Short Communications

Repair of integumental lesions in the decapod crab, *Carcinus maenas* (L.), in the presence of molting hormone

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Summary. Epicuticle is not included as any part of the repair cuticle that closes integumental lesions in crabs receiving multiple injections of molting hormone (20-hydroxyecdysone) before and/or after damage to the integument. It is suggested that deposition of the exclusively lamellate material of repair cuticle is mediated by some epidermal factor(s) rather than by external, humoral influences.
Key words. Wound repair; cuticle; integument; molting hormone; *Carcinus*.

Repair of integumental lesions in the decapod crab, *Carcinus maenas* (L.), is accomplished by the production of considerable amounts of new cuticle directly below the site of injury and at its periphery[1,2]. In common with the repair cuticle produced by the few other arthropods studied in this respect[3–5], the repair cuticle of *Carcinus* lacks an epicuticle, the characteristic outermost layer of arthropod cuticle. The effects of integumental damage on the titer of circulating molting hormone does not appear to have been investigated to date. However, it is unlikely that the repair process is mediated by the hormonal influences that initiate the periodic formation of a new cuticle in the normal molt cycle of a crustacean[6,7]. Explanation of repair phenomena on the basis of their stimulation by molting hormone seems implausible, in view of the observed incomplete and highly localized character of the repair product. However, it might be that exogenous molting hormone, administered near the time of injury, modifies the repair process such that an epicuticle is produced. This communication describes the responses of *Carcinus* epidermis to molting hormone injected in various doses and at various times before and after wounding of the integument.

Methods. Male *Carcinus maenas*, average maximal cephalothorax width 46.4 ± 12.3 mm and average wet weight 29.4 ± 14.7 g, were maintained in a re-circulating seawater system as described previously[2]. Holes were drilled through the integument of the dorsal cephalothorax with a 2-mm diameter carbide dental burr. The location of the holes was chosen to avoid muscle attachments to the cuticle. Molting hormone (MH; as beta-ecdysterone, Sigma Chemical Co.) was injected through an arthrodial membrane of one of the pereiopods. Dilutions of a stock solution of MH in 95% ethanol were made in filter-sterilized seawater such that each amount injected fell in the range 1–2 μg g^{-1} animal wet weight. The total amounts injected in series of multiple injections (see below) were greater than those found by Buchholz and Adelung[8] to be ineffective in accelerating the onset of premolt but similar to those stimulating premolt cuticle development in the lobster, *Homarus americanus*[9]. Control injections consisted of dilutions of 95% ethanol in seawater. Tissues were removed from the wound site and fixed in aqueous Bouin; serial 8-μm sections were stained in Mallory. The epicuticle, if present, was evident in the sections as a distinct outermost layer stained by the Orange G component. No crabs died during the first two experiments; mortality was about 25% in the third experiment, in which the dosages were the largest used.

In all experiments, numbers of crabs equal to those noted below served as controls. Three sets of experiments were conducted with intermolt crabs, as follows:

1) a) Three crabs were injected (1 μg g^{-1}) on days 0, 2, 4, 9, 11, 15, and wounded on day 7; tissue was fixed on day 20; b) three crabs were wounded on day 0 and received injections on days 2, 5 and 7; tissue was fixed on day 14. 2) a) Six crabs were injected (1μg g^{-1}) on days 0, 2, 4, and wounded on day 6; tissue was fixed on day 20; b) six crabs were wounded on day 0 and injected on days 2, 4 and 6; tissue was fixed on day 14. 3) Ten crabs were wounded on day 0 and injected (2 μg g^{-1}) on days 2, 4, 6, 8, 10, and 12; tissue was fixed on day 21. The experiments thus exposed crabs to increased titers of MH before and/or after integumental wounding.

