### REVIEW

# Vertebrate-type Hormones in Crustaceans: Localization, Identification and Functional Significance

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## **INTRODUCTION**

In a 1983 review of the literature relating to invertebrate neuropeptides Greenberg and Price [49] referred to invertebrates as having two sets of One set they called "native neuropeptides. neuropeptides." These are the ones that were originally isolated from invertebrates and have been shown to have specific physiological or biochemical roles in these animals. In contrast, the compounds that Greenberg and Price called "naturalized neuropeptides" are those originally discovered in vertebrates and later identified in invertebrates by various techniques, including chromatography, radioimmunoassay, and immunocytochemistry. At the time Greenberg and Price wrote their review very little information had been obtained about what the physiological and biochemical roles of these "naturalized" compounds might be, and for many of them their roles remain obscure. Peptides are now known to be the largest class of neuroregulatory compounds in both invertebrates and vertebrates [92]. The object of this reivew is to present the more recent evidence for the presence in crustaceans of not only vertebrate-type neuropeptides but of all vertebrate-type peptidergic, proteinaceous, steroid, and lipidderived hormones. However, the classical neurotransmitters, such as norepinephrine and dopamine, are not treated herein because (a) they are so ubiquitious in the animal kingdom that concluding whether one or more of them is vertebrate-type or invertebrate-type does not seem justified and (b) their presence and roles in crustaceans have been extensively reviewed in a recent publication from this laboratory [40]. Where information is available concerning possible physiological roles of these vertebrate-type compounds in crustaceans, that information will be presented also.

In this review we shall use "hormone" as it has been defined by Norman and Litwack [88]. They stated that "any substance that operates at the cellular level, generated either externally or internally, which conveys to that cell a message to stop, start, or modulate a cellular process will come under the purivew of modern endocrinology." Also, Norman and Litwack divided hormones into three classes, based on the distance of action: (a) endocrine hormones, which are the classical hormones such as lutenizing hormone that travel to distant target cells; (b) paracrine hormones, such as the classical aminergic neurotransmitters (e.g. acetylcholine), which travel only a short distance to neighboring cellular targets; and (c) autocrine hormones, such as prostaglandins, that are synthesized and released by the same cell on which they act.

Invertebrate nervous systems, including those of crustaceans, are useful models for analyses of neuronal functioning, including peptidergic

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14

#### M. FINGERMAN, R. NAGABHUSHANAM AND R. SAROJINI

neurotransmission. Technical improvements in such techniques as high performance liquid chromatography, peptide sequencing and radioimmunoassay have greatly enhanced our ability to identify the vertebrate-type hormones in invertebrate tissues, even when these compounds are present in extremely low concentrations.

#### **ENDORPHINS**

#### Localization and identification

The presence of substances in the mammalian brain that have opiate-like activity was clearly established in 1975 when Hughes et al. [57] published the structures of two such endogenous opioids, the pentapeptides methionine enkephalin and leucine enkephalin extracted from the porcine brain. The name "endorphin" is often used, as we have done here, for the entire class of compounds endogenous in mammals that have morphine-like activity [43]. Since 1975, endorphins have been found in several non-nervous organs of vetebrates such as the gut, adrenal medulla, and pancreas [43]. The first demonstration of the presence of an opioid in an invertebrate came from a study with a locust, Locusta migratoria. Radioimmunoassays (RIA) showed its nervous system contains both methionine enkephalin-like and leucine enkephalin-like substances [50]. With respect to crustaceans, the first opioid study was done in 1981 by Mancillas et al. [75]. Through immunocytochemistry they found leucine-enkephalin-like immunoreactivity in all the retinular cells of the spiny lobster, Panulirus interruptus. In addition, such immunoreactivity was also apparent in nerve fibers in chiasm 3 that pass from the medulla terminalis to terminate in the medulla interna. No immunoreactivity against antibodies of another opioid, beta-endorphin, was found. Later, other crustaceans were similarly studied. Jaros et al. [58], using the shore crab, Carcinus maenas, obtained immunocytochemical evidence for a leucine enkephalin-like substance in the eyestalk, i.e. in the sinus gland, lamina ganglionaris, medulla externa, medulla interna, and medulla terminalis. In addition, high performance liquid chromatography (HPLC) of sinus gland extracts of Carcinus

maenas revealed the presence of substances that appeared to be methionine enkephalin, leucine enkephalin, and methionine enkephalin-Arg<sup>6</sup>-Phe<sup>7</sup> [58]. Likewise, Fingerman et al. [39] showed by immunocytochemistry the presence of leucine enkephalin-like and methionine enkephalin-like material in the eyestalk of the fiddler crab, Uca pugilator. Immunoreactivity to both substances was apparent in the retinular cells, lamina ganglionaris, sinus gland, optic peduncle, the three chiasms and medulla terminalis. However, in the X-organ of the medulla terminalis, immunoreactivity against only the methionine enkephalin antibodies, but not leucine enkephalin, was seen. Using the mantid shrimp, Squilla mantis, Marino et al. [77] showed that the subesophageal ganglia contain a peptide that is opioid-like because it is able to displace D-Ala<sup>2</sup>-D-Leu<sup>5</sup>-enkephalin (DA-DLE) from rat brain membranes in a receptor binding assay, and gives positive results in the standard guinea pig ileum assay for opioids. In this test, opioids inhibit the twitch response of the electrically stimulated ileum. Furthermore, the action of this opioid-like substance is inhibited by naloxone, an opioid receptor blocker. This peptide, through use of proteolytic enzymes, gave evidence that it is synthesized as part of a larger inactive peptide and subsequently released in the active form. Because this opioid does not coelute with methionine enkephalin or leucine enkephalin, nor does it cross-react with antibodies to methionine enkephalin or give positive results in RIA for methionine enkephalin, leucine enkephalin, or beta-endorphin, Marino et al. suggested this peptide from Squilla mantis is a "native" neuropeptide. Piccoli et al. [91], also using the DADLE receptor binding assay, found opioid-like DADLE displacement with extracts of brain from Carcinus maenas and brains plus dorsal organ from Squilla mantis, suggestive of the presence of opioid peptides. In an abstract, Dircksen et al. [27] reported that leucine enkephalin-like immunoreactivity, as seen by light and electron microscopic immunocytochemistry, was apparent in the pericardial organs and their segmental nerve connections to the thoracic ganglia of Carcinus maenas and in the pericardial organs of the crabs Cancer pagurus, Portunus puber, and Maja squinado.

