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HORMONAL INVOLVEMENT IN THE MOLTING PROCESS IN THE SOFT TICK, ORNITHODOROS TURICATA DUGÈS

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HORMONAL INVOLVEMENT IN THE MOLTING PROCESS IN THE SOFT TICK, *ORNITHODOROS TURICATA* DUGÈS

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CHAPTER I

INTRODUCTION

The recognition of an endocrine system in the invertebrates has opened a new area in hormonal research, comparable with that of the vertebrates. Since the early work in invertebrate endocrinology, an extensive amount of research has been done in the Phylum Arthropoda and, even more specifically, in the Class Insecta. The many reviews (e. g., Scharrer, 1948; Wigglesworth, 1954; Turner; 1955, Van der Kloot, 1960) published on hormonal agents in insects show that these substances are instrumental in inducing and maintaining physiological processes such as molting, growth and differentiation, pigment migration, metabolism, and reproduction.

In the Phylum Arthropoda, neurosecretory systems which produce various hormones have been described in the following Classes: Chilopoda, Crustacea, Insecta and Onychophora. In the Class Arachnida, the Xiphosura have been reported to have neurosecretory

cells which secrete chromatophorotropins (Scharrer and Scharrer, 1954). Neurosecretory centers have been reported to occur in Araneidae, although their functional significance is still unknown (Gabe, 1954; Legendre, 1953, 1954).

Among the Arthropods, endocrine regulation of the cyclic growth phases of insects has received the most attention. These phases, which are initiated by chemical constituents circulating in the hemolymph, periodically result in the growth of a new cuticle and the shedding of the old one. It has been generally accepted that these chemical constituents are hormonal in nature and action. With the exception of the gonads, the structures responsible for the elaboration of these hormones are parts of neurosecretory systems, and in some cases the organ not only elaborates hormones but stores them as well. An example of this latter type of organ is the corpus cardiacum located in the head region of certain insects.

In some insects there are three hormones concerned with the process of molting and metamorphosis. First, the brain hormone secreted by the pars intercerebralis of the brain (dorsal brain), as in Rhodnius (Wigglesworth, 1951, 1952a) or Platysamia (Williams, 1947, 1952, 1958), which stimulates secretion by the prothoracic glands. Second, the growth and differentiation hormone (GDH) secreted by the prothoracic glands, which brings about growth and final metamorphosis. Third, the juvenile hormone (JH) secreted by the corpus

allatum, an organ which is part of the nervous system and located just caudal to the brain. The JH delays metamorphosis and allows gradual rather than rapid changes to occur during growth and development of insects.

Wigglesworth's experiments (1934, 1954) have shown that before molting can occur in Rhodnius prolixus, the insect must be replete with a blood meal. Molting does not occur in bugs that have been starved or partially fed periodically. When the insect is fully engorged, the stretching of the abdomen apparently stimulates the neurosecretory cells of the pars intercerebralis of the brain to release a substance which stimulates the prothoracic glands to secrete GDH. There is evidence leading to the presumption that the GDH from the prothoracic glands acts on the corpus allatum in the nymph to produce the JH which in turn inhibits the imaginal discs from differentiating into the adult form, thus allowing the retention of the nymphal characters. There is a lapse of time, however, before the hormone concentration of the blood is sufficient to bring about these results. Since there is a balance between precocious metamorphosis and gradual development, approximately equal amounts of the GDH and the JH are found during the first four nymphal stages (Scharrer, 1946).

Wigglesworth (1940) has shown that when brain tissue has been transplanted from an engorged Rhodnius nymph, during the critical period (period of high hormone titer), into the abdomen of totally

decapitated nymphs (both brain and corpus allatum removed) just after feeding, these decapitated forms are induced to molt into diminutive adults thus indicating that absence of the corpus allatum results in precocious development. When, under the same conditions as described above, brains are transplanted into the abdomen of partially decapitated nymphs (only brain removed, with corpus allatum intact) just after feeding, the nymphs molt to the next instar only.

Additional evidence for the effect of JH has been reported by Wigglesworth (1952b). For instance, if the blood of a third-stage nymph with intact corpus allatum, which has just passed the critical period, is joined to a fourth-stage nymph decapitated one day after feeding, the fourth-stage nymph does not develop into a fifth-stage (as it would under normal conditions), but develops the characters of the fourth-stage nymph instead. This is the result of a higher concentration of JH in the third-stage nymph. Characters intermediate between fourth- and fifth-stage nymphs develop when fourth-stage nymphs fed twenty-four hours previously are joined to fourth-stage nymphs fed eight hours previously. The development of this intermediate condition is believed to be the result of the premature introduction of the JH into the molting cycle. The JH is greatly reduced or entirely absent in the fifth instar, so that the GDH is no longer restrained from activating the imaginal discs to differentiate into the adult characters.

According to Wigglesworth (1934), if Rhodnius is totally decapitated immediately after a blood meal, at any one of the nymphal instars, the insect may live more than a year but will not molt. If the pars intercerebralis is removed after the critical period, leaving the corpus allatum intact, metamorphosis is inhibited and molting occurs (Wigglesworth, 1936). Implantation of the corpus allatum causes the retention of nymphal characters, while extirpation of this gland, at any time before the fifth nymphal instar, will result in precocious development into the adult form (Wigglesworth, 1936).

