

ON THE HORMONAL INHIBITION OF MOULTING IN DECAPOD CRUSTACEA

II. THE TERMINAL ANECDYSIS IN CRABS

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(Text-figs. 1-5)

When a crab moults it usually, though not invariably, increases in volume and in linear dimensions. The interval between successive moults or ecdyses frequently varies with the temperature and becomes greater as the individual grows larger. An exception is to be found in some crabs which have a regular moulting season, moulting perhaps once or twice a year. In some species at least the cycle of growth and moulting terminates with a final terminal anecdysis, beyond which the crab never progresses. This lays an upper limit to the size which the animal may attain. Teissier (1935) was, so far as I am aware, the first to draw attention to this phenomenon, whereby a crab—in Teissier's investigations the spider crab, *Maia squinado*—never moults again once it has entered upon this final instar. The occurrence of this terminal anecdysis has been demonstrated in the grapoid crab *Pachygrapsus crassipes* (by Hiatt, 1948), the female blue crab *Callinectes sapidus*, and the green shore crab *Carcinus maenas* (by Carlisle, unpublished); it may be inferred from the somewhat scanty population statistics for other species of crabs, e.g. *Portunus depurator*, which have a definite size limit. The terminal anecdysis is, however, unknown in prawns which may go on moulting and increasing in size until they are overtaken by death, and the same is evidently true of the lobster, *Homarus gammarus* (L.) and the edible crab, *Cancer pagurus*, which may, apparently, increase in size indefinitely; reports of 'giant' crabs and 'giant' lobsters are not infrequent in the lay press and in the fishing periodicals.

In this paper the nomenclature of the moult cycle proposed by Carlisle & Dohrn (1953) will be adopted with addition of the expression 'terminal anecdysis'. Briefly the stages are as follows.

Proecdysis: a period of preparation for the moult, often accompanied by a raised blood-calcium level.

Ecdysis: the act of casting the shell or moulting.

Metecdysis: a period of recovery from the moult, when the animal is still partially soft but is rapidly hardening the new exoskeleton.

Diecdysis: a brief period between the end of one metecdysis and the beginning of the next proecdysis.

Anecdysis: a long period between the end of a metecdysis and the beginning of the succeeding proecdysis; this period often separates groups of moults which are themselves separated by diecdyses, but in species which moult seasonally, it is the normal stage separating two ecdyses.

Diecdysis and anecdysis are often both called intermoult periods, but their endocrine basis appears to be different (see Knowles & Carlisle, 1956) so that separate names are required for them. The term 'intermoult period', moreover has often been used to indicate the total period between two moults, including metecdysis and proecdysis. 'Terminal anecdysis' is a period at the end of the life of a crab when the physiological condition resembles a normal anecdysis, but the animal is incapable of further moulting. It will be noticed that this condition is strictly an anecdysis, but it can hardly be called an intermoult, for it does not lie between two moults. Some differences between animals in non-terminal and in terminal anecdysis will become apparent during the course of this paper.

The problem which has been investigated in the work now to be described is that of the failure to undergo further moults once terminal anecdysis is reached. The approach has been endocrinological.

MATERIALS AND METHODS

THE CRABS

Two crabs have been the subject of these investigations, the oxyrhynchan *Maia squinado*, the spider crab, and the brachyrhynchan *Carcinus maenas*, the green shore crab. All crabs were collected in the Plymouth area. *Carcinus* of the Plymouth population are biometrically and physiologically distinct from two races which occur on the opposite coast of the English Channel and it is probable that other local populations are likewise distinct (see D emeusy & Veillet, 1953; D emeusy, 1953; Carlisle, 1955); numerical data and dimensions refer specifically to the Plymouth population unless some other locality is mentioned.

Maia squinado has been the subject of intensive biometrical study by Teissier (1934-55), who has shown that it never moults again after the moult of puberty. Sexual maturity is achieved at this moult; copulation takes place while the female is still soft after the moult and is not possible thereafter. No further growth takes place. The terminal anecdysis, therefore, is the anecdysis succeeding upon the attainment of sexual maturity. It follows that female *Maia* can only breed once. The earlier moults are separated by long intervals, and the intermoult period appears to be of the nature of an anecdysis, rather than a diecdysis. So far as we are aware at present *Maia* only moults during late July and early August (always excluding the very young crabs). As a seasonal moult, therefore, its intermoult period is almost certainly an anecdysis.

