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EFFECTS OF EYESTALK REMOVAL ON RHYTHMIC LOCOMOTOR ACTIVITY IN *CARCINUS*

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INTRODUCTION

In decapod Crustacea the focus of attention for studies on the control of rhythmicity has centred upon the neuro-endocrine system of the eyestalk. Kalmus (1938), Roberts (1941), Schallek (1942), Edwards (1950), Valente & Edwards (1955) and Powell (1965) have all recorded the disappearance of rhythmic locomotor activity after eyestalk ablation in various decapods. Bliss (1962) was also unable to detect the normal rhythm in several *Gecarcinus* after eyestalk ablation; in one case she found that a circadian rhythm persisted, but with a dramatically changed periodicity. On the basis of this last observation Bliss (1962) and Roberts (1965) have suggested that reported losses of rhythmicity may, in fact, be illusory, unless adequate methods are employed to detect possible changes in frequency.

Another consideration to be borne in mind in eyestalk-ablation experiments, relates to the necessity of distinguishing between the effects of post-operative shock and effects upon a possible rhythmic control mechanism or mediator. This is perhaps particularly relevant in view of conflicting reports relating to the level of activity of decapods from which eyestalks have been removed. Whereas eyestalk-ablated *Cambarus* were shown to exhibit increased activity (Roberts, 1941; Schallek, 1942), total activity is reported to be decreased in ablated *Potamobius* and *Cambarus* (Kalmus, 1938), *Trichodactylus* (Valente & Edwards, 1955), *Uca* (Edwards, 1950) and *Carcinus* (Powell, 1965). The present paper attempts to reconcile these conflicting reports by studying *Carcinus maenas* L. at various times following eyestalk removal and after other experimental procedures. An important treatment adopted relates to the observation that arrhythmic, but otherwise normal *Carcinus*, can be induced to show a rhythm by chilling (Naylor, 1963). Present work uses this observation as a test of the ability of crabs to show rhythmicity after they have been given time to recover from surgical operations. The procedure of frequency analysis is used to test for rhythmicity in the results.

MATERIALS AND METHODS

Crabs used in these experiments were males of 3–6 cm. carapace width. Some were freshly collected from the shore, but, since the normal tidal rhythm is apparent for only 3–4 days in such crabs (Naylor, 1958), their rhythmicity would be expected to disappear before they had recovered from eyestalk-ablation operations. Most crabs used were therefore laboratory stock animals in which the rhythm investigated is that which is apparent after a period of chilling. By chilling for 6 hr. at 4° C. tidal rhyth-

micity can be re-initiated in aquarium crabs, the first peak occurring at the time of return to normal temperatures and subsequent peaks at approximately 12·4 hr. intervals thereafter (Naylor, 1963). Surgically operated crabs were tested by this procedure after being given various periods of recovery.

Usually for removal of eyestalks the crabs were first anaesthetized for 5–10 min. in iced sea water and one eyestalk was removed with a sharp, fine-tipped scalpel. Crabs were then left in running sea water for 2 days before the second eyestalk was removed in a similar manner. The wounds were not cauterized but bleeding was not excessive in crabs anaesthetized in iced water, and specimens were left to recover for a further 6 days in running sea water before being used in the experiments. Retinae were removed with fine scissors after anaesthetizing the animal in iced sea water and again keeping the crab in running sea water for a subsequent recovery period of 6 days.

In eyestalk-chilling experiments the animals were immobilized between Perspex plates bound by rubber bands and suspended in a tank of fast running sea water in a vertical position. The eyestalks of the crab were arranged to project above the surface of the water, and pieces of narrow-gauge rubber tubing were fitted over the eyestalks and attached but not sealed to the orbits. Iced sea water at a temperature of 1° C. was then dripped down the tubes for a period of 10–11 hr., at the end of which time the crabs were transferred to actographs at 15° C. Control animals for these experiments were clamped between Perspex plates and kept in running sea water for 10–11 hr., but were not chilled.

Extracts of eyestalks used for injection experiments were prepared with freshly removed eyes homogenized in a little boiled sea water and centrifuged. The supernatant fluid was diluted to a concentration equivalent to two eyestalks per 0·25 c.c. of solution. Experimental animals were injected through the membrane at the base of the last peraeopod with 0·25 c.c. of eyestalk extract, while controls received similar quantities of muscle extract or boiled sea water.

