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Title The Endocrine Control of the Early Stages of Oöcyte Maturation in Leucophaea Maderae (Fab.)

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A procedure was developed for the isolation and maintenance of isolated abdomina of the cockroach, Leucophaea maderae. The degree of injury and loss of body fluid was best minimized by ligating the animals immediately following the final molt. The nutritional needs of the isolates were satisfied by filling the animals' crops with a glucose or casamino acid-glucose solution prior to separation of the abdomina. Isolates prepared in this manner quickly recovered from the operation and remained quiescent with normal heart beat and cercal response for periods extending to several months.

It was necessary to provide the isolates with an amino acid solution for maximum responsiveness of the reproductive organs to implanted corpora allata. Response was evaluated on the basis of accessory gland activation and oöcyte growth. The nymphal diet

did not contribute to the responsiveness of the accessory glands or oöcytes. Nymphs fed only sucrose prior to the final molt did not provide abdomina responsive to corpora allata unless the newly molted adults were force-fed with amino acids prior to isolation. The amino acid requirement could also be satisfied by injection of the isolates. Abdomina isolated from nymphs fed dog food and force-fed either saline solution or whole casein were not responsive.

A time course study of the responsiveness of the oöcytes to corpora allata implants showed that the capacity for response coincided with the normal mating time of intact females. Oöcytes were unresponsive before the sixth day following ecdysis and thereafter reacted fairly uniformly.

Inactive corpora allata obtained from pregnant females returned to activity when implanted in allatectomized intact females. However, such glands produced no response in the reproductive organs of isolated abdomina within the same period of time. It was concluded that reactivation of the corpora allata was due to humoral stimulus present in intact females but absent in the isolates, rather than to an intrinsic cyclic activity as other workers had suggested.

Bioassays of materials with known gonadotropic activity were attempted. Farnesol was tested as an external smear on the cuticle of isolated abdomina, and a juvenile hormone extract was

tested by injection. Neither of these materials initiated the growth of oöcytes, and the maximum accessory gland response observed was very slight.

Examination of the evolution of radioactive CO_2 following the injection of isotopically labelled glucose and acetate indicated that the respiratory activity of isolates was reproducible and that abdomina continued to function uniformly for several weeks following isolation. The incorporation of acetate-C^{14} into lipids was more variable than its oxidation, but possibly not more so than would be expected with intact animals. The levels of incorporation were similar to those which have been reported for intact roaches.

No correlation was observed between the action of the corpora allata on the reproductive organs and the oxidation or incorporation into fatty acids of acetate-C^{14}. This supports the hypothesis that increases in oxygen utilization which have been observed in the presence of corpora allata are not due to a direct action by the hormone upon respiratory metabolism, but arise indirectly as a result of other events.

The incorporation of leucine-C^{14} into the proteins of the blood and fat body was examined with respect to the response of the reproductive organs of isolates to implanted corpora allata. In these preliminary experiments it was not possible to detect the presence of corpora allata from measurement of the specific

activity of these proteins. Although the highest specific activities were observed in animals with developing oöcytes, the proteins of many unresponsive and control animals were labelled nearly as extensively. It is suggested that protein synthesis occurs in all isolates, with some variation, and that when the corpora allata are able to initiate oöcyte development, this synthesis is increased due to homeostasis.

THE ENDOCRINE CONTROL OF THE EARLY STAGES OF OÖCYTE MATURATION IN LEUCOPHAEA MADERAE (FAB.)

by

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INTRODUCTION

The reproduction of its kind is the final measure of the ability of an organism to integrate successfully a complex array of physiological, nutritional, metabolic and environmental processes. In insects as in other animals this is ultimately achieved through the secretions of several endocrine glands; but the controls and responses involved are so complex and interdependent that their resolution is very difficult. Since maturation and reproduction are continuing processes, experimentation in this area has usually required the use of intact organisms. An approach frequently followed is the extirpation of the endocrine gland and subsequent examination of the target organ or process, following the replacement of an active agent.

The extraordinary tolerance of insects to extreme surgical procedures has been used to advantage in many experiments. However, the majority of studies of the endocrinology of egg development in insects has been carried out in the manner described above. In this approach, maintenance of the normal condition of the animal is attempted and alterations are as limited as possible. In some cases where the roles of several hormones were assessed, glandular areas were cauterized in conjunction with the removal or replacement of other glands (27). In a blood-sucking bug, Rhodnius prolixus, separation of the endocrine organs and the reproductive system has been

effected by decapitation (4, 68, 69). More extensive alterations have been attempted in only very few cases (2, 77). The present study reports the development of a simplified biological unit in which maturation and the early stages of reproduction are possible.

The endocrine organs involved in oögenesis in most hemimetabolous insects--the neurosecretory cells of the brain (NSC) and their connected secretory outlet, the paired corpora cardiaca (CC) and the corpora allata (CA)--are located in and immediately behind the head (Figure 1). The target organs, which for the present purposes are the sex organs, are located in the abdomen together with most of the fat body and digestive tract, the heart, the excretory system and segmentally operative respiratory organs. This natural separation of the organs of function from those which control their activity allows the possibility of autonomous function under experimental conditions. The intervening thoracic segments are primarily locomotory in function, and their removal would greatly reduce the number of extraneous factors due to activity and sensory input.

The abdomen of the silkworm, Hyalophora cecropia, has been successfully isolated by Williams and maintained until maturation of the oöcytes was complete (77). However, the separation of the thorax and abdomen was carried out during diapause when the effects of such a procedure would be lessened. Adult female cecropia do not feed but carry sufficient reserves for oögenesis; in addition, the adult is

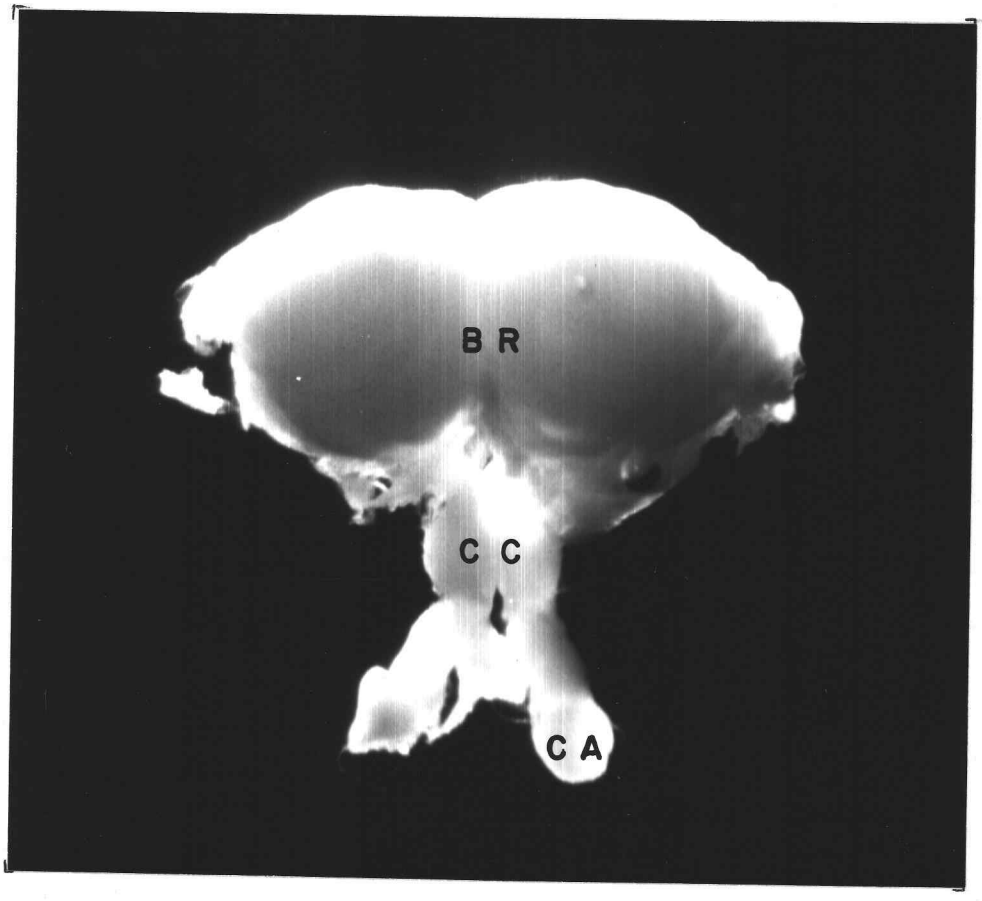

Figure 1. Photograph of the dorsal aspect of the brain-corpus cardiacum-corpus allatum complex of Leucophaea maderae. The portion of the brain shown is the protocerebrum (BR). The optic lobes have been removed. The elongate corpora cardiaca (CC) are attached to nerves which emerge from the venter of the brain (53). The spherical corpora allata (CA) are attached to the posterior ends of the CC by nerves which are not visible in the photograph. The actual width of the brain is 1.67 mm.

capable of egg maturation immediately following ecdysis and requires no endocrine organs for this process. In a complex active animal such as a cockroach the inability to obtain nutrients following the imaginal molt and the trauma involved in a drastic operation such as severance of the body are more serious problems. The profit to be gained from such a biological unit would lie in its simplicity. In addition, the surgical manipulations would be reduced, since single or multiple effects of the components of the endocrine system could be studied with only the addition of factors--their removal having been complete at the time of isolation.

To this end the development of a procedure for the isolation of the abdomen of a common laboratory insect, the Madera or wood roach, Leucophaea maderae, has been undertaken. The functioning of the various organ systems in the isolated abdomen was observed. A pre-feeding requirement for survival of the isolate and for maturation of the oöcytes was found. The endocrinology of the initiation of egg development and its timing were studied. The variation between isolated abdomina in biological and metabolic function was evaluated in several ways, as was the capability of individual isolates to perform in a uniform manner over a period of time. This involved, in addition to microscopic examination, the introduction of several isotopically labelled substrates and the study of their utilization.

HISTORICAL REVIEW

The role of the CA in reproduction in insects was first shown in 1936 by Wigglesworth (72), who found that the deposition of yolk in the oöcytes of Rhodnius is dependent upon their presence. Subsequently this relationship was found to hold true for a variety of species (34, 44, 52, 71). It soon became evident that a large number of contributing factors are involved in the regulation of the activity of the CA. In several species of mosquito, activity is dependent upon a blood meal, whereas other species are able to produce eggs without this prerequisite (21, 36, 37). Unfed Rhodnius are unable to form oöcytes beyond the immature stage, and a similar requirement has been found in some additional species but is absent in others (23, 31, 32, 36, 37, 52). Johansson was able to bring about egg development in starved female Oncopeltus (31) and Leucophaea (32) by implantation of active glands from fed donors. The CA of starved animals are apparently inhibited by the brain, since the glands can be activated and yolk deposition will ensue if the nerves supplying the CA are severed (33).

In cockroaches which bear their eggs in a brood sac until eclosion, the presence of an oöthecum results in inhibition of the CA (15, 16, 17). In several oviparous insects the presence of eggs which are close to maturity reduces the activity of the CA (10, 23, 24, 27, 35, 56, 62, 73). Mating provides stimulus to the CA of several

roaches (1, 16, 18, 22, 46, 48, 49, 50), apparently via nerve transmission to the brain (18).

In addition to its role in the integration of afferent stimuli, the brain is also directly involved in oögenesis through the neurosecretory cells located in the protocerebrum. In Calliphora the NSC are ultimately responsible for ovarian development, and none will take place if they have been extirpated; whereas some females from which the CA have been removed (allatectomy) are able to produce eggs (62). However, many insects will produce eggs in the absence of the NSC provided the CA are present and active (9, 13, 14, 69, 75).

Highnam (25, 26) indicated that NSC of Schistocerca which appear to be accumulating stainable material are inactive, in the sense that their product is not being released into the hemolymph (30). Conversely, cells which do not show large amounts of stainable material are found in females in which egg development is in the active stages. Yolk protein is the first material to be laid down in actively growing oöcytes (26), and a rise in blood protein levels is correspondent with an increase in the activity of the NSC (29). It appears that specific proteins are synthesized as a result of NSC activity (29). Telfer reported a specific female blood protein which is incorporated into developing oöcytes of the cecropia silkworm (60).