To show the interaction of GDH and JH, Wigglesworth (1940), united a headless adult with two partially decapitated fourth-stage nymphs and two totally decapitated fifth-stage nymphs after the critical period. The fifth-stage nymphs provide GDH for the adult while the fourth-stage nymphs with the corpora allata provide, in addition to GDH, the JH. All individuals molt. The adult retrogresses to a nymphal stage; the fifth-stage nymphs become supernumerary instars (sixth-stage) and the fourth-stage nymphs become fifth-instars.

The evidence for the prothoracic glands as the source of the GDH was developed by Wigglesworth (1951, 1952a) in the following manner. Brains were transplanted from fourth- or fifth-stage nymphs which had just passed the critical period into the isolated abdomen (thorax and head removed) of fourth-stage nymphs, twenty-four hours after feeding. These animals failed to molt, which fact shows that the brain is not the source of the GDH.

Experiments on the hemimetabolous Orthoptera (Scharrer, 1946) show the same results as those observed in Rhodnius when the corpora allata are extirpated. Also in holometabolous insects the corpora allata have the same function and the animals show the same reaction in similar studies (Bounhiol, 1943). The JH is diminished or absent in the last larval molt so that the GDH is no longer inhibited and the imaginal discs are allowed to differentiate (Wigglesworth, 1954).

In holometabolous lepidopterans there are three structures responsible for the activation of molting as well as growth and differentiation; the corpora allata, the brain, and the prothoracic glands (Williams, 1947, 1952). The corpora allata, which are posterior projections of the brain, secrete the JH (Williams, 1958). The brain secretes a hormone which induces the prothoracic glands to secrete GDH. The neurosecretory cells (Williams, 1951-1952) of the brain are composed of a median and a lateral group located in the inner mass of each cerebral lobe of the brain. However, only the median group of cells corresponds to the pars intercerebralis cells of the hemipterans. The lateral group of cells has not been described in any other order of insects. In Lepidoptera, both the median and lateral groups of neuro secretory cells are necessary for the production of the brain hormone. One group of cells without the other renders the brain non-functional. The prothoracic glands located in the prothorax secrete the GDH. These structures degenerate and disappear either before or soon after the molt to the adult stage.

In the larval stages of the Lepidoptera the JH from the corpora allata prevents differentiation of the imaginal discs and thus permits the retention of larval characters until that moment when the JH normally diminishes, allowing pupation to occur. Williams (1950, 1958) has shown that extirpation of the corpora allata in all but the last larval stages of moths (Platysamia cecropia and Telea polyphemus) results in precocious pupation and metamorphosis, while removal of the corpora allata in the last larval stage produces no such effect.

According to Williams (1952), the lepidopteran brain secretes a hormone which stimulates the prothoracic glands to release the GDH. When the GDH has reached a sufficiently high concentration the imaginal discs differentiate and the larva is transformed into the pupal or adult form. As mentioned previously, secretion from the corpora allata seems to alter the effect of GDH and thereby governs the time in the life cycle of the insect at which imaginal-disc differentiation takes place.

Williams (1950, 1952), experimenting with Platysamia cecropia, ligated the larvae behind the head and again behind the thorax before they had spun cocoons. The animals lived for several months, but metamorphosis was completely arrested. The same procedure was applied to other larvae just after the spinning of the cocoons. In these animals the thoraxes pupated, but the abdomens did not. Ligations done three days after completion of cocoons resulted in pupation of

both the thorax and the abdomen. From these results Williams concluded that: (a) two hormones are needed, and (b) there is a critical period for each of these hormones. The brain hormone is released from the brain just after the spinning of the cocoon. Three days after completion of the cocoon the critical period of the GDH occurs. After pupation the GDH acts to inhibit production of the brain hormone, which accounts for the period of dormancy or over-wintering of this insect.

Williams' (1946) experiments have shown that it is the brain which must be subjected to a temperature of 3 - 5° C. for a period of one and one-half months before metamorphosis can be completed. Unchilled pupae will not develop further but implants of brains from chilled pupae will induce metamorphosis in unchilled pupae. Also, parabiotic union of an unchilled pupa with a chilled one will be followed by metamorphosis in both.

The endocrine systems of members of two orders of the Class Insecta have been discussed here because these two insects have different life cycles and both display a similar basic, intricate, hormonal regulation of molting, growth, and differentiation.

Up to the present time there apparently have been no reported studies on the mechanism of molting control in the Arachnida. The fact that in members of this group, as in the Insecta and Crustacea, molting affects all parts of the body simultaneously suggests some kind of central control. Moreover, in at least one species of tick,

Ornithodoros turicata, molting does not occur unless the animal has fed (Hopla, C. E., personal communication; personal observations); and in another, O. parkeri, full engorgement is apparently necessary to guarantee molting (Davis, 1941). These observations suggest that the mechanism by which molting is initiated may be similar to that found in the hemipteran Rhodnius, as described above.

The studies on the soft tick, Ornithodoros turicata, which are reported here had the following purposes: to investigate the mechanisms responsible for the control of molting in this animal; to ascertain whether there are identifiable neurosecretory structures which show histologic changes temporally related to the molting cycle; and, in particular, to discover whether there are hormones or neurosecretory substances produced in the brain which have an influence on molting.

Preliminary to studying the effects of experimental interference on the molting process, it was necessary to establish the number and duration of instars under standardized laboratory conditions, as described in Chapter II. Morphologic and histologic studies concerned with identification of presumptive neurosecretory centers and detection of cyclic alterations in their activity are described in Chapter III. The ligation experiments (Chapter IV) were undertaken to determine whether evidence could be found for the localized production of a hormone or hormones responsible for the initiation of molting. Finally, since the findings reported in Chapters III and IV indicated the brain as a

probable site of molt-initiating hormone, the effects of implanting nymphal brains into adult ticks were investigated (Chapter V).

