THE EFFECTS OF JUVENILE HORMONE ON THE EARLY EMBRYONIC DEVELOPMENT OF THE FLESHFLY, SARCOPHAGA BULLATA.

by

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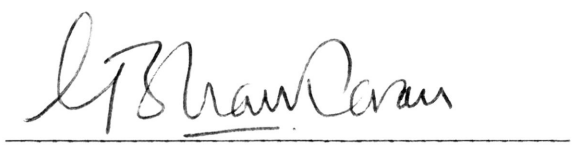

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ABSTRACT

Juvenile hormone (JH), a sesquiterpene compound, acts as a morphogenetic hormone in insects; JH permits expression of the larval genome and inhibits expression of the adult genome. Previous experiments have indicated that development is arrested prior to blastoderm formation in insect eggs treated with JH during the early stages of embryonic development. In the present study, the effects of a highly active JH analog, methoprene, were tested on the eggs of the fleshfly, Sarcophaga bullata (Diptera).

Preliminary studies were performed to establish the membrane permeability of S. bullata eggs to the RNA inhibitors 1% benzamide and actinomycin D. Development of newly fertilized eggs treated with the RNA inhibitors was arrested at the blastoderm stage, indicating that the membrane is permeable. Subsequently, development of eggs treated with 35 µg methoprene/µl acetone was also arrested suggesting that the membrane is permeable to methoprene. A volume of 2µl-35µg methoprene/µl acetone per group of 10 eggs represented the critical concentration. The critical time of methoprene effects on embroyonic development appeared to be between 0 hours, ie. fertilization, and 4 hours. These results will be discussed in relation to current hypotheses on JH action.

DEDICATION

This paper is dedicated to my parents, Jack and Gladys Baker, without whose unselfish love, total devotion, and unerring guidance I would have never obtained my present level of education and understanding of life--I owe them more than ever could be repaid in full.

ACKNOWLEDGMENTS

I would like to express my gratitude to the researchers and the technicians of the Institute of Developmental Biology. Both Dr. Bhaskaran and Dr. Dahm command my greatest respect and my sincere appreciation for the valuable time and patience they expended while introducing me to various aspects of insect endocrinology research and while helping me to solve technical problems. I would like to thank Vicki Riddle for her valuable assistance in maintaining the fly cultures and Karen Kuers for her assistance in the preliminary phases of this investigation. Finally I have the greatest regard and highest respect for those graduate students who willingly offered both their time and their support throughout this research effort.

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INTRODUCTION

Wigglesworth (1935, 1936) first discovered that the corpus allatum of insects secretes a hormone, juvenile hormone (JH), which determines the nature of the molt during post-embryonic development. JH was determined to exert a morphogenetic effect; JH determines the nature of the insect molt depending on the JH titer in the hemolymph. Many papers have been published since then which describe the various effects of JH and JH analogs on the most diverse structures and functions of the insect organism and on the various biochemical processes, and which describe the chemical structure of the hormone itself. In fact, considering the insect organism as a whole, not one structure, function, chemical process, or component has been found to be independent of JH (Novak, 1975). However, despite extensive research, the mechanism of JH action remains unknown. Several hypotheses have been proposed to explain the morphogenetic effects of JH. The three stages in the development of a holometabolous insect (eg. S. bullata) may be diagrammed as follows (Willis, 1969):

XABCD/XEFGHI/XJKLMNO
Larva Pupa Adult

It has always been assumed that these three stages share some specific synthetic activies, the Xs in the diagram, and that each stage has some

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The style and format of this paper follow that of Developmental Biology.

unique activities, the other letters. Wigglesworth (1940) proposed that JH acts as a juvenilizing agent, ie. it "activates" the larval genome. In this diagram, a pupa treated with JH would be expected to perform larval syntheses (XABCD). However, only a few cases of "reverse metamorphosis" have been demonstrated (Willis, 1974), and Wigglesworth attributes this failure to developmental "inertia" (Wigglesworth, 1976).

Williams (1953) has described the action of JH as "status quo"; JH prevents the onset of new sequences of syntheses and favors the repetition of the region-specific temporal patterns of syntheses used in the previous stage. With regard to the status quo hypothesis, a pupa treated with JH would repeat pupal syntheses (XEFGHI), precisely the events one observes (Williams, 1961). Expanding upon Williams' hypothesis, Willis proposes that JH prevents cells from progressing in their developmental program by preventing the increase in polyamine levels which are necesary for reprogramming chromatin (Willis 1981a, 1981b).