Carcinus maenas has also been used in other studies involving enkephalins. Amiard-Triquet et al. [1] by immunofluorescence showed the presence of methionine enkephalin-like material in the hepatopancreas (midgut gland) of this crab. The immunofluorescent material was located mainly basally in hepatopancreas tubule cells of crabs taken from nonpolluted water. However, in crabs from a polluted site or those exposed to Cd, Pb, Cu or Zn for 1-3 weeks the immunofluorescence was located mainly apically in these tubule cells. No immunoreactivity was seen in the lumina of these tubules, which suggests that this immunoreactive material is either not secreted into the lumen or loses its immunoreactivity upon being secreted. Also with Carcinus maenas, Dircksen [26] found by immunocytochemistry that leucine enkephalin-like immunoreactive axons are present in the pericardial organs, neurohemal organs that are cardiostimulatory. These immunoreactive axons enter the pericardial organs via segmental nerves 1, 3 and 7. No colocalization of this material with proctolin, FMRFamide or crustacean cardioactive peptide was seen. More recently, both methionine enkephalin and leucine enkephalin were found in extracts of the thoracic ganglion of Carcinus maenas [73]. Their presence was established by RIA, HPLC and sequence analysis of the opioids in thoracic ganglion extracts. An immunocytochemical study by Rothe et al. [94] of the eyestalks of Carcinus maenas revealed immunoreactivity to both methionine enkepahlin and leucine enkephalin in the sinus gland (more leucine enkephalin than methionine enkephalin being present in this neurohemal organ); only leucine enkephalin in the medulla terminalis, lamina ganglionaris, medulla externa and medulla interna; and only methionine enkephalin in the chiasm between the medulla interna and medulla terminalis. In addition, by HPLC analysis a substance that coelutes with leucine enkephalin was identified in sinus gland extracts.

With another crab, the land crab, *Gecarcinus lateralis*, Leung *et al.* [70] by HPLC and RIA analyses found a methionine enkephalin-like substance in the eyestalk, and methionine enkephalin-like and leucine enkephalin-like substances in the brain. Colleti-Previero *et al.* [22] found three

peptidases in the hemolymph of the crayfish, Astacus fluviatilis, that rapidly degrade leucine enkephalin.

Another opiate first found in mammals is called  $\beta$ -endorphin. It is a 31-amino acid polypeptide. Sephadex G-50 column chromatography of extracts of the hepatopancreas of the red swamp crayfish, *Procambarus clarkii*, revealed three peaks of immunoreactive  $\beta$ -endorphin-like material. These immunoreactive peaks were identified by immunocytochemistry and RIA [52]. Cells of the hepatopancreas of this crayfish contain enzymes capable of degrading  $\beta$ -endorphin [123].

## **Functions**

### A Behavior

The first study of a possible role of an opioid in any crustaceans was that of Maldonado and Miralto [74] with *Squilla mantis*. They found that the defensive response, rapid flexure of the abdomen, which can be experimentally induced by an electrical shock, can be modified by morphine. This drug increases the threshold current required to elicit the response. The loss of sensitivity is dose-related. Naloxone blocks the action of this opioid; coinjection of morphine and naloxone does not result in a loss of sensitivity.

In a more recent study of locomotor activity of *Gecarcinus lateralis*, Martinez *et al.* [79] found that when the crabs were first placed into the activity monitoring chambers locomotor activity increased. FK 33 824, a stable methionine enkephalin analog, markedly enhances this initial activity; but naloxone blocks this excitatory action of the opioid.

In studies with another crab, *Chasmagnathus* granulatus, Lozada et al. [72] found that this crab assumes a defensive posture, with both chelae extended and the body elevated, when an electric shock, 50 Hz, one second duration, and of at least 8 V, is given. Morphine produces a dose-dependent reduction in the sensitivty of this crab to the electrical shock. When naloxone was co-injected with morphine no reduction in sensitivity occured, which suggests that opioid receptors are indeed involved in this reduction of sensitivity to the electric shock. In other behavioral studies with this crab [10, 117], it was found that a danger stimulus in the form of a passing shadow elicits an

escape response that habituates after repeated stimulation, and that this habituation appears to be mediated by endorphins. Support for this suggestion that habituation is the result of endorphin release is the observation that after an habituation session there is an analgesic effect on the defensive response of this crab to an electrical shock.

#### **B** *Pigmentary effectors*

Crustaceans have two types of pigmentary effectors: (a) chromatophores which are responsible for color changes and (b) the retinal pigments, which control the amount of light impinging on the rhabdom. Translocation of the pigments in the chromatophores and at least in the distal and reflecting pigments is clearly regulated by neurohormones.

#### (a) Chromatophores

The physiology of crustacean chromatophores has been reviewed recently [38]. In several species dual control of the chromatophores by pigmentdispersing and pigment-concentrating neurohormones has been demonstrated. In this laboratory we have been particulary intersted in identifying the neuroregulators that control the release of crustacean neurohormones. In the fiddler crab, Uca pugilator, 5-hydroxytryptamine stimulates release of red pigment-dispersing hormone while norepinephrine stimulates release of black pigment-dispersing hormone, and dopamine stimulates release of red pigment-concentrating hormone and black pigment-concentrating hormone. Methionine-enkephalin, but not leucineenkephalin, was found to stimulate black and red pigment concentration in intact Uca pugilator, but not in isolated legs [93]. Methionine-enkephalin also stimulated the release of black and red pigment-concentrating hormones from isolated eyestalks [13, 93]. The opioid antagonist, naloxone, blocked the effects of methionine-enkephalin in intact crabs and on isolated eyestalks. Methionine enkephalin, like the classical aminergic neurotransmitters, has no direct effect on these chromatophores, as evidenced by the lack of effect on the chromatophores in isolated legs. Presumably, therefore, these compounds exert their effect only indirectly, by stimulating release of the appropriate specific chromatophorotropic neurohormone. However, surprisingly,  $\beta$ -endorphin was found to

produce black pigment dispersion in both intact *Uca pugilator* and in isolated legs [93]. Additional evidence for the involvement of opioid-like peptidergic substances in crustacean color changes was provided by Martinez *et al.* [80], who found that although injection of the stable methionine-enkephalin analog FK 33 824 alone into *Gecarcinus lateralis* has no effect on the chromatophores, whereas coinjection of this analog with eyestalk extract strongly potentiates the black pigment-dispersing and red pigment-concentrating actions of the eyestalk extract. This potentiation is blocked by naloxone.

#### (b) Distal retinal pigment

Norepinephrine produces light adaptation of the distal retinal pigment of *Uca pugilator* whereas dopamine induces dark adaptation [62, 64], presumably by stimulating release respectively of the light-adapting and dark-adapting neurohormones. Methionine enkephalin, like dopamine, produces dark adaptation of this pigment, but leucine enkephalin has no effect on this pigment [63].