The species, O. turicata, was chosen as the object of these investigations because of its availability and because it is easily reared in the laboratory. It is able to live for an extended period of time without feeding, and the various instars can be obtained at will by feeding and waiting a few days for molting to occur.

CHAPTER II

LIFE CYCLE OF ORNITHODOROS TURICATA

Materials and Methods

Ticks of the species O. turicata were obtained from two sources for this study. One strain was provided by Dr. Cluff Hopla, University of Oklahoma, Norman, Oklahoma. This strain had been collected from prairie-dog burrows in Oklahoma in 1951 and had been reared in the laboratory since that time. The other strain was donated by Dr. Gordon E. Davis of the Rocky Mountain Laboratory, Hamilton, Montana.

The ticks were reared in a constant-temperature chamber at 29° C. To prevent dessication of the ticks a saturated atmosphere was maintained by placing a pan of water in the chamber.

The ticks were confined to vials cut from 7 mm. glass tubing into 12.5 cm. lengths. The ends of the vials were fire-polished, then stoppered with small wads of absorbent cotton. Cotton was chosen as suitable material for plugging the vials because it allowed sufficient gaseous exchange between the internal and external environment of the vial. To facilitate handling, the vials were held in wooden racks

(35 cm. long X 3, 75 cm. wide X 3, 1 cm. high) which contained twenty-six holes, each 1 cm. in diameter.

Guinea-pig blood was the source of food for the ticks. A guinea pig, shaved with animal clippers, was strapped, ventral side up, to a pine plank. A strip of 2.5 cm. adhesive tape was placed just posterior to the front legs and one strip was placed just anterior to the hind legs. The ends of these strips were thumbtacked securely to the pine plank. Ticks were confined, during feeding, in the tick-holders described by Francis (1938). The metal screw-top container used in this study was 5 cm. in diameter. The container was fitted into the center of a 7.5 cm. adhesive strip in which a circular aperture had been cut. The adhesive tape fitted snugly around the flange of the container, anchoring it firmly within the circular aperture. This apparatus was placed over the abdomen of the guinea pig and thumbtacked tightly to the pine plank. Ticks that were ready for feeding were placed in the container and the lid secured. All ticks were allowed to consume blood to repletion, at which time they automatically released from the guinea pig. The fed ticks were then returned to the proper glass vials and replaced in the constant-temperature chamber.

Adult ticks were fed and sexed. A male and female were confined to each of several glass vials for establishment of a tick colony. Records were kept on the time necessary for the ticks to feed to repletion, the time required for egg laying and hatching, the days required for

each instar to molt after feeding, and the number of molts preceding sexual maturity.

Results

Data on the time span in days between egg laying, hatching, feeding and molting in O. turicata are presented in Table 1.

TABLE 1

LIFE CYCLE OF ORNITHODOROS TURICATA

Condition	Range (Days)
Adult feeding to egg laying	7 - 14
Egg laying to hatching	7 - 13
First feeding to molt	5 - 7
Second feeding to molt	5 - 9
Third feeding to molt	8 - 11
Fourth feeding to molt	10 - 13
Fifth feeding to molt	11 - 15

The feeding time for O. turicata was fifteen to twenty minutes for the earlier instars and thirty to sixty for later stages. These ticks molted five times before attaining sexual maturity. It was observed that the period between feeding and molting in this tick was lengthened when either temperature or humidity was decreased. Under the rear-

ing conditions of this experiment the life cycle, from egg to adult, was approximately two and one-half months.

CHAPTER III

MORPHOLOGIC AND HISTOLOGIC OBSERVATIONS

Materials and Methods

The following techniques were employed to ascertain hormonal function in molting. First, morphological and histological studies were made to determine the possible site of a hormone secretion. A few ticks of the third-instar were fixed in Bouin's solution before feeding. The remaining siblings were fed to repletion and a few of these engorged ticks were fixed on successive days through the period of molt. Ticks that were fixed in Bouin's solution for twenty-four hours were transferred directly to 80% ethanol (Foot, 1933). The preserved ticks were taken through the ethanols to methyl salicylate, through a mush of methyl salicylate and paraffin, and then imbedded in paraffin. Serial sections were cut at eight micra. Three stains were used: Mallory's triple connective-tissue stain, Gomori's chrome-hematoxylin-phloxin stain and Harris' hematoxylin stain. Mallory's triple connective-tissue stain gave the best results for cellular differentiation.

Approximately 150 ticks were sectioned and stained by one or another of the above mentioned stains. The majority of these ticks

were in the third instar. However, a few adults and second-instar nymphs were also prepared for histological comparison. All structures within the ticks were carefully studied and any histological changes which occurred after feeding and preceding ecdysis were noted.

The primary purpose for using the three aforementioned stains in this experiment was to reduce the possibility of staining artifacts in the suspected endocrine organ, the brain. Mallory's triple connective-tissue stain gave the best clarity and differentiation and was therefore chosen to be used consistently in the staining procedure. Thus the majority of histological observations were based upon preparations made with this stain.

