The Role of Crustecdysone in the Moulting Crayfish

Crustecdysone (20-hydroxyecdysone), isolated from the crayfish *Jasus lalandii*[1] and insect pupae[2–6] undoubtedly plays a role in initiating moulting in insects[7] and it is reasonable to expect that it has a similar function in crustaceans. We now report experiments which lend some support to this view.

Male and female specimens of the fresh-water crayfish, *Procambarus simulans*, were maintained in shallow dishes at approximately 20 °C and 12 h daylight. The animals were not fed during the experiments. Strong transillumination was used to detect the presence or absence of gastroliths and allowed the size of gastroliths and the degree of new setal development to be determined. These observations and an examination of the appearance of the exoskeleton allowed a close estimation of the stage reached in the moult cycle.

In a preliminary series of experiments, groups of intact and of one-eyed animals at a late intermoult (C_4) and at an early premoult (D_0) stage and of approximately 1.5 g body weight were injected with solutions of crustecdysone in van Harreveld's saline ranging in concentration from 0.025–0.250 μg/g body weight. No injection schedule could be found that accelerated moulting and no animal moulted within 3 weeks. Three daily injections of 0.250 μg/g crustecdysone killed the animals in 6 days. While these experiments clearly indicate that an increased blood titre of crustecdysone in crayfish at an intermoult stage is not effective in initiating moulting, it does not exclude the possibility that at an appropriate time crustecdysone plays a role in initiating the moulting process.

During the intermoult stages, the X-organ of the crustacean eyestalk produces a moult-inhibiting hormone (MIH) which is thought to act by inhibiting the synthesis of moulting hormones in the Y-gland[8]. It is also possible that the MIH inhibits the action of the moulting hormone as well. An attempt to test this hypothesis has been undertaken by studying the effect of crustecdysone on animals after eyestalk ablation.

Animals weighing from 0.45–0.60 g (towel-dry) were used. All were in early premoult (D_0) stage, as gastroliths were present but new setal formation had not begun. Mortality due to eyestalk ablation was reduced to less than 5% when the eyestalks were removed one at a time at an interval of 24 h. Animals moulted in 6–10 days after eyestalk removal. The animals were injected 36 h after removal of the second eyestalk with 0.02 ml of a solution of crustecdysone in van Harreveld's saline at a dose of 0.0125–0.150 μg/g body weight. Control animals received 0.02 ml of either pure saline or pure peanut oil.

In Figure 1, it is seen that at all concentrations of crustecdysone, the time spent in premoult decreased; the concentration of 0.050 μg/g causing a more marked acceleration than the lower concentrations of 0.0125 and 0.025 μg/g body weight. Higher dosages of 0.050, 0.075 or 0.150 μg/g caused little or no further acceleration of the moulting process.

In a second series of experiments on eyestalkless animals, the timing of the injections was altered. The animals, 1.3–1.5 g in weight, were in stage D_0, but the gastroliths were smaller than those of the animals used in the previous experiments. A crustecdysone dosage of 0.025 μg/g body weight was used. In Figure 2 it is seen that injections given 48 or 72 h after eyestalk removal are more effective than an injection after 24 h. No further increase was obtained with a series of 2 injections given at both 24 and 72 h. Essentially the same results were obtained with a dosage of 0.05 μg/g.

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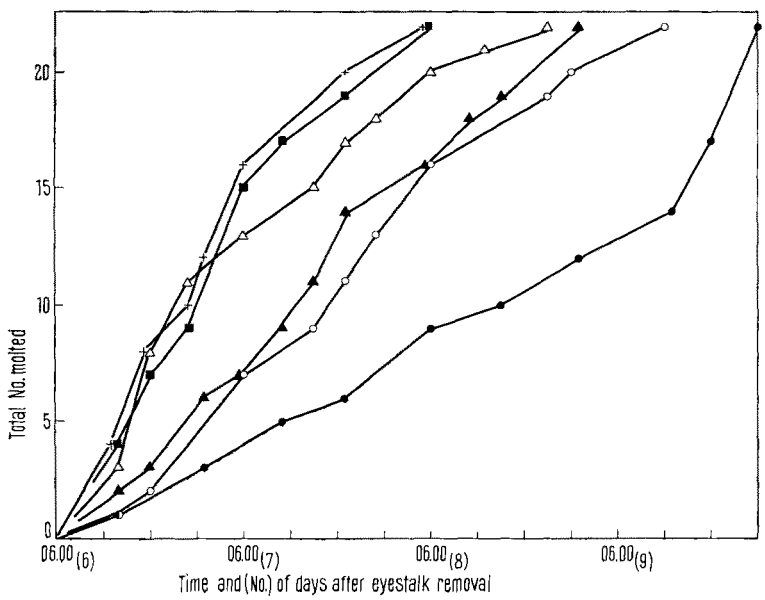

Fig. 1. Effect of concentration of crustecdysone on rate of moulting. • control, 0.02 ml van Harreveld's saline; o 0.0125 μg/g; ▲ 0.025 μg/g; △ 0.050 μg/g; ■ 0.075 μg/g; + 0.150 μg/g.

