timing of the breaks for the insects tested.

We have demonstrated that the diapause of a large percentage of European core borer and codling moth larvae can be prevented in the field by extending the day length artificially to 17 hours. Normally these nondiapausing insects die because they are unable to survive the winter conditions and the lack of food (*2*). In addition, there could be mortality of overwintering diapausing insects so that the combined effects of prevention of diapause by light manipulation and the natural mortality over the winter could cause a great reduction in the population emerging in the spring.

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References and Notes

1. A. S. Danilevskii, *Photoperiodism and the Seasonal Development of Insects* (Oliver and Boyd, London, 1965), pp. 36–124; E. Bünning and G. Joerrens, *Z. Naturforsch.* **15b**, 205 (1960); J. deWilde, *Annu. Rev. Entomol.* **7**, 1 (1962); C. S. Pittendrigh and D. H. Minis, *Amer. Natur.* **98**, 261 (1964); P. L. Adkisson, *Science* **154**, 234 (1966); R. J. Barker, *Experientia* **19**, 185 (1963); S. D. Beck, *Biol. Bull.* **122**, 1 (1962); R. C. Dickson, *Ann. Entomol. Soc. Amer.* **42**, 511 (1949). In a few species, such as the silkworm, *Bombyx mori* L., the reverse is true and "long days" induce diapause [M. Kogure, *J. Dept. Agr. Kyushu.* **4**, 1 (1933)].
2. Unpublished observations in our laboratory.
3. G. W. Ankersmit, *Entomol. Exp. Appl.* **11**, 231 (1968).
4. The Saran screen cages were from the Chicopee Manufacturing Co., Cornelia, Georgia.
5. *Sunrise and Sunset at Washington, District of Columbia, Table No. 1061* (Nautical Almanac Office, U.S. Observatory, Washington, D.C., 1969).
6. K. Diem, *Statistical Tables* (Geigy Chemical Corporation, Ardsley, New York, 1962), pp. 85–103.
7. We thank T. Brindley, B. Butt, and F. R. Lawson (Entomology Research Division) for their help, and K. H. Norris (Market Quality Research Division) for his assistance in selecting the light sources.

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Molting in Land Crabs: Stimulation by Leg Removal

Abstract. *If the Bermuda land crab,* Gecarcinus lateralis, *loses numerous walking legs or both chelipeds, it undergoes almost immediate preparations for molting with attendant limb regeneration. Injections of the arthropod-molting hormone, ecdysterone, have no effect in either intact animals or those missing legs.*

The ability to experimentally control the molt cycle of Crustacea is important for at least three reasons: (i) it provides information on the physiology of molting; (ii) it facilitates studies of growth and regeneration; and (iii) it has potential economic importance. Molting in Crustacea is thought to be controlled by the interplay of a so-called molting hormone, the steroid ecdysterone (see *1*) and the molt inhibitory hormone (MIH) (*2*). Although MIH has not been isolated from X organs, it is thought to be produced there and stored in the sinus glands (*2*). Both X organs and sinus glands are located in the eyestalks, and one means of causing precocious molts is removal of both eyestalks (*3*). Although extirpation of eyestalks is effective as a trigger in the land crab *Gecarcinus lateralis* (*4, 5*), after surgery the animals become lethargic and do not generally survive ecdysis. At best, under certain conditions (when a shallow dish of dilute seawater at a salinity of 15 parts per thousand is available as the only source of water) some specimens live for a week after ecdysis (*6*). The behavior of animals without eyestalks in the period before ecdysis and their greatly decreased viability after ecdysis suggest that they are not entirely normal. Moreover, since the eyestalks are not regenerated, even if the animals survive in the laboratory, they could not live long if they were returned to nature.

We and others have observed that land crabs missing many legs (walking legs, or chelipeds, or both) prepare for molting sooner than animals missing only one or two legs. (During the premolt period the missing legs are regenerated.) In a study of environmental conditions influencing ecdysis, Bliss (*7*) noted that under unfavorable conditions over a 6-month period during which only one of nine control animals molted, five of five animals lacking all but two walking legs molted. We describe here a systematic study of the effect of the removal of appendages (walking legs, or chelipeds, or both) on the initiation of molting. We have also compared these effects with those of ecdysterone injected into either normal animals or animals with a number of limbs removed.

