

Endocrine Control of Vitellogenesis in *Drosophila melanogaster*: Effects of the Brain and Corpus Allatum

ALFRED M. HANDLER AND JOHN H. POSTLETHWAIT
Department of Biology, University of Oregon, Eugene, Oregon 97403

ABSTRACT The endocrine control of yolk deposition in *Drosophila melanogaster* was studied by ligation and transplantation techniques. Endocrine events associated with the initiation of vitellogenesis were found to be synchronized with eclosion rather than the completion of adult development. Decapitation experiments showed that a cephalic event occurring at about the time of eclosion is necessary for each animal to initiate vitellogenesis. The morphogenetic effect of the head could be replaced by a juvenile hormone analog (JHA).

In addition to the cephalic event, a thoracic factor is required for each follicle to initiate vitellogenesis, since preparation of isolated abdomens before 16 hours after eclosion prevented vitellogenesis. In abdomens isolated after this time, no early vitellogenic stages were formed. The suppression of vitellogenesis in isolated abdomens was reversed by implanting corpora allata or by treating these preparations with JHA, but not by implanting corpora cardiaca. Ovaries that were artificially induced to mature by treating isolated abdomens with JHA still displayed the normal complement of ovarian proteins after electrophoresis in polyacrylamide gels.

These results show that a circadian clock triggers vitellogenesis via a cephalic signal at eclosion, which in turn triggers events in the thorax or abdomen. The cephalic signal can be superseded by juvenile hormone, whose presence is necessary for each follicle to become vitellogenic.

In insects, as in vertebrates, maturation of oocytes is hormonally controlled and depends upon the production of yolk proteins by extra-ovarian tissues (Englemann, '70; Wallace and Bergink, '74). Nervous and neuroendocrine activity play important roles in controlling the onset and continuation of vitellogenesis in insects, although the precise function and timing of this activity varies in different species. For example, it has been proposed that synthesis of yolk protein in *Sarcophaga* (Wilkins, '69) is controlled by juvenile hormone (JH)¹ secreted by the corpus allatum (CA), while uptake of this protein into the oocyte is controlled by factors secreted by the medial neurosecretory cells (MNC) of the brain. In contrast to *Sarcophaga*, the roles of the CA and MNC are reversed in *Schistocerca* (Highnam et al., '63; Hill, '62). In *Calliphora* (Thomsen and Lea, '68), the MNC have a gonadotropic effect, while CA activity may indirectly affect

vitellogenesis by stimulating MNC activity. In *Aedes aegypti*, an egg development neurohormone produced by the brain may have an indirect role in stimulating yolk protein synthesis (Lea, '72; Hagedorn, '74), while the function of the CA is to allow development of pre-vitellogenic oocytes up to the resting stage (Gwadz and Spielman, '73).

The species specific interactions of different neuroendocrine functions are made more bewildering by controversy over the role of these organs in *Drosophila*. Vogt ('43, '46) has shown that in *D. hydei* the central part of the larval ring gland (which later develops into the adult corpus allatum) can induce vitellogenesis in adult females deprived of their corpus allatum-corpora cardiaca complex;

¹ Abbreviations used: CA, corpus allatum; CC, corpus cardiaca; JH, juvenile hormone; JHA, juvenile hormone analog; L:D, light:dark; MNC, medial neurosecretory cells; WPP, white prepupa formation.

and reciprocally, that the adult corpus allatum can locally inhibit the appearance of imaginal characters when transplanted into metamorphosing animals. Vogt's ('40a,b, '41) interspecific transplants also argue that the adult derivatives of the ring gland are required for vitellogenesis in *D. melanogaster*, but that the CA hormone is qualitatively different in different *Drosophila* species. Decapitation experiments performed by Bodenstein ('47) showed that *D. melanogaster* can support egg maturation without the brain, which suggests that the brain is not of vital importance. On the other hand, Bouletreau-Merle ('74, '76), on the basis of extirpation experiments in *D. melanogaster*, concluded that the gonadotropic hormone primarily responsible for egg maturation comes not from the corpus allatum, but rather from the brain.

Since the evidence for *D. melanogaster* is contradictory for the functions of the CA and brain hormones during oogenesis, the primary objective of this study is to assess the action and timing of factors originating from the brain and CA, for the initiation and continuation of egg maturation in *D. melanogaster*.