Carcinus maenas on the contrary moults throughout the greater part of the year, refraining from doing so only during the coldest part of the winter, January to March. The moults are separated by diecdyses during the summer and autumn; the only anecdyesis is that during these three cold months. I have shown (Carlisle, 1954, 1955, and unpublished data) that the endocrine control of these two types of intermoult period is somewhat different. A detailed biological account of a brachyrynchan crab, *Pachygrapsus crassipes*, whose moulting cycle follows the same pattern is given by Hiatt (1948). Like *Pachygrapsus*, *Carcinus maenas* ends its life in anecdyesis, a terminal anecdyesis after which it never moults again. In the Plymouth population almost all *Carcinus* over 70 mm carapace breadth and all over 75 mm are in the terminal anecdyesis. The largest of the males are regularly 86 mm in carapace breadth, never in my experience exceeding this figure by even 1 mm. The females are smaller than the males. Analysis of the dimensions of egg-bearing females reveals a bimodal curve of size. I take this to indicate that there are two age-groups breeding each year. In the laboratory I have reared a crab caught when egg-bearing, with a carapace breadth of 23 mm, through four moults to a second breeding and egg-bearing a year later when the carapace breadth was 51 mm. After egg-bearing it moulted once more to a carapace breadth of 63 mm when it entered terminal anecdyesis. The moult of puberty takes place in *Carcinus* at about 16 mm carapace breadth (Cornubert, Dèmeusy & Veillet, 1952), and the animal undergoes about ten more moults before entering on the terminal anecdyesis. The contrast with *Maia* is obvious. It will be shown in the course of this paper that the endocrine basis of terminal anecdyesis differs profoundly in the two species of crabs.

THE ENDOCRINE ORGANS

The X-organ-sinus gland complex of the eyestalk has been described in various species of decapod Crustacea by Bliss, Durand & Welsh (1954), Passano (1953) and Carlisle (1953) among others. It has repeatedly been shown to be the source of a moult inhibiting hormone (see especially Passano, 1953), but it does not apparently secrete a moult accelerating hormone, such as is found in the natantian decapods (see discussion in Knowles & Carlisle, 1956).

The Y-organ described by Gabe (1953) has been shown by Echaliér (1954, 1955) to secrete a moult-promoting hormone. It is possible that the moult-inhibiting hormone of the eyestalk acts not on the tissues, but on the Y-organ, to restrain it from producing the moult-promoting hormone.

In this study attention was directed to these two endocrine complexes.

All extracts of the endocrine organs have been made in distilled water, by grinding up the fresh organ with fine silica sand in a fused alumina mortar and pestle. The resulting suspension was then either filtered or centrifuged, before it was injected intramuscularly.

Previous studies have shown that eyestalk removal and removal of the

X-organ-sinus gland complex have identical effects on moulting (see especially Passano, 1953). As far as the moult-inhibiting hormone of this complex is concerned eyestalk removal is simply a cruder way of effecting ablation of the X-organ-sinus gland complex. Both types of operation have been performed in this investigation, and the results have been strictly equivalent. Eyestalk removal has been performed by cutting through the base of the stalk with fine pointed scissors. One eyestalk has been removed at a time, the other left to the next day in order to reduce the mortality. Removal of the X-organ-sinus gland complex was effected by the method described by Passano (1953), with the obvious modifications necessitated by the slightly different anatomy of the species used. The Y-organ was removed by the method of Echali er (1954).

ASSAY OF EXTRACTS

All assays were performed upon non-egg-bearing female *Leander serratus* of the size-range 55–70 mm overall length. Individuals were chosen which were in diecdysis at the time of injection. Intact individuals were employed and each was injected with a single dose of 0.15 ml. of extract or control saline. Each dose level was administered to twenty prawns and the death-rate during the assay was of the order of 4%. In no group was more than a single test animal lost during the course of an assay. Five days after the injection the prawns were examined under the binocular microscope to determine what proportion of each group had begun proecdysis. The moult-inhibiting hormone of the X-organ-sinus gland complex inhibits the onset of proecdysis while the moult-promoting hormone of the Y-organ promotes its onset. The statistical analysis followed the recommendations of Finney (1952). Graphical illustrations of assays (Figs. 1, 2, 4 and 5) will explain their layout. No units of activity are established: all assays are strictly comparative, devoted to comparing the titre of hormone in the glands in one stage of life with that in another.

RESULTS

Moult-inhibiting hormone *Carcinus maenas*

A comparative assay of sinus glands taken from the eyestalks of male *Carcinus* in the terminal and in earlier anecdyse is illustrated in Fig. 1. It will be seen that the titre of moult-inhibiting hormone per gland is roughly four times greater in the sinus glands of crabs taken during the terminal anecdyse than during earlier anecdyse. Statistical analysis of the data gives the ratio between the effectiveness of the two as 4.25 with 1% fiducial limits at 3.44 and 5.79. It is to be noted that crabs in the terminal anecdyse are larger than younger crabs, so if the data are presented in terms of mg of glandular tissue rather than numbers of glands, the ratio is diminished. Weight for weight the sinus glands of the older crabs are 2.68 times more effective than those of the younger crabs in preventing the initiation of proecdysis in *Leander serratus*,

and the 1% fiducial limits are 1.38 and 3.64. On either basis then, the sinus gland contains more moult-inhibiting hormone during the terminal anecdyasis than during earlier anecdyases.

The difference is even greater between the terminal anecdyasis and diecdysis. Here the ratio of titres of moult-inhibiting hormone (on a weight for weight basis) is 7.92 with 1% fiducial limits at 6.75 and 9.37. This emphasizes once more that during diecdysis there is a lesser production of moult-inhibiting hormone by the eyestalk than during anecdyasis; the endocrine status is different.

