ENDOCRINE
CONTROL IN
CRUSTACEANS

BY

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THE SERIES

AUTHORS' NOTE

EACH of us, while accepting a general responsibility for the whole, is primarily responsible for a different section of this book. Each has contributed that portion which deals with subjects in which he has had most experience and has contributed original observations. Thus Chapters 1, 2 and 3 are the work of Sir Francis Knowles and Chapters 4, 5, 6 and 7 are the work of Dr David Carlisle, though each section has been modified in the light of the other author's criticisms. Consequently any theories put forward may be personal ones, though for the sake of simplicity we have used the first person plural throughout.

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Introduction

DURING the past twenty years a number of reviews have marked the progress of our understanding of endocrine control in crustaceans. Some of these reviews have dealt exclusively with crustacean endocrinology (e.g. Kleinholz, 1942; Brown, 1944; Panouse, 1947; Brown, 1952; Knowles and Carlisle, 1956). Other reviews have considered crustacean hormones in a general survey of invertebrate endocrines (e.g. Koller, 1929, 1938; Hanström, 1939; Lerma, 1936; Scharrer, 1954, 1955; Gabe, 1954; etc.). It is not the intention of this book to present yet another comprehensive survey of the literature but rather to select studies which mark significant advances in our knowledge of endocrine control in crustaceans. We hope that by so illustrating the changing trends of research in crustacean endocrinology since its inception until the present day we may present the pattern of our knowledge in relation to the past discoveries on which it is based and may predict possible future trends.

The study of crustacean endocrines began in 1928 with the independent discoveries by Perkins, working on the prawn *Palaemonetes*, and Koller, who studied the shrimp *Crangon*, that the colour changes of crustaceans were controlled by chemical substances circulating in the blood stream. These conclusions were based on experiments involving an interference with the blood flow or injections of extracts. Both authors found that extracts of the eyestalks when injected into dark animals resulted in an intense and prolonged paling due to concentration of pigment within the chromatophores. During the first decade following this discovery of the hormonal control of colour change in crustaceans investigators directed their attention mainly to the following problems:

(1) Localization and identification of the organ in the eyestalk responsible for the production of the body-lightening hormone.

(2) A more intensive study of the chromatophores of crustaceans and the factors influencing them.

The name of Professor Hanström will always be associated with the discovery of the first known endocrine organ in crustaceans, namely the sinus gland. He and his pupils in a series of researches carried out from 1931 to 1937 showed that there were two structures in the crustacean eyestalk which both on histological and physiological grounds could be suspected of hormone secretion. Their experiments were based on the extraction of different sections of eyestalks of various crustaceans and the effects of these extracts on the chromatophore system. A definite correlation was found between the presence of the sinus gland and the abundance of a chromactivating substance; a correspondence between the X organ and colour change was less definite, though there did seem to be some relationship.

During the 1930's Kleinholz added the distal and reflecting retinal pigments to the other pigment movements shown to be under hormonal control. He found that injections of eyestalk extracts into animals in darkness brought about a typical light-adaptation of the eye. In the field of colour change investigation was mainly directed on the number of hormones which might be implicated in the very diverse movements of pigments within the chromatophores. Brown (1935 *a*, *b*) observed that the four pigments found within the chromatophores of *Palaemonetes* showed independent behaviour in the responses to various backgrounds and concluded that at least four hormones must be operating to account for this independence. On the other hand, Abramowitz (1937 *b*) carried out a number of reciprocal injection experiments between different species and concluded that there was but a single pigment-activating hormone and that the evident independent movements of the pigments might be explicable in terms of specific differences of the end organs. He made attempts to clarify the problem by chemical analysis of the colour change hormone extracted from the eyestalks of the crab *Uca*, but he was unable to obtain a sufficient amount of material to enable him to reach any definite conclusions. The material which he was studying brought about, when injected, a darkening of eyestalkless *Uca*. Carlson (1935) had drawn attention to this difference between the effects of eyestalk extracts on chromatophores in crustaceans; an eyestalk extract which paled *Palaemonetes* appeared to darken *Uca*, and vice versa. Clearly these

results could be explained either by a 'unitary hormone hypo-thesis' in terms of differences of chromatophore response, or by a 'multiple hormone hypothesis' if it could be shown that all the crustacean eyestalks investigated contained two substances, one a *Palaemonetes*-lightening substance and the other a *Uca*-darken-ing substance. The arguments for and against these two hypo-theses were summarized by Kleinholz (1942) in his review.