Activity was recorded in continuous dim red light using tilting-box actographs (Naylor, 1958), most crabs being kept in moist air at 15° C. The procedure of frequency analysis, used to confirm the presence or absence of rhythmicity in the data, is described elsewhere (Williams & Naylor, 1967). In the periodograms so obtained, marked excursions from the general trend of values for the coefficient of variability are indicative of rhythmicity of that frequency in the original data.

RESULTS

Eyestalk removal

In freshly collected crabs rhythmicity is lost following bilateral eyestalk ablation, the crabs showing high initial activity, and becoming virtually inactive after about 12 hr. (Fig. 1). Such crabs were, however, often moribund after about 48 hr. in the actographs, and in most experiments ablated crabs were given a recovery period in sea-water aquaria before activity was recorded. Table 1 compares the average hourly activity of recuperating eyestalkless crabs with that of normal animals. In these experiments crabs were placed in actographs on days 6, 9, 12 and 15 after the start of the experiment and their total activity was recorded for each 24 hr. period. In winter there was no significant difference between the activity of eyestalkless and normal crabs, but

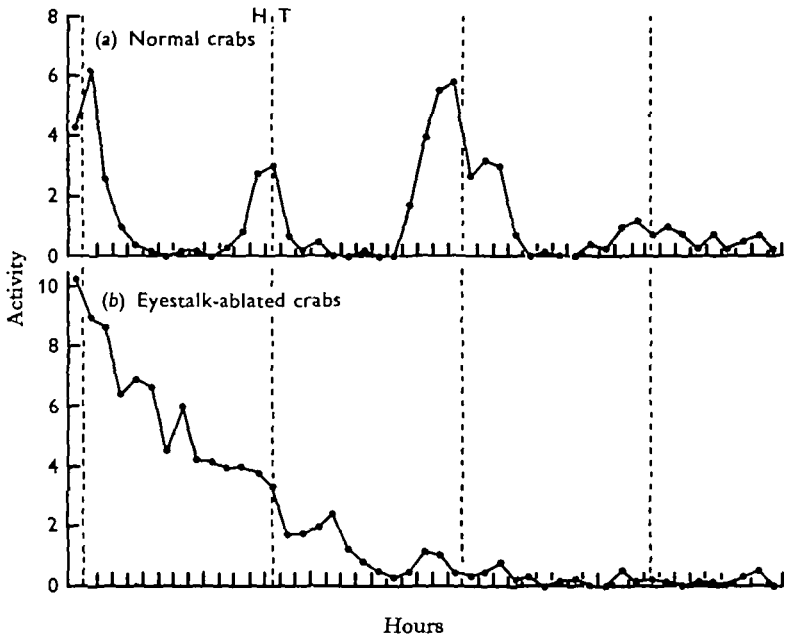

Fig. 1. Average hourly activity in constant conditions of (*a*) four freshly collected crabs and (*b*) twenty crabs from which both eyestalks were removed immediately after collection. (Vertical dotted lines indicate times of high tide.)

Table 1. *Average hourly activity of eyestalkless* (E^-) *and normal* (E^+) *crabs on days* 6, 9, 12 *and* 15 *after the second eyestalk was removed from the experimental animals*

		Number of crabs	Hourly activity values: Mean	Standard deviation	Range	Variances	Comparison of means
			Summer crabs				
Day 6	E^+	9	0·8	0·55	0·2–2·2	≠	$P < 0{\cdot}001$*
	E^-	10	4·8	2·28	0·0–7·4		
Day 9	E^+	9	0·8	0·45	0·3–1·7	≠	$0{\cdot}01 < P < 0{\cdot}02$*
	E^-	12	3·0	2·51	0·0–9·0		
Day 12	E^+	9	0·8	0·63	0·0–2·0	≠	$0{\cdot}002 < P < 0{\cdot}01$*
	E^-	12	3·3	2·39	0·0–7·0		
Day 15	E^+	9	0·7	0·55	0·1–2·0	≠	$0{\cdot}002 < P < 0{\cdot}01$*
	E^-	10	3·0	2·02	0·1–6·3		
			Winter crabs				
Day 6	E^+	12	1·8	1·15	0·3–4·4	=	$P > 0{\cdot}1$
	E^-	12	1·0	1·37	0·0–3·9		
Day 9	E^+	10	1·9	1·07	0·7–3·8	=	$P > 0{\cdot}1$
	E^-	13	1·5	1·27	0·0–4·4		
Day 12	E^+	9	1·1	·71	0·2–2·7	=	$P > 0{\cdot}1$
	E^-	13	1·4	1·08	0·2–3·2		
Day 15	E^+	11	0·9	·26	0·6–1·4	≠	$P > 0{\cdot}1$
	E^-	10	1·4	1·03	0·4–3·7		