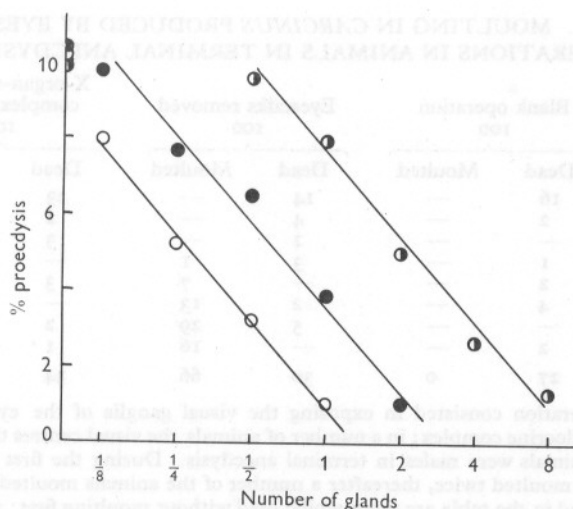


Fig. 1. Pooled results from a number of assays of the moult-inhibiting hormone of the sinus gland of *Carcinus* on *Leander*. The abscissa gives the number of glands supplying the extract which was injected into each individual; the ordinate gives the percentage of *Leander* which were found to be in proecdysis 5 days after the injections were made. All the *Leander* were in diecdysis at the beginning of the assay. Donors in terminal anecdyasis—open circles; donors in non-terminal anecdyasis—black circles; donors in diecdysis—half-black circles.

The high titre of moult-inhibiting hormone present in the sinus glands of *Carcinus* during the terminal anecdyasis suggests that it may be part of the cause of this phenomenon. Removal, therefore, of the source of the hormone should test this hypothesis. This may be performed most simply by removing the whole eyestalk or by removing the X-organ-sinus gland complex, leaving the visual centres of the eyestalk intact. Both operations have led to the same result. All crabs in terminal anecdyasis so operated have immediately entered proecdysis, as evinced by the heightened blood-calcium level and the modifications of the structure of the shell and cuticle. Eventually all which have survived long enough have moulted. The numbers are summarized in Table 1. During twelve months following the operation one crab whose eyestalks had been removed and two from which the X-organ-sinus gland complex had been

removed moulted three times, while one whose eyestalks had been removed moulted four times. This last crab at death had a carapace breadth of 132 mm, compared with the largest specimens caught wild in Plymouth which regularly have a carapace breadth of 86 mm, and one large specimen from the Scilly Islands which measured 92 mm. It is noteworthy that *Carcinus* in terminal anecdyasis have never survived more than 7 months in the laboratory.

There can be little doubt that the moult-inhibiting hormone of the eyestalk in *C. maenas* is a major agent in preventing ecdysis once the crab enters

TABLE 1. MOULTING IN *CARCINUS* PRODUCED BY EYESTALK OPERATIONS IN ANIMALS IN TERMINAL ANECDYSIS

No. operated	Blank operation		Eyestalks removed		X-organ-sinus gland complex removed	
	100		100		100	
Week	Dead	Moulted	Dead	Moulted	Dead	Moulted
1	16	—	14	—	23	—
2	2	—	4	—	2	—
3	—	—	2	—	3	—
4	1	—	3	1	—	4
5	2	—	—	7	3	8
6	4	—	2	13	—	9
7	—	—	5	29	2	23
8	2	—	—	16	1	19
Total	27	0	30	66	34	63

The blank operation consisted in exposing the visual ganglia of the eyestalk, without damaging the endocrine complex; in a number of animals the visual centres themselves were damaged. All animals were males in terminal anecdyasis. During the first 8 weeks of the experiment none moulted twice, thereafter a number of the animals moulted a second time. The dead recorded in the table are those which died without moulting first: a small number died after starting or completing the moult; these are counted among the animals which moulted.

upon its terminal anecdyasis. To stimulate further moulting it is merely necessary to remove the gland system responsible for secreting this hormone. It has earlier been shown (Brown & Cunningham, 1939; Passano, 1953) that injection of this hormone, or implantation of the gland, is sufficient to prevent the onset of proecdysis and subsequent ecdysis in crabs which have been deprived of the X-organ-sinus gland complex or of the eyestalks.

The Y-organ

It is not known for certain whether the moult-inhibiting hormone of the eyestalk acts directly on the tissues in restraining the onset of proecdysis, or whether, as Echaliér (1954, 1955) and Gabe (1953) suggest, it acts in preventing the Y-organ from secreting a moult-promoting hormone which is essential for the processes of proecdysis and ecdysis to take place. Echaliér has shown that extirpation of the Y-organ in *Carcinus* leads to a cessation of all the processes of proecdysis, so that a crab lacking this organ cannot enter

proecdysis, nor continue further with it if it was part way through this stage when the operation was performed.

The Y-organs of 20 male *Carcinus* in the terminal anecdysis had a mean weight of 12.1 ± 1.1 mg, while 20 male *Carcinus* in the penultimate diecdysis had Y-organs with mean weight 13.0 ± 1.2 mg. When the weights, however, are expressed as a ratio of the body weight they become 10.9 ± 0.99 mg% and 21.1 ± 1.95 mg%. The difference appears to be significant at the 1% level. Relative to body size therefore the crabs in terminal anecdysis have smaller Y-organs than those slightly younger.

