CHAPTER I

INTRODUCTION

Presently, marine prawn culture has been developed to an agricultural industry in the coastal seawater of Thailand. Marine prawn, particularly *Penaeus monodon*, is one of the most importance economical species in Thailand. Clearly, prawn products earn a lot of foreign currencies over the recent years.

One of the obstacles to establish prawn aquaculture as an industry is the insufficient supply of good quality wild broodstocks, the need of broodstock culture and research is necessary. Moreover, the ability of reproduction, maturation and spawning of pond reared prawn is less than that of wild prawn. This problem needs to be solved to ensure that a complete prawn culture cycle in the captivity or impoundment is possible.

Many decapod crustaceans, control of ovarian maturation, spawning, and moulting is a major problem in developing commercial aquaculture program. Various methods have been developed to solve the problem. The methods may involve manipulation by;
1) environmental manipulation utilizing temperature, photoperiod, salinity, substrate and population density, 2) nutrition, 3) eyestalk ablation and 4) treatment with exogenous hormones

such as estrogens, progestins and ecdysone by injecting or oral feeding.

To assist in the development of breeding techniques for *Penaeus monodon*, more basic studies should be done on endocrine controling reproduction. Control of reproductive maturation in penaeid prawns could help to ensure availability of broodstock, spawners and better quality seed supply for commercial hatcheries and grow out ponds. This study will provide some basic data on ovarian development of *P. monodon*.

Moreover, biological studies show that growth, reproduction and moulting of crustacean are closely related. Most of them can mate only when a moulting takes place in the female or when the female has a soft exoskeleton, especially in the close thelycum prawn as *Penaeus monodon* (Primavera, 1985). The study will provide some basic data on moulting of *P. monodon*. The present study, hormonal manipulation by injection is developed to understand ovarian development and moulting of *Penaeus monodon*. The study is conducted under the following objectives:

1. To find effects of progesterone and β-estradiol17 on ovarian development of *Penaeus monodon*.

2. To find effects of 2-deoxyecdysone and β-ecdysone on moulting of *Penaeus monodon*.

Review of literatures

Giant tiger prawn, *Penaeus monodon* Fabricius, is one of the largest penaeid prawns in the world. It is the fastest growing of all prawns tested for culturing. The prawn can reach up to 250 g body weight in a year and is a commercial important sea food in Southeast Asian and other part of the world, particularly Japan and USA.

Mainly characteristics of *Penaeus monodon* (Figure 1) is described by Motoh (1981). The thelycum of female is closed one, that make a reproductive mating difficulty in an intermoult stage. It is clear that mating success of *Penaeus monodon* can take place only pre-moult stage of female. Boonyarath Pratoomchat (Personal recommendation, 1991) recommend that mating success in *P. monodon* can be done by artificial insemination with in 8-10 hrs after female moulting.

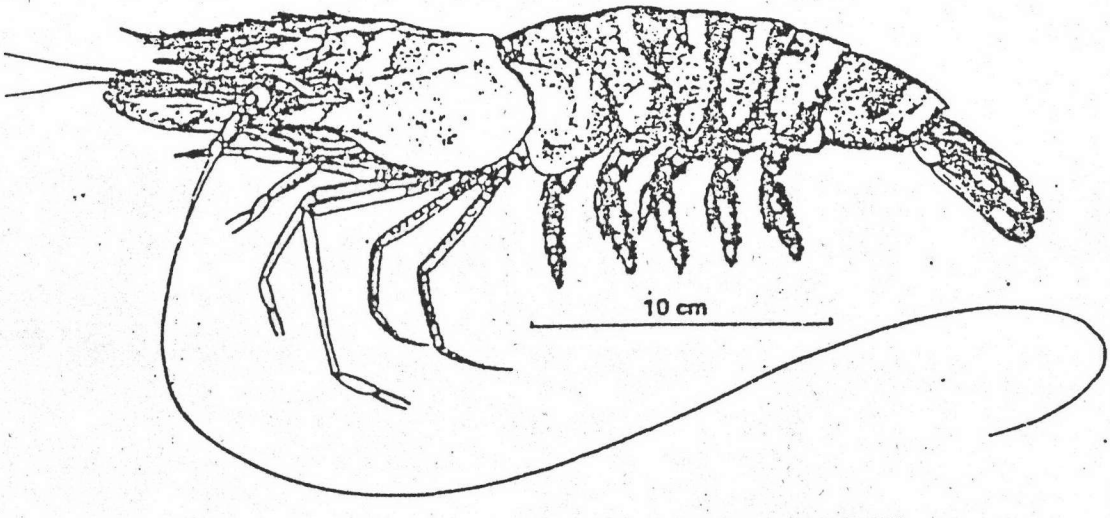

Figure 1. Adult female of the giant tiger prawn *Penaeus monodon* (Source: Motoh, 1981).