During the 1930's observers who removed the eyestalks from crustaceans noted that ecdysis was affected. A number of investigators reported that the intermoult period was shortened and that there appeared to be less calcium in the moulted exo-skeleton. The evidence, however, was held by Kleinholz to be inconclusive, and in his review of the first decade of crustacean endocrinology he came to the conclusion that pigment move-ments were the only functions in crustaceans that were indubi-tably under hormonal control. His review marks the end of the first phase of crustacean endocrinology, namely the discovery that the pigment movements within chromatophores and in the eyes of crustaceans were under hormonal control, and the localization of the sinus gland and the X organ as potent sources of pigment-activating substances.

It is interesting to compare the reviews by Kleinholz (1942) and Brown (1944). Although these two publications are only separated by a few years, they differ in certain important respects, of which perhaps the most significant (in view of sub-sequent advances) is the emphasis laid by Brown on the evidence for the presence of chromactivating substances in the central nervous system. Perkins (1928) had found no influence of extracts of the central nervous system on *Palaemonetes* red pig-ment, but Brown (1933) repeated this experiment and reported that a pigment-concentrating substance was in fact present in the central nervous system. His results were criticized on the grounds that in extracting the nervous system he might have included material present in the blood stream, and that this material might have originated in the eyestalks. A few years later, however, it was shown (Knowles, 1939) that extracts of the nervous systems of crustaceans whose eyestalks had been removed three to four weeks previously had an activity upon the light-reflecting white chromatophores; this clearly could not be attributed to sinus-gland activity. The nervous system of the thorax was indicated as an especially potent source of a

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chromactivating substance, and in 1940 Brown and Ederstrom extended these observations by showing through injection experiments on *Crangon* that the most effective extracts of this region could be prepared from the post-oesophageal trito-cerebral commissure. Later (1946) Brown attempted to localize the source of the chromactivating substance in the commissure and reported that the greatest activity was found along the median faces of the circumoesophageal connectives and in the commissure itself; he reported that there was a concentration of chromactivators in that part of the commissure in which two slight swellings could be seen and in which a bluish green particulate substance and some cell bodies could be observed if a freshly dissected commissure was examined in sea water. He did not, however, pursue these investigations further, although he had a few years before written in his review that 'histological search of the nervous system for secretory elements is much needed'.

It is interesting to note the contrast between the evolution of crustacean and vertebrate endocrinology. The endocrine organs of the vertebrates were described in considerable detail long before experiments on their function were performed, and so in the early physiological experiments on vertebrate endocrines the selective ablation of an organ suspected of endocrine activity was comparatively easy. Crustacean endocrinology, on the other hand, was founded on simple physiological experiments, and the evidence for blood-borne hormones preceded histo-logical studies of their probable sources by many years. In short, vertebrate endocrinology was founded on anatomy but crustacean endocrinology was founded on physiology, and it is interesting to observe that the physiological approach continued for many years in the development of crustacean endocrinology. Thus, although by 1947 Hanström and Panouse were each able to present fairly comprehensive surveys of the form and gross anatomy of the sinus gland in crustaceans, there was little evidence then concerning the microscopic anatomy of secretory cellular elements either in the sinus gland or any other crus-tacean endocrine organ.