Egg maturation depends upon feeding during the adult stage in many flies (Thomsen, '52; Wilkens, '68; Lea, '69). *D. melanogaster*, on the other hand, can support egg development for at least several days without feeding (Bodenstein, '47). This led us to wonder if vitellogenesis in *D. melanogaster* is more like saturniid silkmoths, which do not depend upon a signal given during adulthood, but rather, initiate ovarian maturation during the pupal stage (Pan et al., '69). Alternatively, a signal initiating vitellogenesis might occur at the beginning of adult life, after emergence from the puparium. Assessing the role of eclosion is complicated by the controversy as to whether eclosion in *D. melanogaster* consistently occurs soon after the completion of adult development (Harker, '65), or if eclosion is controlled by a circadian rhythm and occurs independently of development. According to the latter view developed from studies of *D. pseudoobscura* and *D. melanogaster*, eclosion may occur long after development has been completed (Pittendrigh, '54; Pittendrigh and Skopik, '70). Due to these conflicting results, the second objective of this study is to repeat the above experiments in order to determine for ourselves whether the development of the adult is independent of the time of eclosion, and if so, whether the processes which trigger

vitellogenesis are synchronized with eclosion or the termination of adult development.

A constellation of electrophoretically revealed proteins are accumulated by oocytes that mature naturally (Gelti-Douka et al., '74; Gavin and Williamson, '76a,b). Maturation of oocytes can be artificially induced in preparations that surgically or genetically lack CA activity by treatments with a JH analog (Postlethwait and Weiser, '73; Postlethwait and Gray, '75; Postlethwait et al., '76). The third objective of this work is to find whether bona fide egg proteins are caused to appear in oocytes which are artificially induced to mature by exogenous treatment with a synthetic juvenile hormone analog.

MATERIALS AND METHODS

Animals

The Oregon-R wild type stock of *Drosophila melanogaster* was cultured on standard cornmeal-molasses-agar medium at $25 \pm 0.5^\circ\text{C}$. Flies were kept on a 12-hour light: 12-hour dark photoperiod and all freshly eclosed flies were collected during a 5-minute period within one hour after lights-on unless noted otherwise. Animals were maintained after surgery in a small Petri dish on tissue paper saturated with Ephrussi-Beadle ('36) Ringers to which phenylthiourea was added. At appropriate times, ovaries were dissected and the developmental stage of each vitellogenic follicle was scored according to King ('70). Follicles were grouped into three stages of maturation, early (stage 8-9); intermediate (stage 10-12); and late or mature (stage 13-14).

Surgical treatments

Decapitation was performed at specified times by severing the neck with iridectomy scissors. Due to excessive loss of hemolymph from animals decapitated before eclosion, these animals were first ligated around the neck (after removal of the operculum) with nylon monofilament. The neck was then severed anterior to the ligature. Animals decapitated before eclosion were collected initially as white prepupae (WPP) at the end of the light phase in a 12L:12D photoperiod. As indicated in figure 3(12L), more than 90% of the females consistently eclose over a 2-hour period at the beginning of the light phase, 108 hours after WPP formation. Decapitation of pharate adults was performed relative to this time.

Isolated abdomens were prepared by looping

nylon mesh monofilament around the first abdominal segment. The thorax and head anterior to the ligature were then removed and discarded.

Transplantations were performed as described by Ursprung ('67). CA and CC glands were dissected from 3-day-old Oregon-R virgin females in Chan and Gehring ('71) Ringers to which a few crystals of phenylthiourea, streptomycin, and penicillin were added. The CA-CC complex was teased away from the aorta with tungsten microneedles. In cases where the CA or CC were transplanted individually, the CA was carefully teased away from the CC. After isolating glands from three animals, the glands were put into fresh Ringers and transplanted into a ligated abdomen isolated from a newly emerged female fly. The abdomens were then incubated for three days and scored for vitellogenic oocytes.

Hormone applications involved treating ligated abdomens and decapitated female flies with 0.15 μ g of the juvenile hormone analog, ZR-515 (isopropyl 11-methoxy-3,7,11-trimethyl dodeca-2,4-dienoate, a gift of the Zocon Corp.) dissolved in acetone. Hormone was topically applied onto the dorsal side of the abdomen. ZR-515 has been shown to both inhibit adult development (Postlethwait, '74) and to stimulate vitellogenesis in the female sterile mutant, *apterous*,⁴ of *D. melanogaster* (Postlethwait and Weiser, '73).