Ovary Development of *P. monodon*

Development of the ovary in *Penaeus monodon* is described by Motoh (1981), Yano (1985) and Khoo (1988) and can be divided into five stages as shown in Figure 2:

Stage 0. Invisible ovary outline through the dorsal exoskeleton.

Stage 1. Spent stage or Undeveloped stage: The ovary is transparent with no distinguishable outline. Ovary normally consist mainly of oogonia and perinucleolar oocytes.

Stage 2. Development stage: The ovary is visible as thin opaque line along the dorsal central axis. Early yolk vesicle oocytes made up most in this stage.

Stage 3. Maturing stage or Nearly ripe stage: The ovary is visible as a thicker opaque line and larger than the previous stage. At the beginning of stage 3 and late stage 2 ovary, most oocytes are in the late yolk stage.

Stage 4. Ripe stage: The ovary is turgid, broad and densely opaque. The outline is distinct. Spawning is imminent. The oocytes are in the cortical vesicle and germinal vesicle breakdown stage.

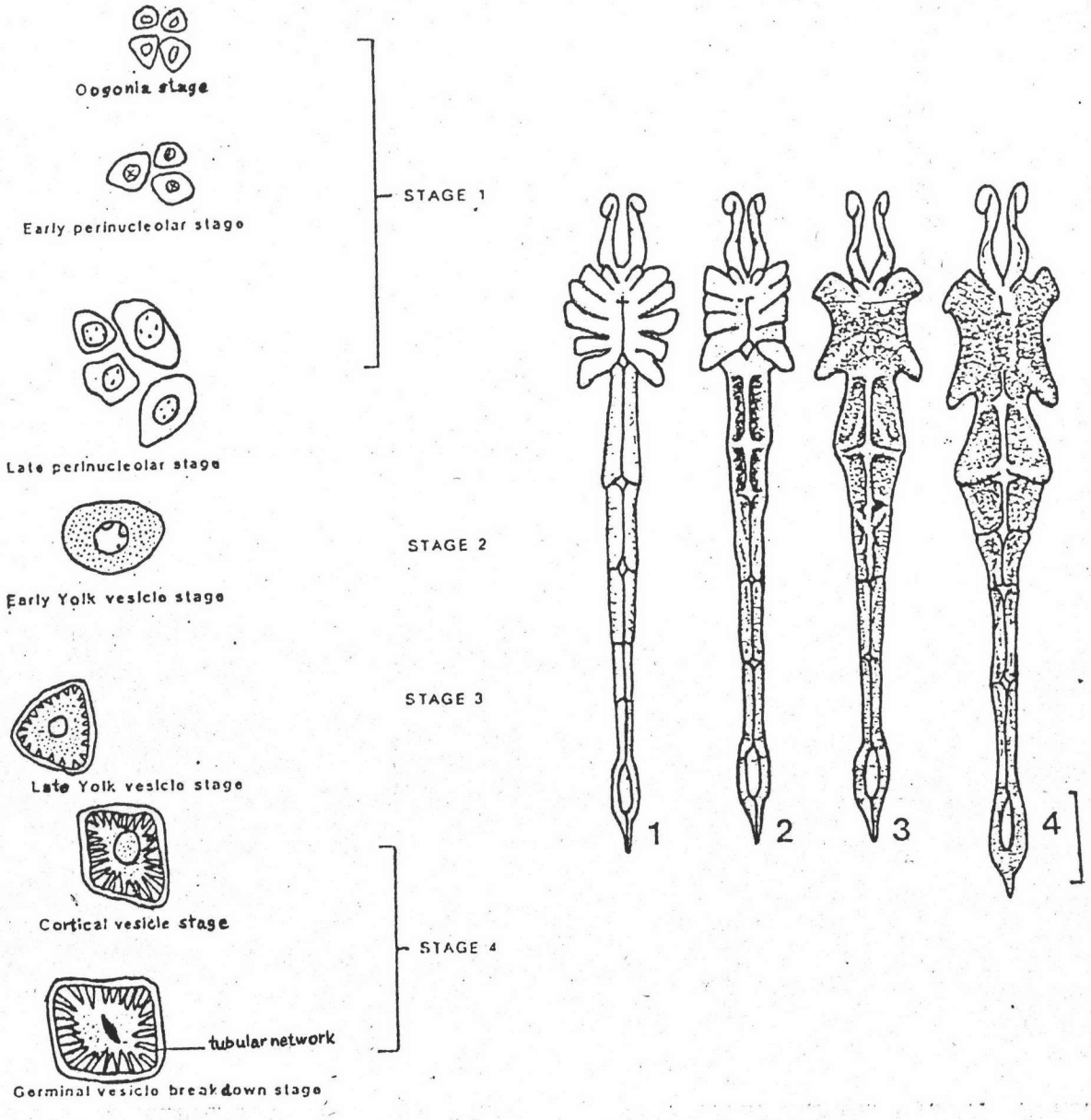

Figure 2. Diagramatic representation of oocyte development in *P. monodon*. 1, Undeveloped or spent stage; 2, developing stage; 3, nearly ripe stage and 4, ripe stage. Scale represent 20 mm (Source: Motoh, 1981; Khoo, 1988).