The second decade of crustacean endocrinology included a considerable number of physiological experiments based on ablation and the injection of extracts. As a result of these methods Brown and his collaborators were able to bring forward

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evidence that the crustacean nervous system and sinus glands contained a number of distinct chromactivating substances and that the 'unitary hormone hypothesis' must be abandoned. Brown and Scudamore (1940) succeeded in separating two different active principles from the eyestalks or sinus glands of several different species of Decapoda by extracting them with 100 % ethyl alchohol and then with sea water. In every species the alcohol-soluble fraction had a relatively strong effect upon the red pigment of *Palaemonetes* and a very weak effect upon the black pigment of *Uca*. The residue after alcohol treatment had a very strong effect upon the latter, but a weaker effect upon the red pigment of *Palaemonetes*. In 1940 Brown and Ederstrom showed that, in addition to the *Palaemonetes*-lightening hormone and the *Uca*-darkening hormone, a third hormone which dispersed the dark pigments in the chromatophores of the telson and uropods could be extracted from the post-oesophageal commissure region of *Crangon*. This tail-darkening hormone could be separated from the *Palaemonetes*-lightening hormone because of its relative insolubility in alcohol. It seemed clear that the postulation of at least three hormones was necessary to explain the control of the movements of dark pigments within crustacean chromatophores, and injection experiments had also indicated the possibility of a separate hormone controlling the white pigments of the light-reflecting chromatophores (Knowles, 1939).

During the 1940's a number of investigators studied the effect of eyestalk removal on the frequency of moulting. Undoubtedly the removal of the eyestalks stimulated precocious moulting (Brown and Cunningham, 1939; Abramowitz and Abramowitz, 1939, 1940; Kleinholz and Bourquin, 1941; etc.), but the effects of eyestalk removal did not seem to be the same at all stages of the moult-cycle. Drach (1944), working on *Leander serratus*, found that removal of the eyestalks was only effective in precipitating the moult if the operation was performed during the inter-moult period, and that it had no significant effect if performed during the main period of pre-moult. The problem of the control of moult was further complicated by the observations of Scudamore (1947) who supplied evidence that the central nervous system of the crayfish contained a moult-accelerating principle. Pyle (1943) showed that the staining reaction of the sinus gland altered at the time of moult and

concluded that the sinus gland released substances concerned in the moult-cycle.

Two further instances of suspected hormonal control were discovered during the second decade of crustacean endocrinology, namely a possible gonad-controlling principle and a diabetogenic principle. Removal of the eyestalks in immature female crustaceans or in mature females at a time when the animals were not breeding resulted in a rapid increase in the size of the ovary (Panouse, 1943, 1944, 1946, 1947; Brown and Jones, 1949).

During an investigation of endocrine influence on carbohydrate metabolism in crustaceans Abramowitz, Hisaw and Papandrea (1944) detected that injection of extracts of the eyestalks of crabs into crabs of the genus *Callinectes* produced a pronounced increase in the amount of blood sugar, and that the greater part of the activity of the eyestalks resided in the sinus gland. On the other hand, neither they nor Kleinholz and Little (1948, 1949) could detect hypoglycaemia after ablation of the eyestalks or sinus gland removal. It had previously been reported that stress of various kinds led to hyperglycaemia in crustaceans. For example, handling, asphyxiation, the injection of brine and other forms of injury all produced forms of hyperglycaemia. If the sinus glands were removed or the nerves leading to them were cut, however (Kleinholz and Little, 1949; Kleinholz, Havel and Reichart, 1950), asphyxia no longer caused hyperglycaemia, and it seemed likely that the effects of stress were mediated through the sinus gland. In this connexion it is interesting to note that some evidence for a heart-accelerating hormone was brought forward during the second phase of crustacean endocrinology. Welsh (1937) showed that eyestalk extracts accelerated the rate of heart beat, and Scudamore (1941) found that extracts of the sinus glands alone also had this effect; he observed, however, that a heart-accelerating effect could also be obtained from extracts of the nerve cord, so arousing the suspicion that the heart-accelerating activity of eyestalk extracts might be explicable in terms of a heart-accelerating effect of nervous tissue as a whole.