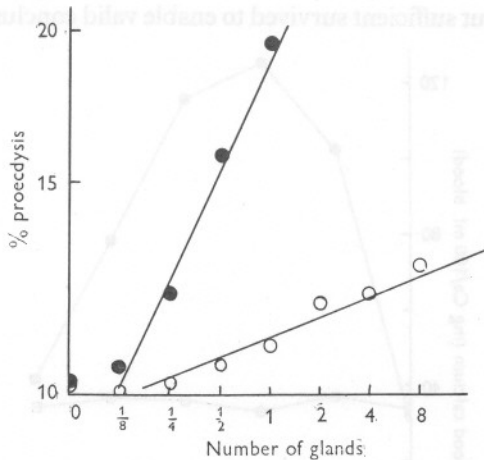


Fig. 2. Pooled results of a number of assays of the moult-promoting hormone of the Y-organ of *Carcinus* on *Leander*. The abscissa gives the number of glands supplying the extract which was injected into each individual; the ordinate gives the percentage of *Leander* which were found to be in proecdysis 5 days after the injections were made. Both scales are logarithmic. All the *Leander* were in diecdysis at the beginning of the assay. Donors in terminal anecdysis—open circles; donors in diecdysis—black circles.

An assay of the content of moult-promoting hormone in the Y-organ of *Carcinus* is illustrated graphically in Fig. 2. The Y-organs from crabs in diecdysis are significantly more potent ($P < 0.001$) in this respect than organs from crabs in the terminal anecdysis.

A single injection of an extract of Y-organ into *Carcinus* in terminal anecdysis resulted in a fourfold rise in blood-calcium level, lasting about 5 days (see Fig. 3). This may be taken as indicative of the beginning of proecdysis, albeit temporary. Accordingly I carried out a prolonged programme of injecting such *Carcinus* twice a week with Y-organ extracts. The extracts were prepared from younger crabs which were in the period of proecdysis, when *a priori* reasoning led me to believe that the titre of the moult-promoting hormone would be highest. Each crab received at each injection the extract of two

Y-organs. After 2 weeks on this regime 20 crabs all showed a heightened blood-calcium level, about four times that in the controls injected with an extract of leg nerve. One crab moulted after 37 days, three more in the next fortnight and one more on the 58th day. On the 60th day the remaining 12 crabs (three had died) were sacrificed, together with the 16 surviving control crabs. All of the latter were still in anecdyosis, while all of the former were in late proecdysis. Repeated injections of Y-organ extract had therefore induced a further moult in crabs which had entered upon the terminal anecdyosis.

Bilateral removal of the Y-organ from *Carcinus* in terminal anecdyosis leads to no obvious effects. Bilateral eyestalk ablation following this operation led to a high mortality but sufficient survived to enable valid conclusions to be drawn.

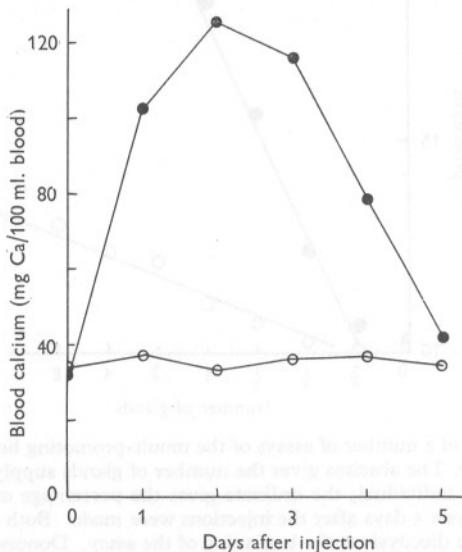


Fig. 3. The effect of injection of an extract of the Y-organ of *Carcinus* on the blood-calcium level of the same species during the terminal anecdyosis. The controls (open circles) received an injection of extract of leg nerve.

Sixty days after the last stage of this double operation was completed not one of the 13 crabs which had survived so long had begun proecdysis; all were still in anecdyosis. This may be contrasted with the results of simple eyestalk removal (see Table 1), when the crabs immediately began proecdysis, and had mostly moulted 60 days after the operation. We may conclude that the Y-organ is necessary for proecdysis to proceed, even in the absence of the moult inhibition afforded by the hormone of the eyestalk. It seems probable that the moult-inhibiting hormone of the X-organ-sinus gland complex of the eyestalk acts primarily in restraining the Y-organ from producing the moult-promoting hormone. It seems likely, however, that it has also a direct action on the tissues, for otherwise the slight titre of moult-promoting hormone

produced by the inhibited Y-organ of the terminal anecdyis (see Fig. 2) would surely lead to a very slowly progressing proecdysis unless the tissues are actively inhibited to counterbalance its effects.