Survival of all experimental animals was based on three criteria. These criteria included (1) visible muscular contraction or bodily movement; (2) movement of the gut tissue and/or flow of material through the Malpighian tubules; and (3) pulsation of the ovaries. Satisfaction of any one or all of these criteria was accepted as an indication of viability of the organism. It is possible that certain animals might have been able to exhibit any one of the above behaviors, and yet be too weakened to support ovarian maturation. Therefore we believe that results considering successful egg maturation as a percentage of surviving animals are probably conservative.

Monitoring development and eclosion

Two groups of animals (15 to 25 animals per group) were collected as white pre-pupae at five different times during a 12L:12D photoperiod; 25°C:24°C thermoperiod. An 8-watt cool white fluorescent bulb (F8T5/CW) placed 18 cm from the animals provided illumination.

Animals were kept in an incubator at all times except when examined. The opercula of the first group of animals were removed at 48 hours after WPP formation, whereupon the animals were examined at approximately 20-minute intervals for the duration of the pupal period. The times at which three cuticular structures appeared were recorded. These structures included ocellar tormogen sclerotization, orange eye color, and wing pigmentation.

The second group of animals was also kept under a 12L:12D photoperiod; 25°C:24°C thermoperiod. During the last light phase which occurred previous to 98 hours after WPP formation, these animals were sexed and placed into an eclosionometer within the incubator. The eclosionometer (Lumme and Lankinen, '73) records emergence by collecting ball bearings pushed by eclosing flies into a fraction collector. For this experiment eclosion was monitored at 20-minute intervals. Different populations of flies may be designated by different colored ball bearings.

Electrophoresis

Ovaries were dissected from freshly eclosed females, untreated 3-day-old females, and ZR-515 treated isolated abdomens incubated for three days. Oviposited eggs were collected over a 2-hour period and kept at 4°C. All samples were homogenized in Ephrussi-Beadle Ringers solution and incubated at 50°C for two hours in 2% recrystallized SDS, 2% β -mercaptoethanol, 0.002 M Tris-HCl buffer at pH 8.0. Samples were then centrifuged at 35,000 *g* for 20 minutes. The supernatant was collected, assayed for protein (modified from Schaffner and Weissman, '73) and brought to 5% glycerol, 0.01% bromphenol blue prior to loading onto the gel.

SDS gel electrophoresis was performed using a system modified from the standard alkaline analytical disc electrophoresis system of Davis ('64). The upper reservoir buffer was changed to 0.053 M Tris base, 0.053 M glycine, pH 8.9, and both upper and lower reservoir buffers contained 0.1% SDS. The resolving gel was poured as an exponential gradient of 7.5-15% acrylamide (BioRad). The gels were 1.5 mm thick and 13 cm long. Gels were run at 30 mAmps/gel for three hours, at which time the dye front had reached the bottom of the gel. Gels were stained for protein with Coomassie blue.