Vitellogenesis in crustaceans has been divided into 3 stages (Muesy and Charniaux-Cotton, 1984; Tan-Femin and Pudadera, 1989). Previtellogenesis (or stage 1) is the stage when storage of free ribosomes and development of rough endoplasmic reticulum vesicles are detected. The second stage is primary vitellogenesis (or stage 2-3), when the rough endoplasmic reticulum vesicles have granular materials inside. The third is secondary vitellogenesis (or stage 4), when uptake of vitellogenin by oocytes occurs. Vitellogenin is a lipoglycocaroteno-protein. It is immunologically identical to vitellin, the egg yolk protein (Charniaux-Cotton, 1985).

Endocrine control of ovary development

The sinus gland in the eyestalk of decapods is known as the source of the gonad inhibiting hormone (GIH). Eyestalk ablation gets rid of GIH results in precocious development of the ovary or the development of oocytes. This was demonstrated for the first time in 1943 by Panous (quoted in Fingerman, 1987). Nakamura (1988) demonstrated that the medulla externa of the eye ball of *Penaeus japonicus* plays a major role in the inhibitory machanism of ovarian development. On the other hand, gonad stimulating hormone (GSH) is found in the brain as well as in the thoracic ganglia of decapods (Otsu, 1963; Gomez, 1965; Kulkarni *et al.*, 1979). In *Uca pugilator*, Eastman-Reks and Fingerman (1984) found a relationship between the amount of GSH and the reproductive stage of female crabs.

Khoo (1988) summarized that environmental factors such as temperature, pH, light and crowding density are affected maturation and spawning in decapod crustaceans. This relation can be explained as Figure 3. Begin with environmental factors stimulating the neurosecretory centers (brain, thoracic ganglia and the X-organ-sinus gland complex as shown in Figure 4) to secrete either the GIH or the GSH. Charniaux-Cotton and Payen (1988) demonstrated that the GIH is released by the sinus glands but the brain and thoracic ganglia appear to contain a gonad stimulating substance. Presently, the target organ of GIH and GSH is unknown. However, Khoo (1988) assumed that the target organ is the ovary. Charniaux-Cotton and Payen (1988) proposed that GSH may act directly on the ovary or on an intermediate organ or both. The production and release of GIH and GSH are cyclic and antagonistic to each other. The stimulated ovary secretes a vitellogen stimulating ovarian hormone (VSOH) (Junera *et al.*, 1977). VSOH then stimulates a vitellogenin synthesizing tissue located in the ovary to synthesize and release vitellogenin (yolk protein). The vitellogenin may be rapidly transferred from the hemolymph to the oocyte surface through the follicular epithelium extracellular space and through the tubular network (Charniaux-Cotton and Payen, 1988).

In certain species of decapods, the vitellogenin is believed to be synthesized by the ovary or/and the hepatopancreas. Yano and Chinzei (1987) indicated that the ovary of *Penaeus japonicus* is the site of vitellogenin synthesis. Moreover, by using immunohistochemical techniques in the crabs, *Carcinus maenas* and