Maia squinado
Moult-inhibiting hormone

A comparative assay of sinus glands taken from the eyestalks of male *Maia* in the penultimate and the terminal anecdyis is illustrated in Fig. 4. It is evident from this that the titre of moult-inhibiting hormone is much lower—about eight times lower—during terminal anecdyis than during the penultimate anecdyis, despite the larger size of the older animals. Statistical

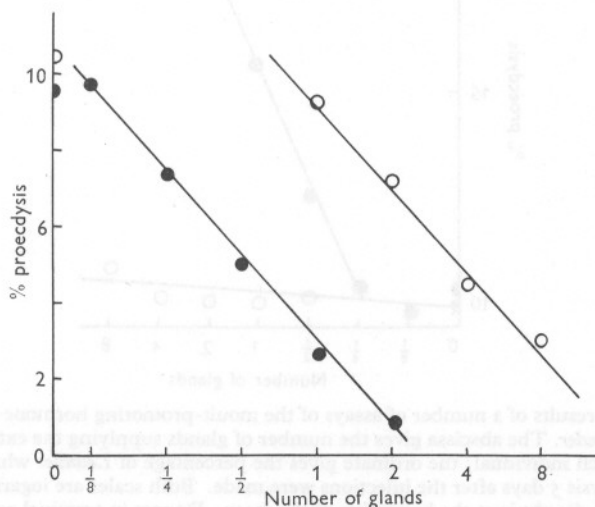


Fig. 4. Pooled results of a number of assays of the moult-inhibiting hormone of the sinus gland of *Maia* on *Leander*. The abscissa gives the number of glands supplying the extract which was injected into each individual; the ordinate gives the percentage of *Leander* which were in proecdysis 5 days after the injections were made. All the *Leander* were in diecdysis at the beginning of the assay. Donors in terminal anecdyis—open circles; donors in non-terminal anecdyis—black circles.

analysis of the data gives the ratio between the titre per gland as 7.31, with 1% fiducial limits at 6.03 and 9.22. Expressed as titre per unit weight of tissue the ratio is 16.02 with 1% fiducial limits at 14.11 and 20.05. A similar assay using female *Maia* as donors gave comparable results.

There can be little doubt that the failure to moult again once the terminal anecdyis has begun is not a result of excessive production of the moult-inhibiting hormone of the eyestalk—a situation quite contrary to that which we have found in *Carcinus*. In *M. squinado*, therefore, we must look elsewhere for the cause of the cessation of moulting.

The Y-organ of *Gabe*

During the penultimate ecdysis this organ is large and prominent, whereas during the terminal ecdysis it is much diminished in size so that it is often difficult to find upon dissection. During the penultimate ecdysis the Y-organs of 20 male *Maia* had a mean weight of 57.36 ± 7.32 mg, while those of 20 male *Maia* in the terminal ecdysis had a mean weight of 3.35 ± 0.92 mg. The difference is statistically very significant, with $P < 0.001$.

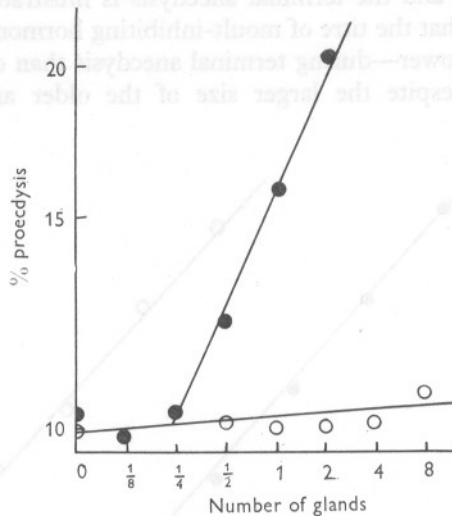


Fig. 5. Pooled results of a number of assays of the moulting-promoting hormone of the Y-organ of *Maia* on *Leander*. The abscissa gives the number of glands supplying the extract which was injected into each individual; the ordinate gives the percentage of *Leander* which were found to be in proecdysis 5 days after the injections were made. Both scales are logarithmic. All the *Leander* were in diecdysis at the beginning of the assay. Donors in terminal ecdysis—open circles; donors in non-terminal ecdysis—black circles.

An assay of the moulting-promoting hormone of the Y-organ of male *Maia* is illustrated graphically in Fig. 5. The regression line for the activity of the extracts prepared from the glands of crabs in the terminal ecdysis has a gradient which does not differ significantly from the horizontal, i.e. there is no noticeable effect of these extracts upon moulting. In contrast, the extracts prepared from glands of crabs in the penultimate ecdysis clearly have a pronounced effect in promoting the onset of proecdysis. The gradient of the regression line is 9.74 ± 1.33 , which differs very significantly from zero ($P < 0.01$).

A single injection of the extract prepared from ten Y-organs from *Maia* in the penultimate ecdysis, injected into each of ten crabs in the penultimate and ten in the terminal ecdysis, led in all of them to a transient threefold rise in the blood-calcium level, quite comparable to that found in *Carcinus* (Fig. 3).

This may be taken as a sign that the injection has led to a transient initiation of proecdysis in both groups of animals. A single injection of a similar extract prepared from the 25 Y-organs of *Maia* in the terminal anecdysis had no effect on the blood-calcium level in any of ten crabs.