#### C Blood glucose

The crustacean hyperglycemic hormone (CHH) is found in the sinus gland. In several crustaceans CHH release has been found to be triggered by 5-hydroxytryptamine [40]. Recently Lüschen et al. [73] and Rothe et al. [94] provided evidence that synthetic leucine-enkephalin, endogenous sequenced leucine-enkephalin isolated from thoracic ganglia of Carcinus maenas, and purified leucineenkephalin-like material (not yet sequenced) from sinus glands of Carcinus maenas inhibit CHH release. Injection into intact Uca puglilator of synthetic leucine-enkephalin or either the sinus gland or thoracic gland material from Carcinus maenas results in a decrease in the hemolymph glucose concentration. Also, eyestalks of Carcinus incubated in the presence of synthetic leucine enkephalin release less than the basal level of CHH as compared with eyestalks incubated in saline alone. Injection into intact fiddler crabs of naloxone prior to injection of leucine-enkephalin blocks the hypoglycemic action of this opioid. In contrast, leucine-enkephalin does not affect the hemolymph glucose concentration in eyestalkless fiddler crabs, which is consistent with the hypothesis that the enkephalin is acting by reducing CHH

output from the sinus glands in the eyestalks.

## HYPOTHALAMIC AND HYPOPHYSIAL HORMONES AND NEUROPHYSIN

#### Localization and identification

In what appears to have been the first effort to identify a "naturalized" peptide in a crustacean, Martin and Dubois [78] by use of an immunofluorescence technique demonstrated the presence of a somatostatin-like substance in the brain, subesophageal ganglion, and ventral nerve cord of the isopod, Porcellio dilatatus. However, no localization of this somatotropin releaseinhibiting hormone-like material was apparent in the sinus glands. Van Herp and Bellon-Humbert [120], using the prawn, Palaemon serratus, provided immunocytochemical evidence for neurophysin-like and arginine vasopressin-like substances in the organ of Bellonci, a component of the eyestalk. However, whereas the neurophysin-like material was present at all times, the vasopressinlike substance was present only at ecdysis. Later, Van Deijnen et al. [119] did an immunocytochemical study of the eyestalk of the crayfish, Astacus leptodactylus, with antibodies against melanocytestimulating hormone, vasotocin, and oxytocin. The immunoreactivity they observed to antivasotocin was the most widespread while that to antioxytocin was the least widespread. The sinus gland reacts positively to antimelanocyte-stimulating hormone and antivasotocin. In addition, cells or fibers reactive: (a) to antivasotocin are in chiasms 1 and 2, the medulla externa, medulla interna, the medulla terminalis neuropil and X-organ of the medulla terminalis, (b) to antialpha-melanocytestimulating hormone are in the lamina ganglionaris, chiasm 1, medulla externa, medulla interna and X-organ of the medulla terminalis; and (c) antioxytocin in the neuropils of the medulla externa and medulla terminalis. In a related study, Mattson and Spaziani [82], using arginine vasopressin antiserum in an RIA with eyestalk extract of the crab, Cancer antennarius, found vasopressinlike peptides are indeed present.

Mizuno and Takeda [84] used immunocytochemistry to identify in several crustaceans neurons immunoreactive to arginine vasotocin/arginine vasopressin antibodies. Such neurons were seen in brains of the sea louse, Gnorimosphaeroma rayi, and the crab, Hemigrapsus sanguineus, but not in the brain or subesophageal ganglion of Procambarus clarikii or the crab, Helice tridens. Mizuno and Takeda [83] also looked for oxytocin-like immunoreactivity in the nervous systems of these four crustaceans, but none was seen. In a preliminary study done earlier Takeda et al. [108] examined only isopod central nervous systems by immunocytochemistry with these neurohypophysial hormone antibodies. Arginine vasotocin/arginine vasopressin immunoreactivity was apparent with Ligia exotica, Porcellio scaber, and Armadillidium vulgare, but no oxytocin immunoreactivity was apparent.

A human somatotropin-like substance was identified by RIA in the hemolymph and extracts of the stomach and hepatopancreas of *Palaemon serratus* [113]. The amounts in the three tissues varied with the molting cycle, being highest in all three tissues during proecdysis.

#### **Functions**

Few attempts have been made to identify possible roles in crustaceans of hypophysial compounds. Sarojini et al. [99] found that oxytocin elvates the hemolymph glucose concentration in the freshwater crab, Barytelphusa cunicularis and the prawn, Macrobrachium kistnensis. The glycogen levels in the hepatopancreas, muscles and integument of both species showed concomitant decreases, which suggests that these glycogen stores were hydrolyzed to provide the glucose that appeared in the hemolymph. Later, Mattson and Spaziani [82] reported that lysine vasopressin, arginine vasopressin, vasotocin, and oxytocin mimic the action of molt-inhibiting hormone, inhibiting ecdysteroid production by Y-organs of the crab, Cancer antennarius. Charmantier et al. [17] found that larval and postlarval lobsters, Homarus americanus, grow more rapidly when injected with human somatotropin. This hormone did not bring on precocious molting activity, but the specimens that received the hormone became longer and weighed more than the controls. The hormone appeared to stimulate tissue growth without in18

#### M. FINGERMAN, R. NAGABHUSHANAM AND R. SAROJINI

fluencing the water content of these test individuals.

Zukowska-Arendarczyk [127] tested ovine follicle-stimulating hormone and luteinizing hormone on the sand shrimp, Crangon crangon. Both intact and eyestalk ligated females were used. Eyestalk ligation was used to eliminate any influence of the gonad-inhibiting hormone of the sinus gland in the eyestalk. However, the responses when these gonadotropins were injected were qualitatively the same among the ligated and non-ligated individuals, although the ligated individuals did show accelerated vitellogenesis and oocyte growth as compared with the non-ligated shrimp. Folliclestimulating hormone causes the number of somatic cells in the ovary to increase but does not affect the oogonia or oocytes. In contrast, luteinizing hormone affects only the germinal epithelium; the oogonia increasing in number and size and the oocytes undergoing accelerated vitellogenesis, but the somatic cells are not affected. So, both hormones stimulate this ovary, but in different ways.

Although chorionic gonadotropin is not an hypophysial hormone, in mammals it is functionally similar to luteinizing hormone, and this for this reason is treated here. Bomirski and Klek-Kawińska [9] found that human chorionic gonadotropin stimulates oogenesis in Crangon crangon. Similarly, Souty and Picaud [106] found that human chorionic gonadotropin stimulates vitellogenesis by the fat body in the isopod, Idotea balthica. Maruo et al. [81], using RIA and a radioreceptor assay, found a human chorionic gonadotropin-like substance in the stomach and hepatopancreas of the lady crab, Ovalipes ocellatus. However, when these investigators tested this substance from the crab stomach in a mouse uterus bioassay, this substance exhibited no biological activity.