Thomsen and Møller (65, 66) implicated the NSC in the stimulation of increased proteolytic enzyme production in the gut of

Calliphora. L' Helias (39) observed reduction of protein synthesis by Dixippus following allatectomy. On the other hand, Sláma (58) found that in Pyrrhocoris the CA appear to affect hemolymph protein concentration indirectly through the induction of ovarian development; whereas Highnam, et al.(27), who observed similar responses in Schistocerca, were able to implicate directly the NSC in the stimulation of protein synthesis.

Pfeiffer (44) was able to show that in Melanoplus the CA exert an effect on metabolism apart from the uptake of proteins by the ovaries. Normally the fat body in this insect enlarges during egg maturation. This increase will occur in ovariectomized animals, and in allatectomized females the fat body becomes hypertrophied. Maturation apparently involves increases in water, glycogen and the utilization of stored fat in response to the CA hormone and independent of any ovarian effect. Vanderberg (68, 69), using Rhodnius decapitated in such a way as to exclude the CA, found a reduction of incorporation of leucine into proteins and of uridine into RNA in comparison with control animals, which were decapitated but retained the CA. DNA labelling, however, was the same in animals allatectomized by decapitation and in the controls. Coles (4, p. 323), using the same technique, found that two specific blood proteins are formed in the fat body and absorbed by the oöcytes. Following decapitation only small amounts of these proteins appear, and it was suggested that,

" the most economical hypothesis to explain these results is that the brain hormone is acting on the chromosomes of the fat body, and that different genes are responding to different concentrations of the hormone." In support, results of Wigglesworth are cited (74, 76) which indicate that the effect of decapitation of larval Rhodnius is upon the release by the fat body of these two specific proteins. Unfortunately, Coles' short note includes no data and only scant description, and it cannot be determined whether he actually means the brain hormone (produced by the NSC) or the product of the CA, which was the case in Vanderberg's experiments.

Several attempts have been made to determine whether the CA hormone has a general metabolic effect, as was suggested by Pfeiffer (44), or is limited to a more specific gonadotropic action upon the sex organs. Sägesser (51) found that CA removal resulted in increased oxygen uptake by Leucophaea even when the ovaries and accessory glands had been removed. In Pyrrhocoris, however, castration eliminates elevation of oxygen consumption brought about by CA reimplantation (43). Thomsen noted an increase in oxygen consumption in Calliphora when CA were implanted (63).

Wigglesworth, in a recent review (76), agrees that there is considerable evidence for direct action by these hormones upon target organs; but he suggests the possibility that homeostatic responses could be the cause of many observed metabolic changes. He cites

several areas of study in which this possibility has merit, particularly those which report increased oxygen uptake (43, 51, 63, 64) in the absence of demonstrable direct action by the hormone. The change in preference of Calliphora females from a diet high in carbohydrate to one rich in protein during periods of active egg development also may be a homeostatic response. Doane (11, 12) attributed differences noted in the size of the fat body of the " female sterile adipose" mutant of Drosophila following implantation of normal ovaries, to a feed-back mechanism originating in the ovaries. In Schistocerca (27) and in Calliphora (38) a feed-back from the CA to the NSC has been shown, and Highnam, et al. (27) point out that reports of metabolic changes following allatectomy could be explained on the basis of malfunction of the neurosecretory system, since allatectomy in Schistocerca reduces the release of secretion by the NSC.

In immature insects the CA control the degree of maturation which results from each molt through the production of the juvenile hormone. A great deal of effort has been concentrated upon the elucidation of the chemical nature of this hormone. Several bioassay procedures have been developed which rely upon the retention of immature characteristics in the presence of hormonal activity by insects which have undergone a molt. It has been found that isoprenoid derivatives, particularly the methyl ether of farnesol, are able to duplicate the effects of active extracts of juvenile hormone.

According to Wigglesworth (75) the identity of the juvenile hormone with the hormone responsible for oöcyte development (gonadotropic hormone) is virtually established by the activity of extracts and of farnesol in both roles. Chen et al. (3) were able to induce yolk development in allatectomized Periplaneta with a surface application of farnesol, and in a similar manner Highnam et al. (28) partially prevented the resorption of oöcyte yolk which occurs when Schistocerca females with well developed oöcytes are allatectomized. Egg development has also been stimulated in allatectomized Cimex with surface smears of farnesol (9). Juvenile hormone extract of cecropia silkworms has also been successfully used in the initiation of yolk deposition (3).

There appear to be differences in the manner in which various species coordinate oögenesis with the many other facets of growth and development. However, it may be that these actually represent modifications of common modes of integration which have been adapted so as to suit the life history of each species. As stated by Wigglesworth (76), Thysanura undergoes alternating phases of moulting and reproduction throughout its life, and coordination is of critical importance; whereas in the reproduction of a parthenogenetic insect such as Dixippus, which feeds and reproduces continuously, regulation is unnecessary. In the latter species the CA are not implicated in reproduction. Similarly, the cecropia silkworm does not require hormonal regulation following the imaginal molt.

The interpretation of the roles assumed by the individual organs involved in reproduction in insects is made difficult by the diversity in the experimental animals used and in the approaches made to the study of the problem. Several feed-back systems have been found, and quite likely other homeostatic responses are involved as well. In addition there is the possibility that one endocrine gland may supplement or even duplicate the role of another. Wigglesworth (74) suggested that the NSC may produce the precursors from which the CA hormone is made. It is generally concluded that the CC receive the secretory products of the NSC and control their release. Some of this material may pass on to the CA. The CA are definitely inhibited by the brain and may be stimulated as well; in at least one insect (27) the release of hormone by the NSC seems to require the presence of active CA and their connectives. In another species (62) the NSC seem to supplant entirely the CA in the initiation of oöcyte growth. It is clear that limitation of the number of factors involved is a desirable goal in this field.

MATERIALS AND GENERAL METHODS

Casein and casamino acids were obtained from Difco Laboratories; glucose-U-C^{14}, acetate-1-C^{14} and leucine-U-C^{14} from the New England Nuclear Corporation; 1, 4-bis-2-(5-phenyloxazolyl)-benzene (POPOP), 2, 5-diphenyloxazole (PPO), thixotropic gel powder and toluene-C^{14} from the Packard Instrument Company; and Tween 80 and Span 80 from the Atlas Powder Company. The dog food used was a commercial brand, Gaines Meal.

Maintenance of the Insect Colony

Adult Leucophaea maderae were obtained from the Oregon State University Entomology Department and were transferred to a chest of drawers with screened fronts and glass tops. Each of two drawers contained from 75-100 adults. The young nymphs produced each week were removed by aspiration and 50-60 were placed in each of two 2. 5 gallon ice cream tubs with screen centers in the lids. Both young and adult insects were nourished by dog food and water only. Breeding drawers and rearing tubs were checked weekly and food and water added as needed. The presence of mature males in the rearing tubs signaled the approaching final molt of the female nymphs, because the males attain maturity after approximately five months and seven instars, whereas the females pass through eight nymphal instars over a period of approximately six months. All the

insects were then sexed and the large female nymphs accumulated in additional modified drawers. They could then easily be observed in large numbers and newly molted females removed as experimental demand required. The remaining mature females were removed weekly and placed 30 per 2. 5 gallon tub with an equal number of males. In this way there was a constant supply of recently mated females of similar ages which served as donors of active CA.

For the past year all insects have been held in a 11' x 6' x 8' room with temperature controlled at 80 ± 2° F, relative humidity at 50 ± 2%, and photoperiod at 14 hours of light per 24 hours. The quality of light was as close to daylight as is available with fluorescent tubes. Prior to this time only photoperiod was controlled.

Surgical Equipment

Dissection instruments used were of the type sold by most scientific supply companies under the description "ultra-fine". These tools were washed in 65% ethanol before and during every operation. Measurement of oöcytes was made with an ocular scale calibrated against a stage micrometer.

For forced-feeding a standard two ml syringe was used to which was attached a Luer-type 30 gage stainless steel needle. A piece of polyethylene tubing measuring 0. 011" I. D. x 0. 024" O. D. (number 10) x 0. 85" long was forced over the needle so that about 0. 75" extended beyond the tip. By warming between the fingers and repeated

bending, a curve in the tubing was obtained which most closely resembles that of a long-shanked fish hook from the eye to the deepest part of the hook (Figure 2, p. 20).

ISOLATION OF ABDOMINA

Preliminary Experiments and Considerations

In order to provide any substantial advantage over the use of intact experimental animals, the procedure for the isolation of abdomina would have to be simple, involving a minimum of damage and loss of body fluid, and the life span of the isolates sufficient for egg maturation.

In preliminary experiments the abdomina of females 8-12 days of age were cut free of the thorax with scissors. The resultant wounds were extensive and it was necessary to cover the area with melted paraffin. The results were evaluated on the basis of the heart beat and by the cercal response, which should be quick and pronounced. The heart beat is normally quite regular, with a rate of 60 beats per minute ± 10 and an amplitude of approximately two mm, and is easily observed under low magnification. Although survival among these animals was erratic, there was sufficient success to warrant continued efforts, particularly following the observation that abdomina were more viable if the wound was held closed and very rapidly sealed.

It was realized that ligation, rather than cutting, would offer several advantages, both in reducing the area of open wound and in facilitating the procedure. However, the cuticle of the roach is

pliable enough to permit this approach only for a short period following ecdysis, and very large numbers of nearly mature female nymphs would be required for adequate selectivity. On the other hand, limitation of selection to newly molted females which have not yet begun the process of hardening and darkening of the cuticle would restrict physiological variation to a clearly defined stage. By performing the isolation at the time when the cuticle is soft, the constriction would be so small as to completely eliminate fluid loss and the healing processes aided. Also, ligation would effectively separate the wound area from the abdomen itself.

The principal disadvantage to this approach is that the insect must be obtained during a period which, in relation to its life span, is extremely short. It was hoped that individual broods of nymphs reared under constant conditions would provide reasonable numbers of individuals maturing with some degree of synchrony. The result, however, was that the final molts of any 20 animals were scattered over a period of a month. It was found that a rearing program producing 100 to 150 newly hatched nymphs per week would provide, six months later, a sufficient number of large nymphs to give the selectivity required. Since two or three hours are required for the molt to be completed to the point of full wing expansion, a check of the holding drawers two or three times a day detected those molting within a 12-14 hour period. Up to one dozen experimental animals could be obtained each day in this manner.

The adoption of thread-ligation at ecdysis quickly led to improved results and permitted the experiments which are discussed immediately below. Survival of isolated abdomina was usually 80-90% of the total number ligated when the final procedure was used (see below), and nearly all of those which died did so within the first three days. Depending upon the care with which selection was made, 50-75% of the abdomina isolated could be expected to be satisfactory for experimental purposes. The most common failure observed was a weakening of the heart beat, manifested by flaccid stroke and reduction in the amplitude. It was noted that successful isolates did not seem to deteriorate for a long period of time. That is, according to the criteria expressed above, the condition of abdomina which survived well the first few days could be expected to remain quite constant. Many have been kept several months and a few were held for nearly five months and still appeared to be in good condition. The experiments utilizing labelled substrates which are discussed later also pertain to this subject.

Forced-feeding immediately prior to the isolation was found to contribute to the success of the operation. Abdomina taken from individuals which had not been force-fed prior to isolation survived only at the rate of 20% to the 20th day (N=10 animals) and appeared to be desiccated upon dissection. When force-fed 0.15-0.30 ml of saline, desiccation was prevented and survival was improved (50%,

N=18). Since glucose is absorbed freely from the gut at rates apparently governed by its conversion in the hemolymph to trehalose, the principle blood sugar (67), it was considered to be a desirable material for forced-feeding. By providing each isolate with 0.15-0.30 ml of a 10% solution of glucose the 80-90% survival rate mentioned above was achieved.