The hypotheses proposed by Wigglesworth, Williams, and Willis are based on studies performed on insects in post-embryonic stages of development; however, any hypothesis for the mechanism of JH action must account for observations at all levels of embryonic development, including early embryonic development. In the present study, I propose to test the Willis hypothesis using the embryonic system; it is hoped that the results of this study will provide critical evidence either supporting or refuting this hypothesis.

LITERATURE REVIEW

The relative titers of ecdysone and JH in the insect's hemolymph determine the morphogenetic changes during insect metamorphosis. The neurosecretory cells (NSC) of the brain stimulate the production of both homones. In particular, during ecdysone production, the lateral NSC in the brain release prothoracicotropic hormone (PTTH), which is discharged into the corpora cardiaca (CC) or the corpora cardiaca/ corpora allata (CC/CA) for storage and subsequent release into the hemolymph. PTTH released from the CC or the CC/CA stimulates the prothoracic glands to produce ecdysone; ecdysone initiates all molts and causes body tissues to differentiate into adult structures. On the other hand, during JH production, allatotropins released by the medial NSC stimulate the CA to produce JH. Whereas, ecdysone initiates the epidermal changes required for actual molting, the JH titer determines the nature of the ensuing molt. Thus high JH titers will result in the retention of larval characteristics, low JH titers will result in pupal transformation, and an absence of JH will result in adult formation (Schneiderman, 1961). The JH titers as effecting morphogentic action are presented diagrammatically in Figure 1.

In order to understand the effects of JH on early embryonic development, one must first understand normal embryonic development. Genetic research indicates that all cells of an organism contain the full genetic compliment characteristic of that organism; however, in each cell type only a specific battery of genes are active. This process of cell differentation occurs only after fertilization and then only

after a period of repeated cell divisions in the embryo. Thus the cleavage nuclei located in the yolk mass during the early embryonic states of normal insect development are totipotent, ie. are not programmed for a specific sequence of syntheses.

It is believed that activation of specific gene loci in specific groups of blastodermal cells results from the interaction of the genome with cytoplasmic determinants. The existence of cytoplasmic determinants has been based on partial ultra-violet (u.v.) irradiation experiments in Drosophila. Bownes and Kalthoff (1974) demonstrated that u.v.-irradition of specific regions of the early Drosophila embryos produced defective embryos, the defect corresponding to the initial site of irradiation. Moreover, experiments performed by Bownes and Sanders (1976) indicated that u.v.-irradiation of the anterior pole of Drosophila eggs during the nuclear multiplication stage produced embroys with defective anterior structures; they concluded that the u.v. light must damage some factors at the anterior portion of the egg which are essential for the subsequent interactions needed to form the anterior portion of the embryo. Furthermore, the general belief that the cleavage nuclei are not activated until the blastoderm stage has been supported by the work of Riddiford and others. In experiments conducted by Riddiford (1971), evidence indicated that the mRNA provided by the maternal genome during oogenesis was sufficient for development until the blastema stage. Therefore, the transition from the blastema stage to gastrulation signals the synthesis of new RNA. In addition, nucleoli, structures which synthesize rRNA, do not appear in the cleavage nuclei until blastoderm formation. Consequently, inhibition of

new RNA synthesis results in developmental arrest.

JH, as well as JH analogs and numerous other substances have been shown to produce ovicidal effects if applied in sufficient concentrations before the blastema stage, possibly by either directly or indirectly inhibiting new RNA synthesis (Riddiford, 1969; Sivasubramanian, 1979a, 1979b). Experiments conducted by Riddiford (1971) indicated that the earlier the exposure of the eggs to JH, the sooner abnoramalities were encountered in subsequent development. For example, when Cecropia eggs were treated with JH immediately after oviposition, development was blocked at the blastoderm stage (Riddiford and Williams, 1967; Riddiford, 1970b; Riddiford, 1971). However, once the eggs were fertilized and oviposited, JH no longer blocked development at the blastoderm stage, but only later at blastokinesis, a series of morphogenetic movements occurring midway through embryonic development and signaling the beginning of the first instar of larval differentiation. When JH was applied to Cecropia eggs before blastokinesis, the organisms which hatched showed delayed effects during larval life. When JH was applied after blastokinesis, most individuals hatched, larval life proceeded normally, but metamorphosis was blocked. Thus, the relationship observed between the time of JH application and the stages at which development is arrested indicates the existence of specific hormonal effects rather than non-specific pharmacological effects (Sivasubramanian, 1979). Hence it is likely that at the blastema stage, a stable alteration in the nuclei, induced through the mediation of a cytoplasmic determinant, results in primary differentiation of the cleavage nuclei (Bhaskaran, Ramakrishnan,

and Adeesan, 1970), and that any interference with this alteration results in a disruption of embryogenesis.