### GASTRIN/CHOLECYSTOKININ

### Localization and Identification

Gastin and cholecystokinin are peptides that have the same five amino acids at their carboxyl end [68]. Consequently, antisera raised against either of these peptides generally are immunoreac-

tive to both. It is now recognized that both gastrin and cholecystokinin belong to a single peptide family called the gastrin/cholecystokinin (G/ CCK) family. Not only do gastrin and cholectystokinin function as vertebrate gastrointestinal hormones, but both are also present in vertebrate nervous systems, probably functioning there as neurotransmitters. By RIA Larson and Vigna [67] showed the presence of G/CCK-like material in the digestive tract of *Cancer magister* (the highest concentration occurring in the stomach), and also in the digestive tract of Upogebia pugettensis. This material was not detected in the hepatopancreas of Cancer nor in the digestive tracts of two barnacles, Balanus sp. and Pollicipes polymerus. G/CCKlike material of *Cancer magister* was analyzed by Sepahdex G-50 chromatography [66] from the stomach, hemolymph and carapace. Three immunoreactive molecular forms of G/CCK-like peptides were found.

Scalise *et al.* [102], using immunocytochemistry, provided supporting evidence for the presence of G/CCK-like material in the stomach of *Cancer* magister. This material appears to be synthesized in the gastric epithelial cells and then secreted into the procuticle which lines the stomach lumen. Bellon-Humbert *et al.* [6] reported that by immunocytochemistry and with an antibody to cholecystokinin 8 they observed that neurosecretory cells in the medulla externa and medulla terminalis X-organ of *Palaemon serratus* are immunoreactive to this antibody.

Van Deijnen et al. [119] who, as mentioned above, used a wide variety of mammalian antisera to test for "naturalized' peptides in eyestalks of Astacus leptodactylus found immunoreactivity against a gastrin (C-terminal) antibody in the medulla externa nd medulla terminalis X-organ. They [119] also used an antibody to cholecystokinin that was raised against the middle portion of the molecule which does not crossreact with gastrin. This anticholecystokinin revealed the presence of reactive cells in the lamina ganglionaris, medulla externa, medulla interna, medulla terminalis (both in the X-organ and the rest of the ganglion), and also in the sinus gland. By immunofluorescence and RIA Turrigiano and Selverston [114] showed G/CCK-like material is in the input nerve and neuropil of the stomatogastric ganglion, commissural ganglia, brain and eyestalks *Panulirus interruptus*.

Favrel et al. [37], by immunocytochemistry, showed the presence of G/CCK-like peptides in the eyestalk and stomach of Palaemon serratus. In the eyestalk, cells in the medulla externa and medulla terminalis X-organ and the sinus gland were labelled. In the stomach, epithelial cells and the cuticle were labelled also. Like with Cancer magister, Sephadex G-50 fractionation of eyestalks, stomach and hemolymph of Palaemon serratus revealed multiple molecular forms of G/CCKlike material. The quantity of G/CCK-like peptides, as measured by RIA, in the eyestalks showed no significant variation with the molting cycle, but in the hemolymph the concentration was highest, during late proecdysis, just before ecdysis; and for the stomach concentration peaks were apparent during postecdysis and during proecdysis. Van Wormhoudt et al. [121], using shrimps, Penaeus japonicus and Penaeus stylirostris, and an RIA for G/CCK-like peptides found, after fractionation on a Sephadex R G-50 SF column, four molecular forms of these peptides in the hemolymph of each shrimp. The amount of each form varies with the species. Four G/CCK-like peptides that cross-reacted with a specific Cterminal G/CCK antiserum were also isolated from extracts of the stomach of the lobster, Nephrops norvegicus, by Favrel et al. [35]. Their molecular weights are in the range 1000-2000 daltons. These four peptides show very weak crossreactivity with human gastrin, about 0.03%. Amino acid analysis showed none of these peptides has the same five terminal amino acids at the carboxyl end as human gastrin and cholecystokinin, these five being necessary for full immunoreactivity. Instead they had only three or four of the requisite amino acids.

## Functions

Because of the established roles of gastrin and cholecystokinin as regulators of digestion among vertebrates, studies of the possible roles of G/ CCK-like peptides in crustaceans have centered on similar functions. Larson and Vigna [66] reported that G/CCK-like material from *Cancer magister*  does not stimulate secretion of either gastric acid or pancreatic amylase in the rat. This suggests that the crab material while sufficiently like vertebrate G/CCK to react with the antisera is nevertheless sufficiently different so as to be incapable of eliciting a response in the rat. Working with *Palaemon serratus*, Favrel and Van Wormhoudt [36] found that injection of gastrin 17 results in increased incorporation of leucine into proteins of the hepatopancreas.

Turrigiano and Selverston [114] found that electrical stimulation of the stomatogastric input nerve, which contains G/CCK immunoreactive fibers, results in the release of G/CCK-like peptide into the stomatogastric ganglion of Panulirus interruptus. Also, application of cholecystokinin to this ganglion results in increases in the spike frequency and number of spikes per burst of the pyloric rhythm and also activates the gastric mill. In view of these results, these investigators suggested that in this lobster G/CCK-like peptide functions as a neuromodulator in the stomatogastric ganglion. When Penaeus japonicus and Penaeus stylirostris are fed, the amount of G/ CCK-like material in their hemolymph increases [121]. This increase indicates that these peptides may help regulate the digestive process. Also, in Panulirus interruptus the hemolymph concentration of G/CCK-like peptide increases after feeding (up to fourfold), and the effect lasts about four hours [115]. Concomitant with this increase is an increase in gastric mill activity. Also, the cholecystokinin antagonist, proglumide, when injected within the first two hours after feeding, causes a prolonged decrease in gastric mill activity.

SedImeier and Resch [103] tested the efficacy of gastrin and cholecystokinin *in vitro*, using the hepatopancreas of the crayfish, *Orconectes limosus*. Both gastrin and cholecystokinin were found to stimulate release of amylase and proteases into the incubation medium. Favrel *et al.* [35], using three of the four G/CCK-like peptides they isolated from extracts of the stomach of *Nephrops norvegicus*, found that two of the three stimulate protease secretion *in vitro* from the hepatopancreas of *Orconectes limosus*.

## INSULIN

#### Localization and identification

Although early studies [41, 53, 69] showed that injected mammalian insulin does not affect the hemolymph glucose concentration in crustaceans, interest in the possible presence and roles of insulin-like peptides in crustaceans continues. Davidson et al. [25] reported that the hepatopancrease of Carcinus maenas shows insulin-like activity in a mouse diaphragm glycogen synthesis test, and that some cells in the hepatopancreas, but not in the gut, of Carcinus maenas and Homarus gammarus have staining characteristics of mammalian insulin-secretory pancreatic islet beta cells. But Davidson et al. [25] cautioned about hastily drawing firm conclusions when using light microscopic staining features and histochemical staining reactions devised for mammalian cells on tissues of invertebrates. Likewise, Falkmer [34] had earlier found in a study with the use of light microscopy that such insulin-producing cells are absent from the gut of the blue crab, Callinectes sapidus, also.