(* Means significantly different at the 2% level or less.)

in summer a highly significant increase in activity was evident in ablated crabs (Table 1). *Carcinus* is normally arrhythmic in winter and exhibits overt locomotor rhythmicity only during the summer months.

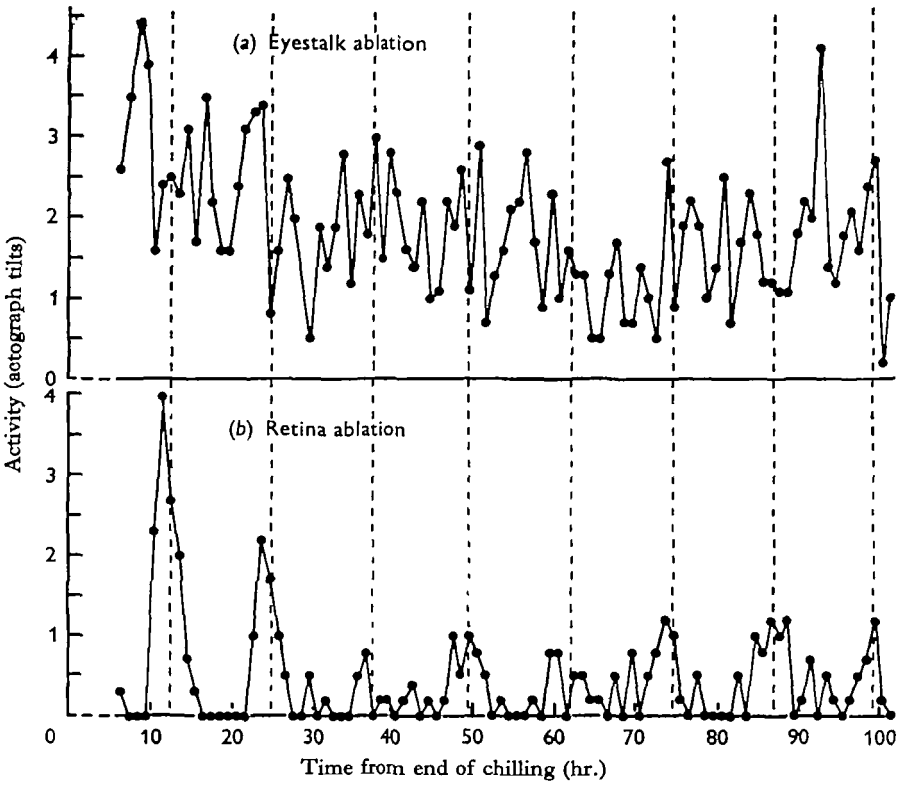

Fig. 2. Average hourly activity after chilling at 4° C. for 12 hr. of (*a*) twenty crabs from which eyestalks were removed and (*b*) five crabs from which retinae were removed. (Vertical dotted lines at 12·4 hr. intervals from end of chilling period.)

In addition to the increased activity observed in eyestalkless crabs in summer, no crab without eyestalks, at any time of the year, was observed to show rhythmic locomotor activity. Figure 2*a* shows the average activity over 4 days of sixteen eyestalkless crabs given 6 days to recover from eyestalk ablation and then chilled to 4° C. for 12 hr. There is no evidence of tidal rhythmicity in that figure, and frequency analysis of the data reveals no rhythmicity in the 6–18 hr. frequency range (Fig. 3*a*). In contrast, control animals from which only the retinal portion of the eye stalk was removed showed clear evidence of rhythmicity. Activity peaks roughly coincide with 12·4 hr. intervals marked off after the end of the chilling period (Fig. 2*b*), and very high values for the coefficient of variability appear in the 12–13 hr. range of the frequency analysis of these data (Fig. 3*b*). From these experiments there is preliminary evidence that the region of the eyestalk proximal to the retina is a source of locomotor inhibition which may be involved in the control of rhythmic locomotor activity.