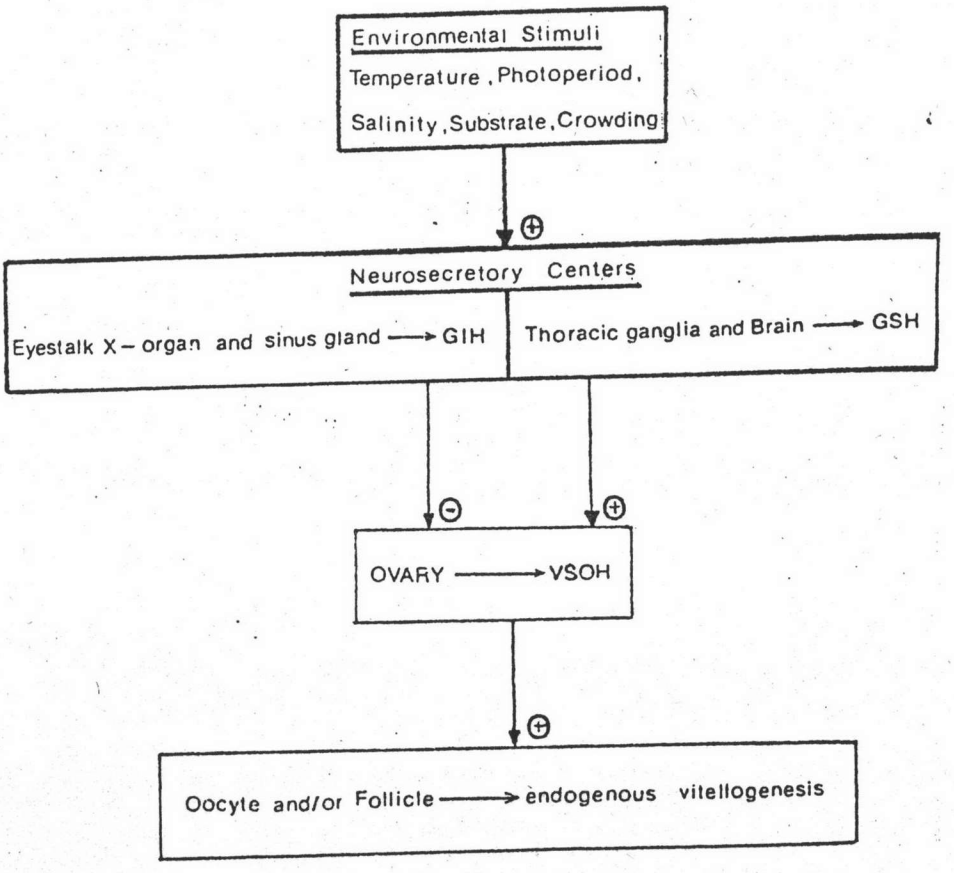

Figure 3. Diagram of hormonal control of vitellogenesis in Decapod Crustacean;

-----> demostrated evidence

\+ stimulatory

\- inhibitory

GIH Gonad inhibiting hormone

GSH Gonad stimulating hormone

VSOH Vitellogen stimulating ovarian hormone

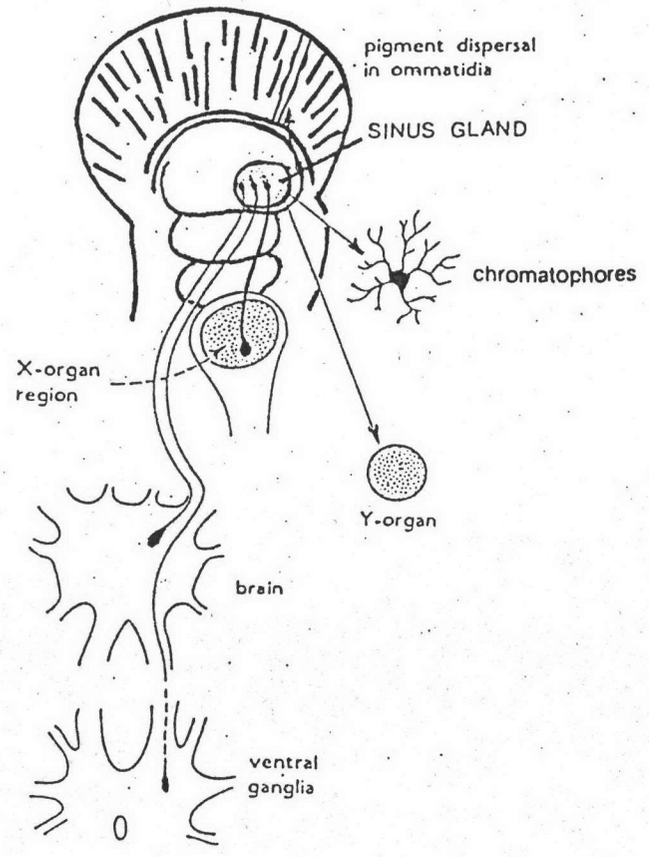

Figure 4. Diagram of neurosecretory system of decapod crustaceans (Source: Highnam and Hill, 1989).

Libinia emerginata Paulus and Laufer (1987) found that the vitellogenin localized in hepatopancreas specialized cell and designated vitellogenocyte.

Crustacean ovary is capable of producing steriods similar to sex steroid hormones of vertebrates (Burns *et al.*, 1984). Donahue (1940) found estrogens in the ovary of *Panulirus argus*. Estrone and estradiol-17β are also found in the ovaries of *Parapenaeus fisurus* (Jeng *et al.*, 1978). Khoo *et al.* (1986) measured estradiol titers in the hemolymph of *Macrobrachium lanchesteri* and found a relationship between estradiol and ovarian cycle.

Chan (unpublished, quoted in Khoo, 1988) detected significant levels of endogenous 20 α-dihydroprogesterone, dehydroepiandrosterone and progesterone, and 17α-hydroxyprogesterone in the ovary by using radioimmunoassay and high concentrations of estradiol-17β (Figure 5) in the mid-gut gland of *Penaeus japonicus*. Moreover, Chan demonstrated that the presence of enzymes are capable of transforming steroids.