Sanders [96], using an RIA and guinea pig anti-insulin serum showed that the hepatopancreas, gut and hemolymph of Homarus americanus contain insulin-like peptides. The hepatopancreas contains the highest concentration of immunoreactive material while the eyestalk has none. Fractionation of hepatopancreas, hemolymph and gut on a Sephadex G-50 column revealed molecular heterogeneity of the insulin-like material. The hepatopancreas contains the highest proportion of high molecular weight insulin-like material, the gut and hemolymph having relatively more of the lower molecular weight insulin-like material. In view of these results, Sanders [96] hypothesized that a large insulin-like peptide is synthesized in the hepatopancreas, secreted into the gut where it is broken down to smaller units, reabsorbed into the hepatopancreas and then secreted into the hemolymph.

## **Functions**

Sanders [97], using *Homarus americanus*, found that bovine insulin, hepatopancreas extract, and hemolymph induce glycogenesis in abdominal

muscle of this lobster in vitro, but gut extracts have no such effect. However, injection of these insulin-like peptides from the hepatopancreas does not affect the glucose concentration in the hemolymph of Homarus americanus [98]. High glucose concentrations in the hemolymph did not stimulate release of insulin-like peptides into the hemolymph, nor did hepatopancreas extracts speed the rate of glucose clearance from the hemolymph. These findings are consistent with the earlier results from several laboratories mentioned above [41, 53, 69] that mammalian insulin has no effect on the hemolymph glucose concentration in crustaceans. This crustacean insulin-like material presumably functions to regulate glycogenesis in muscle cells, but has no role in hemolymph glucostasis. Insulin does not stimulate release of amylase or proteinase from the isolated hepatopancreas of Orconectes limosus [103]. Consistent with the results of Sanders [96, 97] are the observations of Baker and Carruthers [4, 5] that porcine insulin stimulates glucose transport into muscle cells of the barnacle, Balanus nubilis. Baker and Carruthers also found with these muscle cells that insulin lowers the cytosolic ionized calcium level, reduces the cyclic AMP content and increases the cyclic GMP content. These observations are consistent with the earlier observations of Bittar et al. [7] that insulin stops sequestration of soldium ions in muscle cells of the barnacles, Balanus nubilus and *Balanus aquila*, and also increases the activity of the guanylate cyclase system. Insulin injected into these cells produces an increase in the rate of sodium efflux and injection of guanosine triphosphate into muscle cells pre-exposed to insulin also produces an increase in sodium efflux.

### SUBSTANCE P

#### Localization and identification

In the vertebrate central nervous system substance P apparently serves as a neurotransmitter that mediates sensory input, e.g. transmission of pain impulses to the brain. By immunocytochemistry substance P-like material was found in the eyestalk of *Panulirus interruptus* [75]. This immunoreactivity material is in the lamina ganglio-

naris, medulla externa, medulla interna, medulla terminalis (in the X-organ as well as the rest of this ganglion) and sinus gland. In eyestalks of *Uca pugilator*, substance P-like immunoreactivity was seen in the retinular cells, lamina ganglionaris, medulla externa, medulla interna, sinus gland and optic peduncle [39].

Goldberg et al. [46, 47] by immunocytochemistry identified cells immunoreactive to substance P antibody in the stomatogastric nervous systems of *Panulirus interruputs, Homarus americanus* and *Cancer borealis*. In these three decapods the neuropil of the stomatogastric ganglion is strongly immunoreactive, but none of the stomatogastric ganglion somata showed any immunostaining. Also, in the connective ganglia some somata and the neuropil were positively immunoreactive. Marder [76] noted that the stomatogastric ganglion of *Cancer irroratus* shows the same pattern of substance P-like immunoreactivity as do the stomatogastric ganglia in these other three decapods studied by Goldberg et al. [46, 47].

Sanderman et al. [95] by immunocytochemistry demonstrated substance P-like immunoreactivity in cells of the brains of the crab, Leptograpsus variegatus, and the crayfish, Cherax destructor. Four large neurons in the brain of each of these crustaceans are immunoreactive, two in the protocerebrum and two in the deutocerebrum. These paired immunoreactive protocerebral cells are, more specifically, in the dorsal cell clusters that lie on each side of the median protocerebrum. Each of these cells extends through the ipsilateral and contralateral olfactory lobes to terminate among the lateral somata of the olfactory lobe, not in the neuropil. The cells from each side of the brain are mirror images of each other. Each deutocerebral cell runs only ipsilaterally, terminating within the neuropil of the olfactory lobes.

## **Functions**

Substance P was found to increase the amounts of amylase and proteases released *in vitro* from the hepatopancreas of *Orconectes limosus* [103].

## CALCITONIN and CALCITONIN GENE-RELATED PEPTIDE

### Localization and identification

Calcitonin is a small polypeptide secreted by the ultimobrachial glands in jawed fishes, amphibians, reptiles and birds; and in mammals by the so-called C cells of the thyroid gland. As a hormone, calcitonin produces hypocalcemia in vertebrates. Calcitonin is also present in the vertebrate central nervous system, presumably functioning there as a neuromodulator. The gene that codes for calcitonin also produces another polypeptide, called calcitonin gene-related peptide (CGRP). This substance is present in central and peripheral nervous systems of vertebrates, where it appears to function as a neuromodulator, and in several other regions of the vertebrate body, including the heart, spleen and urogential tract as well [89].

Bellon-Humbert *et al.* [6] by use of immunocytochemistry and antibodies against human, pig and salmon calcitonins demonstrated the presence of a calcitonin-like substance in the medulla terminalis X-organ of *Palaemon serratus*. Arlot-Bonnemains *et al.* [3] by RIA with anticalcitonin antibodies from salmon showed the presence of a calcitonin-like polypeptide in the hemolymph of *Palaemon serratus*. The amount of calcitonin-like material in the hemolymph varies with the molting cycle, being highest during postecdysis, and lowest during intermolt The hemolymph calcium level, however, was maximal during proecydsis and lowest during postecdysis.

Using RIA, a radioreceptor assay and antisalmon and antihuman calcitonin antibodies, Funchereau-Peron *et al.* [42] found a calcitonin-like peptide in the eyestalks of *Nephrops norvegicus, Homarus americanus, Palaemon serratus,* and *Penaeus vanamei.* In addition, other organs of *Nephrops* were examined. The hepatopancreas of this lobster contains a higher concentration of calcitonin-like peptide than any of the eyestalks examined from these four species. The foregut of *Nephrops* also contains a high concentration, likewise higher than in these eyestalks, but less than in the hepatopancreas. This calcitonin-like material of *Nephrops* that was detected by the antisalmon

calcitonin antibodies exists in two forms. One has a molecular weight of 4500 daltons, somewhat higher than vertebrate calcitonins. Salmon calcitonin is 3432 daltons, and human calcitonin is 3418 daltons. However, in addition to this 4500 dalton molecule, a higher molecular weight calcitoninlike substance was also found. The latter was found by gel filtration to have a molecular weight greater than 5,000 daltons.