We have found that the second phase of crustacean endocrinology (1940–50) was marked by three main advances: (a) the discovery that various aspects of metabolism, growth and development were probably under hormonal control, (b) evi-

dence that a number of different hormones were involved in the pigment movements of crustaceans, (c) suggestive evidence that chromactivating substances were produced in the central nervous system. These advances were all founded on the physiological approach, namely on experiments of ablation and the injection of extracts. The detailed structure of the secretory tissues was still incompletely known.
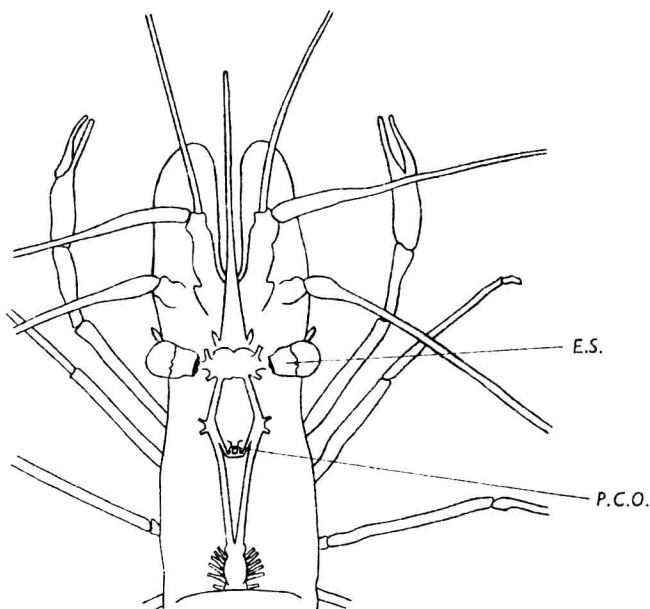

Fig. 1. The position of the principal neurosecretory release centres in crustaceans. *E.S.* Eyestalk, which contains the sinus gland and other neurosecretory systems. *P.C.O.* Post-commissure organs.

The third phase of crustacean endocrinology began in the year 1951. In that year, for the first time, the distribution of chromactivating hormones was identified with secretory droplets lying along the course of nerves leading to blood sinuses. The first direct proof of neurosecretion in crustaceans was offered by Knowles (1951) who showed that the greatest chromactivating potency in the post-oesophageal commissure region lay not in the commissure itself as had previously been suspected by Brown and his collaborators, but in two lamellae which lay adjacent to, and attached to, blood sinuses. Secretory

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droplets could be detected in these lamellae and along the course of two fine nerves leading to these lamellae from the commissure. A few months later Enami (1951 *a, b*), in a very detailed survey of the presence of chromactivating substances and secretory elements in the crab *Sesarma*, showed that secretory droplets could be detected along the course of the nerves leading from a cluster of cell bodies in the medulla terminalis to the sinus gland. Enami, however, did not consider that these histologically demonstrable droplets represented the hormonal material, but believed that this was manufactured by secretory elements in the sinus gland itself. His results, however, indicated that the materials necessary for the production of hormones by the sinus gland were supplied to it along the nerves leading towards it, whether or not these materials were transformed by cellular elements in the sinus gland.

Enami's discovery came at a most opportune moment to explain very puzzling results which were being obtained by various investigators who compared the effects of eyestalk removal with those obtained by removing the sinus glands only. Brown and Cunningham (1939) had shown that it was possible to detect the sinus gland in the living eyestalk by reason of its bluish white opalescence, and Brown (1942), Panouse (1946) and Kleinholz (1947) had devised methods for removing the sinus gland without damage to the other tissues contained in the eyestalk. Using these techniques, Bliss (1951) and Passano (1951) found that removal of the sinus gland alone had no apparent effect on moulting, though there was an undoubted precipitation of pre-moult in animals in which the whole eyestalk had been removed. Clearly these results could be explained by the hypothesis that the sinus gland contained a moult-inhibiting hormone, but that the major source of production of this hormone lay not in the sinus gland but elsewhere. This problem was finally resolved by Passano (1953) who found that the effects of bilateral eyestalk ablation could be duplicated by removal of the sinus glands together with the nerve supplying them, including the cell bodies of these neurones located in the medulla terminalis. Conversely, implantation of the sinus glands and the associated neurones completely inhibited precocious moulting in animals from which the eyestalks had previously been removed. His experiments indicated that a moult-inhibiting hormone is produced in the neurones leading to the