Ovaries in the early stages of development have been found to be principally involved in the deposition in the oöcytes of specific yolk proteins by the follicle cells (60, 68). Large increases in blood protein concentration were shown to occur concurrently with oöcyte development (27, 29), and a source of amino nitrogen was desired for the isolates. A requirement for free amino acids by the isolated abdomen was felt to be possible in the light of the finding by Thomsen and Møller that in Calliphora the product of the NSC has an effect on the production of proteolytic enzymes in the gut (66). The amino acids from acid-hydrolyzed casein were used because they provide the full spectrum of amino acids and a low sodium chloride content and were not injurious to isolates when injected at 3% concentration. Twelve animals injected with 20 µl were as healthy after ten days as controls which were not injected. A solution of 3% casamino acids and 2% glucose was as satisfactory from the point of view of survival as glucose alone (90%, N=10). Raising the amino acid level to 5% reduced viability to 33% (N=6), and a 15% solution was completely lethal (N=6).

The Isolation Procedure

Forced-feeding

On the basis of the preliminary trials the following procedure was developed for the successful isolation and maintenance of abdomina.

Newly molted females were removed from the holding drawers and anaesthetized with CO_2 while the cuticle was still white and soft, but after the wings had fully expanded. The animal was held by the prothorax between thumb and forefinger with the head at right angles to the plane of the body and the clypeus resting on the inside tip of the thumb (Figure 2). The syringe with the polyethylene tube attached, described in the Materials section, was held in the other hand. With the aid of a binocular microscope under low magnification the tip of the tubing was gently pushed under the clypeus. The angle formed between the syringe and the animal was about 45^{o}, with the curve of the tubing pointing downward toward the thumb. The clypeus was lifted with gentle downward pressure of the syringe which levered the tubing upward against the thumb. When the tubing was well under the clypeus, it passed above the tips of the mandibles and between their bases. The tip of the tube was gently forced upward and into the end of the pharynx with a downward movement of the syringe accompanied by forward pressure. The curve of the tube then passed around the

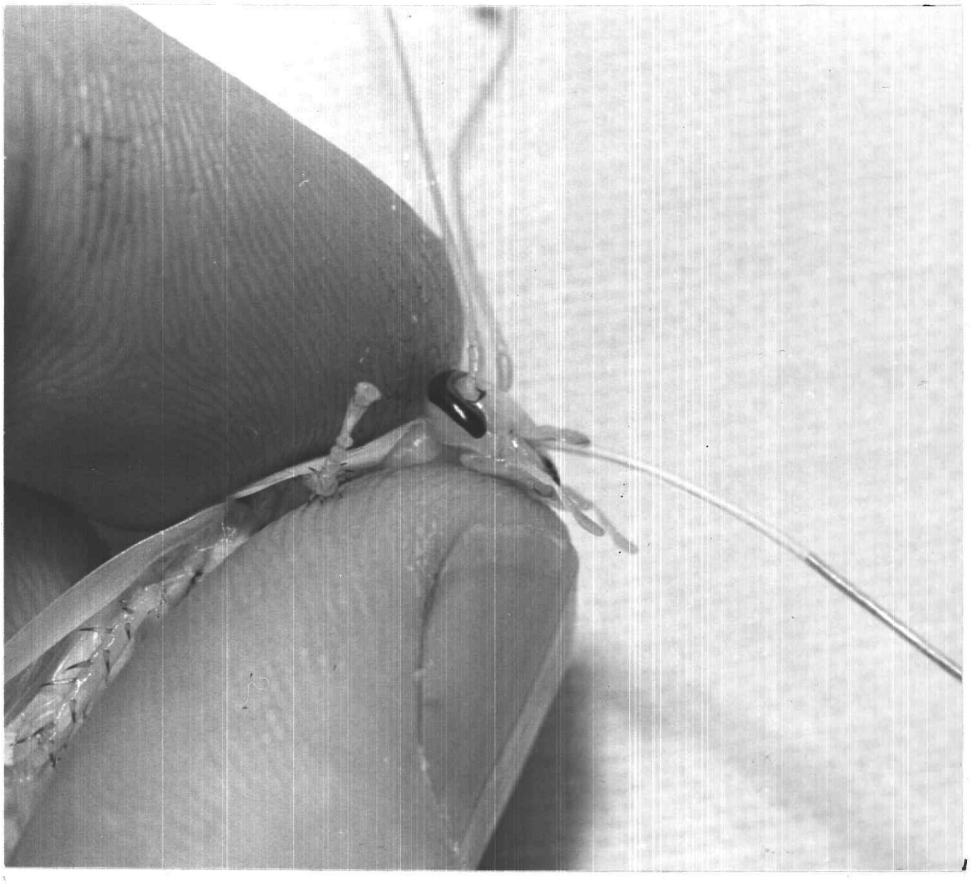

Figure 2. Photograph of a recently molted female L. maderae being force-fed.

angle formed between the buccal cavity and the pharynx, and the entire length entered into the esophagus. The desired volume of liquid was then expressed into the crop. Up to 0.3 ml was usually retained without regurgitation. Once regurgitation commenced it was usually extensive and could not be overcome.

Ligation

In order to position the thread for ligation it was necessary to remove the wings, which were simply clipped off. It was also advantageous to remove the legs. With the insect on its venter, a loop of thread with a surgeon's knot was placed over the tip of the abdomen. By reaching through the loop with forceps, the tip of the abdomen was grasped and lifted upward and anteriorly, lifting the first abdominal segments away from the metatarsi. The thread was placed as far forward as possible against the metacoxae (Figure 3) and the knot tightened while simultaneously pressing on the thorax with one finger to insure the entire crop being forced behind the ligature. The thorax was then cut free with scissors and the tissue around the knot trimmed. Bleeding through the knot seldom occurred unless the cut tissue came in direct contact with another surface before healing. Isolated abdomina are shown in Figure 4.

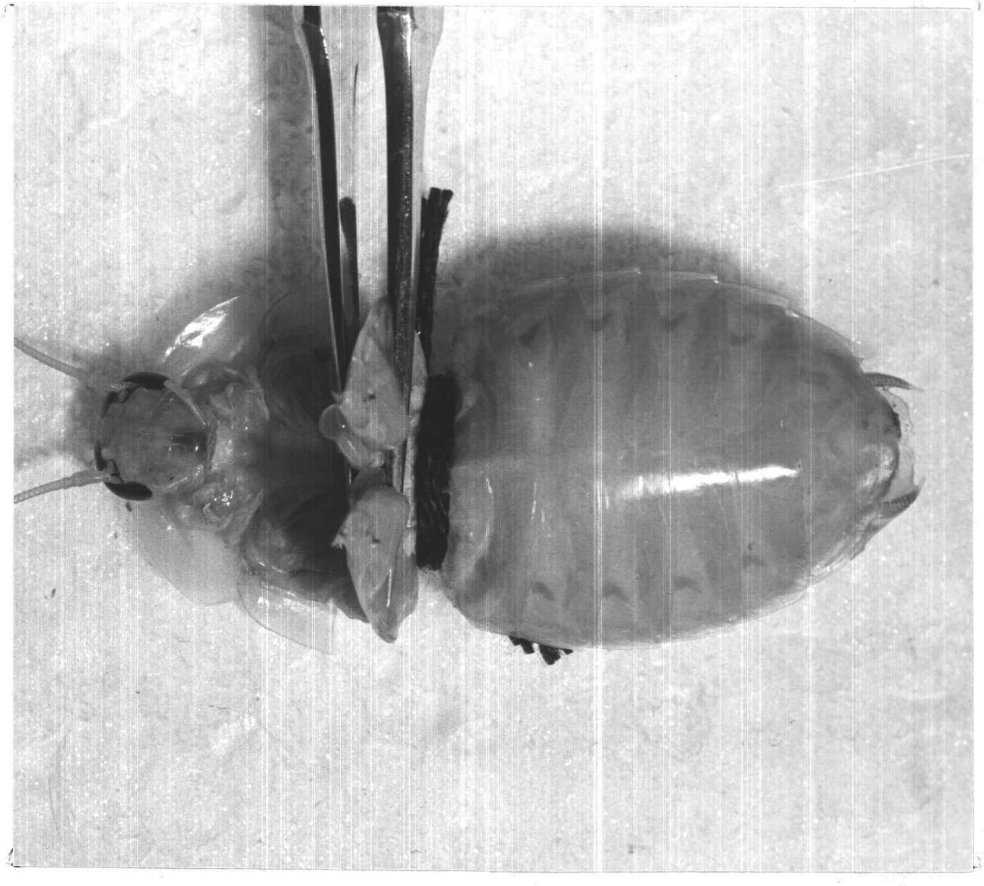

Figure 3. Photograph of the ventral aspect of a female L. maderae with the legs and wings removed and a thread positioned for ligation of the abdomen. A coarse black thread is shown here for contrast and visibility. The metathoracic coxae are held with forceps so that the position of the ligation can be seen.

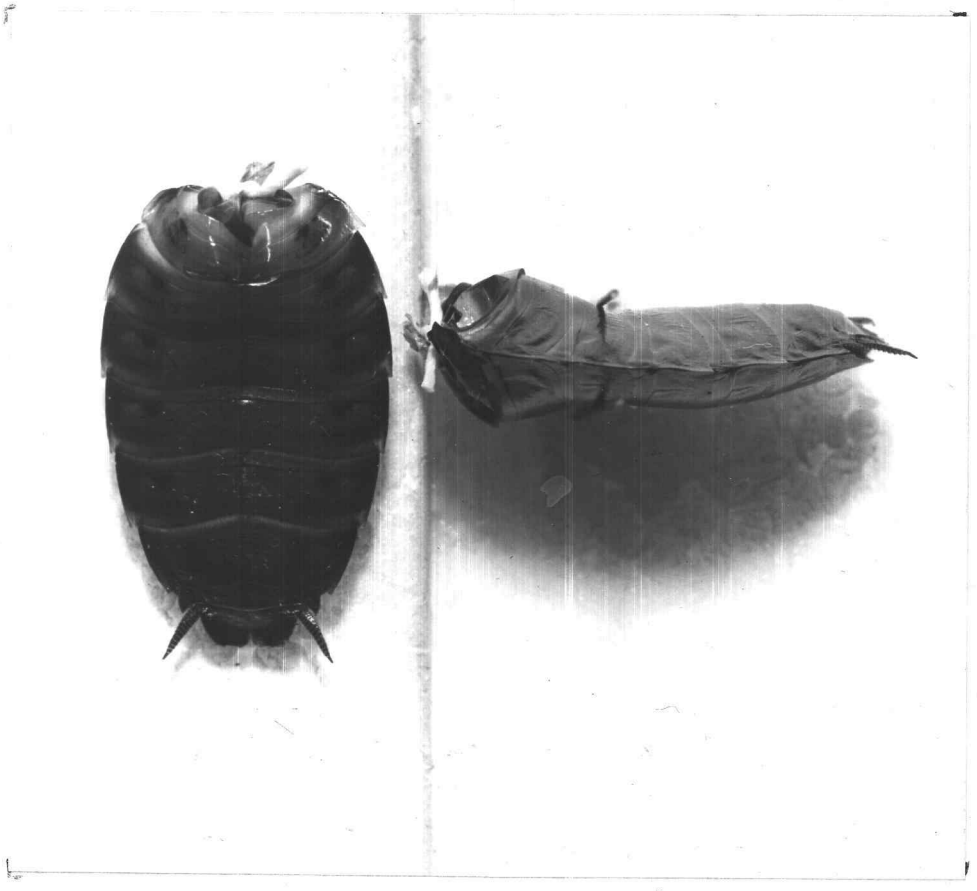

Figure 4. Photograph of the dorsal (left) and lateral (right) aspects of isolated abdomina of L. maderae. Abdomina average 2.3±0.2 cm in length and 855 ± 175 mg in weight.

Activity of the Isolated Abdomina

Immediately upon recovery from anaesthesia the isolated abdomina entered a short period of muscular activity involving both lateral and longitudinal contractions. Within an hour this activity ceased and the isolates remained quiescent unless stimulated by touch or heat. Darkening and hardening of the cuticle appeared to proceed normally and by the tenth hour the area of ligation was hard and the cut surface dry. As observed under low magnification, activity from this time on appeared to remain at an unvaried low level unless the isolate was physically stimulated. The heart beat usually remained strong and continuous and the digestive tract could be seen to undergo rhythmic activities. According to Davey (5, 6, 7, 8) acceleration of the heart beat is mediated both by innervation from the ventral nerve cord and retrocerebral complex and by endocrines released from the CC. It is evident, however, that normal rhythmic activity is possible for long periods in the absence of stimulus from the anterior region.