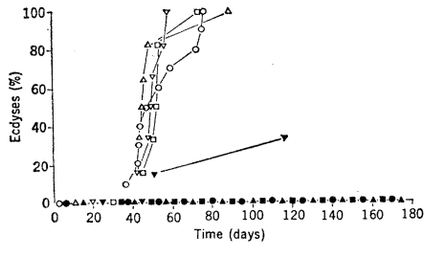

Fig. 1. Graph showing percent of animals which underwent ecdysis after the injection of ecdysterone in the presence of (missing only one walking leg, L^+) or absence of eight (L^-) walking legs. Sixteen animals received 0.1 ml of 0.5*M* NaCl (○, L^-; ●, L^+); 12 animals received 6 μg/g ecdysterone (□, L^-; ■, L^+); 12 animals received 18 μg/g ecdysterone (△, L^-; ▲, L^+); 12 animals received 6 μg/g ecdysterone on days 0, 1, and 3 (▽, L^-; ▼, L^+). Treatment occurred on day zero.

Gecarcinus lateralis, the animals used in this study, came from the Bermuda Biological Station. In the field, they live in deep burrows and ordinarily emerge only at night and after a heavy rain. Their normal molting season in nature is not known. Over the years we have observed that crabs brought into the laboratory and kept in community tanks undergo ecdysis 5 to 10 months after arrival. In a large group of crabs kept in individual containers, some molt sooner than 5 months after their receipt (*7*). However, we find that for adults the intermolt period is 180 to 300 days after adaptation to the animal-room conditions. Regardless of the time of year in which they are collected, we have seen them undergo ecdyses in all months of the year, although relatively few ecdyses occur in June or July.

All experiments were performed on animals weighing 20 to 40 g. If the eyestalks of such animals had been removed, they would have reached ecdysis in 6 to 8 weeks (*5*). All animals on their arrival in the laboratory were induced to autotomize (*8*) one walking leg; its removal does not influence the length of the molting cycle and the regeneration of this limb is a convenient and easily monitored external sign of an approaching ecdysis (*7, 9*). Some animals were induced to autotomize other limbs as well (Table 1). Ecdysterone (*10*) at a final concentration of 1 mg/ml was dissolved in a drop of ethanol and brought to volume in 0.5*M* NaCl. The amount of this solution injected into an animal ranged from 0.18 to 0.54 ml and was less than 1.5 percent of the animal's weight in all cases.

Data from a number of experiments are summarized in Table 1, and the complete data from one experiment (group 3) are shown in Fig. 1 so that the synchronous behavior of one popu-

lation can be compared with the lack of molting of another. In this experiment, 28 animals were made to autotomize all eight walking legs (the chelae were left on). They were divided into four groups, of which one group of ten received an injection of 0.1 ml of 0.5*M* NaCl. One group of six received 6 μg of ecdysterone per gram of body weight; still another received 18 μg per gram in a single injection; the final group received a total of 18 μg per gram as three injections of 6 μg per gram each on days 0, 1, and 3. Another set of 24 animals with only one leg missing was divided into four groups of six animals each and similarly injected with ecdysterone. After treatment, all animals were placed in individual containers.

In all groups missing limbs, animals molted after a mean elapsed time of 53 days. Inspection of the curves in Fig. 1 or of the means and standard deviations of the individual groups in Table 1 shows that the four subgroups do not differ from each other; that is, ecdysterone at the doses given has no influence on accelerating molting in animals missing limbs. Rather, it is the loss of limbs which is the effective stimulus to ecdysis. The same conclusion may be drawn from the other experiments given in Table 1, where several combinations of limb deprivation are seen to be equally effective, regardless of the amounts of ecdysterone administered to the animals. These procedures do not leave the animal incapacitated and the survival rate after ecdysis is as high as that of normal animals. The very large standard deviations for all groups of animals that retained their limbs (on the order of months rather than days or weeks) indicate that they have not been synchronized at all by the ecdysterone. In group 3, of the animals that retained their legs and received three sequential doses of 6 μg/g each, only two have molted. However, 5½ months (165 days) after the start of this experiment, 22 of 24 of these animals show no external signs of preparation for molting, although such signs are easily discernible 30 to 40 days before ecdysis (*7*, *9*).