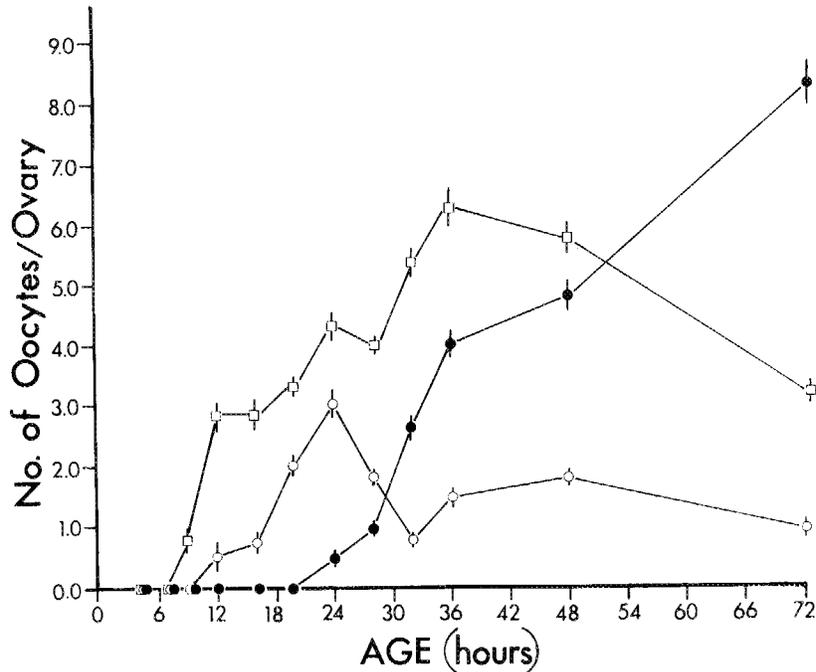


Fig. 1 Appearance of early, intermediate, and late stage oocytes in unoperated control animals raised at 25°C. Animals were dissected at the times indicated. No fewer than ten animals (20 ovaries) were dissected for each point. Follicle stages are indicated by ● late stage; ○ intermediate stage; and □ early stage. Data is plotted as the mean \pm S.E.M.

RESULTS

Normal schedule of ovarian maturation

To interpret experimental treatments, we first needed to know the normal schedule of ovarian maturation. Egg development was therefore assayed in untreated virgin female flies at various times after eclosion. At the time of eclosion, the ovary contains pre-vitellogenic oocytes ranging from stage 1 to 7 (King, '70). Figure 1 shows that early vitellogenic oocytes (stages 8 and 9) were first apparent at nine hours after eclosion. Intermediate stage oocytes (stages 10 to 12) were first seen at 12 hours, while late stage oocytes (stages 13 and 14) first appeared at 24 hours after eclosion. By 32 hours after eclosion, all animals exhibited mature oocytes.

Effect of photoperiod on developmental rate

Although yolky oocytes are not observed until several hours after emergence, events initiating vitellogenesis may have occurred much earlier. We have considered the possibility that initiation begins either at the completion of adult development, or at the time of

eclosion. Presently, there is conflicting evidence which considers whether eclosion in *D. melanogaster* occurs soon after the completion of adult development as the "next step" in ontogeny, or if eclosion is controlled independently of adult development. Pittendrigh and Skopik ('70) support the latter view due to their observations in *D. pseudoobscura* and *D. melanogaster*. Their results indicate that eclosion may occur soon after the end of adult development only if the eclosion gate is open (the "allowed phase"). If adult development is completed when the eclosion gate is closed (the "forbidden phase"), eclosion may be delayed for as long as 18 hours (Pittendrigh, '54; Skopik and Pittendrigh, '67). Harker ('65) also observed eclosion rhythms in *D. melanogaster*, but her explanation to account for this phenomenon is different. Harker ('65) concluded from work with *D. melanogaster* that the rate of adult development is controlled by the environmental light cycle and that eclosion occurs directly after the completion of adult development. According to this view, eclosion rhythms are due to the cumulative effect of several circadian oscillators, and

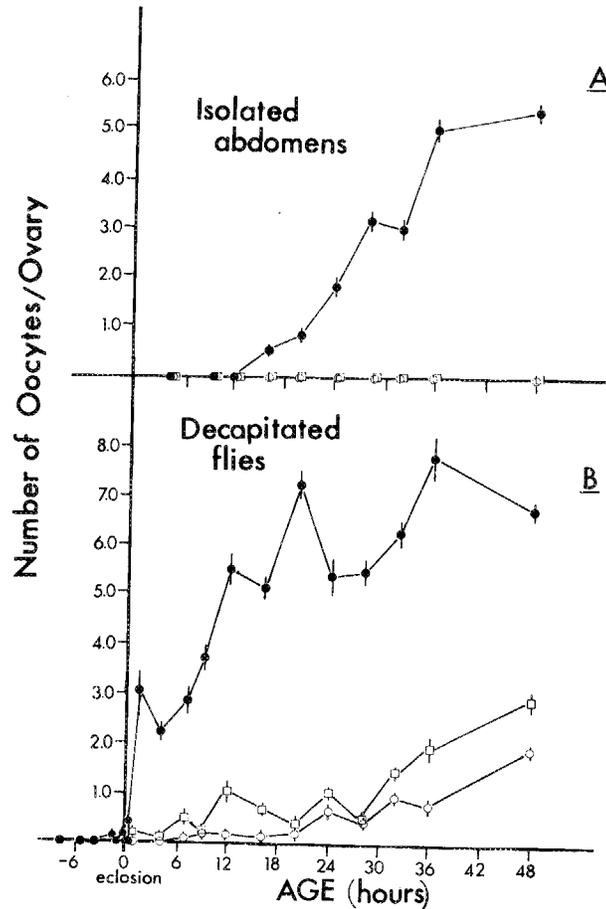


Fig. 2 Appearance of early, intermediate and late stage oocytes in (A) isolated abdomens and (B) decapitated female flies. Operations were performed at the times indicated before or after eclosion and ovaries were dissected at 72 hours after eclosion. No fewer than ten animals (20 ovaries) were dissected for each time point. Animals decapitated previous to five minutes after eclosion revealed 0.0 early and intermediate stage oocytes/ovary (data not plotted). Follicle stages are indicated by ● late stage; ○ intermediate stage; and □ early stage. Data is plotted as the mean \pm S.E.M.

eclosion is not independent of development.