The excretory system continued to function and occasionally small amounts of dry feces were found, indicating that the insect was maintaining water balance. Respiratory movements were only occasional, and it is assumed that since the organisms were at a low level of activity, free diffusion through the spiracles provided sufficient gas exchange in the absence of active ventilation movements.

That respiration was active is demonstrated in the labelled acetate experiments described later.

Blood circulation was checked by injecting a small amount of amaranth, a dye which is soluble in insect blood and not readily taken up by the tissues. Ten µl of a 2% solution injected at the extreme anterior produced reddening easily visible under low magnification and direct illumination. The color slowly diffused posteriorly, and by ten minutes blood collected from the cerci was colored. Samples taken after 20 minutes were uniformly colored as measured colorimetrically.

THE PHYSIOLOGY OF THE INITIATION OF EGG DEVELOPMENT

The Normal Reproductive Physiology of Leucophaea

Each of the two ovaries in the abdomen of this insect consists of 20 or more tubules containing numerous undeveloped oöcytes and terminal follicle cells. The length of the terminal oöcytes at the time of the imaginal ecdysis is 0.97 ± 0.01 mm (48). When the CA become active, the ovarioles begin the deposition of yolk in the terminal oöcytes and these increase in size. At maturation the oöcytes average 5.56 ± 0.02 mm in length. Most females mate nine days after ecdysis (46) at which time the oöcytes average 1.08 ± 0.01 mm (48). Engelmann (18) found that the oöcytes of mated females develop sooner and within a more predictable period of time following ecdysis than do virgins. Once yolk deposition has begun the oöcytes rapidly increase in size and become mature in 20-25 days (48). The eggs then pass into the uterus, are fertilized and are enclosed in an oötheca formed principally of proteins secreted by the accessory (colleterial) glands, designated as A_2 by Engelmann (15). The oötheca contain an average of 33.8 eggs (78) and are retained by the female until the eggs hatch. This is referred to as the period of pregnancy and lasts 91.8 days (48).

During gestation it is critical to the animal that no new oöcytes

develop, since the abdomen could not contain both an oöthecum and developing oöcytes. Engelmann found that the CA in pregnant females are inhibited (15) and that inhibition is exerted by the brain by way of nerves leading to the CA (18). When the eggs hatch, the CA resume activity and the new terminal oöcytes begin to develop. The number of nymphs hatching may vary from 18 to 42 (47). Roth and Stay (48) found that passage of the second group of matured oöcytes into a new oöthecum occurs 27.8 ± 0.3 days after hatching of the first.

Scharrer (52) found that the accessory glands are activated by the CA and that they function independently of the ovaries. Several workers have since used the presence of secretory material in the tubules of the accessory glands as a criterion for activity of the CA (2, 32, 46). Measurements of fixed specimens of accessory glands have been made (52); but it is possible to make valid, although somewhat subjective assessment of their condition by observation with the aid of a dissecting microscope. This technique was used in the experiments reported here.

In order to clarify the evaluations of sex organ response to CA hormone which will be made later, it is necessary to describe now the relationships between increasing degrees of accessory gland response and the growth of terminal oöcytes. This will require the presentation of some data which are drawn from experiments described later in the text, but this is justified by the absence of

quantitative descriptions of this process in the literature.

In the immature female, and in the animal without active CA, the accessory glands are small and the numerous tubules are flattened and transparent (Figure 5). In isolated abdomina which did not receive CA and were used as controls in later experiments, the oöcytes averaged 0.96 ± 0.06 S.D. (N=24). Isolates which did receive CA but did not show any accessory gland response, designated as negative, contained oöcytes which measured 0.97 ± 0.09 mm (N=16). These measurements compare favorably with the figure reported by Roth and Stay (48) for the average length of terminal oöcytes of females immediately following ecdysis, which was 0.97 ± 0.01 mm.

The second condition observed is referred to herein as enlarged. As the accessory glands respond to CA hormone they increase in length and diameter and begin the deposition of material within the tubules, which gives them a white opaque appearance. This condition never occurs in the absence of CA, and it precedes measurable growth of the oöcytes, which does not become obvious before they have grown to 1.15 mm or more in length. Enlarged glands may be distinguished from completely responsive glands by their flattened aspect and the lack of color. In isolated abdomina implanted with active CA in which the accessory glands were designated as enlarged, the terminal oöcytes averaged 1.01 ± 0.09 mm

(N=11), which falls within the range of the control animals.

Full activity of the accessory glands, designated here as positive, was usually observed in conjunction with obvious increase in length of the terminal oöcytes. In isolates in this condition the oöcytes usually measured more than 1.16 mm in length and averaged 1.45 ± 0.28 mm (N=28), and the accessory glands were completely turgid and filled with bluish-appearing secretory products (Figure 5).

These measurements were all made on the tenth day after the addition of CA activity to the isolated abdomina. The average length of the oöcytes of a normal female Leucophaea ten days after mating was 2.50 ± 0.08 mm (N=20), indicating that yolk deposition in the virgin isolate, as in the virgin intact female (17), is not as rapid as in the mated female.

Figure 5. Photograph of the left colleterial glands of three female L. maderae. At top center are glands of the type classified as negative. The glands at bottom left show the enlarged condition and those on the right are positive. The bluish color of the positive glands causes the photograph to appear less opaque than they do under visual observation. The length of the longest tubule in the positive gland is 3.06 mm.

Oöcyte Development in the Isolated Abdomen

Methods

CA were obtained from females whose terminal oöcytes were between 1. 67 and 5. 0 mm in length. During this period deposition of yolk is occurring and the CA are active. The insects were individually anaesthetized and the abdomina opened for examination of the oöcytes. A suitable donor, from which the thorax and abdomen had been removed, was held on paraffin in a petri dish by a bent pin forced firmly across the anterior margin of the prothoracic shield with the pressure applied by a pair of crossed pins. This pressure, which held the frons firmly against the paraffin, completely exposed the cervical region and greatly reduced the flow of blood from the prothorax. The cervical membrane and the narrow longitudinal sclerite which lies on its dorsum were then cut adjacent to the head and the sclerite pulled to one side, where it was held by the pressure of the pronotal shield. Since the donor was being sacrificed, the tissue overlying the CA was simply removed and the area flooded with saline. With the aid of a dissecting microscope, high magnification, and intense illumination, the CA were located and freed of the connective tissue which held them in place, and the recurrent nerve was severed. Each CC was grasped at its mid-point and pulled free. Both CA could then be lifted out together in undamaged

condition by holding the CC with the forceps. The glands were placed in saline until the desired number were accumulated.

Intact glands were implanted into isolated abdomina through a small triangular opening (approximately 4 mm^2) which was cut in the left side of the fifth ventral sclerite. The glands were inserted through the opening with forceps and the cuticle replaced and sealed with melted paraffin. Loss of body fluid rarely occurred during this procedure. Later dissection showed that the glands remained free in the pericardial sinus close to the site of insertion and in apparent good condition.

In some experiments it was necessary to introduce nutrient solutions or substrates into the isolates. This was accomplished with a 30 gage stainless steel needle inserted through the intersegmental membrane between the fourth and fifth tergites just mediad to the muscle insertion area. The needle was moved anteriorly until the tip was immediately below the second or third tergite, thus preventing the escape of expelled fluid through the entry wound. Bleeding was rarely observed.

The procedures involved in the forced-feeding and isolation of abdomina were accomplished as described above. Animals were obtained from the holding drawers and had normal dietary histories unless stated otherwise. Where dietary restrictions were to be imposed prior to the final molt, animals were maintained individually

in glass containers in order to eliminate cannibalism. It was necessary to hold large numbers of nymphs in this manner, since it was impossible to anticipate when individuals would commence the final molt. The duration of this holding period was as long as 30 days, and animals which attained maturity before the fifth day were discarded.

The Initiation of Oöcyte Development

In the first attempts to initiate egg development in the isolated abdomen, CA were removed from selected donors and homogenized in two drops of saline in an all-glass micro-homogenizer. The homogenate was then drawn into a syringe and injected into an isolated abdomen. Unfortunately, at the time these experiments were carried out the greater sensitivity of the accessory glands to CA hormone was not appreciated and all response evaluations were made on the basis of oöcyte growth. Nonetheless, since 18 animals were tested and none showed any growth of the terminal oöcytes beyond that of the control animals, it can be assumed that homogenates do not exhibit gonadotropic effect. At least two pairs of glands were injected at each time, and three abdomina received five injections of homogenate over a 15-day period. Five were injected twice and ten were injected once, with examination following seven to ten days after the first injections and three to five days after the last

injections. Most animals were at least eight days old (post-isolation) at the beginning of the experiment.

Two sets of intact glands were then implanted into three abdomina five days after isolation, and ten days later one had developing oöcytes, the largest of which was 1.4 mm long. Five abdomina were held ten days after isolation and implanted with two pairs of glands. Examination ten days later showed that three of these had well developed oöcytes. The accessory glands of these eight animals were examined and four of them showed positive response. An additional eight animals were then implanted with three pairs of CA nine days after isolation, and four of these responded on the basis of the accessory glands, two having oöcytes undergoing yolk deposition. Seven abdomina were implanted nine days after isolation with active CA which had been cleaned as completely as possible of any adhering fragments of CC tissue. Four of these had positive accessory gland response and two had developed large oöcytes after a ten-day holding period. Implantation of only CC had no effect on the sex organs (N=6). These results were interpreted as indicating that the attached cardiacal tissue does not contribute to the gonadotropic effect. Due to the fragility of the CA, much more care must be taken during manipulation and implantation when no cardiacal tissue is present to serve as a 'handle'. More donors are required since more glands are damaged and made unsatisfactory for experimental use. The

CC have been shown to elevate the rate of heart beat when implanted into roaches (5) and to cause an increase in blood protein levels in the desert locust (27); but these effects were not of more than 24 hours duration. For these reasons it was felt that inclusion of fragments of CC tissue in the implantations carried out in this study was acceptable and would not interfere with evaluations of CA activity.

The Amino Acid Requirement for Oöcyte Development

The nymphal feeding requirement and the importance of the post-ecdysial feeding were examined with abdomina isolated from animals which had been held on a diet of cube sucrose and water prior to the final molt. After ecdysis, selected animals were force-fed 10% glucose, a solution of 3% casamino acids and 2% glucose, or a 2% whole casein and 2% glucose solution. According to the manufacturer's label the last two solutions would be equal in nitrogen content.

A group of abdomina from sugar-fed, glucose force-fed animals was injected with 50 µl of casamino acid-glucose solution three days after isolation. For comparison, animals held on the normal diet were force-fed saline, glucose or casein-glucose upon isolation. Controls were fed normally and force-fed casamino acid-glucose. In this experiment all animals were held for 8-10 days after isolation of the abdomina, implanted with two pairs of active CA and held an additional ten days before dissection. The results are given

in Table I. Animals to which only sugar was available both before and after ecdysis were not able to respond to active CA with either yolk deposition in the oöcytes or complete activation of the accessory glands. The introduction of amino acids into such isolates by injection made response possible, and a forced-feeding of amino acids raised these responses almost to normal levels. Whole casein, however, did not contribute to the responsiveness of animals fed either dog food or sucrose as nymphs. The significance of the post-ecdysial feeding as compared to the nymphal diet is seen in the animals fed chow as nymphs and force-fed saline before isolation. In this group no oöcyte development occurred and only very little accessory gland response, whereas over 50% of those force-fed amino acids responded positively and over 33% developed large oöcytes.

Time-course of Sex Organ Responsiveness

It has been noted that females usually do not mate prior to approximately the ninth day after ecdysis and that up to this time the oöcytes do not begin the deposition of yolk (46). However, the ability of the oöcytes of females younger than this to respond to active CA had not been examined. To do this the holding period following isolation and prior to implantation of CA into isolates was varied. After being held an additional ten days the animals were

Table I. Effect of nymphal diet and force-fed nutrients on the response of the reproductive organs to corpora allata.