There is now a substantial evidence to suggest that steriod hormones may play an important role in the regulation of vitellogenesis. Kanazawa and Teshima (1971) demonstrated that cholesterol (Figure 5) is a precursor of steroid hormone production in spiny lobster *Panulirus japonicus*. The other steroid hormone that have been reported to be involved in crustacean

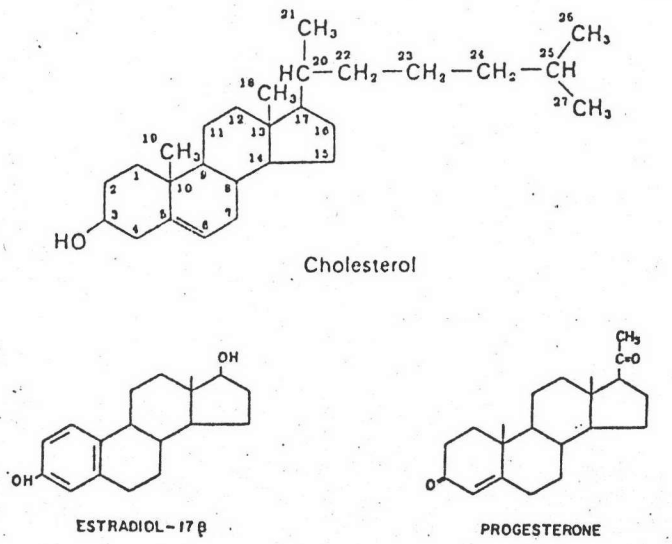

Figure 5. a) The formula for cholesterol. The carbon atoms are numbered in the standard manner (Source: Highnam and Hill, 1978). b) The formular of estradiol-17β and c) The formular of progesterone.

reproduction the progesterone groups. The ovary of *Portunus triculatus* shown a potential to convert (4^{-14C}) progesterone to 17L-hydroxyprogesterone, testosterone and deoxycorticosterone due to the presence of some specific enzymes (Teshima and Kanazawa, 1971).

Exogenous treatments with vertebrate steroid hormones have also been demonstrated to an effect on ovarian maturation in prawn. Vitellogenesis is stimulated by progesterone (Figure 5) in both of freshwater prawn *Macrobrachium kistnensis* (Sarojini *et al.*, 1985), and marine prawn *Parapenaeopsis hardwickii* (Kulkarni *et al.*, 1979). A similar response has been identified on addition of estrone and estradiol in *Macrobrachium lamerri* (Sarojini *et al.*, 1986), and 17 α-hydroxyprogesterone in marine penaeid prawns (Nagabhushanam *et al.*, 1980; Yano, 1987). It is believed to be the first criterion for oocyte development. Yano (1985) used progesterone for inducing ovarian development and spawning in *Metapenaeus ensis*.

Moulting in crustacean

Moulting cycle of crustacean, is one of the most fascinating aspects of their physiology. The life of an crustacean consists of successive periods of apparent growth when the animal escapes from its limiting, hard exoskeleton and expands a new and soft cuticle to provide room for subsequent body enlargement. Long time ago some scientists believed that moult (ecdysis) was a periodic event interrupting the normal life of the animal. Now, it is recognized that the various stages of the moulting cycle are more or less

continuous; the recovery from one moult being followed by storage of metabolic reservation and preparation for the next moult. The crustacean moult cycle can be most easily divided into four stages : premoult, moult, postmoult, and intermoult (Lockwood, 1967; Tombes, 1970).

Prior to ecdysis a new cuticle of *Penaeus* spp. is partially formed below the existing exoskeleton and the medial layers of the latter are then largely re-absorbed. The part that remains is cast off at moult and a rapid increase in body size occurs before the new cuticle is hardened (Lockwood, 1967).

It is important to understand moulting cycle stages in prawn so that a manipulation to control moulting could be done precisely. The characterization of the moulting stages of penaeid prawn had been described by Smith and Dall (1985). They catagoried moulting stages as:

Stage A Immediate postmoult can be described into; Stage A_1, begins immediately after the prawn has flicked clear of exocuticle. The cuticle is slippery and has a very soft membranous consistency. The setae and setal bases are filled with a cellular matrix and Stage A_2, the cuticle though still membranous gradually becomes firmly. The cellular matrix continues to retract from the distal end of setae as shown in Figure 6.