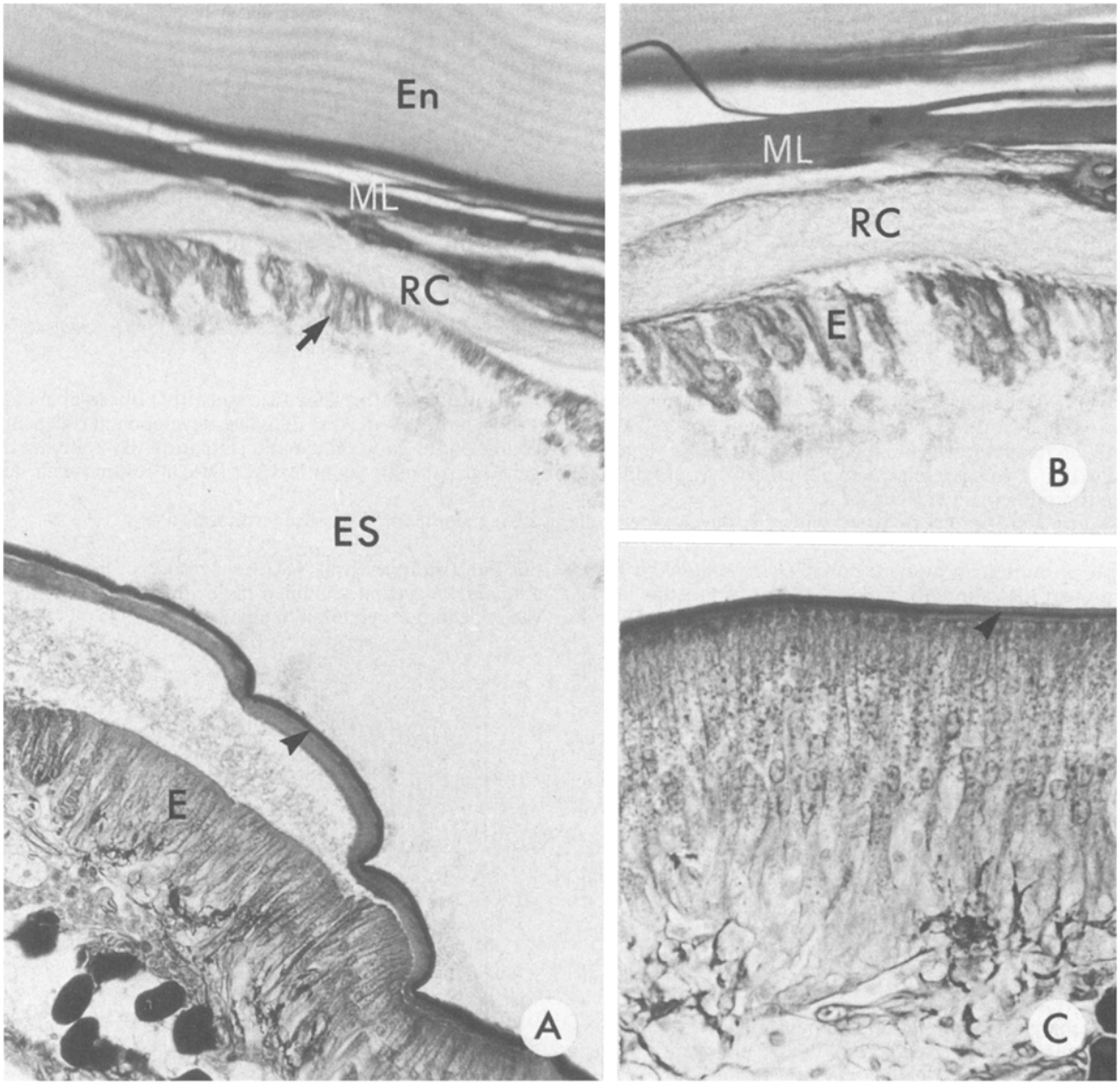
Results and discussion. In the time intervals used in this study, distinct repair cuticle was produced by the majority of both MH- and solvent-injected animals (86% and 76% respectively). Four crabs were found to be in premolt when their integument was examined. The presence of an epidermis immediately below the repair cuticle and another epidermis under the new (premolt) cuticle shows that these animals entered premolt during the 14–21-day interval between wounding and tissue fixation (fig.).

Presumably the epidermis beneath the premolt cuticle in the wound region originated either by tangential division of cells previously depositing repair material or by migration of cells from the adjacent undamaged integument. New epicuticle was present as the outermost layer of the premolt cuticle; epicuticle was not included in the repair cuticle produced by these animals (fig.) neither was it included in the repair cuticle of any of the other crabs.