In order to determine whether egg maturation is signalled by the completion of adult development, or by emergence, we felt it necessary to satisfactorily determine whether these two events are actually independent of each other in our stocks of *D. melanogaster*. This was accomplished by a modified repetition of Harker's ('65) and Pittendrigh and Skopik's ('70) experiments. Mixed-sex populations of animals synchronized at the white pre-pupal stage were collected at five different times during a 12L:12D photoperiod. The times chosen were those which gave the largest developmental time differences in Harker's ('65) experiments. The time of ap-

pearance of three cuticular markers (ocellar tormogen sclerotization, orange eye color, and wing pigmentation) was monitored in these animals, in addition to the time of eclosion. Figure 3 indicates that animals entering the prepupal stage at different phases of the photoperiod developed external structures after a given time interval had elapsed, independent of the phase in the photoperiod that pupariation occurred. For example, sclerotization of the ocellar bristle socket occurs at 53 ± 2 hours no matter when in the photoperiod the animals pupariate. Each developmental event appeared in a population within a 2- to 7-hour period. This variation may be due in part to the fact that males consistently take longer to

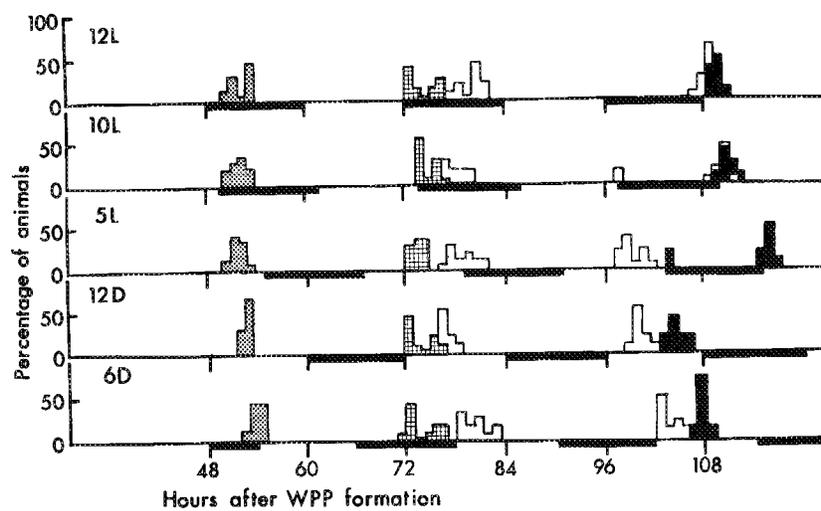


Fig. 3 Time of cuticular structure development, and eclosion, of animals collected as white pre-pupae at different phases of a 12L:12D photoperiod. WPP were collected synchronously at the given number of hours into the light (L) or dark (D) phase. Cuticular markers are designated as dotted bars for ocellar tormogen sclerotization; cross-hatched bars for orange eye color; and shaded bars for wing pigmentation. Time of eclosion is designated as white bars for females, and black bars for males. Dark periods are indicated by black bars under each panel. Light periods occur during the intervals between the black bars. Animals were continuously reared under a 12L:12D photoperiod.

TABLE 1

Ovarian maturation in animals eclosing at different times after prepupae formation

Vitellogenic stage	Hours to eclosion		P
	98-99 hours	107.5-108.5 hours	
Early	4.59 ± 0.33 ¹	4.56 ± 0.20 ¹	0.93 ²
Intermediate	3.91 ± 0.72	3.61 ± 0.54	0.75
Late	0.46 ± 0.13	0.73 ± 0.18	0.25

¹ Mean number of follicles per ovary ± S.E.M.

² Student's t test, d.f. = 12.

develop than females. These results contradict Harker's ('65) conclusion that the rate of adult development in *D. melanogaster* depends on the phase in the photoperiod that pupariation occurs.