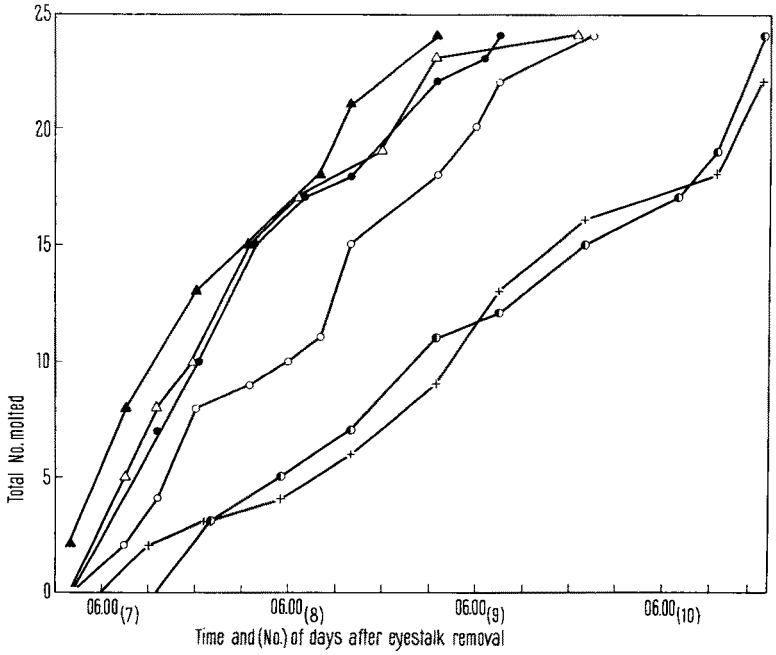

Fig. 2. Effect of time of dose after eyestalk ablation on rate of moulting. All experimental animals received crustecdysone in concentrations of 0.025 µg/g body weight. ◐ control, 0.02 ml van Harreveld's saline injected at 24 and 72 h; + control, 0.02 ml peanut oil at 24 and 72 h; o crustecdysone at 24 h; • at 48 h; △ at 72 h; ▲ at 24 and 72 h.

From the above experiments, it is evident that in the absence of eyestalks, crustecdysone in very small amount reduces the time taken to moult. However, the effect of crustecdysone is appreciable only after a period of 24 h or more following eyestalk removal. This time may be required for the blood titre of the MIH to drop and allow the animal to become responsive to the effect of crustecdysone. Also crustecdysone may initiate only some of the processes associated with moulting and it may be unable to exert its effect until other hormones have been synthesized by the Y-gland in response to the removal of the source of inhibiting hormone.

Résumé. La crustecdysone injectée dans l'écrevisse, *Procambarus sinulans* ne cause pas la mue chez l'animal intact mais l'accélère après ablation des pédoncules oculaires.

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Studies on Lens Regeneration in *Xenopus laevis*

Lens regeneration from the dorsal iris following lentectomy is a unique feature and is restricted to a number of species among the urodeles[1,2]. In the anurans, however, the 'lens potency' of the iris is lost during larval life[3].

The results of studies on regeneration from the dorsal iris in adults are not in agreement with each other, and if regeneration was found the conclusion was doubted[1,4].

Campbell[5] reported that in the adult *Xenopus laevis* lens regeneration from the dorsal iris following lentectomy occurs in 25% of the cases, and the time required for this is approximately 6 months. On the other hand, Overton and Freeman[6] and Freeman[7] showed that in the metamorphosed *X. laevis* lens regeneration appears to be absent, though it occurs from the inner cell layers of the cornea during the larval life. It was decided, therefore, to reinvestigate the problem of lens regeneration in adult *X. laevis*.

Methods. Sexually mature animals were purchased from a dealer in the Netherlands. Lenses were removed from the eyes under the dissecting binocular microscope while the animals were kept under anaesthesia with MS 222. From 5–188 days after the operation the eyes were extirpated for investigation. They were fixed in Bouin's solution and treated according to the usual histological procedures. Serial sections were cut to 10 µ thickness; they were stained with haematoxylin and eosin.

Results and discussion. As is shown in the Table the complete removal of the lens in the adult *X. laevis* was achieved successfully in a few cases only. In most of the cases some lens epithelium was retained within the collapsed capsule, as could be confirmed histologically

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