Results

In the living O. turicata the brain is a white spheroidal structure slightly flattened dorsoventrally, and is approximately 320 to 350 micra in length as determined by serial section measurement. It is located immediately caudal and ventral to the hypopharynx. The brain (Figures 1, 3, 4) surrounds the esophagus; the latter progressing obliquely through the brain, from a ventral-anterior position through the mid-line of the brain to a dorsal-posterior position where it emerges.

Histological study of the brain reveals that it is composed of two different types of tissue (Figure 2). The innermost portion is composed of a compact fibrous mass, the neuropile, which in turn is

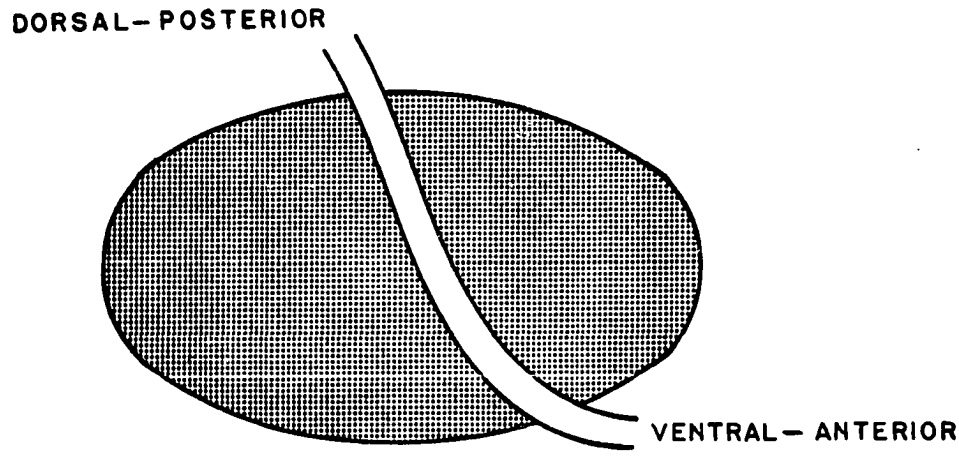

FIG. 1. SCHEMATIC DIAGRAM (LATERAL ASPECT) OF O. TURICATA BRAIN AND ESOPHAGUS

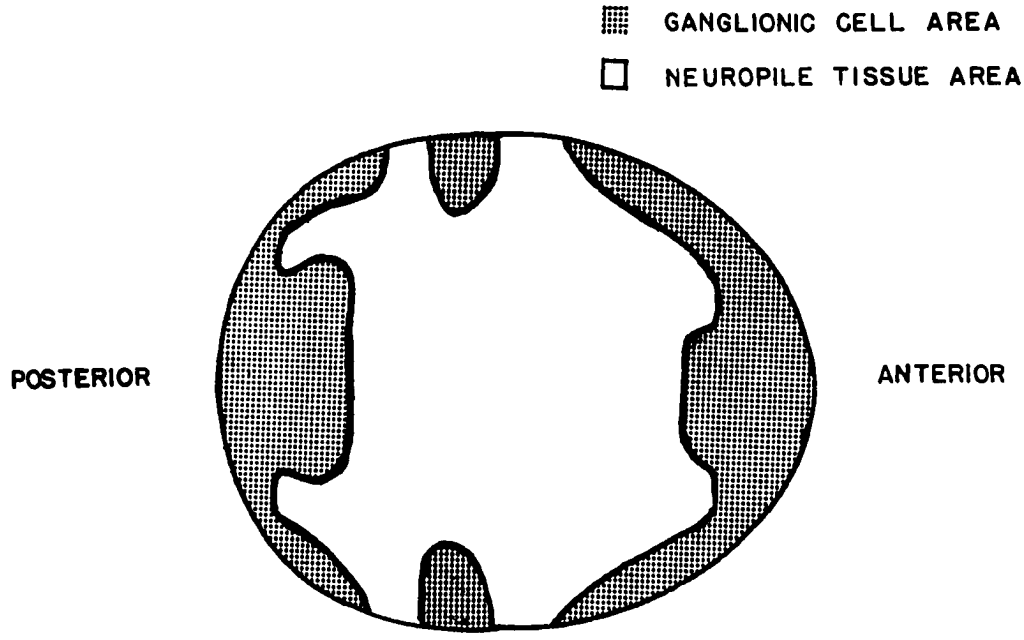

FIG. 2. SCHEMATIC DIAGRAM (HORIZONTAL SECTION) OF O. TURICATA BRAIN SHOWING INNER NEUROPILE TISSUE AND PERIPHERAL GANGLIONIC CELL AREAS

surrounded by large ganglionic cells with deeply staining nuclei as shown in Figure 4. The entire brain and nerve trunks are sheathed in a neurilemma which also invests that portion of the esophagus which passes through the brain.

By fixing ticks before and on successive days after feeding, a time-graded series was prepared for study of possible histological changes in the periphery of the brain which might be associated with storage and secretion of a hormonal agent. The nuclei of the ganglionic cells of second- and third-instar ticks prepared three to four weeks after molting were basophilic. However, these nuclei were acidophilic in ticks that were fixed immediately after feeding and the acidophilic condition persisted in the brain of ticks that were fixed at intervals up to four and five days after feeding. After this time lapse, scattered basophilic nuclei began to reappear. There was a gradual decrease in the number of acidophilic nuclei which paralleled the progress of exuviation. Finally, there was complete disappearance of acidophils and the nuclei again became basophilic. Adult ticks that were prepared for histological study without having been fed prior to fixation revealed basophilic nuclei of the brain just as had the second and third instars prepared under the same conditions. Adult ticks fixed one to two days after having been fed failed to show assumption of the acidophilic nuclear condition and the nuclei remained basophilic. No histological change was observed in the brains of adult ticks under the stated

conditions comparable to that which occurred in the second and third instars. No histological change was observed in the neuropile tissue of the brains of any of the ticks under observation.