Injection experiments

In summer, eyestalkless crabs were injected with extract of eyestalks removed from donor crabs during the quiescent stage of their activity rhythm. Average hourly activity values fell gradually during the period of 12 hr. after injection, both in experimental animals and in controls, but crabs injected with eyestalk extract were generally less active than controls injected with muscle extract (Fig. 4*a*) or boiled sea water (Fig. 4*b*). The fluctuation of mean hourly activity values of the controls of Fig. 4*a* probably relates to the smaller number of crabs used in those experiments (eight controls), compared with the sixteen controls in Fig. 4*b*. Using a method of paired comparisons of means, the differences between experimental animals and controls are significant at the 1 % level in Fig. 4*a* and at the 0·1 % level in Fig. 4*b*.

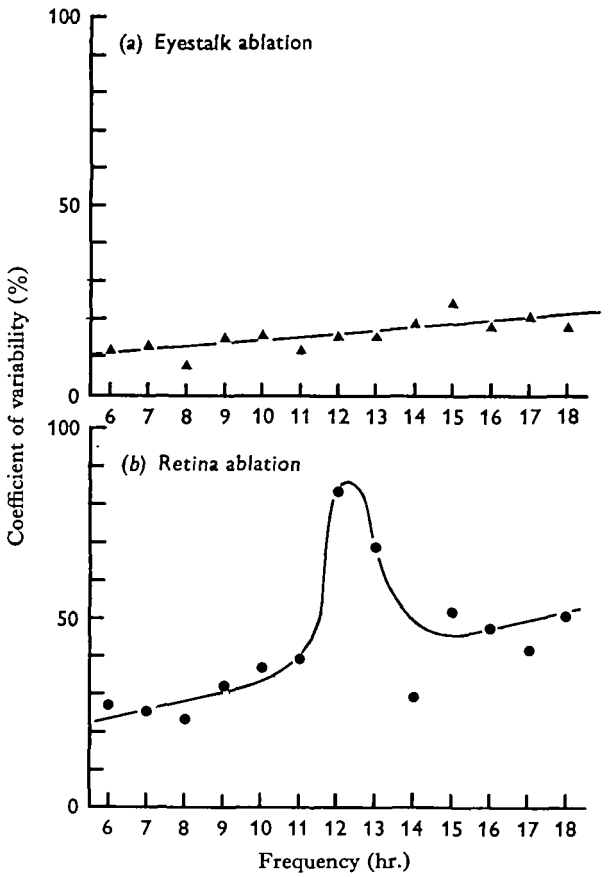

Fig. 3. Periodogram analyses of data in Fig. 2, scanning for possible rhythms of 6–18 hr. frequency.

Eyestalk chilling

If eyestalks play a part in the regulation of locomotor activity, chilling the eyes while maintaining the body at normal temperatures might be expected to give a result similar to that obtained when chilling the whole animal. Figure 5*b* shows that this is true, the

induced, approximately tidal, rhythmicity (Fig. 6*b*) being phased from the end of the chilling period. Control animals immobilized in a similar manner to the experimental animals, but not chilled, showed no rhythm (Figs. 5*a*, 6*a*).