The doses of ecdysterone used in these experiments were equal to or greater than those reported effective in causing molting preparations in crayfish (*11*) or insects (*12*). Although doses of 100 to 200 μg/g triggered molting

Table 1. Effect of leg removal, or injections of ecdysterone, or both, on interval between molts.

Animals (No.)	Limbs missing (No.)	Kind	Ecdysterone (μg/g)	Mean time to molt [days ± S.D. and (range)]
		Group 1		
15	1	Walking	6	154 ± 39 (115-236)
16	2	Chelipeds	6	60 ± 10 (46-85)
	4	Walking		
7	2	Chelipeds	0	72 ± 15 (60-101)
		Group 2		
4	1	Walking	6	171 ± 72 (84-235)
5	1	Walking	12	152 ± 58 (78-222)
6	2	Chelipeds		
	4	Walking	6	56 ± 11 (41-73)
6	2	Chelipeds	12	53 ± 6 (46-64)
	4	Walking		
		Group 3		
6	1	Walking	0	>195
6	1	Walking	6	>195
6	1	Walking	18	>195
6	1	Walking	6,6,6*	(50-)
10	8	Walking	0	55 ± 15 (35-77)
6	8	Walking	6	54 ± 11 (43-75)
6	8	Walking	18	52 ± 18 (43-88)
6	8	Walking	6,6,6*	51 ± 6 (42-59)

* Injected on days 0, 1, and 3.

preparations in the pillbug *Armadillidium* (*11*), we have not used such high doses with our larger animals because of the prohibitive cost.

Although extracts from the green crab *Carcinus maenas* induce molting in insects (*13*), Crustacea are relatively unresponsive to the presently available preparations of molting hormone (*14*, *15*). Moreover, molting hormone may be lethal to insects (*12*). *Rhyncosciara* injected with minimal effective doses of ecdysterone undergo cytologically normal molting preparations; yet many do not survive ecdysis (*16*). In the first attempt to test the physiological effectiveness of ecdysterone in Crustacea (*14*), a cumulative dose of less than 1 μg/g killed one species of crayfish (*Procambarus simulans*) without causing precocious molting in intact animals; the hormone had a slightly enhancing effect on animals in which molting preparations had already been triggered by removal of the eyestalks (*14*). Precocious ecdyses in intact specimens of *Procambarus* sp. were produced with doses of 6 to 12 μg/g, but higher doses were lethal (*10*). The lethality of the presently available preparations of ecdysterone might be due to a contaminant, although there is no definite evidence for this. The relative ineffectiveness in Crustacea is not necessarily indicative of lack of physiological potency of these hormone preparations; in animals with an intact X-organ sinus gland complex, the secretions of MIH may not be readily overcome by the injection of exogenous hormone.

As we report here, the loss of appendages triggers molting nearly, if not equally, as effectively as the extirpation of the X-organ sinus gland complex with its resulting removal of MIH. The most obvious conclusion is that the walking legs are the source of MIH; however, we do not think this is likely. Perhaps the loss of body mass shuts off the secretion of MIH. Although the mechanism whereby this is achieved is not known, the adaptive advantage to the animal is clear, since the earliest ecdysis means the promptest recovery of lost limbs.

