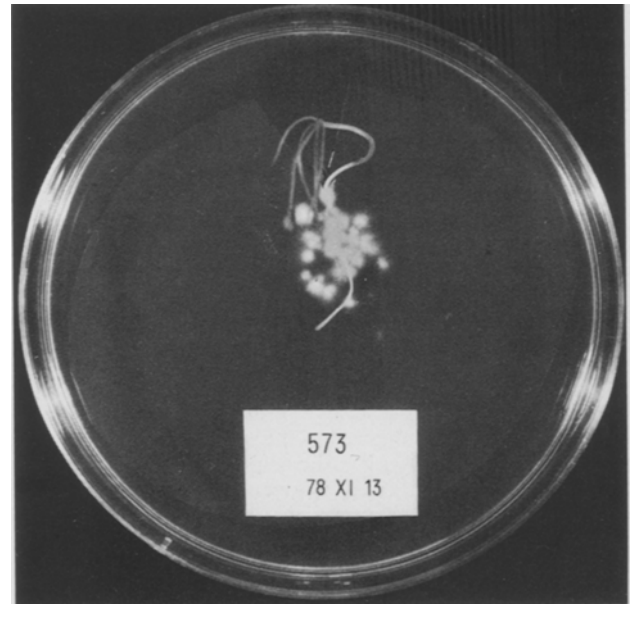
It was thought that increased knowledge of the conditions for spore germination in *Hebeloma* might also contribute to the understanding of the spore germination mechanism in other, chiefly micorrhiza-forming genera, e.g. *Inocybe* and *Cortinarius,* of the family Cortinariaceae, in which spore germination in vitro has not yet been achieved. In some other Homobasidiomycetes the germination rate could be increased by using *Rhodotorula* yeast as an activator organism and/or by adding activated charcoal[4]. Therefore these measures and some others were tested for their efficiency in improving the germination of spores from a number of *Hebeloma* species.

14 spore collections from fruit-bodies of *Hebeloma* were obtained in the autumn of 1978 and preserved in petri dishes under sterile conditions in darkness at 4 °C. Most of them came from habitats in the neighbourhood of Uppsala, Sweden. After spore-casting, the fruit-bodies were dried and sent to Dr G. Bruchet, Lyon, for determination. All of them could be identified as to section, subsection and stirpes, but some could not be given a definite species name because of the inadequacy of the material. However, the collections comprised at least 5 different species representing as many subsections or stirpes.

The spore germination tests were made on plates of a semisynthetic nutrient agar medium[4]. About 0.1 ml of a suspension containing 500,000–1,000,000 spores per ml was spread over the agar surface by means of a glass rod. In some experimental series a colony of *Rhodotorula glutinis* (Fres.) Harrison was inoculated onto each plate, or activated charcoal was dusted over half of the agar surface. The plates were sealed with parafilm and incubated in darkness at 25 °C. Because of the generally very sparse and varying germination, which did not take place simultaneously, no efforts were made to estimate the percentage germination exactly.

Spores of all tested collections, except No. 568, germinated at least in 1 experiment (cf. table). The first germinations could usually be observed only after 2 or 3 weeks. New ones then gradually appeared, even after an incubation time of up to 5 months. In all cases the percentage germination was below 0.1%. In certain spore collections, e.g., in Nos. 1 and 569, many of the small mycelia formed from the germinated spore died for unknown reasons before they had reached a size visible to the naked eye.

None of the 14 tested strains reacted positively to *Rhodotorula,* which confirms the results of Bruchet[5] gained with the



A pine seedling growing on an agar plate, the entire surface of which is evenly sown with spores of *Hebeloma mesophaeum,* strain No. 573. A thin cellophane foil covers almost the whole agar surface including the root of the seedling. The culture was incubated for 4 weeks by daylight at about 20 °C. Germinations have occurred chiefly close to the root. They have given rise to mycelia visible to the naked eye after 2–3 weeks.