Minute, irregularly shaped, acidophilic granules were observed in the hemolymph of one group of third-instar ticks that had been fixed for histological study. These granules appeared in the hemolymph of ticks that had been fixed two, three, four and five days after feeding. The granules were observed in that portion of the aorta which comes into contact with the anterior portion of the brain.

CHAPTER IV

LIGATION EXPERIMENTS

Materials and Methods

The technique of ligation was employed in an effort to determine whether hormonal function was responsible for molting. The ligatures in these experiments were teased from a very fine nylon thread and were thoroughly coated with beeswax. A total of over 300 second-, third-, and fourth-instar ticks was ligated just behind the brain area on successive days after feeding. In each of several experiments, six to ten ticks from the same sibling group were set aside for controls, and the remaining ticks were ligated as described. Controls and ligated ticks were observed daily for signs of exuviation (whitening of the integument, splitting of the anterior integument, and shedding of the cast). The number of days between ligation and molting (if it occurred) was recorded. A few ticks by accident were ligated immediately behind the fourth pair of legs.

Results

If the ligature was sufficiently binding, the suspected brain hormone would be isolated in the anterior region and molting should

occur only in that region. Table 2 summarizes the results of tick ligations.

TABLE 2

SUMMARY OF TICK LIGATIONS

Category	No. of Third Instar	Per Cent	No. of Fourth Instar	Per Cent
Total ligated	72		68	
Lost ligature	3		0	
Died	28		22	
Failed to molt	0		11	
Molted anterior to ligature	8	19.5	11	31.4
Molted completely	25	61.0	17	48.6
Complete molt. Ligated after critical period	8	19.5	7	20.0
Total No. of ticks that molted	41	100.0	35	100.0

The percentages in this table exclude those ticks that died, lost their ligature or had failed to molt (Plate II; Figure 11) by the time all controls had molted.

The critical period or the period of sufficient hormone circulating in the tick has not been conclusively demonstrated at this time. However, the ligated ticks showed no indications of exuviation until the fourth or seventh day after feeding. These observations indicate that the critical period for third-instar ticks was perhaps between four to six days after feeding and for the fourth-instars, five to seven days after feeding (See Appendix). Inasmuch as it was necessary to remove these ticks from the constant-temperature chamber daily for observations, thus changing the environmental conditions, the molting time for the ticks was increased by one to three days over the normal pattern shown in Table 1. Therefore, the "critical period" category in Table 2 is a presumptive one. Nor was it possible to be certain whether complete molting occurred because of incomplete ligation or, in spite of effective ligation, because the critical period had been reached.

Third- and fourth-instar tick ligations resulted either in molting anterior to the ligature only (Plate II; Figures 6, 7, 8); in complete molting (Plate II; Figures 12, 13) of the tick; or in no molting at all (Plate II; Figure 11). None of these experimental animals molted posterior to the ligature without having first molted in the anterior region. Of the third-instar ticks ligated, 19.5 per cent molted only in the anterior region and of the fourth instars ligated, 31.4 per cent molted only in the anterior region. Those ticks that were accidentally

ligated behind the fourth pair of legs molted and lost the posterior end of the body with cast (Plate II; Figures 9 and 10). Although second-instar ticks were ligated, they seemingly were too delicate to withstand the trauma of the ligature and nearly all failed to survive long enough to yield significant information.

CHAPTER V

TRANSPLANTATION EXPERIMENTS

Materials and Methods

To further establish hormonal control of molting, the suspected endocrine organ, the brain, was transplanted from third-instar ticks which had been fed 48 to 72 hours prior to the operation, into adults which had been fed 24 hours previously.

The procedure for transplanting was as follows: a piece of masking tape with the adhesive side exposed was taped to a glass slide. The glass slide served as an operating board and facilitated handling. The tick to be sacrificed was placed ventral side up on the adhesive portion of the tape, and the slide with the adhering tick was placed under a dissecting microscope. With iridectomy scissors, a mid-ventral incision was made at the junction of the first pair of legs in the donor (third-instar) tick. A small bore glass pipette (approximately the diameter of the brain) with a rubber tube attached was used to aspirate away the body fluid and expose the brain. Insect Ringer's solution was used to keep all exposed tissue moist. The adult tick to receive the transplant was placed, ventral side exposed, just beside the donor

tick. One of the fourth pair of legs was cut off close to the body. Fine pointed watch-makers forceps was used to transplant the brain from the third instar into the opening provided by excision of the leg of the adult recipient. The brain was assisted into the body cavity of the adult tick with the fire-polished end of a tiny glass rod about the diameter of the brain. The body fluid of the adult tick clotted rapidly, closing the opened stump of the leg. Three or four brains were transplanted into each adult tick.

Multiple transplants were made for two reasons: (1) some ticks do not molt after feeding and it was feared that some of the donor brains would not have been properly stimulated for hormonal secretion, and (2) to be assured of getting at least one brain properly implanted since these brains are extremely small and very difficult to see even with the aid of a dissecting microscope.