We must conclude, then, that the absence of the moult-promoting hormone of the Y-organ during the terminal anecdysis in *Maia* is concerned in some way with the cessation of moulting. In this species the cessation of moulting is not a result of excessive production of moult-inhibiting hormone by the X-organ-sinus gland complex of the eyestalk. The cause of the functional degeneration of the Y-organ in the terminal anecdysis of *M. squinado* must be sought elsewhere than in the effects of the moult-inhibiting hormone of the eyestalk.

DISCUSSION

In *M. squinado* the last moult is that at which it attains sexual maturity, a state comparable with that found in insects. Once maturity is reached there is no further growth. Crustacea, however, suffer from one disability from which insects are free: copulation can only take place when the female has a soft integument and the male a hard one, that is, when the female is in the first stage of metecdysis and the male in some later stage of the moult cycle. In *Maia*, therefore, copulation is only possible when the female has just completed her final moult and the male has completed his some time previously. But the final moult in *Maia*, in Plymouth waters, takes place at a fixed time of year, in late July or August. It is thus impossible for the newly moulted male of that year to copulate with a newly moulted female: copulation must be between a male whose final moult took place one year or more previously and a newly moulted female. The heaps of *Maia*, which may be observed in July and August, are mixed heaps of moulting and copulating crabs (see Appendix). It is not until about 6 months after copulation that the eggs are finally fertilized, by sperm stored in the bursa copulatrix, and oviposited onto the pleiopods where they remain for about 9 months before hatching. A male, therefore, survives at least 12 months and a female at least 15 months after the final ecdysis. Nevertheless, once a *Maia* has entered upon the terminal anecdysis it may be considered as aged, for it is no longer capable of regenerating damaged tissue or limbs, or of repairing more than the most superficial injury. It is noteworthy in this respect that a *Maia* in terminal anecdysis is very much less ready than a younger crab to autotomize a damaged or captive limb. A mature *Maia* may be suspended by one leg out of water, even if the leg is damaged severely, without autotomizing the limb: it will die in this situation without casting the leg. A younger *Maia*, whose powers of regeneration are much greater, will cast the limb so treated and escape back to water with great rapidity. Echali er (1955) has reported that in the absence of the Y-organ, a crab cannot regenerate missing limbs, nor even produce the bud with which such regeneration normally begins.

The experiments reported in this paper suggest that in *Maia* the terminal anecdysis is brought about by the degeneration of the Y-organ. Correspondingly, no further ecdysis can begin, nor can regeneration of damaged parts. It seems likely that in the absence of predation, the death of a mature *Maia* is brought about by an accumulation of minor damage to the shell and tissues. It must also be remembered in this context that moulting in Crustacea often seems to serve as a mode of excretion (see Richards, 1951), since the exoskeleton stores waste material and especially nitrogen, which is discarded from the body on moulting. A crab in terminal anecdysis, therefore, is unable to use this method of excretion and may thus be constrained to accumulate toxic waste products in the tissues.

TABLE 2. INCREASE IN VOLUME OF *MAIA SQUINADO* AT MOULTING

Moult	Mean increase
Fourth before prepuberty	2.17
Third before prepuberty	2.15
Second before prepuberty	2.20
Before prepuberty	2.19
Prepuberty	2.26
First after prepuberty	2.20
Second after prepuberty	2.19
Last (puberty)	1.89

The increase in volume at each of the last eight moults is expressed as the ratio of the volume after moulting to that before it. Each ratio is the mean of at least fifteen observations and includes data of my own, supplemented by computations from the data of Teissier, 1934-55.

If the immediate controlling factor of terminal anecdysis in *Maia* is the degeneration of the Y-organ, we yet have no evidence as to the causation of this degeneration. All that can be said is that the inhibitory action of the X-organ-sinus gland complex is not the cause. The causative factor must be sought earlier in the life history. A clue to what is involved may be seen in the size increase which *Maia* undergoes at each moult. For most of the moults the relative increase in volume (in the male) is *ca.* 2.18 (see Table 2). At two moults only is the relative increase significantly different—the moult of prepuberty, when the gonads begin to increase in size and show the first signs of approaching maturity, and the moult of puberty three moults later, that is to say, the final ecdysis when the animal becomes sexually mature. At the prepuberal moult the relative increase in volume is *ca.* 2.26, greater, that is, than at other moults, while at the puberal moult the relative increase in volume is *ca.* 1.89, correspondingly less than at other moults. I have shown previously (Carlisle, 1955) that increase in volume at moulting is a function of the activity of the water balance hormone of the X-organ-sinus gland complex. It is possible that this is implicated in the control of the final ecdysis and may have some influence on the Y-organ, though such a concept must remain a mere speculation for the moment.