Nymphal diet	Forced-feeding	Injection	N[a]	Number with large oöcytes[b]	Accessory gland response		
					Positive	Enlarged	Negative
sucrose	glucose	------	10	0	0	4	6
sucrose	glucose	amino acid[c]	14	3	4	3	7
sucrose	amino acid	------	11	3	4	4	3
sucrose	casein	------	5	0	1	3	1
dog food	saline	------	9	0	1	2	6
dog food	glucose	------	3	0	0	2	1
dog food	casein	------	5	1	2	2	1
dog food	amino acid	------	17	6	9	3	5
dog food	amino acid	amino acid	11	4	5	3	3

[a] N = number of experimental animals.

[b] Oöcytes larger than 1.15 mm in length

[c] A solution of 3% casamino acids - 2% glucose.

examined for accessory gland and oöcyte response. It was found that very little response occurred until the sixth day (Table II). At this age the number of positive accessory gland responses rose to approximately 50% and remained at this level thereafter. It will be noted, however, that there was less reliability in oöcyte response. While the maximum response was usually 25-33% of the implanted abdomina, occasionally up to 50% responded with developing oöcytes.

An attempt was made to determine whether this variation in response and its limitation to a maximum of 50% was due to nutrient deficiency or to differences in CA activity. Eleven animals were force-fed casamino acid-glucose solution and their abdomina isolated. Ten days later they were implanted with two pairs of CA and injected with 50 µl of casamino acid-glucose solution. After ten days they were examined and no increase in response was observed. Four developed growing oöcytes and five had positive accessory glands. An increase in the number of pairs of CA to three had been shown earlier to give the same response level as two. Therefore, the possibility was examined that the responsiveness of the sex organs might be enhanced by early implantation of glands combined with a longer holding period prior to examination. Active CA were placed into eight abdomina on the third day after isolation and the holding period increased to 17 days. The results were similar to those obtained with the normal procedure--two developed large oöcytes and four displayed positive accessory glands.

Table II. Responsiveness of reproductive organs with respect to post-molt age.

Post-isolation[a] holding period	N	Number with developing oöcytes	Accessory gland response		
			Positive	Enlarged	Negative
0 - 1	6	0	0	2	4
2 - 3	7	1	1	0	6
4 - 5	6	1	1	1	4
6 - 7	17	4	8	3	6
8 - 9	17	6	9	3	5
10 - 11	9	2	4	0	5
12 - 14	5	1	3	1	1
15 - 25	15	7	7	3	5

[a] Number of days after molting and isolation and before implantation of glands.

Implantation of Brains

Roth (45) has pointed out that the maximum rate of oöcyte development in Leucophaea apparently requires the combined stimuli resulting from both mating and feeding. He suggested that mating provides additional stimulus to CA which have already been partially activated by the brain as a result of feeding. The fact that the average level of positive accessory gland response in isolated abdomina was only 50% of the implanted animals also suggested the possibility that the CA require continuing stimulation from the brain in order to maintain high levels of activity. It is possible to remove the entire protocerebrum of the cockroach and to maintain intact the nervi corpora cardiaci, the CC and the CA. In this manner the entire neurosecretory complex can be transplanted with a minimum of disruption of its integrity. Seven attempts were made to implant such intact systems into isolated abdomina. It was necessary to cut a much more extensive opening into the abdomen than for CA implants, and extensive loss of haemolymph occurred. The tissue of the brains was badly damaged as a result of the manipulations involved in attempting to force the implants into the abdomina. Later dissection showed that the brains had almost completely disintegrated and only the CA and CC remained intact. As a result of the severe treatment the condition of six of the abdomina was very poor after ten days.

The single isolate which survived in good condition did not develop large oöcytes but did contain positive accessory glands.

Activity of CA in Relation to the Reproductive Phase of the Donor

In Leucophaea the CA of females bearing oötheca are inactive. By placing CA from pregnant females into ten-day-old isolated abdomina, it was possible to determine whether these glands, their nervous connectives to the brain having been severed, would return to activity. Eight abdomina were implanted with two pairs of CA from pregnant donors following the usual forced-feeding, isolation and holding procedures. None of these showed any indication of accessory gland response, either positive or enlarged, or ovarian development ten days later. Four abdomina implanted with four pairs of inactive glands also failed to respond in any way. The report by Engelmann (18) that inactive CA placed into allatectomized intact females will return to activity was verified. Five animals treated in this manner all developed large oöcytes (1.89 ± 0.01 mm) and active accessory glands in ten days, whereas mock-operated allatectomized females were negative (N=3).

Mock Gland Implantations

In all of the above experiments and in those to be discussed, abdomina were included which were treated in the normal manner except that small pieces of leg muscle, comparable in size to two

pairs of CA, were implanted instead of glands. A total of 18 animals were mock-operated; upon examination only one had accessory glands which might have been classified as possibly enlarged, and they contained no secretion. None of the isolates showed positive accessory glands or oöcyte enlargement.

Bioassay of Gonadotropically Active Materials

Farnesol, applied as an external smear, and injected juvenile hormone extracts of the cecropia silkworm have been reported to initiate yolk deposition in the oöcytes of allatectomized Periplaneta (3) and in decapitated Cimex (9). These materials were tested on Leucophaea isolates in the following manner. Fifty µl of farnesol were applied to the dorsal surfaces of 11 abdomina on the tenth day following isolation. The activity of a juvenile hormone extract, generously supplied by Dr. Carrol M. Williams, was examined by injection in 27 isolates. The material was received as a crude extract carried in peanut oil and was tested in this form. Eleven abdomina received ten µl, eight received 20 µl, three received 30 µl and five received 50 µl of the extract. Ten days later these, and the farnesol test animals, were compared with control animals which had been injected with 50 µl of peanut oil.

The known toxicity of farnesol was reduced by the method of application as has been reported by others (3). Nine of the 11

treated abdomina survived to the tenth day and were observed to be in good condition. Four possessed accessory glands which were slightly enlarged and contained some secretory material. All of the others were negative in this respect and none displayed any evidence of yolk deposition in the oöcytes. Evaluation of the cecropia extract is obscured by its toxicity to the isolates. All of those which were injected with 30 or 50 µl of material died soon thereafter. Nine of the 11 which received ten µl survived and one showed slight accessory gland response. Six of the isolates treated with 20 µl lived and four evidenced slight accessory gland response. Three of these gave some indication of anomalous oöcyte activity. Although the eggs had not increased in length, they were twice the diameter of normal undeveloped oöcytes (0.40 mm). All of the control animals survived and none showed any sex organ development.

UTILIZATION OF C^{14} LABELLED SUBSTRATES

Methods

Three isotopically labelled materials, glucose-U-C^{14}, acetate-1-C^{14} and leucine-U-C^{14}, were administered to isolated abdomina for examination of the variation in oxidative metabolism in isolates and to study the utilization of the last two materials in the presence of CA hormone. Procedures for rearing the insects, preparation of the abdomina, implantation of glands and injections were carried out as described above. Blood samples were taken by clipping one of the cerci and collecting drops in 0.2 ml of distilled water.

Collection of Radioactive CO_2

For purposes of comparison of the respiratory function of different animals and treatments the radio-respirometer described by Wang et al., (70) and by Silva et al., (57) was used. The instrument, as adapted for these studies, consisted of a 50 ml round-bottomed flaskinto which the abdomen, injected with labelled substrate, was placed. The animals were kept at constant temperature (30° C) for the duration of the experiment by immersing the flasks in a conventional Warburg water bath. Each flask was connected with aeration tubing which supplied a metered amount of air (seven ml per minute). The air then passed through an outlet leading to a CO_2 trap consisting

of a sparger which was immersed in ten ml of a mixture of ethanol and monoethanolamine (2:1). The sweep gas could be routed via a three-way stopcock into either of a pair of these traps for each animal flask, and the flow could be diverted from one trap to the other by switching the stopcock, thereby permitting the sampling of gas at desired intervals without interrupting the flow through the animal flask. The trap solution for each time period was removed from the trap with a small amount of absolute ethanol as a rinse and the volume made to 15 ml with the alcohol.

Lipid Extraction

At the end of the experiment CO_2 trapping was terminated; the animals were quickly dissected, the terminal oöcytes measured and the condition of the accessory glands evaluated. The abdomina were then killed in ten ml of absolute ethanol. Each was homogenized in an all-glass homogenizer, the homogenate placed in a 50 ml flask and the volume brought to 20 ml with 25% KOH. Boileasers were added and the solution allowed to saponify under nitrogen on a steam table for 8-12 hours. Shake extraction of unsaponifiable lipids was carried out three times with 20 ml volumes of diethyl ether, the extracts pooled and dried overnight by adding 100 g of anhydrous sodium sulfate. Sufficient sulfuric acid was added slowly to the aqueous fraction to bring the pH to two, and the saponified material was

extracted with three 20 ml volumes of petroleum ether (BP 30-50°). These extracts were also pooled and dried. The ether fractions were then reduced to dryness on a rotary evaporator at 60° or less. The saponified material was redissolved in petroleum ether and the unsaponified fraction in absolute ethanol, and the volumes were made to ten ml.

Protein Extraction

The incorporation of label from leucine-U-C^{14} into the proteins of blood and fat body was measured by precipitating the proteins from aqueous solution and determining the number of disintegrations per minute (DPM) per μg of protein present.

Blood samples were drawn from the cerci of each isolate and the sample of blood in water was immediately frozen by immersion in a bath of dry ice and acetone. The abdomen was then dissected, the condition of the sex organs noted and the fat body removed and placed in saline in a watch glass. Contaminating material such as tracheal and malpighian tubule fragments was picked out with forceps, and the tissue was placed on filter paper in a suction funnel and rinsed with a continuous fine stream of saline for 30 seconds. Suction was continued until all extra liquid was removed. The tissue was then placed in a small all-glass homogenizer and homogenized in one ml of 10% trichloroacetic acid (TCA). The

homogenate was drawn off, the homogenizer rinsed with an additional two ml of 10% TCA and the liquid combined in a conical centrifuge tube and frozen in the dry ice bath.

Twenty-four hours after homogenization the samples were thawed and three ml of 10% TCA added to the blood solutions. Extractions of both blood and fat body protein were carried out in the same manner. All samples were stirred on a vortex type mixer and centrifuged at low speed. The liquid layer was decanted and the nucleic acids extracted from the precipitate with a hot wash (90^{o}) of 5% TCA with mixing. The proteins were again sedimented with centrifugation and the liquid decanted. Successive washes followed by centrifugation were carried out to remove excess TCA and to extract lipids, using one wash with absolute ethanol and two with chloroform-methanol (2:1), respectively. The volumes of all extractions and washes were three ml for the blood samples and five ml for the fat body.

Following the final wash the protein was dried under a fine stream of nitrogen and dissolved in warm (50^{o}) 0.1 N NaOH. The blood samples were made to one ml and the fat body to ten ml. Protein concentration was measured using a modification of the Folin technique as given by Lowry (40). Values were obtained by comparison with standard curves of crystalline bovine serum albumin. After color development samples were read in a Beckman

Spectronic 20 spectrophotometer at 500 mμ against a saline blank.

Measurement of Radioactivity

Five ml of each CO_2 trap solution were pipetted into a 20 ml screw-top vial with ten ml of a scintillation mixture consisting of ten ml of toluene, 60 mg PPO and 1.5 mg POPOP. The solutions were counted in a Tri-Carb two-channel liquid scintillation counter at a high voltage setting of 1250 volts. A known amount of toluene-C^{14} was added as an internal standard to selected samples and these vials counted again to determine the absolute efficiency of the counting system.

$$\frac{a-b}{c} \times 100 = \text{percent efficiency}$$

a = observed counts per minute (CPM) due to unknown and internal standard

b = observed CPM due to unknown

c = known DPM of internal standard

The radioactivity of the $C^{14}O_2$ was expressed as the percent of the administered activity. This can be most easily done by standardizing the substrate and calculating a factor, D, which when multiplied by the observed CPM of the sample gives the %IR (percent of administered activity recovered per interval of time).

$$D = \frac{(100)\,(d)}{(y)\;\;(z)}$$

d = dilution of the sample

y = DPM of administered activity

z = counting efficiency of the sample

Five ml samples of the lipid extracts were assayed for radioactivity in the same manner as the CO_2 fractions. However, in this case the counting efficiency of every vial was measured with an internal standard, since considerable variation in the amount of color quenching may occur in lipid extracts. The total DPM in each extract were calculated and the results expressed as the percent of administered activity recovered per fraction or per unit mass of lipid. For graphical presentation the CO_2 recovery data were plotted as cumulative %IR.

