

## Phenolic Tanning and Pigmentation of the Cuticle in *Carcinus maenas*

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### SUMMARY

1. The histochemistry of the cuticle in different stages of the moult cycle of *Carcinus maenas* is described.
2. The newly formed cuticle of a crab exposed at moulting shows evidence of phenolic tanning in the epicuticle.
3. The subsequent tanning of the pigment layer of the endocuticle is confined to a brief period immediately after moulting.
4. The polyphenol oxidase of the epicuticle involved in tanning disappears soon after moulting, but some time later an oxidase is again indicated, this time in the pigment layer. It appears to be secreted by the tegumental glands.
5. From the concurrent appearance of a polyphenol oxidase and tyrosine and the subsequent formation of dihydroxyphenols and melanin-like substances in the cuticle, it is suggested that the oxidation products of tyrosine are responsible for the pigmentation of the cuticle.
6. Pigmentation of the cuticle is discussed in relation to phenolic tanning in Crustacea and Insecta.

### INTRODUCTION

RECENT work has emphasized the fundamental similarity of the crustacean cuticle to that of insects (Drach, 1939; Pryor, 1940; Dennell, 1947 *b*). The last-mentioned author in addition to confirming the occurrence of phenolic tanning in Crustacea has suggested that its mechanism may be essentially similar to that in insects. Notwithstanding the homology of the cuticle in the two groups mentioned above it is seen that in Crustacea tanning of the cuticle is much abbreviated, the prime cause of hardening being calcification (Dennell, 1947 *b*). In insects it has been shown (Pryor, 1940; Fraenkel and Rudall, 1940, 1947; Dennell, 1946, 1947 *a*) that the hardening of the cuticle is due to the passage into it of a polyphenol, the oxidation product of which forms cross-linkages within the protein phase of the cuticle. The other concomitant changes are the reorientation of the chitin crystallites, a loss of solubility of proteins and a darkening effect. It has been pointed out (Pryor, 1940; Pryor *et al.*, 1947) that hardening and darkening of the cuticle of insects are the result of tanning, and it therefore appeared of interest to discover the relation existing in Crustacea between tanning and the formation of melanin-like pigments in the cuticle.

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## METHODS

The material used in this work was the merus of the walking legs of *Carcinus maenas*, obtained from Plymouth. Paraffin, frozen, and hand sections were prepared for study of the cuticle. Dehydration was carried out with dioxane to avoid the undue hardening that may result from treatment with higher grades of ethyl alcohol. The stains used were Mallory's triple stain and Masson's trichrome stain. The oxidases of the cuticle were studied using the nadi reagent (a mixture of dimethyl-para-phenylenediamine and  $\alpha$ -naphthol (Lison, 1936) on frozen and hand sections. Melanin-like substances were detected by the solvent action of ethylene chlorhydrin. In addition, a number of other histochemical reagents were used for the detection of fats, phenols, and amino-acids, and are mentioned in appropriate places in the text.

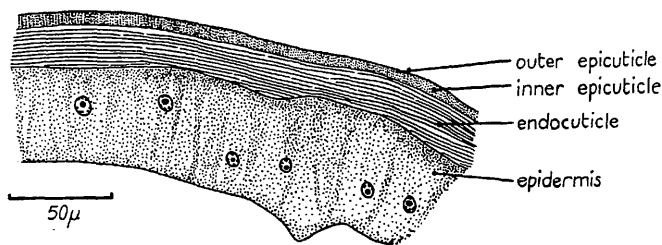


FIG. 1. Section through the newly formed cuticle of *Carcinus maenas* in the process of shedding the old shell.

## PHENOLIC TANNING AND DARKENING

Dennell (1947 *b*) described the cuticle of Crustacea in the light of recent work on that of insects and brought forward evidence of the occurrence of tanning in the epicuticle and the outer layers of the endocuticle. The quinones responsible for tanning have been shown to be formed by the oxidative activity of a polyphenol oxidase which is located in the epicuticle in the very early stages after moulting. From the disappearance of the oxidase soon after moulting it appears that the tanning of the cuticle is a brief process confined to a period immediately following the formation of the new cuticle. With a view to throwing more light on this aspect of the problem, the cuticle was studied from its appearance at the time of moulting.

The newly formed cuticle of a crab at the time of shedding the old shell (fig. 1) consists of a thin epicuticle and a homogeneous endocuticle staining red and blue respectively with Mallory's triple stain. Overlying the red epicuticle is a very thin blue-staining membrane, the outer epicuticle, whose integrity as a discrete layer is borne out by its separation from the inner epicuticle due sometimes to mechanical factors and noticed in fixed prepara-

tions of the cuticle. The inner epicuticle even before the old shell is cast shows evidence of tanning. At this stage in frozen and hand sections the untreated epicuticle shows an amber coloration but the endocuticle is colourless. That the amber coloration of the epicuticle is due to tanning is indicated by the bleaching of the amber-coloured zone by diaphanol, which has a selective action on tanned regions. The occurrence of tanning in the epicuticle is further confirmed by the results obtained with the Millon and argentaffin reactions, both of which are positive, indicating the presence of aromatic

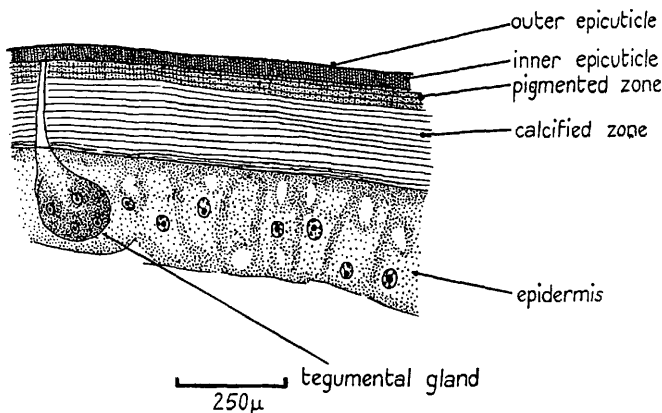


FIG. 2. Section of the cuticle of a soft crab about two days after moulting.

substances. The endocuticle is unaffected by such treatment. Staining of sections with sudan black B. indicates that both the inner and outer epicuticles contain lipid substances. Ferric chloride gives a slight green coloration in the inner epicuticle and in the outer layers of the endocuticle, indicating the presence of a dihydroxyphenol. This is consistent with the observation (Dennell, 1947 *a*) that tanning spreads inwards as a result of a wave of quinone formation induced by the polyphenol oxidase activity in the epicuticle. The presence of polyphenols in the outer layers of the endocuticle as indicated by the ferric chloride test suggests impending tanning of this region.

Sections of the cuticle of a soft crab some time after moulting (fig. 2) show that both the epicuticle and the outer layers of the endocuticle are tanned. That the tanning of the cuticle is completed by this stage in the moult cycle is suggested by the lack of a reaction with ferric chloride, indicating the absence of a dihydroxyphenol. In the absence of an oxidase and extension of the tanned zone at this stage, dihydroxyphenols involved in tanning may not now occur in the cuticle. Fig. 3 shows a section of the hardened cuticle of