Presently three hypotheses have been proposed to explain the mechanism of JH's "anti-metamorphic" action. Wigglesworth (1940b) proposed that JH acts specifically as a juvenilizing agent, ie. it "activates" the larval genome. Evidence supporting this hypothesis came from Piepho's classic experiment in which he demonstrated that adult cuticle transplanted to a larva which was subsequently induced to molt, formed a new layer of pupal cuticle. This experiment demonstrated the regression of adult cuticle to pupal cuticle to larval cuticle under the influence of JH; JH activated the larval genome (Wigglesworth, 1970). However, only a few such cases of "reverse metamorphosis" have been described, and Wigglesworth (1976) attributes these failures to developmental "inertia."

Williams (1953) has described the action of JH as "status quo"; JH prevents the onset of new sequences of syntheses and favors the repetition of the region-specific temporal patterns of syntheses used in the previous stage. Virtually everything known about JH suggested that it was involved in gene-switching on a massive scale, such as would be required for the activation of one gene-switch and the inactivation of another; in short, JH controls sequential polymorphism (Wigglesworth, 1970; Ashbruner, 1970). To provide a mechanism for this massive gene switching, Williams and Kafatos (1971) suggest that three gene-sets, larval, pupal, and adult, are distinguishable by information encoded in the genome and, moreover, that members of each set are identifiable in terms of certain nucleotide sequences

which serve as "promoters." Thus, Williams and Kafatos proposed a model of JH action in which each of the three gene-sets is presumed to be under the control of a certain "master regulatory gene" called MG_L, MG_P, MG_A respectively, as diagrammed in Figure 2.

Recently, Willis has expanded upon the Williams hypothesis and has proposed a new hypothesis for the mechanism of JH action. According to Willis (1981b), JH prevents cells from progressing in their developmental program by preventing the increase in polyamine levels which are necessary for reprogramming chromatin. Her hypothesis is based upon evidence from both in vivo and in vitro experiments on a variety of systems; this evidence may be summarized as follows:

- polyamines facilitate nuclear reprogramming (in frogs)
- polyamines facilitate dissociation of HMG proteins from chromatin (in Tetrahymena)
- cellular levels of polyamines increase with increased ornithine decaroxylase (ODC) activity, the rate-limiting enzyme in polyamine synthesis (in Cecropia)
- ecdysterone increases ODC activity (in Cecropia tissues)
- JH prevents the induction of ODC (in bovine lymphocytes)

By this hypothesis and the supporting experimental evidence, polyamines are considered to be necessary to permit changes in the state of the chromatin changes necessary for cells to undertake new patterns of syntheses. Furthermore, the hypothesis is in accord with two significant characteristics of JH action; namely, the long critical period for JH action and the failure of JH to act in an all-or-none manner at the level of the single cell. However, Willis has failed to obtain any experimental evidence in support of her hypothesis; therefore,

conformation of this hypothesis awaits critical experimentation on embryomic and post-embryonic stages of development in different species of insects.

MATERIALS AND METHODS

Culture Maintenance

Newly emerged S. bullata adults were placed in plastic containers containing fresh liver, sugar cubes, and a water supply. These flies were reared in an incubator maintained at 28°C, R.H. 60%, and a 16:8 light:dark cycle. Fresh liver was placed in the cage daily to insure that the adult females received the necessary proteins and amino acids required for normal egg maturation.

Egg Collection

After rearing the flies on fresh liver and under the aforementioned conditions for four days, adult females were checked for oviposition either approximately 2 hours after the beginning of the light phase of the light:dark cycle or 4-8 hours prior to the dark phase of the light:dark cycle, corresponding to either early morning or early evening under natural environmental conditions, respectively. For the critical concentration and the critical time investigations discussed below, treatments required more precisely timed eggs, ie. determination of exact time of oviposition. Hence, adult females were checked at hourly intervals from the beginning of the light phase on the fourth day until 4 hours later, and from 8 hours prior to the dark phase until the dark phase began.

Actual examination of the adult females involved anesthetizing the culture with either carbon dioxide or ether; subsequently, refrigeration at 2°C for 10 minutes was used since it slowed

the movement of the flies significantly and lacked the longer lasting anesthetizing effects of carbon dioxide or ether. Thus, refrigeration provided a means for examining the culture repeatedly without introducing factors which might have been harmful to egg viability. After anesthetizing the culture, the lower portion of the female's abdomen adjacent to the ovipositor was depressed with forceps. Any eggs emerging from the ovipositor indicated that oviposition had occurred, and the ovipositor was clipped to aid in the explusion of the eggs from the uterus. The collected eggs were placed in a drop of distilled water (d H_2O) in order to prevent desiccation prior to treatment. The adult females which had not oviposited eggs were returned to the incubator and were retested for oviposition at a later time.