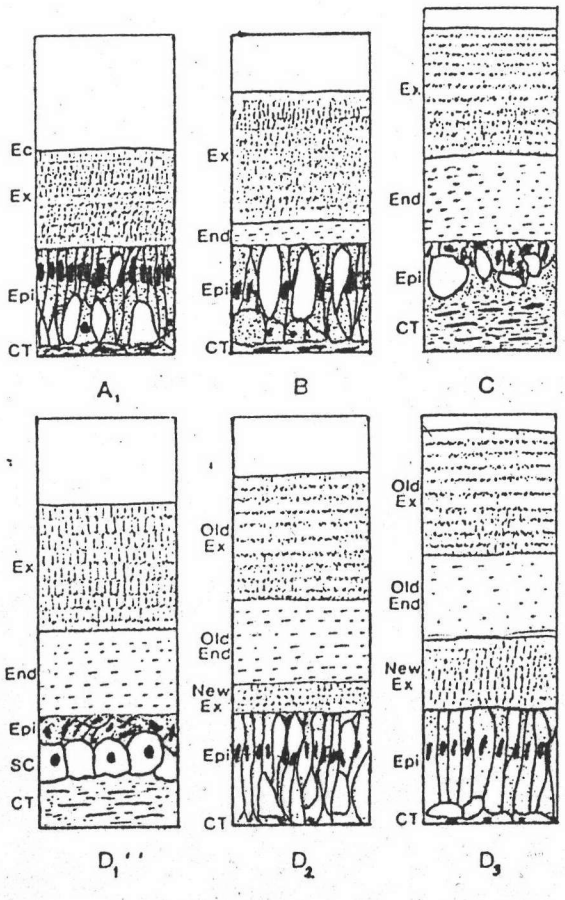

Figure 6. Semi-diagrammatic representations of sections of abdominal cuticle and epidermis. A_1, B, C, D_1'', D_2, D_3 moult stages. Ec, epicuticle; Ex, exocuticle; End, endocuticle; Epi, epidermis; CT, connective tissue; SC, storage cells (Source: Smith and Dall, 1985).

Stage B Postmoult stage, the exoskeleton which at the start has a parchment-like consistency becomes relatively hard though flexible as shown in Figure 6 and 7.

Stage C Intermoult stage begins when the secretion and hardening of the exoskeleton has been completed and may be identified by the presence of setal cones in most of the setae as shown in Figure 6 and 7.

Stage D Premoult stage can be divided into; D_0, defines as retraction of epidermis from the exoskeleton (apolysis). The retraction continues until the epidermis has withdrawn from the setal bases and forms a straight line below and parallel to the setal nodes (Figure 7, D_0). D_1, the period of development of new setae (setogenesis), this stage may be divided into three substages, which are identified by the developmental stages of the new setae. D_1', first signs of invagination of the epidermis at the site of new setae will develop. D_1'', the invagination of the epidermis continues to increased. New setal tips are generally visible (Figure 7, D_1''). D_1''', the period with setae reach maximum invagination and continue to develop. The scalloping of the epidermal edge reaches a maximum and has a uniform undulating appearance. D_2, the stage may be identified by the change in the scalloped appearance of the epidermal edge where the setal shafts disrupt the smooth curves of the scalloping (Figure 7, D_2). D_3, the period of maximum reabsorption of components of the old exoskeleton. It may be recognized initially by the