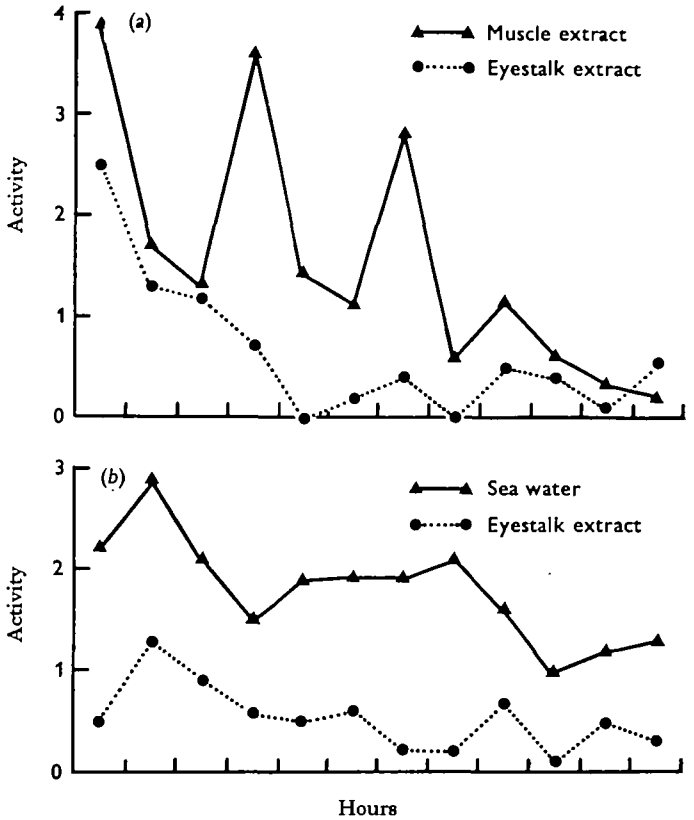

Fig. 4. Average hourly activity of eyestalkless crabs after injection of various extracts: (*a*) eight crabs injected with eyestalk extract and eight controls with muscle extract, (*b*) fourteen crabs injected with eyestalk extract and sixteen controls with boiled sea water.

DISCUSSION

Although Roberts (1965) and Gabe (1966) suggest that the results of Bliss (1962) show that a biological clock controlling the activity rhythm of *Gecarcinus* is not situated in the eyestalk, Bliss herself avoids such a conclusive statement. She offers two alternative explanations of her results by suggesting that either the biological clock does not reside within the eyestalk, or a second rhythmic centre assumes control once the eyestalk mechanism is removed. As Bliss (1962) points out, normal rhythmicity was usually lost on ablation, and in the one animal which showed circadian rhythmicity after ablation, the period of the rhythm changed dramatically from 24·2 hr. to 22·7 hr. after eyestalk removal. In general Bliss's results agree with work on *Cambarus* (Kalmus 1938; Roberts, 1941; Schallek, 1942), *Uca* (Edwards, 1950) and *Trichodactylus* (Valente & Edwards, 1955) in which normal rhythmicity is abolished in ablated animals. Present observations on *Carcinus* also show that rhythmicity is lost after eyestalk ablation and

cannot be induced in recuperated animals by chilling. All workers agree, therefore that normal rhythmicity is altered or lost following eyestalk ablation, which suggests that the eyestalk hormonal mechanism is at least a mediator, if not an autonomous clock, involved in the control of normal rhythmic locomotor activity.

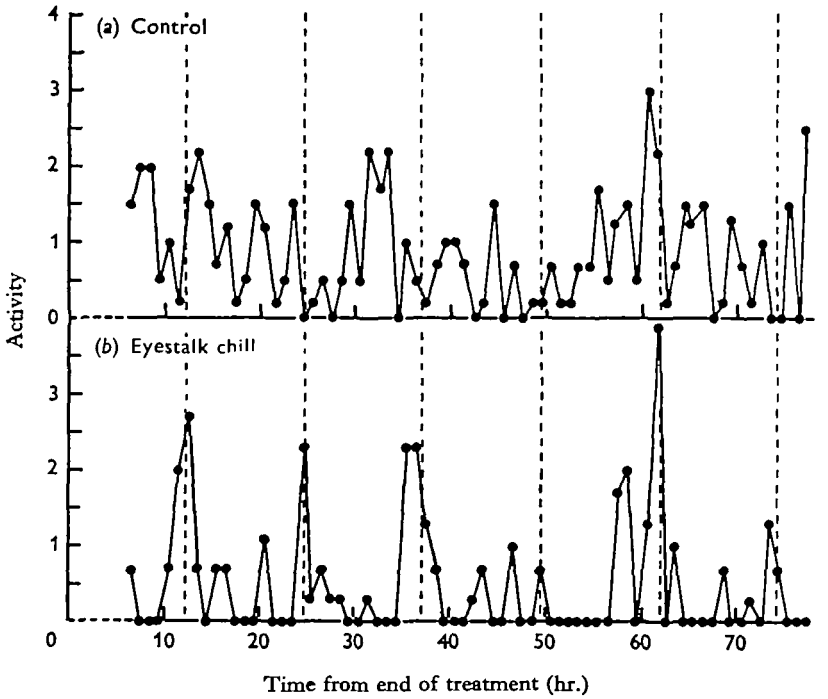

Fig. 5. Average hourly activity of (*b*) three crabs subjected to chilling of the eyestalks and (*a*) three controls clamped in the same manner as experimental animals but not chilled (symbols as in Fig. 2).