Early Embryonic Development

Newly fertilized eggs were divided into six groups and each group was suspended in a drop of d H_2O. The groups were then incubated under the same conditions as the adults for various time intervals. A wet mount of the newly fertilized eggs, designated 0 HR., was prepared and lightly squashed; then, the eggs were viewed under phase contrast. The condition of the egg yolk and the presence or absence of any nuclei were noted. The remaining groups, designated 2 HR., 4 HR., 6 HR., 8 HR., 10 HR., were incubated for the indicated time and subsequently examined as the 0HR. group.

Membrane Permeability

Newly fertilized eggs were collected, divided into seven groups, and placed on a piece of black electrical tape in a small petri dish. Beneath the piece of tape was a Kimwipe moistened with d H_2O, the moisture providing the required humidity. The groups of eggs were treated topically as follows:

1. 0.1 ml d H_2O
2. 0.1 ml Ringer's solution
3. 2 µl acetone
4. 2 µl-35µg olive oil/µl acetone
5. 0.1 ml 1% benzamide solution
6. 2 µl actinomycin D
7. 2 µl-35µg methoprene/µl acetone

Each group of eggs was then incubated under the same conditions as the adults for 48 hours. At the end of the incubation period, the eggs were scored as developed or undeveloped, developed implying the appearance of any structure leading toward a more advanced form. This method of incubation, however, resulted in numerous desiccated treatment groups, particularly those groups treated with acetone, actinomycin D, and methoprene. Thereafter, the eggs were placed directly on Kimwipes moistened with d H_2O, and the groups of eggs were treated by topical application of the above substances.

Critical Concentration

Newly fertilized eggs were collected and divided into seven equal groups. Each group of eggs was placed directly on a Kimwipe moistened with d H_2O and treated topically with 2 µl of the following methoprene in acetone solutions:

1. 0.05 µg/µl
2. 0.5 µg/µl
3. 5.0 µg/µl
4. 12.5 µg/µl
5. 20.0 µg/µl
6. 35.0 µg/µl

In addition, a control group, the group not treated with methoprene, was incubated with the treated groups to insure that the eggs used had been fertilized and were viable prior to treatment. Incubation conditions were the same as for adults, and the groups were examined 48 hours later. At the end of the incubation period, the eggs were scored as developed or undeveloped, the lowest concentration showing 100% inhibition development representing the critical concentration.

Critical Time

Newly fertilized eggs were collected and divided into seven equal groups. Each group of eggs was placed directly on a Kimwipe moistened with d H_2O and treated topically with 2 µl - 35µg methoprene/ µl acetone at the following time intervals:

1. < 2 HR.
2. 2 HR.
3. 4 HR.
4. 6 HR.
5. 8 HR.
6. 10 HR.

In addition, a control group, the group not treated with methoprene, was incubated with the treated groups to insure that the eggs used had been fertilized and were viable prior to treatment. Incubation conditions were the same as for adults, and the groups were examined 48 hours later. At the end of the incubation period, the eggs were scored as developed or undeveloped.

Data Calculation

All calculations of percentage inhibition were determined by a modified Abbott's formula (Babu and Panwar, 1973):

Percentage inhibition =

$$\frac{\text{percent inhibition in treatment - percent inhibition in control}}{\text{100\% - percent inhibition in control}}$$

d H_2O was used as the control in all calculations.

RESULTS

Early Embryonic Development

The eggs of S. bullata are approximately 2 mm long and 1 mm wide, elliptical, and densely pearly white to yellow. The elongate egg is tapered anteriorly and blunt posteriorly; no appendages are present and a chorion and an inner vitelline membrane encase the yolk mass. A thin layer of cortical cytoplasm separates the yolk mass from the vitelline membrane.

Upon maturation the eggs are released from the ovary and move down the uterine tube at which time sperms stored in the spermatheca fertilize the migrating eggs. Subsequently, the eggs are oviposited in the uterus, and cleavage begins at a rapid rate. Cleavage nuclei are concentrated in the anterior third of the egg initially; however, as cleavage progresses the nuclei migrate posteriorly and laterally, becoming evenly distributed throughout the yolk mass. Concomitant with early cleavage, small dark polar granules begin to appear in the periplasm at the posterior pole.