In *Carcinus maenas* we meet with rather a different situation. Here there can be little doubt that the main restraining influence preventing further moulting during the terminal anecdyesis is over-production of the moult-inhibiting hormone of the X-organ-sinus gland complex. It seems likely that its prime action lies in restraining the Y-organ from producing the moult-promoting hormone, but nevertheless I believe that it must have some direct action on the tissues also; for otherwise the tissues would respond sooner or later, however slowly, to the small amounts of moult-promoting hormone which are produced by the incompletely inhibited Y-organ. What factor it is which leads the X-organ to produce the excess amounts of the moult-inhibiting hormone we have as yet no idea. But in *Carcinus* the terminal anecdyesis only differs from earlier anecdyeses as far as I have yet discovered in the higher titre of moult-inhibiting hormone. The control of the terminal anecdyesis in this species seems to be merely an accentuation of the control of the earlier anecdyeses and may well be gradually approached in these successive earlier anecdyeses. *Carcinus* undergoes at least ten moults, eight diecdyses and two or three anecdyeses between the moult of puberty and the terminal anecdyesis (in the male, and probably also in the female). As I have repeatedly pointed out in previous papers (see Knowles & Carlisle, 1956) the control of anecdyesis is different from that of diecdysis, and it is to this difference that we must look for origin of the terminal anecdyesis in *Carcinus*.

One such difference between *Carcinus* in diecdysis and in anecdyesis is in the level of secretion of the moult-inhibiting hormone of the X-organ-sinus gland complex. It will be obvious from a perusal of Fig. 1 that this difference is accentuated when we compare terminal anecdyesis. During terminal anecdyesis the eyestalk appears to be producing the moult-inhibiting factor faster than at any previous time in the life of the crab. There is thus in *Carcinus*, in contrast with *Maia*, a positive inhibition of the initiation of a further proecdysis once the animal has entered upon the terminal anecdyesis. It is obvious, furthermore, that the Y-organ of *Carcinus* is not degenerate like that of *Maia* during the terminal anecdyesis, and correspondingly a *Carcinus* in this stage is more capable of repairing minor damage to the shell. Once the inhibition to moulting is removed by surgical interference *Carcinus* enters upon a new proecdysis, stimulated to this, no doubt, by the moult-promoting hormone of the Y-organ. *Maia*, in contrast, lacks both the inhibiting factor and also the factor which might provoke renewed moulting.

It is evident that the type of control of the terminal anecdyesis which is exemplified by *Carcinus* is more flexible than that found in *Maia*. Among the Portunidae, to which *Carcinus* belongs, there is great variation in the moulting cycle. At one extreme are to be found crabs like *Carcinus* and *Portunus depurator*, which have ten or a dozen moults after the moult of puberty, so that the terminal anecdyesis does not coincide with sexual maturity, but rather begins several years later. At the opposite extreme are crabs such as *Callinectes*

sapidus in which in the female the moult of puberty is the final moult, after which the animal is sexually mature and enters upon the terminal anecdytis, while in the male the moult of puberty appears to be several moults earlier—the copulatory appendages are present throughout the last few intermoult periods. Such variation between closely related species is hardly conceivable with the type of control of the terminal anecdytis found in *Maia*.

This investigation has served to reveal the existence of two distinct mechanisms whereby the terminal anecdytis, which is characteristic of the end of the life-span of most species of crabs, may be regulated. A mental review of the biology of crabs suggests that the mechanism found in *Maia* may well be characteristic of all the oxyrhynchan Brachyura, while that found in *Carcinus* may be characteristic of the brachyrhynchan Brachyura, or at least of the Portunidae. The mechanism found in *Maia* is reminiscent of the control of the final instar of insects and the organs concerned may be homologous (see Gabe, 1953). The dynamic equilibrium which seems to exist in *Carcinus*, on the other hand, appears to be unrelated to anything found in insects and to be a mechanism developed in the one group of crabs.

It must be emphasized that the hormonal influences which this investigation has shown to be effective in regulating the terminal anecdytis are only the immediate causes of this phenomenon. The mediate causes must be sought elsewhere as must the reason for the existence of the phenomenon. The problem of the terminal anecdytis, the ageing of crabs and the factors which cause natural senescence and death in these creatures is no more than touched, but in the hormonal factors concerned in inhibiting moulting we see an influence which is the last agent in the chain of events which brings about senescence.

SUMMARY

In many if not most species of crabs (but not quite all) there is a limit to growth, when no more moulting or ecdysis is possible in normal circumstances. This condition of permanent anecdytis is known as the terminal anecdytis. In the spider crab *Maia squinado*, the last moult is the moult of puberty, when the animal finally attains sexual maturity, and this moult has different biometrical characteristics from the others. In *Carcinus maenas*, in contrast, the moult of puberty takes place when the animal is quite small and may be succeeded by about ten further moults before the terminal anecdytis begins.

The immediate cause of the cessation of moulting in *Maia squinado* is shown to be the degeneration of the Y-organ, which secretes a moult-promoting hormone. In the absence of this gland and its secretion moulting can no longer continue. In *Carcinus* the Y-organ does not degenerate after the final ecdysis and the cause of the cessation of moulting is to be sought in the excessive production by the X-organ-sinus gland complex of the moult-inhibiting hormone. This effectively prevents moulting from proceeding.

Removal of the X-organ-sinus gland complex in *Carcinus* allows ecdysis to continue, so that giant crabs can be produced in the laboratory by this means, and at the same time the life-span may be increased. The operation has no such effect on *Maia*. In either species injection of Y-organ extracts produces transiently the first signs of an approaching moult, in the form of a heightened blood-calcium level. In *Carcinus* repeated injection led to eventual ecdysis. The presence of the Y-organ is shown to be necessary for removal of the X-organ-sinus gland complex to stimulate moulting in *Carcinus*.