In *Gecarcinus* (Bliss, 1962) eyestalk removal not only affects the locomotor rhythm but also precipitates moulting. The activity pattern of eyestalkless crabs is similar to that of unoperated crabs in premoult, all of which show a disturbance of their normal circadian rhythmicity. These findings have been used to suggest that activity changes following eyestalk removal are directly related to the loss of the neuroendocrine system which inhibits moulting (Bliss, 1962; Gabe, 1966). In contrast, *Carcinus* in present experiments showed no indication of precipitate moulting as a result of eyestalk ablation. This is supported by other work, for whereas eyestalk ablation induced moulting in juvenile *Carcinus* (Passano & Jyssum, 1963) and in old specimens in terminal anecdysis (Carlisle, 1957), the same operation did not induce moulting in crabs of intermediate age such as were used in present experiments (Carlisle, 1954). Moreover, unlike *Gecarcinus*, normal *Carcinus* which moult in acktographs maintain their tidal rhythmicity during proecdysis. They show increased total activity only during the 24 hr. or so immediately before the moult, and tidal rhythmicity is evident up to the time of ecdysis. This evidence precludes for *Carcinus* the close relationship between moulting and rhythmic locomotor activity which Bliss suggested for *Gecarcinus*.

Apart from differences relating to the possible initiation of moulting by eyestalk extirpation conflicting reports of the level of activity in eyestalkless decapods may also relate to differences in the lapse of time between ablation and activity recording. Thus the observations by Powell (1965) that activity was low in eyestalkless *Carcinus* would be explained if, as seems likely (Bliss, 1962, in discussion), only a short recovery period was given. Similarly, the reduced activity of eyestalkless *Trichodactylus* may be related to the fact that the animals were placed in actographs only 'several hours' after

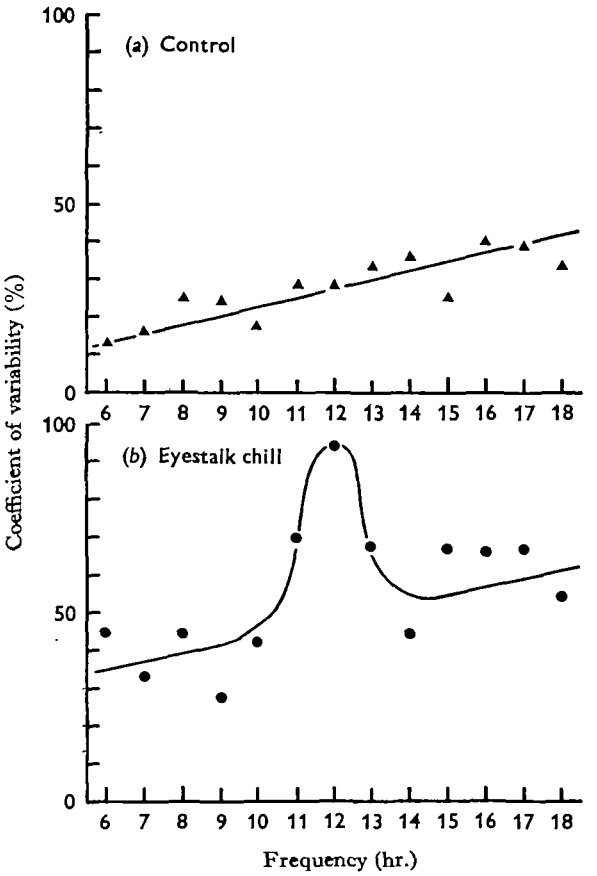

Fig. 6. Periodogram analyses of data in Fig. 5, scanning for possible rhythms of 6–18 hr. frequency.