The cleavage nuclei enter the cortical cytoplasm approximately 8 hours after oviposition, thereby signaling the blastema stage. The nuclei of this state characteristically contain no nucleoli and have not been delineated by cell boundaries. Soon thereafter, cell boundaries begin to form and nucleoli began to appear, and approximately 10 hours after oviposition blastoderm formation is complete. The egg then undergoes gastrulation, organogensis and larval

formation.

Membrane Permeability

Experimental evidence indicated that the embryonic membranes of S. bullata eggs are permeable to small molecular weight molecules, as well as lipophilic molecules. The control groups, ie. those groups treated with 0.1 Ringer's solution, 2 µl acetone, and 2 µl-35 µg olive oil/µl acetone exhibited 0.51%, 0.00%, and 5.17% inhibition, respectively. Thus, topical application of non-inhibiting solutions did not inhibit normal embryonic development significantly. However, treatment with 0.1 ml 1% benzamide solution, 2 µl actinomycin-D, and 2 µl-35 µg methoprene/µl acetone arrested normal embryonic development, these groups exhibiting 100.00%, 92.71, and 96.36% inhibition respectively.

Both benzamide and actinomycin D, which are RNA inhibitors, must enter the nucleus in order to inhibit development; therefore, it appears that the S. bullata egg is permeable to small molecules, including methoprene. The small percentage development observed with actinomycin D and 35 µg methoprecne/µl acetone may be attributed to experimental variation and the age of the eggs at the time of treatment. (See Table 1.)

Critical Concentration

Although 2 µl-35 µg methoprene/µl acetone appeared to represent the critical concentration, these results may be considered inconclusive. This concentration is much higher than those reported by

researchers using similar juvenile hormone analogs; however, S. bullata eggs were not dechorionated or permeabilized as the eggs used in other experiments. Dechorionation and permeabilization was unnecessary with S. bullata, though, since the membrane permeability investigations established that the embryonic membranes are in fact permeable. Due to a time factor, the eggs were not always collected immediately after oviposition; therefore, age at time of treatment must be of critical imporance, the more advanced embryos requiring higher concentrations of JH analog to inhibit development. (See Table 2.)

Critical Time

S. bullata eggs treated at various times after oviposition showed no clear indication of a critical time for the methoprene effects; therefore these results may be considered inconclusive. Preliminary experiments indicate that eggs treated 2 hours after oviposition are completely inhibited by methoprene; whereas, eggs treated at intervals prior to and after the 2 HR. interval show only a small decrease in percentage inhibition. Such a small decrease in the percentage inhibition might be attributed to experimental variation and the age of the eggs at the time of development. However, in order to establish with certainty the critical time of methoprene effects on embryonic development, if any critical time in fact exists, the experiments must be repeated with more precisely timed eggs. (See Table 3).

DISCUSSION

Since this investigation proposed to study the effects of methoprene on early embryonic development, a fly system having a considerable pre-gastrulation developmental period was desired. Blastoderm formation is complete 3.0-3.5 hours after fertilization in D. melanogaster and 2 hours after fertilization in M. domestica; however, blastoderm formation in S. bullata requires 10 hours. Thus by using S. bullata, treatment may be initiated at a particular time ± 30 minutes without introducing significant error; whereas, in D. melanogaster and M. domestica initiating treatment at a particular time ± 30 minutes will result in considerable error since many critical events in embryonic development may occur during this time interval.

Furthermore, the membrane permeability of S. bullata eggs provides another advantage favoring its use over the relatively impermeable membranes of other fly eggs, notably D. melanogaster and M. domestica. Although in many investigations JH and JH analogs have been applied topically to adult female insects (Matolin, 1970; Riddiford, 1971; Enslee and Riddiford, 1977; Sivasubramanian, 1979a, 1979b), thereby preventing embryonic development at the blastoderm stage, very few investigations have reported the same results when treating the eggs topically without dechorionating or permeabilizing the eggs initially. In particular, Limbourg and Zalokar (1973) developed a permeabilizing technique for D. melanogaster in which the eggs were mechanically dechorionated and subsequently permeabilized by immersion in n-octane. Arking and Parente (1980) modified this

technique by dechorionating the eggs by immersion in sodium hypochlorite rather than by mechanical methods. These dechorionating and permeabilizing techniques subject the eggs to harsh chemicals and subsequently damage or remove the egg's protective barrier against the external environment; these techniques may reduce the viability of the eggs, thereby producing "artifactual" results.

The membrane permeability of S. bullata eggs will facilitate future experiments in which the eggs may be incubated with substances other than RNA inhibitors. Since the membrane is permeable to RNA inhibitors it will probably be permeable to other small molecules such as radiolabeled amino acids and nucleic acid precursors. This will permit the study of macromolecular changes in eggs treated with JH.