sinus gland and that the sinus gland might be no more than a reservoir of the hormone. These results were supported by observations on the control of oxygen consumption in crabs. Removal of the sinus glands alone had little or no effect on the level of oxygen consumption or the respiratory quotient in either crabs or crayfish, but eyestalk ablation resulted in an increase of oxygen consumption (Bliss, 1951, 1953). Bliss interpreted her data as implying that a hormone controlling oxygen consumption was formed in some tissues in the eyestalk other than the sinus gland, but was stored and released at the sinus gland. It has subsequently been shown that the sinus gland is the meeting place of neurosecretory fibres from many cell groups which lie in the eyestalk and elsewhere (Bliss and Welsh, 1952; Bliss, Durand and Welsh, 1954; Carlisle, 1953a, b; Potter, 1954; Knowles, 1955). All these authors agree that the material which is produced in the neurosecretory cell bodies is transported along axon fibres, and that these fibres end in the sinus gland in the form of club-shaped terminations. Whether or not the substances brought to the sinus gland are transformed there by cellular elements is still a matter for discussion, and the evidence for and against this view will be considered later. It has become clear, however, during the past decade that most of the known crustacean hormones originate in cellular elements of the central nervous system and that the greater part of the endocrine activity in crustaceans is comparable to the neurosecretion attributed by the Scharrers and others (1954) to the hypothalamus-hypophysial system in vertebrates and the brain-corpus cardiacum system in the insects.

Although most of the endocrine effects so far demonstrated seem to be associated with neurosecretory systems, there are some which are not. Charniaux-Cotton (1954a, b) has described an endocrine gland in an amphipod which seems to be responsible for the differentiation of the primary and secondary male characteristics. Gabe (1953b) described an organ which he called the Y organ, which has been shown to affect sexual development, moulting and development of the regeneration buds of extirpated legs (Echalier, 1955).

Within recent years studies have indicated that a number of metabolic processes in crustaceans are influenced by blood-borne hormones. In addition to the moult-inhibiting substance, the ovary-inhibiting hormone and the diabetogenic principle

there seems to be evidence that an endocrine system is implicated also in the control of chitin formation, the decomposition of lipoids and carotenoids, the rate of oxygen consumption, calcium metabolism, water balance, testicular growth, the development of the male secondary sexual characteristics, and that moulting may be controlled by three or more different hormones, acting at different stages in the moult cycle.

Recently, and at present, a number of attempts have been made to determine the chemical nature of some of the crustacean hormones. It has been shown by means of paper electrophoresis and dialysis experiments that a number of chromactivating substances may be isolated from extracts of sinus glands and post-commissure organs (Knowles, Carlisle and Dupont-Raabe, 1955). Using chemical methods of purification Östlund and Fänge (1956) have isolated a chromactivating substance from the eyestalks of *Pandalus*. All these authors have shown that it is possible to inactivate the purified chromactivating substances by using enzymes which attack peptide linkages, and there seems to be good ground for believing that a number of the crustacean chromactivating substances are peptide in nature.

At the present moment we seem to be nearing the end of the third phase of crustacean endocrinology. During the first phase (1928–38) the presence of chromactivating hormones was demonstrated by injection and ablation experiments, and the sinus gland was indicated as a possible source of these substances. During the second phase (1939–51) extraction and injection experiments indicated that more than one hormone was implicated in the control of pigment movements, and that, in addition to the sinus gland, the central nervous system seemed to contain considerable amounts of chromactivating substances. Moreover, it was shown during this second phase that in addition to pigment movements certain metabolic activities (e.g. moulting, carbohydrate metabolism and ovarian development) should be added to the list of activities under hormonal control. The third phase began in 1951 when it was shown that most of the known endocrine systems in crustaceans were neurosecretory systems and the hormones were produced in modified neurones and transported along their axon fibres to the blood stream. It has been shown that these neurosecretory systems are complex in form and that the hormones produced by them may be numerous and affect various aspects of metabolism in addition