removal of the second eyestalk, and activity was recorded for a maximum of 7 days (Valente & Edwards, 1955). Indeed, there was evidence of gradually increasing total activity during the course of the experiments of the last authors. High levels of locomotor activity are recorded in eyestalkless *Cambarus* kept in acktographs for up to 3 weeks after ablation (Schallek, 1942) and in *Carcinus* in present experiments on days 6, 9, 12 and 15 after eyestalk removal in summer. Evidently previously rhythmic animals which have had their eyestalks removed and have been given time to recover from the operation show continuously high activity, suggesting that the eyestalks are a

source of rhythmic inhibition of locomotion. This suggestion is complemented by the results of experiments involving the injection of eyestalk extract, which inhibits locomotor activity in recuperated, eyestalkless *Cambarus* (Roberts, 1941) and *Carcinus* (p. 111). The injection experiments are consistent with the view that the inhibitory principle is a blood-borne hormone. In addition, preliminary experiments suggest that eyestalk extract from active donors is less effective in suppressing activity in recipients than extract from quiescent donors (Williams, 1966), but difficulties arise in the interpretation of such experiments, since an inhibitory factor may be stored in the eyestalks of active animals without being released into the blood.

Such a mechanism may perhaps be compared with mediation of the locomotor rhythm in *Periplaneta americana* by the suboesophageal ganglion (Harker, 1955, 1956, 1960; Roberts, 1965, 1966; Brady, 1967). Harker's (1960) view that the oesophageat ganglion functions as an endogenous hormonal clock in the cockroach has recently been questioned by Roberts (1965, 1966) and Brady (1967). The last author suggests that the neuro-endocrine rhythms of the nerve-cord ganglia are rather ephemeral and are perhaps controlled by an electrical pacemaker in the brain. In *Carcinus* the apparently rhythmic production of an inhibitory hormone by the neurosecretory cells of the eyestalk has not definitely been shown to be an autonomous process. The only evidence of there being a self-timed clock within the eyestalk comes from the observations thal rhythmicity is rephased by chilling the eyestalks, while the remainder of the crab is kept at room temperature. It should not be overlooked, however, that information concerning the chilling period may be passed from the eyestalk to a rhythmic centre elsewhere in the body, which in turn controls hormone production in the eyestalk. Evidence for nervous involvement in the control of rhythmicity in the crayfish *Cambarus* is given by Schallek (1942), who found that the promotion of activity by eyestalk removal could be duplicated by section of the optic nerves. It should be noted, however, that Schallek (1942) favoured a nervous mechanism as an alternative to endocrine factors, rather than as an additional mechanism involved in the control of rhythmicity.

Whether the eyestalk hormonal mechanism in *Carcinus* is self-timing or not, it is in all probability only part of a complex clock system. Previous work on temperature relationships of the rhythm (Naylor, 1963) has suggested that at least two mechanisms control locomotor rhythmicity in the crab. One of these, postulated on the basis of the short-term effects of temperature change upon the *Carcinus* rhythm, may equate with the eyestalk inhibitory mechanism discussed here. Thus a fall in temperature, which induces a transient advance and enhancement of the next peak of activity (Naylor, 1963), may do so by temporary inhibition of production or release of the eyestalk inhibitory hormone. Conversely, a rise in temperature, which delays and reduces the next activity peak, may temporarily enhance the production or release of the inhibitory hormone. The more deep-seated mechanism which maintains the rhythm despite the transient changes (Naylor, 1963) could be a nervous mechanism which in the light of present results, is expressed via the hormonal mechanism of the eyestalk. Bliss's single result of a crab showing rhythmicity after eyestalk ablation could be explained if in that animal some deep-seated clock expressed its rhythmicity via another neurohaemal centre outside the eyestalk, more or less along the lines of her own alternative explanation of the *Gecarcinus* results (Bliss, 1962).

SUMMARY

1. Male *Carcinus* collected in summer and given several days to recover from eyestalk ablation showed a continuously high level of activity. Injection of eyestalk extract inhibited activity. Unlike control animals from which retinae were removed, experimental animals did not show a rhythm after chilling. Chilling the eyestalks alone elicited a rhythm similar to that appearing when the whole animal was chilled.

2. The results are consistent with the view that locomotor rhythmicity is mediated via an inhibitory hormonal mechanism in the neurosecretory cell groups of the eyestalk. There is no direct evidence that the neuroendocrine mechanism alone constitutes an autonomous clock, however, and it is in any case probably co-ordinated by one or more other rhythmic systems within the crab.

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