Measurement of protein radioactivity was made on 0.5 ml samples of both blood and fat body material. Each sample was suspended in 14.5 ml of thixotropic gel drawn from a stock solution. The gel was prepared by mixing 750 ml of toluene scintillation fluid, 6 ml of Tween 80-Span 80 (1:9), 7.5 ml of glycerol and 20 g of thixin in a blendor. The samples were thoroughly mixed and counted in the scintillation counter. Due to the probability of variation in counting efficiencies using gel, all samples were spiked with an internal

standard and absolute efficiences were determined. The results were expressed as DPM per mg of protein.

Results

Variation in Oxidative Metabolism

Variation in the production of $C^{14}O_2$ was considered as a possible index of the degree to which abdomina differed in their response to isolation. In a similar manner, changes in oxidative metabolism over a period of time would be a measure of deterioration in individual isolates during their existence.

The oxidation of glucose was performed over an extensive period using two abdomina. These were injected with 200 µl of saline containing 25 µM of glucose with a specific activity of 2.8×10^4 DPM per µM. The $C^{14}O_2$ evolved was trapped at 12 hour intervals. The animals were re-injected with the same substrate at 144 hours and trapping continued until the 288th hour. Total $C^{14}O_2$ recovered from the first injection represented 27.8% and 29.2% of administered activity for the two animals. Activity trapped during the second period represented 34.4% and 36.9% of administered activity for these two isolates. The latter figures are higher than the former because some glucose administered at the first injection remained to be oxidized during the second trapping period. Figure 6 presents the time course of cumulative %IR for each animal.

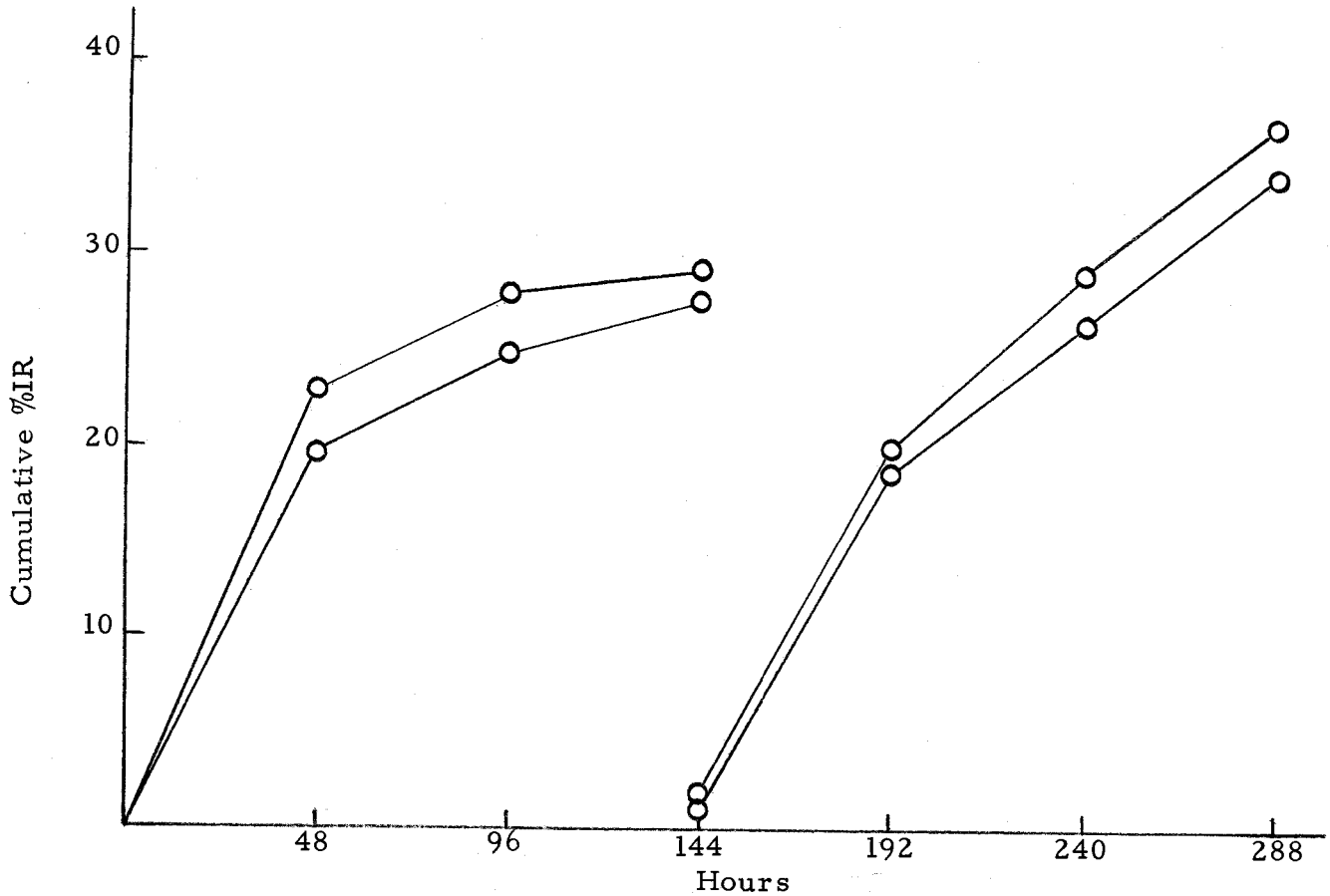

Figure 6. Time course of cumulative recovery of radioactivity in CO_2 from glucose-U-C^{14} from two abdomina, each re-injected at 144 hours.

The oxidation of acetate-1-C^{14} by a replicated series of abdomina also demonstrated the degree of variation between individuals. Six abdomina were isolated after a forced-feeding 150 µl of 10% glucose. Five days later they were injected with 150 µl of saline containing 25 µM of sodium acetate with a specific activity of 6.8×10^4 DPM per µM. The $C^{14}O_2$ produced was trapped at 2, 4, 8, 12 and 24 hours after injection. At termination of trapping the abdomina were sacrificed, homogenized, saponified and extracted as described. The oxidation of acetate as represented by $C^{14}O_2$ evolution followed almost exactly the same time course as that shown in Figure 7, which presents data from a later experiment. The average total percent recovery of administered activity was 68% with a standard deviation of 1.34%. The percentages of administered activity recovered as $C^{14}O_2$ and as activity present in the saponified and unsaponified fractions are shown in Table III. Also included in this table are similar figures for three abdomina treated in an identical manner, but with the substrate containing 50 µM of sodium acetate carrier, and for two abdomina given 100 µM of carrier acetate. Very uniform utilization of acetate was observed with respect to oxidative metabolism. The range in total recoveries from those administered 25 µM was 67-70%. The amounts of acetate oxidized were roughly proportional to the levels administered. The incorporation of label into the lipid fractions was more variable, with a

Table III. Utilization of acetate-1-C^{14} by isolated abdomina.

μM Acetate-1-C^{14}[a]	%-Recovery of administered activity		
	CO_2	Saponified fraction	Unsaponified fraction
25	69.7[b, c]	6.7	< 0.1
25	69.2	2.3	0.3
25	67.0	5.5	0.1
25	68.0	6.1	0.8
25	70.0	11.8	0.1
25	70.0	6.0	0.1
50	69.9	10.0	< 0.1
50	70.8	6.0	0.1
50	61.0	5.0	0.1
100	52.2	3.3	0.1
100	47.6	2.6	0.1

[a] 2.8×10^4 DPM per μM in all cases.

[b] Total percent recovery of administered activity in 24 hours. See p. 37-38.

[c] Standard deviation for 25 μM of carrier = 1.37.

range of percent recovery in the total saponified fractions of 2. 3 to 11. 8. Much less acetate was incorporated into the unsaponified fractions, where total percent recoveries ranged from less than 0. 1 to 0. 8.

Oöcyte Development and Utilization of Acetate-1-C^{14}

The effect of the CA hormone upon the utilization of acetate was measured in four isolates which were force-fed casamino acids-glucose before isolation, held ten days and implanted with two pairs of glands. Four similar animals were mock operated. Ten days later each was injected with 100 µl of saline containing 0. 026 µM of acetate-1-C^{14} with an activity of 1. 88 x 10^6 DPM. Carbon dioxide labelling was examined as previously described. At the termination of the 24-hour trapping period each abdomen was dissected and extracted as described before. Weights of the lipid extracts were determined in tared stainless steel planchets. The specific activities of the saponified and unsaponified fractions are given in Table IV, with the recoveries of $C^{14}O_2$ represented by the total percentages of administered activity which were recovered. Recoveries of radioactivity in CO_2 ranged from 62. 8% to 75. 7% of administered activity, with both extremes of the range represented in control animals. Similar scattering of incorporation into lipids was found; treated and control animals ranged from 90-137 DPM per mg of

Table IV. Effect of corpora allata on the utilization of acetate-1-C^{14}.

Animal	Treatment[a]	Oöcyte length	Accessory gland response	% R[b] CO_2	DPM per mg Saponified	DPM per mg Unsaponified
1	control	0.89	negative	75.7	90	4987
2	CA	1.39	positive	72.3	160	7374
3	CA	0.89	negative	69.6	140	6586
4	control	0.97	negative	72.0	174	5792
5	CA	1.59	positive	64.5	317	10693
6	CA	0.89	negative	66.7	208	11092
7	control	0.95	negative	68.4	287	7087
8	control	0.92	negative	62.8	302	4559

[a] Control animals were mock-operated.

[b] Total percent recovery of administered activity in 24 hours.
See p. 37-38.

saponified material. However, in the unsaponifiable fractions, where recoveries ranged from 4559-11092 DPM, the average values for animals with CA were slightly higher than those for the controls. The time course graph of $C^{14}O_2$ recovery (Figure 7) indicates that the rate of oxidation of acetate is not affected by the CA hormone.

Incorporation of Leucine-U-C^{14} into Protein

Some workers have implicated the NSC in stimulating protein synthesis correlated with the initiation of oöcyte maturation by the CA (27); whereas others have noted increased protein synthesis in the presence of the CA alone (68, 69). It was of interest, therefore, to examine what effect the CA would have on protein synthesis in the isolated abdomen. The incorporation of leucine-C^{14} was used to examine this relationship. Abdomina handled in the routine manner were injected with 20 µl of saline, containing 0.005 µM of leucine-U-C^{14} with an activity of 2.06×10^6 DPM. The animals were sacrificed after two, four or eight hours. Carbon dioxide was trapped at two-hour intervals for the eight hour pulse and one-hour intervals for the shorter pulses. Injections were staggered so that upon termination of the pulse period a sufficient time interval was allowed for taking of blood samples, examination of the reproductive organs and removal and homogenization of the fat bodies. After completion of the first runs it was decided further $C^{14}O_2$ collection was not