Treatment of S. bullata eggs with methoprene during early embryonic development, usually immediately after oviposition, arrests development at the blastoderm stage. Thus, it appears that the early stages of embryonic development in S. bullata are quite sensitive to methoprene. The critical period during which the developing egg of S. bullata is most sensitive to JH or JH analogs needs to be determined.

Although the studies on critical concentration have not produced conclusive results, the results obtained thus far are encouraging. The relatively high concentrations needed to inhibit development might have been due to the presence of the chorion. Therefore, further experiments must be performed on dechorionated eggs before any conclusive statement regarding the critical concentration of methoprene and the critical time of methoprene effects can be made. Future studies will

include investigations on the effects of methoprene on ODC activity and polyamine levels in order to obtain evidence which will substantiate or refute Willis' hypothesis. However, Heby (1981) has indicated that polyamines have a major role in the control of cell proliferation and differentiation. Moreover, Herbst and Dion (1970) have shown in Drosophila that polyamine levels increase significantly concomitant with mRNA synthesis and protein synthesis, both processes requiring gene activation and possibly gene reprogramming. Furthermore, Emanuelsson and Heby (1977) demonstrated that eggs of Ophoyotrocha labronica treated with α-methylornithine, a putrescine synthesis inhibitor, do not develop past gastrulation. Therefore, preliminary evidence suggests that the polyamines levels, and in particular ODC activity, represent critical factors in gene reprogramming and subsequent development.

CONCLUSIONS

Upon examining the experimental evidence the following conclusions have been established:

1. The embryonic membranes of newly fertilized eggs of S. bullata are permeable to small molecular weight substances, as well as lipophilic molecules.

2. Methoprene, a JH analog, inhibits the early embryonic development of S. bullata.

3. Eggs of S. bullata are sensitive to methoprene until the blastoderm stage.

Treatment	µl/ 10 eggs	Reductions	# Treated	# Developed	# Not Developed	% Inhibition
Ringer's Solution	100	11	163	138	25	0.51
Acetone	2	14	196	169	27	0.00
35 µl/µl olive oil in acetone	2	13	192	155	37	5.17
1% Benzamide solution	100	8	114	0	114	100.00
Actinomycin-D	2	7	97	6	91	92.71
35 µl/µl methoprene in acetone	2	13	160	5	155	96.36

Table 1. Permeability of embryonic membranes of newly fertilized S. bullata eggs.

[Methoprene] µg/µl	µl/10 eggs	Replications	# Treated	# Developed	# Not Developed	% Inhibition
0.05	2	6	63	44	19	26.28
0.5	2	2	23	19	4	12.80
5.0	2	5	53	46	7	8.39
12.5	2	6	62	52	10	11.47
20.0	2	6	63	52	11	12.88
35.0	2	9	160	5	155	96.70

Table 2. Critical concentration of methoprene on S. bullata eggs.

Time Interval	[Methoprene] µg/µl	µl/10 eggs	Replications	# Treated	# Developed	# Not Developed	% Inhibition
< 2 HR.	35	2	7	67	5	62	92.12
2 HR.	35	2	7	55	0	55	100.00
4 HR.	35	2	7	61	9	52	84.43
6 HR.	35	2	7	60	10	50	82.41
8 HR.	35	2	7	60	15	45	73.61
10 HR.	35	2	7	63	12	51	79.89

Table 3. Critical time of methoprene effects on S. bullata eggs.