Two separate methods of producing terminal anecdyis exist in crabs, both involving the hormones which normally regulate the moult cycle, but no reason is known for the existence of the phenomenon.

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APPENDIX

A number of reports have appeared in the lay press and in fishing journals of the occurrence of heaps of spider crabs (*Maia squinado*) at various points around the coast. One such account is given of large heaps 'about two feet high and three feet in diameter' on the coast of Jersey during the autumn of 1952, by Le Sueur (1953)¹. He interpreted these heaps, which were observed by fishermen and by the president (an amateur zoologist) of the Société jersiaise, as a means of protection against *Octopus* which will successfully attack crabs larger than themselves. While congregation for mutual defence is certainly a major part of the explanation of such a heap it is by no means all. Some observations which I was able to make during July to September 1956, on a heap of *Maia* at Mothecombe, South Devon (50° 18.3' N., 3° 57.9' W.) have enabled me to supplement this explanation.

Like those in Jersey, the heap at Mothecombe was about 1 m in diameter, or rather more, and 0.5 m or more high. It consisted of about 60 *Maia*. When first observed on 12 July the top of the heap was about 1 m below low tide level, and the depth of water at that point was, therefore about 1.5 m. From the tide tables it is apparent that there was never less than 90 cm of water above the top of the heap, at any stage of the tide or phase of the moon, for the tide on 12 July was a spring tide and low water prediction was 0.5 ft (15 cm) below chart datum (the mean level of low water spring tides throughout the year). The crabs in the heap remained almost completely stationary even when sand was thrown in a cloud over them. The animals of the bottom layer of the heap were clinging to an isolated rock about 1.2 by 0.9 m, which was completely surrounded by sand. This rock stood about 20 cm above the surrounding sand and lay about 60 cm from the main reef which ran close by. A moderately heavy growth of *Laminaria*, chiefly *L. saccharina*, was attached to the rock and fronds rose through the heap of crabs, floating about above their backs, providing a partial cover. About twenty of the crabs were full-grown males, in terminal anecdyosis; the rest were about equal numbers of smaller, immature males and females, of a size which indicated that they were in the penultimate instar. During the course of the next month the heap was swollen by the addition of about a further 20 crabs, making 80 in all. On 25 July a newly cast shell lay beside the heap and I located a soft male in the centre of the heap. During the next three weeks one or two crabs moulted every day, and always the soft crabs were to be found in the centre of the heap, not on the surface. As time wore on it became apparent that each female as soon as she moulted entered into copulation with one of the old, hard males. So that by mid-August six or eight pairs could be observed in copula at

¹ *Bull. Soc. jersiaise*, Vol. 16, pp. 37-8.

one time. By mid-September the heap was beginning to disperse, but had not completely done so when equinoctial gales rendered the sea too opaque to continue observations.

During the summer of 1956 *Octopus* were too rare around the Devon coasts to be a major predator of *Maia*, and so far as I am aware no other common animal of the English fauna is capable of attacking a hard *Maia*. A soft crab, however, is vulnerable to attack from a great many predators, for not only is its shell soft, but its muscular system is inefficient until the skeleton is hardened. Most brachyrrhynchan crabs before and after moulting hide away in crannies amongst the rocks, or buried in the sand. *Maia* from its very shape is incapable of doing either. With most species of crabs, which are carnivorous, the predator which is most likely to attack a soft crab is another crab. *Maia*, however, is not a carnivore and, therefore, will not attack another of the species, even when soft. It is, therefore, feasible for them to congregate together for mutual protection when moulting, an unthinkable happening in a carnivorous species of crab. It seems to me reasonable to suppose that this is in fact the primary purpose of the heaps, to which is inevitably added the secondary purpose of mating. Copulation could equally well take place between pairs of crabs in isolation, as happens in other species of crabs, but if there is communal moulting then it will take place where moulting happens. It seems not improbable that the seasonal moulting of *Maia*, which only takes place in July and August and not at other times of the year, has arisen because of this method of mutual protection that this species has developed.

In support of my contention that *Maia* is not a carnivorous species of crab I will mention observations I have made at Mothecombe on the feeding of this species. In addition to the heap of crabs numerous isolated individuals were observed, mainly animals in terminal anecysis. Many of these, when observed closely, were found to be feeding by browsing on tufted growths, both animal and plant. Such plants as *Enteromorpha*, *Corallina*, *Heterosiphonia* and *Griffithsia*, and animals such as tufted Bryozoa and hydroids appeared to be the main foods of *Maia*. The crabs were often to be seen browsing directly on the ends of the longer tufts, while the shorter organisms were detached from the substratum or broken by the chelae and thence conveyed to the mouth. It is an undoubted fact that *Maia* are often taken in crab pots, enticed there by the bait, but in the wild it seems unlikely that fresh or decaying flesh forms any major part of the diet. The enlarged chelae of the mature male seem to serve especially in fighting, or rather in warding off other males, while coupling with a female. Any fights which may be observed are slow moving affairs and I have never seen any sign of damage to an animal resulting from this cause.