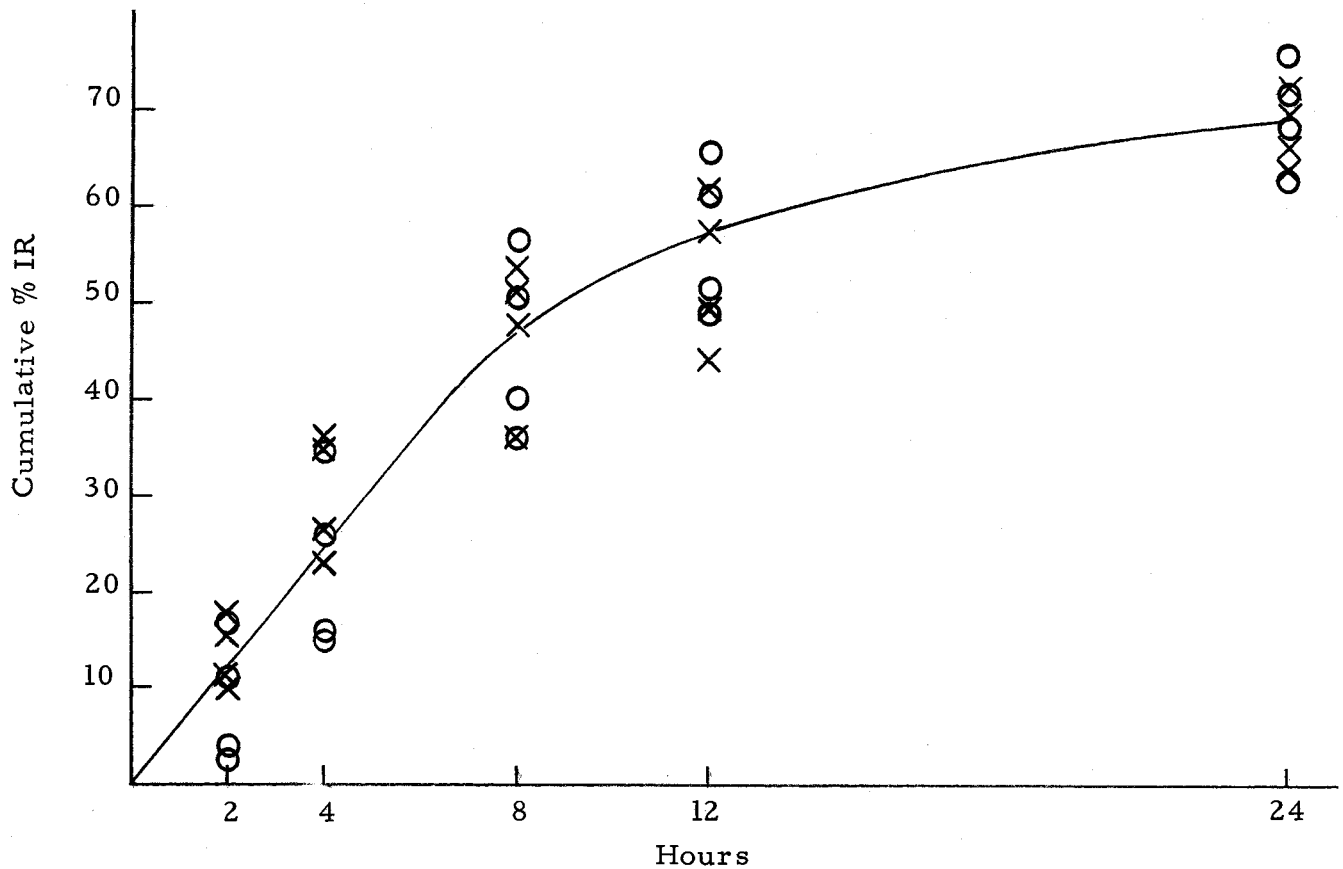

Figure 7. Time course of cumulative recovery of radioactivity in CO_2 from acetate-1-C^{14} from abdomina with corpora allata (X) and without corpora allata (O).

necessary, and 16 additional abdomina were treated for a two hour pulse without this procedure. The data are listed, ranked according to the length of the longest terminal oöcyte, in Tables V and VI. The degree to which individual abdomina responded to the CA implants can be judged by the oöcyte length, developing oöcytes being more than 1.15 mm long, and by the response of the accessory glands. After two hours the amount of leucine-U-C^{14} incorporated ranged from 1,000 to 43,300 DPM per mg of blood protein and from 2,800 to 37,900 DPM per mg of fat body protein. Similar results were obtained at four and eight hours, with some reduction in the range of specific activity. Neither the amount of incorporation of label into protein nor its appearance in CO_2 can be correlated with CA activity.

A trend can be seen in the amount of protein labelling which may indicate a shift from fat body to blood. After two hours 19 of 24 animals had amounts of radioactivity in the fat body equal to or higher than in the blood, whereas at four hours this was true in only one out of eight animals.

Table V. Incorporation of leucine-U-C^{14} into protein in the presence of corpora allata. Two-hour pulse.

Treatment[a]	Oöcyte length	Accessory gland response	% R^b CO_2	DPM per mg protein	
				Blood	Fat body
CA	2.50	positive	---	43,300	37,900
CA	1.45	positive	---	3,400	16,300
CA	1.33	positive	---	10,100	24,800
CA	1.20	positive	---	4,000	27,200
CA	1.17	positive	0.1	27,500	37,100
CA	1.17	enlarged	---	6,900	7,300
CA	1.08	enlarged	0.7	20,800	17,000
control	1.08	negative	---	1,900	4,100
CA	1.06	enlarged	---	2,300	5,400
control	1.06	negative	0.5	10,000	5,100
CA	1.06	negative	---	3,100	13,900
CA	1.03	enlarged	0.2	11,000	17,300
CA	1.03	negative	0.3	6,800	4,300
control	1.03	negative	0.2	7,300	8,200
control	1.03	negative	---	2,300	7,000
CA	1.00	enlarged	0.4	17,700	13,200
CA	1.00	enlarged	---	1,400	7,400
control	0.97	negative	---	8,700	25,100
CA	0.97	negative	---	2,900	5,800
control	0.95	negative	---	2,100	2,800
control	0.92	negative	---	4,000	14,200
CA	0.86	negative	1.0	12,800	13,000
CA	0.86	enlarged	---	3,200	5,200
CA	0.86	negative	---	4,100	4,800

[a] Controls were mock-operated.

[b] Total percent recovery of administered activity in two hours. See p. 37-38.

Table VI. Incorporation of leucine-U-C^{14} into protein in the presence of corpora allata. Four- and eight-hour pulses.

Treatment[a]	Oöcyte length	Accessory gland response	% R[b] CO_2	DPM per mg protein Blood	DPM per mg protein Fat body
Four-hour pulse					
CA	1.14	enlarged	1.8	32,300	8,600
CA	1.08	enlarged	2.0	29,100	13,500
CA	1.08	negative	0.9	8,700	9,900
CA	1.06	negative	1.5	11,500	7,200
control	0.97	negative	1.3	9,200	8,200
CA	0.97	negative	1.9	14,700	8,200
CA	0.95	negative	0.8	20,100	10,100
control	0.86	negative	2.0	11,500	8,500
Eight-hour pulse					
CA	2.00	positive	1.9	30,100	-----
CA	1.45	positive	2.6	39,100	-----
CA	1.36	positive	1.8	62,500	-----
control	1.08	negative	3.4	57,700	-----
CA	1.06	enlarged	3.1	28,100	-----
control	1.03	negative	6.4	33,800	-----
control	1.03	negative	7.2	------	-----
CA	0.83	negative	2.7	29,700	-----

[a] Controls were mock-operated.

[b] Total percent recovery of administered activity in four and eight hours. See p. 37-38.

DISCUSSION

It is apparent that the successful isolation of the cockroach abdomen is dependent upon minimizing the degree of injury and attending to the nutritional requirements of the isolate. This was accomplished by force-feeding the roach with nutrient solution and ligating, instead of cutting the animal, at a time when the cuticle was soft. Bodenstein and Sprague (2) reported the only other attempt to use this approach on a roach (Periplaneta). Their procedure, which omitted forced-feeding, was less successful from the point of view of survival than decapitation. Of six attempts, one animal lived four days, four lived five days and one survived for seven days. Decapitation has also been used by Wigglesworth (76), Vanderberg (68, 69), Davis (9) and Coles (4). In their procedures Rhodnius was decapitated by ligation with a fine wire in the cervical region in such a manner as to include or exclude the CA. Prior to this the animals were given a final blood meal, which would be comparable to a forced-feeding with the roach. The most obvious disadvantage in the use of decapitation is that the large thoracic and subesophageal ganglia, the prothoracic glands, numerous receptors and effectors and particularly the locomotory appendages are included in the experimental unit. Decapitated Leucophaea become very active when stimulated only slightly, and handling them is precluded. The rapid

attainment of almost complete quiescence by the isolated abdomen is, therefore, an advantage not gained by decapitation. The reduction of variation due to activity would be particularly desirable in the examination of energy metabolism or substrate utilization.

Successful isolation of the abdomen of the silkworm, Hyalophora cecropia, was achieved by Williams (77). As has been discussed previously, however, the physiology of this insect is particularly well adapted to this technique. Observation of isolated abdomina of Leucophaea which were prepared by ligation immediately following ecdysis and a forced-feeding indicated that they were able to survive in good condition with consistent results. The data obtained from the oxidation of acetate and glucose substantiate the observations that recovery is rapid and that the subsequent condition of isolates is uniform, even though this insect is normally active throughout its life cycle and requires nutrients at frequent intervals.

The initiation of yolk deposition in oöcytes of isolates required the implantation of intact active glands. Two pairs of glands brought about a positive response by accessory glands in 50% of the isolates and by oöcytes in 25%. Increased numbers of implanted glands did not improve the results. No instance has been found in the literature where homogenates or extracts of small numbers of CA were successful in initiating oöcyte development. However, it would have been better procedure had the homogenates used here been suspended

in peanut oil--a technique commonly employed for the injection of active agents in insects in order to protect against metabolic destruction of the materials. It was also unfortunate that, at the time homogenates were tested, the responsiveness of the accessory glands was not appreciated. Bodenstein and Sprague (2) noted that the function of the left colleterial gland in Periplaneta is under the control of the CA, and they also found that two to four implanted glands were sufficient to elicit the response in decapitated animals.

In Leucophaea a normal regimen is required for the maturation of oöcytes (52, 54). Von Harnack (23) reasoned that since starved females which received active CA implants were able to mature their oöcytes (32), the CA were inhibited via the brain which responded to the lack of nutrients. Larsen and Bodenstein (36) obtained evidence that mechanical distension of the gut following feeding resulted in the activation of the CA in a mosquito. Engelmann (18), however, could not find evidence that such a phenomenon occurs in Leucophaea, and he concluded that the nutrient value of the food was responsible. Although Johansson (32) has shown that starved female Leucophaea are able to develop mature oöcytes if they are implanted with active CA, the data in Table I show clearly that the absence of an adequate supply of nutrients rich in amino acids was definitely deleterious to the responsiveness of the sex organs to the CA hormone. Whether the inability to develop oöcytes or to produce

large amounts of secretion in the accessory glands was due to insufficient nutrients or to reduced activity of the CA when placed in an inadequate milieu cannot be definitely stated; but the fact that accessory gland responses rated as enlarged were fairly constant, regardless of the nutritional status, indicates that the CA remained active. It is possible that full activity of the accessory glands would not be possible in the absence of sufficient protein precursor, since their secretory product is largely protein (15).

The importance of the post-ecdysial feeding was clear-cut and was recently substantiated by Engelmann and Rau (20), who showed by measuring the daily consumption of food that intake was greatest during the first days following the final molt. These workers found that very little food was eaten during the last two weeks of the final nymphal instar or by the adult containing mature eggs. Feeding activity could be correlated with yolk deposition but not with CA activity, since newly molted allatectomized females also consumed large amounts of food.

Roth (45) found that by denervating the CA and then starving females of Leucophaea the inhibitory effect of the brain was removed and oöcytes matured. Similarly, Engelmann (18) implanted active and inactive CA into pregnant females and found that 98% induction of new oöcyte development was caused by the active CA and 63% by the inactive CA. By following repeated cycles of egg

maturation in the presence of such isolated CA he concluded that these glands, when separated from their nervous connectives, show a cyclic activity which is intrinsic. Scharrer (53, 54, 55), Engelmann (15), Johansson (33) and Roth (45) have all suggested that the CA are under nervous inhibitory control because in situ CA of starved or pregnant females are inactive, whereas implants are active. Scharrer (54) concluded the stimulatory control to be via a substance present in the neurosecretory products of the brain. Highnam (26) found that cauterization of the NSC of Schistocerca prevented growth of the terminal oöcytes and Davis (9) and Vanderberg (68, 69) suggested a similar role for the brain in Cimex and Rhodnius, respectively.