Adult ticks were fed 24 hours prior to the operation since it was felt that they would be in better nutritional condition to receive the transplants. Also, the increased size from engorgement facilitated operative procedures.

To provide controls for this experiment, sham operations were performed on adult ticks by removing one of the fourth pair of legs close to the body. The tip of the glass rod was gently forced in and out of the incision in order to simulate the insertion of the brain into the body cavity.

The experimental adult ticks receiving brain transplants from the third-instar ticks and the sham operated control ticks were replaced into appropriately labeled vials and returned to the constant-temperature chamber.

Seven to fourteen days later the experimental and control animals were removed from the constant-temperature chamber and allowed to feed on guinea-pig blood, since molting in the experimental animals was dependent upon whether they fed at this time.

Results

Because of the paucity of adult ticks only a few brain transplants were feasible. The first series of transplants was made on July 25, 1959. Third-instar ticks had been fed on the 23rd of July and three adults (two females and one male) had been fed on the 24th of July. The following day the transplants were made into the adults, and on August 1, 1959, these operated adults were fed again. These ticks fed readily, which was possibly due to the fact that a great amount of body fluid was lost during the operation. Normally, ticks of this stage will not feed so soon after having had a blood meal. All of these adult ticks shed their casts. One tick molted 24 days after the last feeding; one molted approximately 35 days after the last feeding and the last tick to molt, a male, shed its cast 61 days after the last blood meal. Both of the molted female ticks produced normal clutches of eggs.

The second series of transplants was made on September 20, 1959. Third-instar ticks were fed on the 18th of September and four adult male ticks were fed on the 19th of September. The following day brains were transplanted into these adults. During the operation very little body fluid was lost from these adults. Two of these operated ticks died and the remaining two were placed on a guinea pig for feeding on the 28th of September, but they did not feed at this time. These ticks were again placed for feeding on October 7th. One animal partially fed; none molted.

The third series was operated on November 12, 1959. The third-instar ticks were fed on the 10th of November and the adults (two females and one male) were fed on the 11th of November. Very little body fluid was lost from these ticks as a consequence of the operations. All operated ticks lived and were allowed to feed again on November 25, 1959, but they did not feed at this time. Feeding was tried again on December 17th and at this time all three adults fed to repletion and were replaced in the constant-temperature chamber for further observations. During the time lapse preceding the last feeding the females produced one or two normal clutches of eggs. These operated adults of the third series have not molted to date (April 1960).

Five adult ticks (two males and three females) that received sham operations at the time the second series of experimental animals was operated have been fed several times. None molted: three died.

It was observed that ticks which had lost the majority of their body fluid during the operation and still survived were likely to feed sooner after the operation than those which did not lose a lot of body fluid. However, those ticks which did not lose a lot of body fluid survived the operation better. Also with a loss of body fluid there is more available space for transplanted brain tissue and thus better assurance that any pressure exerted on the body of the tick, when being handled immediately after the operation, would not accidentally force the transplants through the clotted hemolymph at the site of the incision.

CHAPTER VI

DISCUSSION

In this study it was observed that the life cycle of O. turicata ticks varied with temperature and humidity. Increased temperature hastened molting and decreased temperature had the opposite effect on this process. A decrease in humidity seemed to increase the length of time required between feeding and molting. Under the conditions of this experiment, the life cycle of this species, from egg through five nymphal stages to adult, was approximately two and one-half months. These findings are comparable to those reported by Francis (1938), Davis (1941, 1943), Herms and Wheeler (1936).

Francis (1938) reported that under non-regulated laboratory conditions the life cycle of O. turicata from egg to adult required a period of nine months and ten days. The number of nymphal stages was, in some cases, four, and in others, five. The longevity of these ticks ranged from five and one-half to six and one-half years. However, when kept at normal room humidity the ticks live only a few weeks to a few months. Survival time may be increased by keeping the ticks in moisture chambers saturated with ammonium chloride.

Davis (1941, 1943) has also studied the life histories of two species of ticks, O. nicollei and O. parkeri, kept in humidity jars at room temperature. Observations showed that higher temperatures hastened molting and lower temperatures delayed it. He reported (1941) that O. parkeri nymphs which were only partially engorged may molt regularly or they may fail to molt until engorgement has taken place. This latter alternative is comparable with the results Wigglesworth (1934) found in partially engorged Rhodnius.

The life cycle of O. hermsi has been investigated by Herms and Wheeler (1936). When these ticks are kept at a constant temperature of 24° C. and a relative humidity of 90%, the incubation period for the eggs is fifteen to twenty-one days; the first molt may occur within the egg, but the larvae remain hexapod until after the first feeding and molt. The larvae are ready to feed about three days after hatching and remain attached for thirty to sixty minutes before releasing. The time required for each nymphal instar to molt varies with the instar. Usually the earlier instars require fewer days until molting occurs than do later instars. Molting takes place about fifteen days after the first feeding; eleven to fifteen days are required for ticks to molt after the second feeding; ten to thirty-two days are required for the fourth molt (third nymphal instar). At this time the adult may appear, but usually a fifth feeding and molt are necessary before sexual maturity is reached. Egg laying begins about thirty days after the last molt.

Altogether, the life cycle of this species of tick requires about four months under laboratory conditions, as mentioned above.

Ticks of the species O. turicata lend themselves well to endocrine studies of molting because they are easily and conveniently reared in the laboratory. They are able to live for an extended period of time without feeding and various instars can be made available by feeding and waiting a few days for molting to occur.