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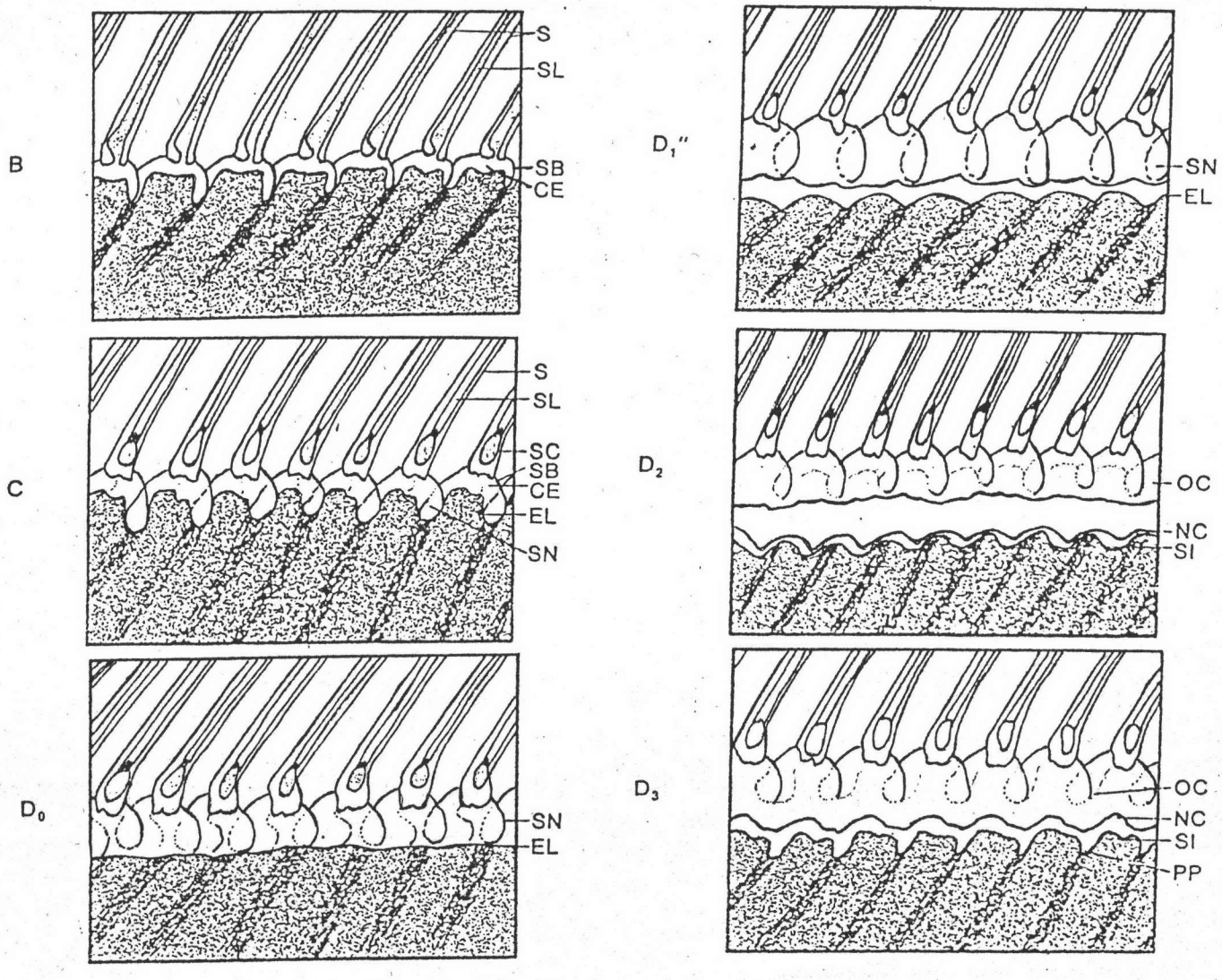


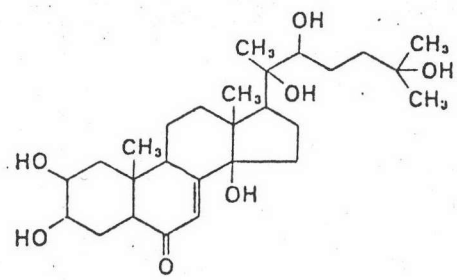
Figure 7. The line drawing of the uropod edge are tracings of the photographs. B, C, D_0, D_1", D_3 moult stage. S, setal shafts; SL, setal lumen; SB, setal base; SC, setal cone; CE, clear cuticular edge of uropod; EL, epidermal line; OC, old cuticle; NC, new cuticle; SI, setal invagination which everts to form seta ecdysis; PP, area visible as pinpoints of light in this stage (Source: Smith and Dall, 1985)

increased distinction of the new setae nodes between the bases of the new setae in the uropod (Figure 7, D_3), and D_4, this period the prawn looses itself within the old exoskeleton and the ecdysial sutures open. The old exoskeleton has become so delicate. It may be broken by lightly rubbing.

Stage E ecdysis, this is the process during which the prawn extracts itself from the old exoskeleton and averts the setae on the new exoskeleton.

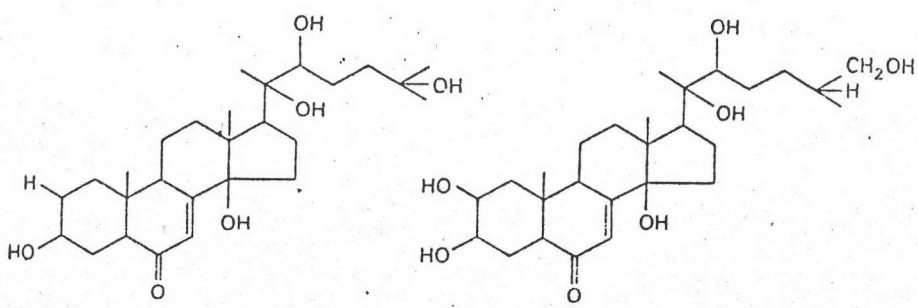
Crustacean moulting hormones

A crustacean steroid hormone, extracted from crayfish homogenate had been isolated and identified, called **crustecdysone** (Highnam and Hill, 1978). The structure of this steroid hormone is as **β-ecdysone (20-hydroxyecdysone, ecdysterone)** shown in Figure 8. A second ecdysone has been isolated in marine crayfish, *Jasus lalendei*, in which the hydroxyl group on C-2 is replaced by hydrogen. The compound is therefore called **2-deoxycrustecdysone** (Figure 9a). In the crab, *Callinectes sapidus* two ecdysones in addition to crustecdysone have been identified. One called **callinecdysone A** (Figure 9b), is an isomer of crustecdysone with a hydroxyl group on C-27 instead of C-25 and is probably identical with the plant steroid **inokosterone** and the other **callinecdysone B** (Figure 9c), has an additional methyl group on C-24 compared with crustecdysone and thus resembles the plant steroid **makisterone A** extracted from *Padocarpus macrophyllus* (Tombes, 1970; Highnam and



20-hydroxyecdysone

Figure 8. The formula for 20-hydroxyecdysone. Note the additional -OH group on carbon atom 20 (Source: Highnam and Hill, 1978).