It is apparent that it is not sufficient simply that MH be present at a time when repair cuticle is being deposited for epicuticle to be incorporated in the repair material. It might be argued that the amounts of MH injected were insufficient to influence the course of repair cuticle deposition. However, the titer of MH was clearly adequate to promote entry by some crabs into premolt. In these cases, epicuticle production was restricted to the premolt cuticle even though the epidermis that had begun to form repair cuticle must have been exposed to increased titers of molting hormone. Even if these crabs had been responding only to a release of endogenous MH some time before the time of injury (rather than to the injected MH), the wounded epidermis nevertheless would be responding in the presence of elevated MH titers; these titers in several decapod Crustacea are high until shortly before ecdysis[7,10].

The observations reported here suggest that, upon being wounded, the crab epidermis embarks upon a synthetic sequence that is insensitive to external (hormonal) influences. The autonomous nature of the repair process has already been noted[1,2]. Repair, once underway, proceeds apparently uninterruptedly so that the resulting repair cuticle can become thicker than the adjacent undamaged cuticle.

The mode of action of MH on crustacean target tissues is not understood[7]. Proteins that bind to MH have been found in the cytoplasm of crayfish epidermis and other tissues[11], sug-



Light micrographs through integument of *Carcinus maenas* in early premolt. *A* Repair cuticle (RC) and epidermis (arrow) are visible just below the old endocuticle (En) and membranous layer (ML). An ecdysial space (ES) separates these layers from the newly-forming premolt cuticle and its epidermis. A distinct epicuticle (arrowhead) is seen as a narrow, densely-stained layer above the new exocuticle. The latter has retracted in places from the epidermis (E), ×200. *B* Repair cuticle produced before separation of the old cuticle from the new (apolysis). The loosely-organized repair cuticle (RC) lies above a degenerating epidermis (E). No epicuticle is present. ML, membranous layer, ×395. *C* An early stage in the development of the new (premolt) cuticle. Exocuticle has just begun to form below the epicuticle (arrowhead). This micrograph is taken from the section seen in *A* but from the opposite side of the wound, ×380.

gesting that MH influences epidermal cell activities in a manner consistent with its steroid nature. However, it is remarkable that many of these activities appear able to start and continue in the presumed absence of appreciable concentrations of MH. *Carcinus* can produce abundant amounts of repair cuticle, closely resembling the normal procuticle in ultrastructural organization (Halcrow and Smith[2], op.cit.), during intermolt; levels of the hormone during this phase of the molt cycle in *Carcinus* are minimal[12]. It seems unlikely, particularly as the repair cuticle lacks an epicuticle, that MH in the hemolymph increases to levels that permit cuticular synthesis by cells at the injury site but not elsewhere. The precise role of MH in stimulating the deposition of new cuticle is therefore open to speculation. It is hypothesized here that the production of lamellate cuticle in pre- and postmolt animals is initiated indirectly by MH through some intermediary, the release of which also can be brought about by injury to the epidermal cells. Also, in the normal molt cycle it is supposed that MH directly stimulates the synthesis of epicuticle but that this synthesis is suppressed in the wound response. The latter response therefore results in the appearance of exclusively lamellate cuticle in the repair material.

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Imaginal wing disc morphogenesis, a sign of diapause development in the European corn borer

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Summary. In the European corn borer, subtle changes in imaginal wing discs during diapause constitute observable indications of diapause development, in experimental as well as in field-grown larvae. Wing disc diapause development is dependent mainly on temperature, and its total achievement is a necessary condition for good diapause termination. By applying these observations, we have improved a method that provides homogeneous populations of larvae that can resume their development rapidly in any season.
Key words. European corn borer; wing disc development; diapause development; diapause termination.

Larvae of the European corn borer, *Ostrinia nubilalis* Hbn. cause great losses in corn yields. Mature fifth-instar larvae enter a photoperiodically induced diapause lasting 7 or 8 months during the winter season[1,2].

In order to differentiate diapausing from non-diapausing field or laboratory larvae, three physiological symptoms of the diapause syndrome have been studied:

– The stainability of the protocerebral neurosecretory cells (NSC) by Victoria blue (VB) and paraldehyde fuchsine (PF), is a phenomenon which is observable in non-diapausing larvae, and which progressively disappears during the first 3 or 4 months of diapause[3].