From histological studies, only the brain seemed to show a definite histochemical change correlated with exuviation. The histochemical change was restricted to the nuclei of these cells. The change of the ganglionic nuclei from the fasting basophilic condition to the post-feeding acidophilic condition is rapid and striking. This secretory cycle, paralleling the phenomenon of exuviation, suggests that the brain is somehow involved in the molting process.

Wigglesworth (1934) reports cyclic changes in the corpus allatum in a similar time-graded series of histological observations on Rhodnius prior to and subsequent to feeding. Cyclic secretory activity in the corpus allatum of some Orthopteran larvae has been reported by Cazal and Guerrier (1946) but these authors do not relate the cycle to physiologic events or to feeding. Wigglesworth (1940) noted large cells with fuchsinophil inclusions in the brain of fourth-stage Rhodnius nymphs fixed before and six days after feeding (at the height of secretion of the molting hormone) but could determine no difference between the cells of the fasting and fed insects.

Thus the histological observations, reported in Chapter III, of neurosecretion and a neurosecretory cycle similar to those in organs which have been demonstrated by others to have endocrine functions, support the assumption that the brain of O. turicata produces a secretion which is somehow concerned with molting.

The results of the ligation experiments indicate the production of a substance involved in molting which can be isolated to the anterior portion of the body. Therefore, the site of the organ secreting this ecdysis-stimulating substance is obviously in the anterior region since sufficiently binding ligatures, made extremely early in the molting cycle, resulted in molting in the anterior region only. Since the hormone could be isolated anterior to the second pair of legs of the tick, and because the greatest portion of the brain is located between the first and second pair of legs and was the only organ to show a definite histochemical change, this structure was concluded to be a possible site of hormone secretion.

Since guinea-pig blood was the source of food for the ticks used in the experiments reported here, the possibility occurred to the author that a substance or substances, circulating in the vascular system of the food source, could directly affect ecdysis in the tick. Gilbert and Schneiderman (1958) have reported that the adrenal cortex of vertebrates has a sterol-like substance with juvenile hormone activity.

If some substance in guinea-pig blood were effecting ecdysis one

would expect engorged and ligated ticks to molt completely rather than to molt anterior to the ligature. Since it was observed in this experiment that binding ligatures resulted in molting in the anterior region alone, the possiblity of a circulating vascular ecdysis-stimulating substance from guinea-pig blood was ruled out. Also, if such a substance were in the food source, one might expect engorged, adult ticks to molt after feeding, but they do not. It was observed that partially fed ticks do not molt. One might suspect that a partially engorged tick would molt if the molting activator were in the food source unless, of course, there was an insufficient amount of the activator substance. However, the most plausible explanation for this non-conformity was insufficient stretching of the abdomen. Molting does not occur in Rhodnius, the blood-sucking hemipteran, unless it is engorged. Wigglesworth (1934) presents evidence that distention of the abdomen is essential for reflex stimulation of the neurosecretory center of the brain.

From the results obtained in these experiments it is evident that hormonal control is involved in the molting process of O. turicata. From my own observations and those reported in the literature, it is definitely established that molting occurs in the nymphal stages only, and, except for the first molt, ecdysis occurs only after a blood meal of sufficient quantity to stretch the abdomen; alternatively, since the esophagus passes through the brain, it is possible that sufficient food

in this tubular structure would result in pressure directly against the brain which might stimulate it to secretory activity.

The data on brain transplanting are sparse, primarily because few adult ticks were available and mortality during and following the operations was very high. Nevertheless, the results of the experiments indicate that the brain is the source of a hormone involved in the molting process. It is entirely possible that this hormone is the same as, or similar to, either the GDH or JH found in insects. However, other experiments will have to be undertaken to establish its identity.

Perhaps, in the tick, the brain secretes a hormone which stimulates another gland to secrete a substance involved in molting and metamorphosis. It is possible that the salivary glands of the tick could be the source of such a hormonal substance in addition to their already established function of secreting an enzyme which prevents coagulation of host blood as the tick feeds.

In insects the sequence of events in hormonal regulation of molting is: the brain hormone stimulates the prothoracic glands to secrete GDH. This substance not only brings about imaginal differentiation but effects termination of the brain hormone secretion and stimulates release of JH from the corpora allata. The JH, in turn, inhibits metamorphosis. In the last larval stage the JH is either insufficient or absent, thus allowing the adult characters to appear. Perhaps in the tick there is a similar sequence of events.

Wigglesworth (1959) has proposed another theory for molting and metamorphosis in insects. He suggests that perhaps the juvenile hormone is a simple chemical substance which determines whether the enzymes responsible for the development of larval characters will be active. He reasonably assumes that enzyme production is controlled by appropriate genes, and theorizes that JH, when present in quantity, could activate the gene-enzyme system of the larva. As JH diminishes, the pupal gene-enzyme system would assert itself, and in the absence of the hormone, the gene-enzyme system of the adult would become active.

It is not yet possible to say just how molting and metamorphosis in the tick is hormonally regulated.

CHAPTER VII

SUMMARY

An attempt was made to determine whether a hormone is involved in the molting process of the soft tick, Ornithodoros turicata Dugès. An estimated 3000 ticks were used in developing experimental techniques and collecting data.

Ticks were reared in a constant-temperature chamber at 29°C. Under the laboratory conditions of this experiment the life cycle of O. turicata, from egg to adult is approximately two and one-half months.