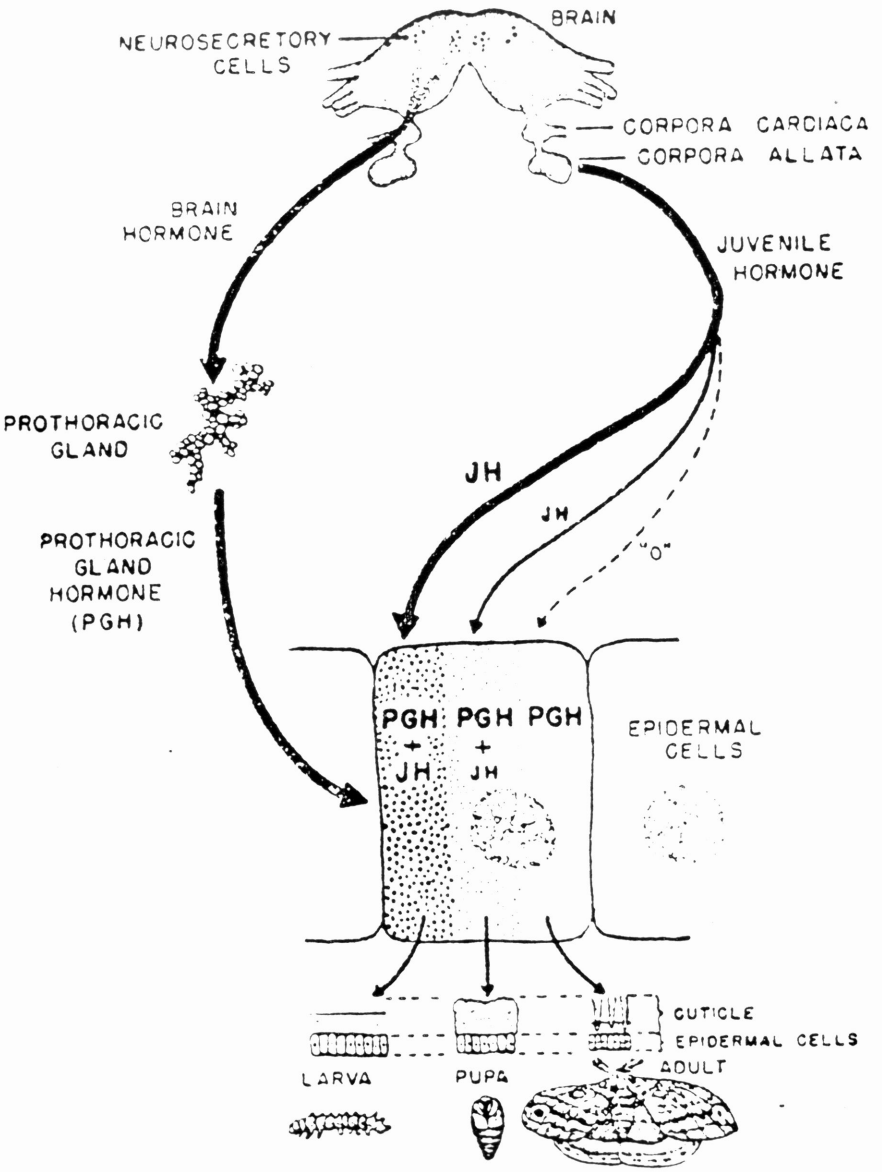


Figure 1. Schematic diagram of the principal endocrine organs of an insect and the mode of action of their hormones (Schneiderman, 1961).