– The hemolymphatic titer of trehalose, measured by gas-liquid chromatography, is a factor that is 5–10 times higher in diapausing larvae than in non-diapausing larvae[3].

– In diapausing larvae, the development of the imaginal wing discs structures seems to be arrested at different early phases. The most advanced, rather poorly differentiated phase is called III3[4].

Before diapause termination, diapausing larvae must undergo a maturation termed 'diapause development'[5]. Hence diapause appears to be 'a dynamic state during which specific physiological processes occur'[6]. We propose that the subtle changes in the morphogenesis of wing discs during diapause, constitute an observable indication of the progression of diapause development in the larvae of *O. nubilalis*.

Previous examples of morphogenetic changes during diapause have been cited[7]. Particularly, in diapausing embryos of *Cnephasia pumicana*, the mesenteron must develop before diapause termination[8].

Post-diapause development is characterized by the reversal of the three physiological criteria for diapause. Firstly, the protocerebral NSC regain their stainability, then the titer of hemolymphatic trehalose decreases, and finally the imaginal wing discs resume development. This succession of events is invariable: wing buds do not develop without preliminary changes at the neurosecretory and metabolic levels in the wild as well as in the laboratory[3].

Evidence of wing disc development during diapause. Field-grown populations from experimental infestations in Versailles (France) were studied from September to June, in 1984–1985 and in 1985–1986. In both cases, as early as October, all collected larvae entered diapause. Their trehalosemia was high and development of their wing buds was arrested at stages I, II and III1 (fig. 2). From January, III2 stages appeared and became more numerous. The first III3 stages were observed at the beginning of March (fig. 2). At this time, protocerebral NSC were weakly or not at all stainable. Trehalosemia remained high. Physiologically, the larvae appeared to remain in diapause.

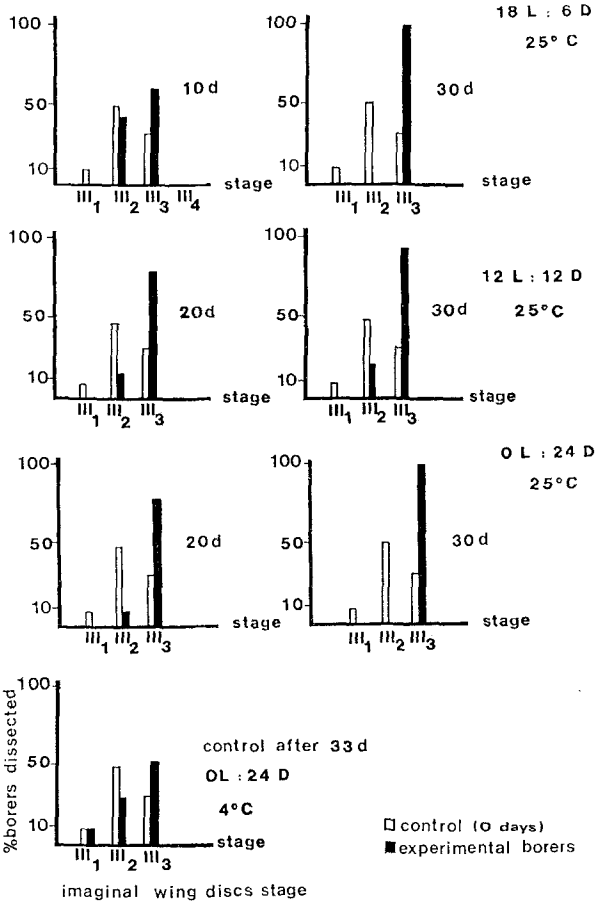

Figure 1. Experimental influence of temperature and photoperiod on the differentiation of the imaginal wing discs during diapause. The development of imaginal wing discs of diapausing control larvae maintained at 4 °C and 0L:24D, was compared with those of diapausing experimental larvae placed at 25 °C, and at different photoperiods (18L:6D, 12L:12D, 0L:24D). Each batch of experimental borers represents 20 larvae, collected in South-West France (Bordeaux).