It may be concluded that:

(1) there is a definite histochemical change in the ganglionic cells of the periphery of the brain of nymphal ticks, subsequent to feeding, which parallels exuviation. This same histochemical change is not observed in adult ticks.

(2) by use of ligatures, molting can be restricted to the anterior region of the tick. Therefore, the presumptive hormone is produced in the anterior end of the tick.

(3) it is possible to induce a supernumerary molt in the adult tick by transplanting nymphal brains into the adult.

These conclusions, in the light of previous experiments on neurosecretory cells, ligation, and transplantation, support the assumption that molting in O. turicata is a hormonally controlled process. Also, it would suggest that a hormone is produced, or produced and stored, in the brain from which it is released upon some stimulus associated with engorgement.

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PLATE I

Fig. 3 Cross-section of anterior portion of O. turicata brain showing ganglionic cell tissue. A small portion of neuropile tissue shows at bottom right.
Bouin's - Mallory, X 630.

Fig. 4 Cross-section of mid-portion of O. turicata brain showing peripheral ganglionic cell tissue and inner neuropile tissue. Esophagus appears at bottom left.
Bouin's - Mallory, X 630.

PLATE I

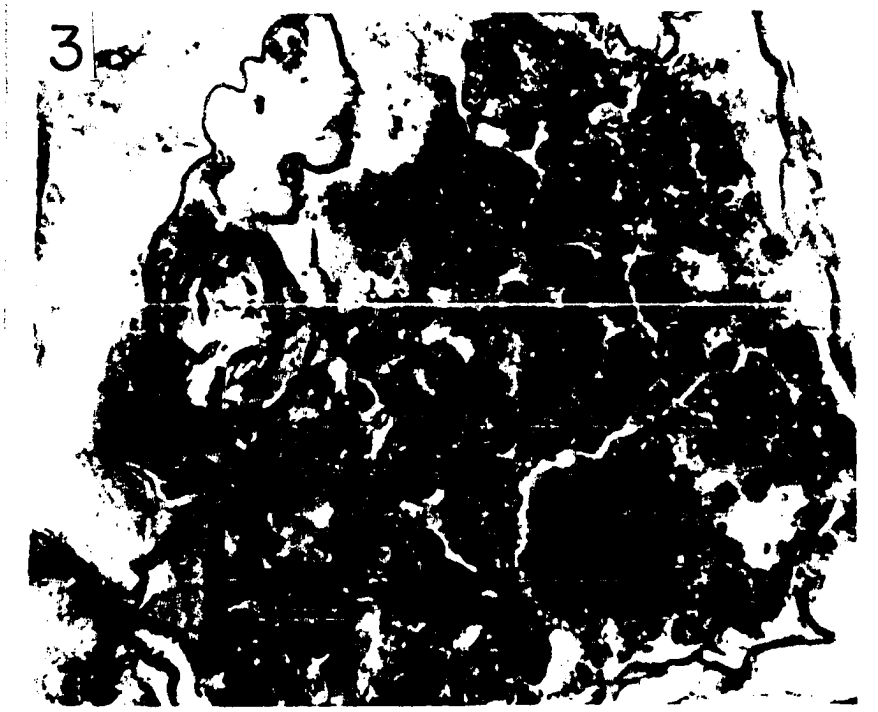



PLATE II

Fig. 5 Normal O. turicata: fifth instar.

Fig. 6 Fifth instar; ligated 24 hours after feeding; showing anterior molting only.

Fig. 7 Third instar; ligated 48 hours after feeding; showing anterior molting only.

Fig. 8 Fourth instar; ligated 24 hours after feeding; showing anterior molting only.

Fig. 9 Third instar; ligated 144 hours after feeding; showing anterior molting and loss of posterior end of body with cast.

Fig. 10 Third instar; ligated 48 hours after feeding; showing anterior molting and loss of posterior end of body with cast.

Fig. 11 Fourth instar; ligated 144 hours after feeding; showing no molting.

Fig. 12 Third instar; ligated 24 hours after feeding; showing complete molting.

Fig. 13 Fourth instar ticks; ligated 240 hours after feeding; showing complete molting.

PLATE II



















RESULTS OF LIGATION OF O. TURICATA.

THIRD INSTAR TICKS

	No. of days after feeding when ligature was applied.										
Category	1	2	3	4	5	6	7	8	9	10	11
Total ligated.	8	8	8	8	8	8	8	4	4	4	4
Lost ligature.	2	0	0	0	0	0	0	0	1	0	0
Died.	0	3	2	3	3	7	3	0	1	2	3
Failed to molt.	0	0	0	0	0	0	0	0	0	0	0
Molted completely.	6	2	4	4	5	4	0	4	1	2	1
Molted anterior to ligature.	0	3	1	1	0	1	1	0	1	0	0

Controls molted in 8 - 11 days after feeding.

RESULTS OF LIGATIONS OF O. TURICATA.

FOURTH INSTAR TICKS

	No. of days after feeding when ligature was applied.									
Category	1	2	3	4	5	6	7	8	9	10
Total ligated.	11	10	10	7	7	7	4	4	4	4
Lost ligature.	0	0	0	0	0	0	0	0	0	0
Died.	3	3	4	2	0	5	0	0	2	3
Failed to molt.	5	1	0	1	3	1	0	0	0	0
Molted completely.	1	1	4	4	3	0	4	4	2	1
Molted anterior to ligature.	2	5	2	0	1	1	0	0	0	0

Controls molted in 9 - 10 days after feeding.