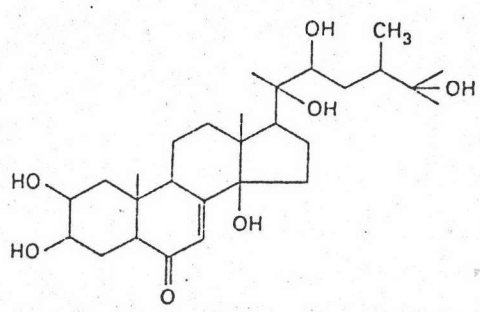


Figure 9. Moulting hormones from crustaceans, additional to crustecdysone. (a) 2-deoxycrustecdysone from *Jasus lalendei*, possibly a deactivation product of crustecdysone. (b) callinecdysone A, and (c) callinecdysone B, both from *Callinectes sapidus*. (Source: Highnam and Hill, 1978).

Hill, 1978).

Endocrine control of moulting in Crustacean

Endocrine control of moulting was not demonstrated until after the basic endocrine mechanism of chromatophore regulation is understood. This coincided with the discovery of the eyestalk as an endocrine organ. Since that time substantial evidence has led to the hypothesis that in adult malacostracans moulting is under the control of at least two hormones; a moult-inhibiting hormone (MIH) produced by the X-organ-sinus gland complex and a moulting hormone (MH) secreted by the Y-organ or moulting gland (Costlow, 1968). The control of ecdysone production in the Y-organ is a two-step hormonal sequence (Highnam and Hill, 1978). It is stimulated by the decreasing secretion of the X-organ-sinus gland complex. The latter is stimulated by other factors like environmental conditions passing through neurosecretory centers and nerve cell ending at the eyestalk area. Then the Y-organ synthesize and release moulting hormone which control the epidermal cell's activity (Tombes, 1970) as shown in Figure 10.

An endocrine demonstration has been repeated often on many different crustaceans but Passano (1961) primarily studied on crabs crabs and found moulting induction by the removal of both eyestalks and then inhibit moulting by the injection of eyestalk extracts into the body. The Y-organs are absent when the eyestalks are removed, moulting does not occur, but if the Y-organ are replaced in

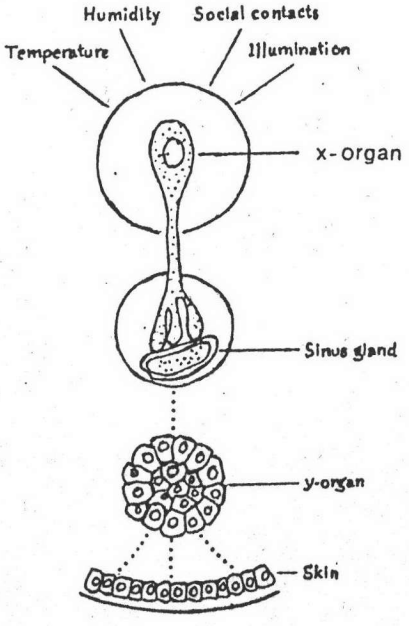


Figure 10. Moulting in Crustacea is under a second-order neuroendocrine mechanism with a neurosecretory moult inhibiting hormone (MIH) and moulting hormones (MH) (Source: Tombes, 1970).

organisms without eyestalks, the moulting processes will be initiated. This evidence suggests that the Y-organ through the production of the MH, plays as a principal role in moulting but is often inhibited by the neurosecretory MIH.

Hormonal effect of moulting has been demonstrated by Putth Songsangjinda and Sumeth Chaiwatharagoon (1988) in *P. merguiensis* with an exogenous injection of **20-hydroxyecdysone** and found a reduction of moulting duration in hormonal treated group. Pornsilp Pholpunthin *et al.* (1987) also found a same result of B-ecdysterone in *Penaeus monodon* and *Penaeus merguiensis*. Clearly, hormonal control plays an important role of moulting in crustaceans and *Penaeus spp.* However, there is not and study of the effect of moulting hormone on different stage of moult. The present study will emphasize in this aspect on *P. monodon* to understand the effect of moulting hormone on moulting cycle.