Graf *et al.* [48], using extracts of an entire amphipod, *Orchestia cavimana*, in an RIA found a calcitonin-like substance, the concentration of which changes with the molting cycle. The maximum amount is present at ecdysis, while the least is found early in intermolt. The concentration increases markedly late in proecdysis. The least is seen early in intermolt.

Arlot-Bonnemains *et al.* [2] with an RIA and human anti-CGRP antibody found immunoreactive material in several tissues of *Nephrops norvegicus*, including the hepatopancreas, foregut, eyestalk, brain and heart. Of the tissues examined, the hepatopancreas has the highest concentration of this CGRP-like material, followed by the foregut, which led these investigators to suggest the CGRPlike substance may function in crustaceans as a regulator of gastrointestinal function.

Sasayama et al. [100] demonstrated by immunocytochemistry with antibodies to salmon calcitonin and rat CGRP the presence of cells immunoreactive to both antibodies in the central nervous system of Armadillidium vulgare. CGRP immunoreactive cells are present in the brain and circumesophageal connectives while calcitonin immunoreactive cells are in the brain only. More recently, Cameron and Thomas [14] by use of RIA showed the presence of salmon calcitonin-like material in several tissues, including the hepatopancreas, brain, heart and hemolymph of the blue crab, Callinectes sapidus. The hepatopancreas contains the highest concentration of immunoreactive material. The concentration of immunoreactive material in the hemolymph varies with the molting cycle, being highest during proecdysis, but the calcitonin-like material in the other tissues does not vary with the molting cycle. The immunoreactive material in the hemolymph and hepatopancreas consists to a great extent of a 27.2

kilodalton protein with an amino acid composition very similar to those of the 17.5 kilodalton human calcitonin precursor and the 22 kilodalton calcitonin-like molecule isolated earlier from the hemolymph and hepatopancreas of Nephrops norvegicus by Van Wormhoudt and Fouchereau-Peron [122]. However, in Callinectes there is also some immunoreactive material of relatively low molecular weight. These high molecular weight substances of 22 and 27.2 kilodaltons likely correspond to the one found by Funchereau-Peron et al. [42] in Nephrops that was larger than 5,000 daltons, whereas the low molecular weight material from Callinectes likely corresponds to the 4,500 dalton calcitonin-like compound found by Fouchereau-Peron et al. in Nephrops [42].

## **Functions**

The roles of calcitonin and CGRP in crustaceans have not been examined extensively. In a study of Palaemon serratus by Arlot-Bonnemains et al. [3] they found that injection of salmon calcitonin during preecdysis does not alter the hemolymph calcium concentration. Sellem et al. [104] investigated the effect of salmon calcitonin on the concentration of calcium in the hemolymph of Orchestia cavimana. They found that the calcium level decreased when the calcitonin was injected during intermolt and early proecdysis but had no effect during late proecdysis or during postecdysis. Normally, the hemolymph calcium level is high during proecdysis because of calcium resorption from the old cuticle, and is low during intermolt. As mentioned above, Graf et al. [48] found that calcitonin-like material is at its lowest concentration in Orchestia cavimana at the start of intermolt and increases rapidly during proecdysis to peak at ecdysis. So, the exogenous salmon calcitonin was effective when the endogenous calcitonin-like activity was low, but ineffective when the endogenous activity was high.

## **MISCELLANEOUS PEPTIDES**

#### Localization and identification

A few "naturalized" peptides have been the subject of only one or two investigations. These

substances will be treated together in this section. Van Deijnen *et al.* [119], in the immunocytochemical study of the eyestalks of *Astacus leptodactylus* already referred to above, found cells immunoreactive to three antisera in addition to those mentioned earlier. Cells or fibers showing immunoreaction (a) to antisecretin are in the lamina ganglionaris, medulla externa, medulla interna, medulla terminalis and sinus gland; (b) to antiglucagon are in the lamina ganglionaris, medulla externa, medulla interna, medulla terminalis and chiasm 1 and (c) to antiglucose-dependent insulinotropic peptide (antigastric inhibitory peptide) are in the medulla externa, medulla interna, medulla terminalis and chiasm 1.

Charmantier-Daures et al. [18] did an immunocytochemical study of the eyestalks of Homarus gammarus that involved antibodies against neuropeptide Y and the atrial natriuretic A few cells that are immunoreactive factor. against the atrial natriuretic factor antibody are present in the medulla externa and medulla terminalis whereas the sinus gland and a few cells and numerous fibers in the medulla interna and medulla terminalis react to neuropeptide Y antiserum.

#### **Functions**

Turrin et al. [116] using an RIA for atrial natriuretic peptide found that the amount of this substance in the hemolymph of the mangrove crab, Ucides cordatus, is related to the environmental salinity. When crabs are transferred from 26 parts per thousand sea water, which is isosmotic with the hemolymph of this crab, to 34 parts per thousand sea water the concentration of this peptide in the hemolymph icreases significantly. These investigators suggested that this peptide has a role in the osmoregulation of this crab, perhaps to accelerate sodium excretion under when the crab is in a hyperosmotic environment. Sedlmeier and Resch [103] in a study already referred to above found that glucagon, like insulin, does not produce in vitro release amylase or proteinase from the hepatopancreas of Orconectes limosus whereas bombesin, like gastrin, cholecystokinin, and substance P does stimulate release of these enzymes in vitro.

#### Localization and identification

This section will be devoted to those derivatives of fatty acids collectively known as eicosanoids, the best known of which are the prostaglandins. Prostaglandins were first identified in mammalian semen and thought secreted by the prostate gland (hence their name), although it is now clear they are secreted into the semen by the seminal vesicles. However, prostaglandins are by no means limited to the reproductive system. Prostaglandins are produced in virtually all mammalian tissues, and function as chemical messengers. Other eicosanids include the leukotrienes, thromboxanes and such hydroxyunsaturated fatty acids as the barnacle hatching substance.