The complete lack of response by the reproductive organs of isolated abdomina implanted with inactive CA indicates that the glands did not return to activity within the ten day duration of the experiment. Engelmann's results with inactive CA were observed after 46 days (18), so it cannot be concluded definitely that the results are in conflict. However, in the present work allatectomized intact females produced large oöcytes within ten days when implanted with CA, whether or not the glands were active. Since inactive CA remained so when placed in isolated abdomina, it must be assumed that a stimulatory effect not present in the isolate was responsible for activation of inactive CA in intact females. It is

therefore suggested that the intrinsic cyclic activity referred to by Engelmann (18) may have been representative of cyclic humoral stimulation by the brain in the absence of an overriding inhibitory nervous control. Lea and Thomsen (38) did note cyclical changes in the NSC of Calliphora which coincided with variations in the size of the CA. Although they attributed the cause to the CA, through a feed-back mechanism, a more complex interrelationship between the NSC, the CA-CC and the reproductive organs may exist. As Wigglesworth points out in his recent review (76), the brain may be responsive to homeostatic changes or other effects caused by feeding or the development of the oöcytes.

In Leucophaea mating also acts as a stimulus to the CA, and mated females mature their oöcytes more rapidly than do virgin females (18). This was also noted in these experiments. The oöcytes of mated females approximately ten days after mating were more than twice as large as those of isolated abdomina and one-third larger than those of allatectomized females ten days after implantation with active glands. According to Roth (45) the females do not mate before the sixth day following ecdysis and the average age at mating is nine days. Engelmann (18), Barth (1) and Roth and Barth (46) presented evidence implicating the CA in the production of a pheromone which induces males to court. The ovaries were shown not to be responsible for the initiation of receptivity since

ovariectomized virgin females mated at the normal time (18). It is of interest, therefore, that the capacity of the ovarioles of isolated abdomina to respond to active implants was not appreciable until the sixth day (Table II). At this age the level of positive responses approached 50%, which is the usual response attained using isolated abdomina with present procedures. It is noteworthy that similar response levels were obtained by Engelmann with inactive implants in pregnant animals (18). This may indicate, as was suggested by Roth (45), that the activity of functioning CA is further enhanced by the brain as the result of additional stimuli such as mating, feeding or the absence of an oöthecum. Roth (45, p. 943) states, "In L. maderae the stimulating effect resulting from copulation is 'stored' if the female is starved after mating, and manifests itself when food is finally supplied. This suggests that mating may result in an increase of gonadotropic hormone which is not released in starved L. maderae until proper nutritional stimulation occurs."

The inability to achieve levels of response above 50% by the sex organs to CA implants remains as the principal limitation of the utility of isolated abdomina. Unless some untested nutritional factor is limiting, the forced-feeding described appears to be adequate, as additional injection did not improve results. Increase in the numbers of CA implanted was similarly ineffective. The inability of early implantations of CA to enhance or accelerate oöcyte

development indicates that both the delay in oöcyte responsiveness and the maximum activity of CA in isolates are relatively fixed factors. In view of the evidence that multiple factors are responsible for the maximum stimulation of the CA, it is now felt that the absence of the secretions of the NSC probably explains the results. It may be that the incomplete response obtained to applications of farnesol can be explained in a similar manner.

Bioassay procedures for gonadotropic activity have all involved one form of allatectomy or another, and it would be advantageous to be able to obtain quantitative estimates using a system such as the isolated abdomen. The results obtained with the juvenile hormone extract were not conclusive, but they indicate that determinations might be possible if the extract were further purified to remove toxic contaminants. Chen et al. (3) obtained definite yolk deposition with a crude silkworm extract, but it is not known how their material would compare with that used in these experiments in either activity or purity. More direct comparison can be made in the case of farnesol. Here again, Chen et al. (3) were able to induce actual oöcyte development in allatectomized Periplaneta, whereas Leucophaea isolates responded to farnesol application only with partial accessory gland activation. Whether this difference was due to species sensitivity, activity of material, sensitivity of the bioassay procedure or to the presence of an unknown factor

present in intact animals and absent in isolates is not known. It has been found that certain farnesol derivatives, principally farnesyl methyl ether, show more juvenile hormone activity than does farnesol (75). The utility of the isolated abdomen as a bioassay unit will not be proved until more active materials and more highly purified extracts have been tested on both isolates and allatectomized intact females. This phase of experimentation awaits further examination of the relationship between the NSC and the CA.

The measurements of respiratory metabolism support the contention that the abdomina maintain a healthy state. Approximately 30% of the glucose introduced into the body fluids was oxidized. Five days later a similar amount was oxidized at about the same rate as the first. Acetate injected ten days after isolation was oxidized at very nearly equal rates by each of six isolates. The values for the incorporation into lipids are somewhat more variable, but the several steps involved in isolation of small amounts of activity reduce the precision with which such measurements can be made. Louloudes et al. (41), in a study of lipid synthesis from acetate-C^{14} in Periplaneta, reported levels of incorporation of label into saponifiable and unsaponifiable fractions very similar to those given here.

Many workers have noted a rise in oxygen consumption in connection with the activity of the CA. Thomsen (63) found the CA

to be necessary for maintenance of oxygen uptake by female Calliphora at maximum levels. Later Thomsen and Hamburger (64) demonstrated that this effect is not due to the direct action of the hormone on the ovaries, since elevated oxygen utilization was not reduced by castration. Using Leucophaea, Sägesser (51) obtained similar results by implanting active CA into females from which he removed both the ovaries and accessory glands. On this basis he theorized that the hormone stimulates respiratory metabolism. This supposition is not borne out by the results reported in Figure 7, in which the high levels of recovery indicate that oxidation of acetate is probably via the TCA cycle, and in which the figures for treated and untreated, responsive and unresponsive abdomina are quite uniform and completely inter-mixed. Engelmann and Rau (20) noted that increased oxygen consumption in the presence of active CA can also be correlated with feeding. They concluded that increased digestive activity was responsible for elevated oxygen uptake, but that the CA was still responsible. However, this is another case in which the roles of the CA, the NSC and possible feed-back mechanisms have not been separated. On the basis of the evidence presented here it appears that the CA are not directly responsible for an increase in respiratory metabolism or in digestion, although the latter point was examined only indirectly.

Although it is unlikely that acetate exists in any but very small concentrations in the hemolymph, it enters the oxidative pathways with facility and is oxidized in amounts roughly proportional to the level administered. It must be noted that the rate of oxidation appears to depend upon the concentration in the hemolymph. Therefore if the effect of the CA were to increase the rate of formation of acetate this would lead to an increase in its oxidation which would be reflected in the QO_2, but would not necessarily be detectable by the method used here.

The presence of the CA had no apparent effect on the incorporation of acetate into fatty acids, but the results may indicate some difference in the incorporation into unsaponifiable lipids.

The significance of proteins in the oögenesis of insects was discussed in a previous section. There is good evidence that complete proteins are taken up from the blood by the developing oöcytes (60, 68) and that specific proteins are incorporated into the yolk and constitute the major part of its protein component (4, 60). However, the roles of the components of the endocrine system in this process have been given different interpretations. Thomsen and Møller (66) suggested that the NSC may have a basic role in the general synthesis of proteins. Hill (29) and Highnam et al. (27) were able to separate the effects of the NSC and the CA in Schistocerca. They implicated the NSC in the stimulation of a general

increase in protein synthesis and the CA in the activation of yolk deposition in the oöcytes. Vanderberg (68, 69), however, concluded that increases in protein synthesis in Rhodnius were attributable to CA activity. On the other hand, Coles (4) theorized that the brain (NSC?) stimulates the synthesis of specific yolk proteins in the fat body of Rhodnius.

This problem was examined in Leucophaea by administering a labelled amino acid and examining the incorporation of label into the proteins of the blood and fat body. The presence of the CA could not be distinguished on the basis of the specific activity of proteins, although hormonal activity was indicated by the effect on the reproductive organs. It will be noted in Tables V and VI that all of the abdomina which responded by developing oöcytes (1.15 mm or larger) displayed leucine incorporation in the upper range. However, several implanted abdomina which responded only to the extent of enlarged accessory glands, and several which were negative, also incorporated label to the same extent. Finally, there were several control abdomina which produced specific activities as high as some of the most responsive implanted isolates. If it is assumed that the CA are directly responsible for protein synthesis, then one might expect to find greater separation between the specific activities of the proteins of responsive individuals and those which were

unresponsive or did not receive CA. This conclusion is only tentative, however, on the basis of these exploratory experiments.

It appears that protein synthesis occurs with some variation in isolates, whether they have CA implants or not. It may be that the CA hormone is able to cause the development of oöcytes only where there is sufficient protein, and that when development occurs, increased labelling of protein obtains due to homeostatic feed-back as material is taken up by the oöcytes. However, it is also possible that the CA are responsible for the production of specific proteins in the blood or fat body. This effect would not be revealed by differences in specific activity of total protein extracts.

The appearance of labelling in the fat body and blood proteins showed a definite shift with time. At two hours, 19 of 24 abdomina developed specific activities in the fat body equal to or greater than in the blood, whereas at four hours this was true in only one out of eight animals. Hill (29) obtained similar patterns in the incorporation of C^{14}-glycine in Schistocerca, where labelling appeared more rapidly in the fat body than in the blood and more slowly still in the oöcytes. While it is tempting to conclude that protein is being synthesized by the fat body and moving via the blood to the ovarioles, much more information is needed before such a relationship can be postulated.

SUMMARY

The development of a procedure for the isolation of the abdomen of a cockroach was undertaken in order to make available a simplified biological unit for examining the endocrinology of oögenesis.

Preliminary trials indicated that separation of the thorax and abdomen with a ligation at the time of the imaginal ecdysis would be the most practicable approach. Subsequent experiments showed that a forced-feeding of the insect immediately prior to isolation of the abdomen was necessary, and that glucose or a casamino acids-glucose solution would provide for satisfactory survival and longevity of the isolates. Observation and examination of the abdomina indicated that they quickly recover from the operation and maintain the functions of their organs at adequate levels for a long period of time. The high degree of uniformity in the utilization of C^{14}-glucose and C^{14}-acetate demonstrated that individual isolates function fairly uniformly and that inter-individual variation in respiratory activity is low. This information, combined with biological observations, gives assurance that isolated abdomina survive and function well and are not simply deteriorating.

It was not possible to stimulate egg development in isolates with homogenates of the CA; however, activation of the accessory glands and initiation of yolk deposition in the oöcytes were possible

with implantation of two pairs of active glands. A forced-feeding of casamino acids prior to isolation or injection of casamino acids into the isolates was necessary to attain maximum response levels of the sex organs to gland implants. This substantiates the information of others that the post-ecdysial feeding is important for the process of oögenesis.

It was found that a post-ecdysial period of maturation of about six days is required by the oöcytes before they are able to respond to the CA hormone. It was not possible to shorten this period or to enhance the response of the reproductive organs by increasing the holding period.

Inactive CA obtained from pregnant females remained inactive in isolates as measured by accessory gland response and oöcyte development, whereas they returned to activity when placed in allatectomized intact females. It was concluded that rather than being activated due to an intrinsic cycle, inactive glands placed in intact animals are stimulated by a factor produced in the anterior region, probably by the NSC. Similarly, it is possible that the lack of continuing stimulation of the CA by the NSC may be responsible for the reduced number of isolates that responded to implantation of active glands (40% less than intact animals).

Recoveries of $C^{14}O_2$ from acetate-injected isolates with and without development of the sex organs did not indicate an effect

by the CA upon respiratory metabolism, as others had suggested. Although incorporation of acetate-1-C^{14} into saponifiable and unsaponifiable fractions was more varied than its oxidation, no definite changes correlated with endocrine effect were observed.

Determinations of the incorporation of C^{14} from leucine into the proteins of the blood and fat body showed that synthesis of proteins occurred in isolates with or without CA implants, and that the highest levels of specific activity were correlated with oöcyte development, but not necessarily with CA activity. It is suggested that when the CA are able to stimulate yolk deposition in the oöcytes, increased protein synthesis follows as a homeostatic response. The higher rate of appearance of specific activity in the fat body proteins over those of the blood lends support to the evidence of others that this organ may be the site of synthesis of the proteins involved in vitellogenesis.

Bioassays of the gonadotropic activity of farnesol and of a juvenile hormone extract of the cecropia silkworm were inconclusive, but they indicated that quantitative evaluation of materials of higher activity or lower toxicity may be possible in the isolated abdomen.

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