We suggest that our observations on triggering precocious molts with concomitant regeneration of appendages could be of economic significance. For example, the size of specimens of the edible Alaskan king crab (*Paralithodes camtschaticus*) caught in the wild decreased significantly between 1954 and 1963 (*17*), indicating that the population is being depleted. Since walking legs are the source of meat in this species and since the animal regenerates limbs (*18*), it might be commercially desirable to remove four to six walking legs at the time of capture and to return the animals to the sea. This procedure would leave the animal not incapacitated, could be predicted to stimulate precocious molting with attendant regeneration of walking legs, and would not deplete the population. This treatment might also be adapted for the production of edible soft-shelled crabs out of season, although in such a case it would not contribute to the conservation of the species.

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References and Notes

1. Ecdysterone (crustecdysone, 20-hydroxyecdysone, β-ecdysone) isolated from the seawater crayfish *Jasus lalandei* caused puparium formation in an insect [F. Hampshire and D. H. S. Horn, *Chem. Commun.* **2**, 37, (1966)].
2. L. M. Passano, in *The Physiology of Crustacea*, T. H. Waterman, Ed. (Academic Press, New York, 1960), vol. 1, p. 473.
3. C. Zeleny, *J. Exp. Zool.* **2**, 1 (1905).
4. D. E. Bliss, *Biol. Bull.* **104**, 275 (1953); D. M. Skinner, thesis, Radcliffe College (1958).
5. D. M. Skinner, *Amer. Zool.* **6**, 235 (1966).
6. D. E. Bliss, personal communication.
7. ———, in *Bertil Hanstrom: Zoological Papers in Honour of his Sixty-fifth Birthday, November 20th, 1956*, K. G. Wingstrand, Ed. (Zoological Institute, Lund, Sweden, 1956), p. 56.
8. Autotomy: In certain Crustacea, muscles at the base of an injured limb are contracted, breaking off the limb at a preformed plane (that is, autotomy plane).
9. D. M. Skinner, *Biol. Bull.* **123**, 635 (1962).
10. Ecdysterone was purchased from the Mann Research Laboratories or was the kind gift of Dr. D. H. S. Horn. We thank Dr. E. Mattingly for checking the efficacy of the preparations by injecting 50 μg/g into *Rhyncosciara*; the expected chromosomal puffs appeared.
11. A. Krishnakumaran and H. A. Schneiderman, *Nature* **220**, 601 (1968).
12. C. M. Williams, *Biol. Bull.* **134**, 344 (1968).
13. P. Karlson and D. M. Skinner, *Nature* **185**, 543 (1960).
14. M. E. Lowe, D. H. S. Horn, M. N. Galbraith, *Experientia* **24**, 518 (1968).
15. D. Adelung, *Zool. Anz.* **30**, suppl., 264 (1967); A. C. Warner, J. R. Stevenson, C. Church, *Amer. Zool.* **9**, 1085A (1969).
16. N. Gabrusewycz-Garcia and E. Mattingly, personal communications.
17. R. B. Nickerson, F. J. Ossiander, G. C. Powell, *J. Fish. Res. Board Can.* **23**, 729 (1966).
18. K. Niwa and H. Kurata, *Bull. Hokkaido Reg. Fish. Res. Lab.* **28**, 51 (1964).

19. Research sponsored by the AEC under contract with the Union Carbide Corporation. D.E.G. is an Oak Ridge graduate fellow under appointment from the Oak Ridge Associated Universities.

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Differentiation of Immature Mucous Cells into Parietal, Argyrophil, and Chief Cells in Stomach Grafts

Abstract. *Microscopic and submicroscopic studies on regenerating gastric mucosa neonatally grafted in the subcutaneous tissue of littermate mice have revealed that immature mucous cells are totipotent; ultimately they transform into mature mucous, parietal, argyrophil, and chief cells in the gastric glands.*

Since Bensley's description of specialized cell types in the gastric mucosal epithelium much uncertainty has existed concerning their origin in the fundic glands. Several investigators have studied their reappearance in gastric mucosa following surgical or thermal injury (*1*). It was stated that the cell types of the stomach are fixed and cells of the foveolar surface do not transform to mucous neck cells, nor do the latter transform into parietal and chief cells (*2*). On the other hand, it has been postulated that such transformations do occur and the mucous neck cells serve largely to replenish short-lived parietal cells (*3*). Much of the difficulty in visualizing these transformations has arisen from the lack of techniques adequate for the demonstration of transitional forms. The regression of specialized cell types in glands bordering a lesion to more nondifferentiated type also makes it extremely difficult to identify newly regenerated glands as opposed to old regressed ones. A technique for the creation of a glandular stomach in the subcutaneous tissue has been established in mice (*4*). By coupling this technique with electron microscopy we have analyzed which cells constitute the precursors for parietal, argyrophil, and chief cells.