The barnacle hatching substance is released by adult barnacles into their mantle cavity where it stimulates hatching of nauplii that underwent their early development in the mantle cavity. While several barnacle tissues can synthesize the hatching substance, the epidermis appears to be the major site of synthesis. Preliminary evidence led Clare et al. [19-21] to suggest that the hatching substance of Semibalanus balanoides is a prostaglandin-like compound. In 1985, the same year as the third publication on the subject by Clare et al. [21], Holland et al. [56] showed that this hatching substance of Semibalanus balanoides is an eicosanoid, but not a prostaglandin. Prostaglandins are cyclic compounds whereas this hatching substance is from the linear, acyclic, pathway of unsaturated fatty metabolism for eicosanoid synthesis, and is therefore more closely related to the leukotrienes. Nevertheless, a histochemical staining technique for prostaglandin synthetase gives positive results with ovaries and developing egg masses of Semibalanus balanoides, and extracts of these egg masses have thin layer chromatography characteristics similar to those of the primary prostaglandins [55]. Holland et al. [56] proved by thin layer chromatography, gas chromatography, and mass spectrometry that the hatching substance of Semibalanus balanoides is 10, 11, 12-trihydroxy-5, 8, 14, 17-eicosatetraenoic acid. Later, Hill et al. [54] showed that the hatching substance of another

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barnacle, Elminius modestus, has a different structure. It appears to be an eicosanoid also, apparently a monohydroxyeicosapenataenoic acid isomer, probably 8-hydroxyeicosapentaenoic acid. Although this substance is different from the hatching substance identified by Holland *et al.* [56] for Semibalanus balanoides, the hatching substance of Elminius modestus is effective not only with Elminius but also with Semibalanus balanoides.

Hampson *et al.* [51] found that hemolymph cells of *Carcinus maenas* are capable of synthesizing eicosanoids such as 5-hydroxyeicosatetraenoic acid, prostaglandin  $E_2$  and thromboxane  $B_2$ . The calcium ionophore A23187 was used to stimulate this eicosanoid production

#### **Functions**

Casterlin and Reynolds [15] used the crayfish, Cambarus bartonii, in one study and in another they [16] used Homarus americanus and Penaeus duorarum. The aim of both studies was to determine whether injection of prostaglandin E1 would alter the preferred temperatures of these crustaceans. This prostaglandin does indeed evoke an increase in the preferred water temperatures. The test chambers were designed so that each specimen could control the water temperature, thereby revealing its temperature preference. The crayfish showed a 3.4°C increase in temperature preference with 0.5 mg of prostaglandin, the lobster  $4.7^{\circ}\text{C}$ , and the shrimp 4.5°C, the lobster and shrimp receiving 0.1 mg. Prostaglandins may have a role in thermoregulation in these crustaceans, even though it would be minor because they are basically ectotherms, perhaps by stimulating a thermoregulatory control center.

The crab, *Rhithropanopeus harrisii*, produces a rhythmic abdominal flexion during and after copulation. This behavior can be induced by exposing males or females to the crab's semen or homogenates or extracts of the crab's seminal vesicles [44]. This abdominal flexion can also be induced by prostaglandin  $F_2$  and prostaglandin  $E_2$ . However, prostaglandins have not yet been identified in crab seminal fluid.

Prostaglandins may have a role in regulating ion transport across crustacean gills. Siebers *et al.* 

[105] found that indomethacin, an inhibitor of prostaglandin synthesis, produces a decrease of the transbranchial potential across isolated gills of *Carcinus maenas* when applied to the basolateral side of the gills. Concomitantly, the rate of chloride ion influx decreased significantly in the presence of indomethacin.

Spaziani *et al.* [107] incubated ovaries of *Procambarus paeninsulanis*, with the prostaglandin precursor arachadonic acid, and then isolated from these ovaries a newly synthesized compound that comigrated on thin layer chromatography plates with prostaglandin  $F_{2a}$ . Synthesis of this prostaglandin was inhibited by indomethacin. The rate of synthesis was higher when ovaries in the early stages of vitellogenesis were used rather than later stages. As suggested by these investigators, prostaglandins may have a normal role in regulating vitellogenesis in this crayfish.

Koskela *et al.* [61], working with the tiger prawn, *Penaeus esculentus*, found that prostaglandin  $E_2$  produces a decrease in the length of the intermolt cycle; but does not stimulate ovarian development. Furthermore, neither  $17\alpha$ hydroxyprogesterone nor  $17\beta$ -estradiol affects the duration of the molting cycle or ovarian development of *Penaeus esculentus*.

#### **STEROIDS**

#### Localization and identification

Several steroids that are ordinarly identified with mammals have been found in crustaceans. Donahue [28], in what appears to have been the first report that a "naturalized" steroid is present in crustaceans, found that the ovaries of Panulirus argus contain an estrogenic substance. Ovarian extracts elicited growth of the vaginal epithelium in mature, ovariectomized rats. Later, Donahue [29] by fluorimetric analysis and the rat vaginal assay he used previously [28] showed that eggs of Homarus americanus, at the time the eggs attach to the pleopods, contain an estrogenic substance. Donahue [31] identified this estrogenic substance as alpha-estradiol. Lisk [71] later reported that eggs of Homarus americanus contain estradiol-17 $\beta$ . Estrone was not found. Whole body extracts

of the euphausiacean, Euphausia superba, were found to contain the steroids progesterone, testosterone and esterone [87]. Jeng *et al.* [59] by use of gas chromatography, RIA, and a bioassay based on weight increases of the mouse uterus found that the ovaries of *Parapenaeus fissurus* contain both estrone and estradiol- $17\beta$ , with estrone being the major estrogen present. In a recent study of the blue crab, *Callinectes sapidus* [101], neither  $17\beta$ estradiol nor estrone was detected in males or females. However, a compound similar to estriol is present in the hemolymph, hepatopancreas, ovaries, testes and eyestalks.

Gilgan and Idler [45] found that the testes and androgenic glands of Homarus americanus are each capable of converting androstenedione to testosterone. This conversion requires the presence of the enzyme  $17\beta$ -hydroxysteroid dehydrogenase (oxidoreductase). Blanchet et al. [8] showed this enzyme must also be present in the testes and vasa deferentia of male Carcinus maenas because extracts of these organs converted androstenedione, dehydroepiandrosterone and estrone respectively to testosterone, and rost-5-ene- $3\beta$ , 17 $\beta$ -diol and estradiol-17 $\beta$ . Burns et al. [11] showed that testes of Homarus americanus can convert progesterone to  $20\alpha$ -dihydroprogesterone. This conversion requires the enzyme steroid 20ketone reductase. The same investigators [12] also showed that testosterone is present in the hemolymph and testes of Homarus americanus.

Tcholakian and Eik-Nes [109, 110] found that the androgenic gland of *Callinectes sapidus* has the enzymatic capability to convert progesterone to testosterone, 11-deoxycorticosterone,  $20\alpha$ -hydroxyprogesterone, and  $\Delta^4$ -androstenedione. Also, intact male crabs converted  $\Delta^5$ -pregnenolone to progesterone,  $\Delta^4$ -androstenedione, testosterone and 11-deoxycorticosterone.