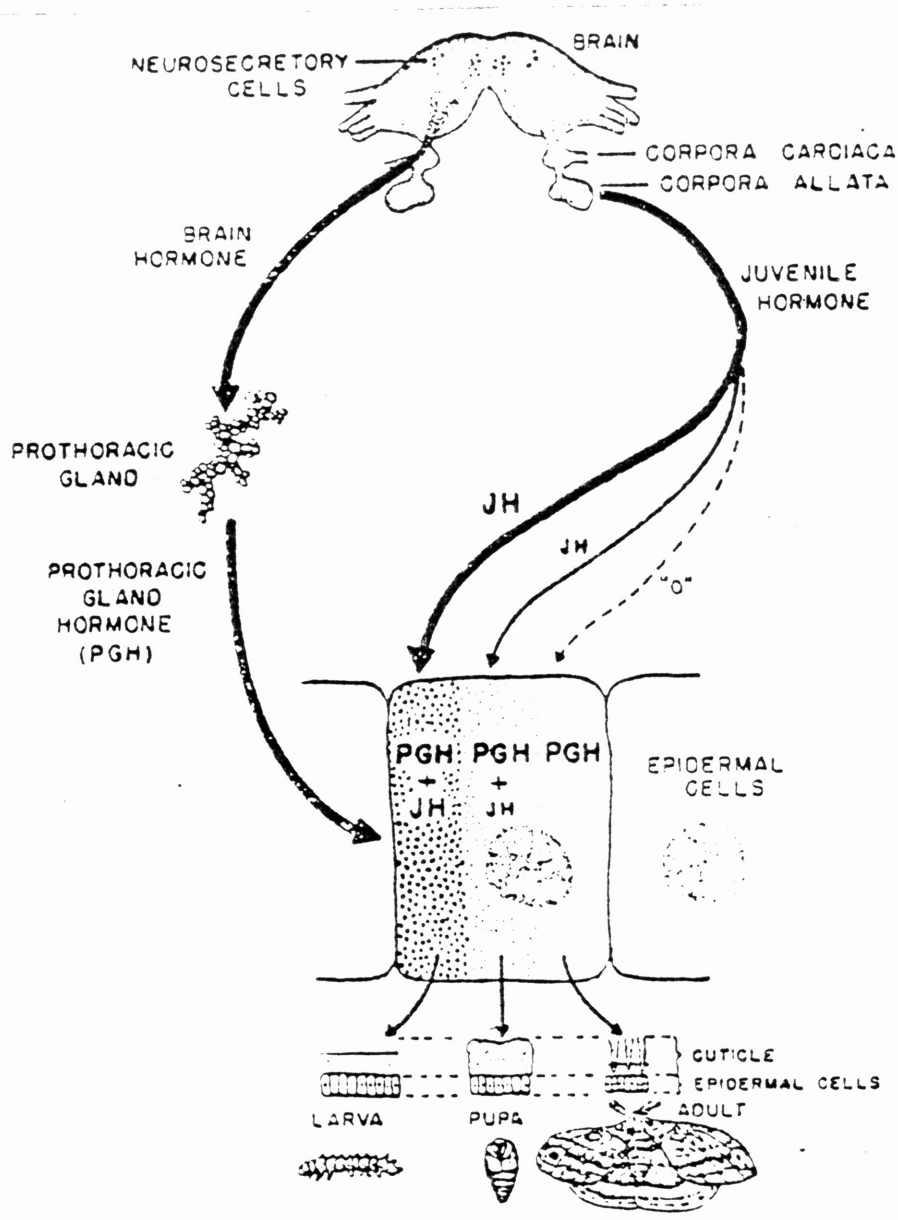


Figure 1. Schematic diagram of the principal endocrine organs of an insect and the mode of action of their hormones (Schneiderman, 1961).

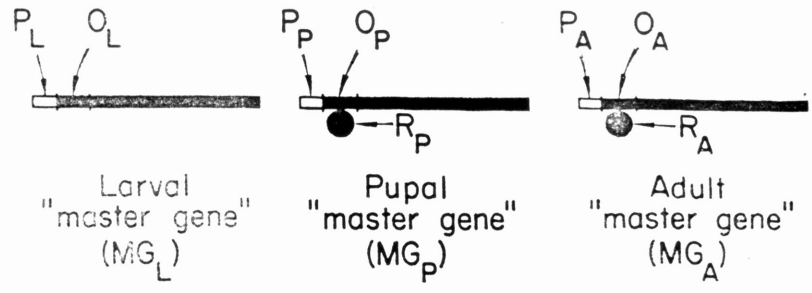
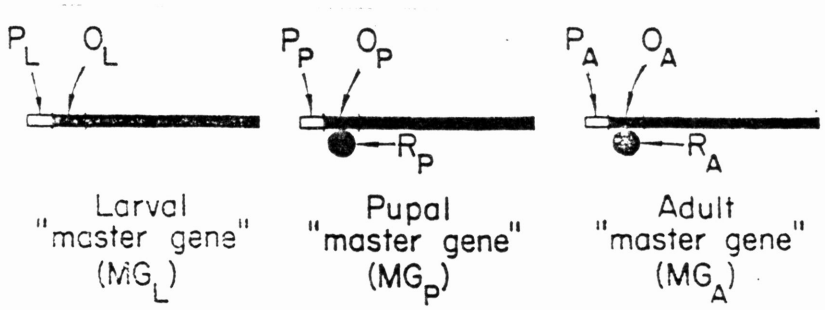


Figure 2. A diagrammatic representation of a hypothetical scheme of gene control during insect growth and metamorphosis. The genome of each cell is viewed as subdivisible into three gene-sets, each controlled by its own master regulatory gene, MG_L, MG_P, and MG_A, respectively. Juvenile hormone (JH) is depicted as a co-repressor which participates in the control of the master genes of the pupal and adult gene-sets. Abbreviations not defined in the diagram are as follows: P_L, P_P, and P_A are the promoters of the corresponding master genes; O_L, O_P, and O_A are the operators.



R_P *(the repressor of the "pupal master gene") is active only in the presence of a high titer of JH.*

R_A *(the repressor of the "adult master gene") remains active in the presence of a low titer of JH.*

Both repressors are inactive in the total absence of JH.

Figure 2. A diagrammatic representation of a hypothetical scheme of gene control during insect growth and metamorphosis. The genome of each cell is viewed as subdivisible into three gene-sets, each controlled by its own master regulatory gene, MG_L, MG_P, and MG_A, respectively. Juvenile hormone (JH) is depicted as a co-repressor which participates in the control of the master genes of the pupal and adult gene-sets. Abbreviations not defined in the diagram are as follows: P_L, P_P, and P_A are the promoters of the corresponding master genes; O_L, O_P, and O_A are the operators.

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