The gastric mucosa of newborn mice of the C57BL/6Ms and dd/I strains consisted of surface epithelium, pits, and a few simple tubular glands. It was ascertained in these mice that the surface and pits were covered with mucous cells which contained fully formed secretory granules, and that fundic glands contained numerous parietal cells which had well-developed intracellular canaliculi and a few argyrophil cells. Mice of both strains, less than 24 hours old, were grafted subcutaneously with the glandular segment of the stomach of littermates of the same sex. Two to three days after the operation the grafts formed tumors that were 1 to 2 mm in diameter, which consisted mainly of degenerating epithelial and mesenchymal cells covered with a single layer of regenerating epithelial cells (Fig. 1A). Examination of serial sections from 12 such grafts revealed that all the regenerating epithelial cells were positive for the periodic acid–Schiff reaction and for mucicarmin staining. Electron micrographs taken from four other grafts also demonstrated that only mucous cells survived as epithelial cells (Fig. 1B). The majority of the cells were characteristic in their possession of many intramitochondrial globules between the inner mitochondrial membranes. During this period bundles of fibroblasts of host origin accompanying numerous capillaries infiltrated the central parts of the necrotic masses of the grafts. The bundles became thicker and turned inside out the epithelium covered masses. Thus, the tumors transformed into small branched canals 4 to 6 days after grafting. A few immature parietal cells with undeveloped intracellular canaliculi and mucous granules appeared in the epithelium during this period (Fig. 2A). These cells may secrete hydrochloric acid (*5*), and this might be correlated with transformation of grafts from tumors into canals to isolate the acid from the subcutaneous tissue of the hosts. Between day 6 and day 14 the canals developed

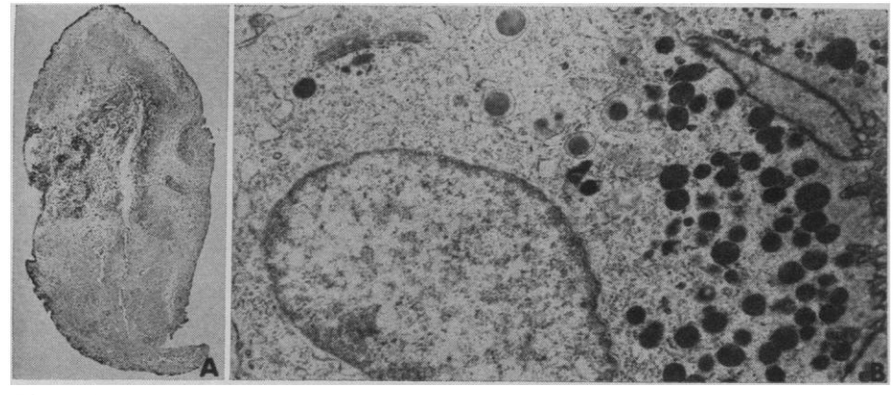

Fig. 1. Stomach graft implanted neonatally, 3 days before, in the subcutaneous tissue of a littermate mouse. (A) Single layer of regenerating epithelial cells covers degenerative epithelial and mesenchymal tissues and central regenerating mesenchymal tissue which contain newly infiltrated vessels from the host (× 29). (B) Immature mucous cells in the regenerating epithelium in a stomach graft as shown in (A). Fully formed mucous granules are present in the apical cytoplasm and mitochondria which contain a few intramitochondrial globules are scattered in the perinuclear area (× 9230).