Teshima and Kanazawa [111, 112] found that the ovaries of the crab, *Portunus trituberculatus*, can convert progesterone to 11-ketotestosterone,  $11\beta$ -hydroxyandrost-4-ene-3, 17-dione, testosterone,  $17\alpha$ -hydroxyprogesterone and deoxycorticosterone. Kanazawa and Teshima [60] in another study of steroids used *Panulirus japonica*. In this study they injected radioactive cholesterol and then found that the hepatopancreas, ovaries and hemolymph contained newly synthesized prog- $17\alpha$ -hydroxyprogesterone, androsesterone, tenedione and testosterone, and in addition the hepatopancreas contained deoxycorticosterone and corticosterone. Vitellogenic ovaries of Penaeus monodon metabolize progesterone mainly to four  $5\alpha$ -pregnane derivatives ( $5\alpha$ -pregnan-3,20-dione,  $20\alpha$ -hydroxy- $5\alpha$ -pregnan-3-one,  $3\beta$ hydroxy- $5\alpha$ -pregnan-20-one and  $5\alpha$ -pregnan- $3\beta$ , 20 $\alpha$ -diol) plus two minor metabolites, 20 $\alpha$ hydroxypregn-4-en-3-one and 1,4-pregnadiene-3,20-dione; whereas vitellogenic ovaries of Nephrops norvegicus metabolize progesterone to only one metabolite, 20a-hydroxypregn-4-en-3one [126].

Ollevier *et al.* [90] analyzed the hemolymph of *Astacus leptodactylus* for nonecdysteroid steroids. In both sexes pregnenalone,  $17\alpha$ -hydroxypregnenalone, testosterone and cholesterol were clearly identified, and there was indication that androstenedione,  $5\alpha$ -dihydroxytestosterone, 11-ketotestosterone and  $11\beta$ -hydroxytestosterone were also present. However, with the techniques they used, dehydroepiandrosterone, progesterone,  $17\alpha$ -hydroxyprogesterone and estrogens could not be detected in either sex, but females alone have  $6\beta$ -hydroxyprogesterone.

Couch *et al.* [23], using RIA, found progesterone but not testosterone, in mandibular organs of *Homarus americanus.* Later Couch *et al.* [24], again with RIA, and using female specimens reported the presence of estradiol- $17\beta$  and progesterone in the mandibular organ, green gland, hepatopancreas, ovary and hemolymph of *Homarus americanus.* However, the estradiol was not detectable in any of these tissues if the female had immature ovaries. Progesterone was always present in the mandibular organs; its concentration is unrelated to the state of the ovaries. But in all other tissues progesterone was not detectable or present only in low concentrations when the ovaries were immature.

Van Beek and De Loof [118] analyzed by RIA total body extracts of adult female brine shrimp, *Artemia* sp. Progesterone and pregnenolone were low during vitellogenesis, but high at the beginning and end of each vitellogenic cycle. The amount of  $5\alpha$ -dihydroxytestosterone was very low at the start

of vitellogenesis, peaking at the end of vitellogenesis. The testosterone level was low during the vitellogenic cycle, and highest in nonvitellogenic ovaries. Estrone was higher during vitellogenesis than at other times, but the fluctuation was not large. Estradiol increased during vitellogenesis, peaking at the end of vitellogenesis. Pregnenalone was present in higher concenterations than any of the other steroids.

In a study designed to detect both conjugated and unconjugated steriods Fairs et al. [32], using chromatography/mass spectrometry, gas examined tissues of Nephrops norvegicus. Found in the ovary in unconjugated form were  $5\alpha$ dihydroxytestosterone, testosterone, pregnenolone and 20α-hydroxypreg-4-en-3-one. Eggs and hemolymph contained unconjugated  $17\beta$ -estradiol while the ovary and hemolymph contained conjugated  $5\alpha$ -dihydrotestosterone. Fair *et al.* [33] then analyzed the steroids in the ovary of Penaeus monodon by the same techniques. Conjugated pregnenolone, conjugated and unconjugated dehydroepiandrosterone, unconjugated progesterone, conjugated  $17\beta$ -estradiol and conjugated and unconjugated estrone were present in these ovaries, peaking during mid to late vitellogenesis, but conjugated testosterone was present only in mature ovaries.

#### **Functions**

Donahue [30], who showed that eggs of *Homar-us americanus* contain an estrogen, looked for a possible role for this substance. Berried females undergo ecdysis only after the larvae have hatched. When he stripped newly laid eggs from the swimmerets, the majority of the lobsters underwent a premature ecdysis. He suggested the eggs contain a moltinhibiting factor. He then proceeded to inject estradiol into male and female lobsters, and found that molting activity was inhibited. Danahue concluded by suggesting that ecdysis of berried females is normally inhibited by egg estrogen and perhaps also ovarian estrogen.

Kulkarni *et al.* [65] found that progesterone stimulates ovarian maturation in the prawn, *Parapenaeopsis hardwickii.* The ovary size, oocyte size, and ovarian fat content all increased. With another species of this prawn, *Parapenaeopsis sty-*

lifera, Nagabhushanam et al. [85] found that 17hydroxyprogesterone induces spawning at 20°C whereas normally spawning does not occur until the water temperature reaches 30°C. Also with male Parapenaeopsis hardwickii Nagabhushanam and Kulkarni [86] observed that testosterone injections produce hypertrophy and hyperplasia of the androgenic glands which develop to a functional state only in male crustaceans. There was a concomitant stimulation of testicular development, presumably under the influence of androgenic gland hormone released as a consequence of the effect of the testosterone on the andorgenic gland.

Yano [124] observed, using the greasyback shrimp, *Metapenaeus ensis*, that progesterone injections induce ovarian maturation and spawning of the eggs from these ovaries. Later, Yano [125] using the kuruma prawn, *Penaeus japonicus*, concluded that injection of  $17\alpha$ -hydroxyprogesterone induces vitellogenesis, as evidenced by an increase in the amount of vitellogenin in the hemolymph of the specimens treated with this hormone.

Koskela *et al.* [61], as noted above, reported that injection of prostaglandin  $E_2$  into *Penaeus esculentus* results in a significant decrease in the length of the molting cycle but does not affect ovarian development. However, neither  $17\alpha$ hydroxyprogesterone nor  $17\beta$ -estradiol affected the molting cycle or the ovaries of this shrimp.

#### **CONCLUDING REMARKS**

Despite the large body of knowledge that has accumulated in recent years concerning the identity of many vertebrate-type hormones in crustaceans, largely as a result of improved immunologic and chromatographic procedures as well as the possibilities offered by recombinant DNA technology/molecular biology, the precise functions and mechanisms of action of these compounds remain to be established. In regard to the vertebrate-type peptides in crustaceans, the biochemical and physiological roles of these peptides (including their receptor systems) in crustaceans need to be firmly established in order to eliminate any doubt as to which ones are normally involved in the vital functions of this large group of invertebrates.

Similarly, with reference to the vertebrate-type steroids in crustaceans we need to firmly establish their roles, and to determine their relative importance vis-a-vis the "native" steroids, the ecdysteroids, in the regulation of molting and reproduction. Not only are ecdysteroids the crustacean molt-promoting hormones, but there is also evidence that at least in some crustaceans, vitellogenesis is ecdysteroid-dependent.

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