In contrast to the results for the development of cuticular structures, the time to eclosion did depend upon the phase in the photoperiod when animals formed white prepupae (fig. 3). Female flies entering the white prepupal stage at 10 hours into the light phase take 12 hours longer to eclose than females collected as white prepupae 5 hours into the light phase. In addition to the variation in the time taken to eclosion between populations collected at different phases, there was a consistent lag in the time taken by males

to eclose. While development of cuticular markers occurs during both the light and dark phases, eclosion is generally limited to the light phase. For animals collected at five and ten hours into the light phase (fig. 3), the majority of animals emerge either before or after, but not during the dark phase. This result for *D. melanogaster* reaffirms Pittendrigh and Skopik's ('70) conclusion that development is not coupled to the circadian oscillation, while the time of adult emergence is. Therefore, animals which have completed development within the eclosion gate may emerge, while slower developing animals are delayed in eclosing until the next gate opens.

If one assumes that the shortest time from pupariation to eclosion is the length of time it

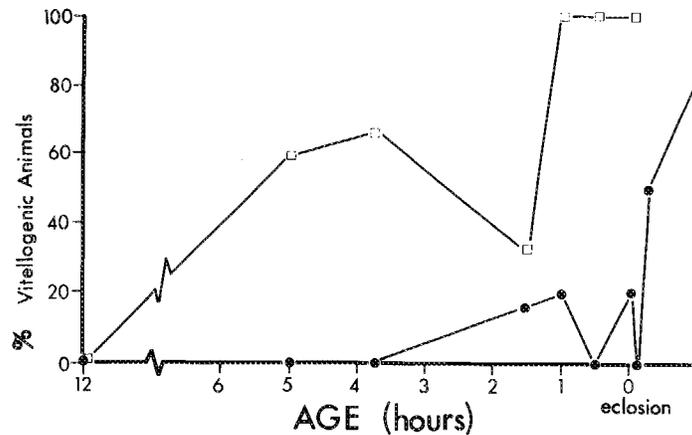


Fig. 4 The effect of JHA on egg maturation in female flies decapitated before eclosion. The percentage of animals exhibiting vitellogenic oocytes is indicated by □ for animals treated with $0.15 \mu\text{g}$ of ZR-515, and ● for non-JHA treated animals. Ovaries were dissected at 72 hours after eclosion.

takes to develop a fully differentiated pharate adult, then in some animals eclosion may be delayed for as long as 12 hours after the completion of adult development.

Initiation of egg maturation

Since the experiment reported above shows that the emergence of the adult fly occurs independent of the time the adult completes development, it was possible to determine if the onset of egg maturation is synchronized to either event. The majority of females collected as white prepupae at the end of the light phase eclose after 107 to 108 hours, while females collected at the beginning of the light phase 12 hours later eclose after only 98 to 99 hours (fig. 3). Thus, we could prepare one group of animals developing 12 hours ahead of a second group, although both groups will eclose within a 2-hour period shortly after lights-on. If egg maturation is dependent upon the time of eclosion, there should be no significant difference in the amount of ovarian maturation between the two populations when assayed 24 hours after eclosion, a time when even a few hours difference is detectable in the randomly pupariating controls (fig. 1). This experiment was repeated seven times. For each experiment, two groups of animals (10-12 animals per group) were prepared. As table 1 shows, there was no significant difference between the mean number of early, intermediate, or late stage oocytes produced by each population when assayed 24 hours after eclosion. Therefore we conclude that the endo-

crine factors that initiate vitellogenesis are associated with eclosion rather than the completion of adult development.

Vitellogenesis in decapitated flies

If the signal which initiates the events resulting in ovarian maturation occurs at the act of emergence, one should be able to identify the tissues involved in some essential aspect of this process. Many insects depend upon the stimulatory action of either neurohormones or nerve action from the brain for egg maturation to proceed. In order to see if a cephalic factor is necessary for the stimulation and continuation of ovarian maturation in *D. melanogaster*, female flies were decapitated at various times before and after eclosion. Egg maturation was assayed in each case 72 hours after the scheduled time of eclosion.

Figure 2B indicates that animals decapitated prior to 1.5 hours before eclosion contained no vitellogenic oocytes at 72 hours after their scheduled time of eclosion. Only 5 out of 38 animals decapitated at times ranging from 1.5 hours before to 2 minutes after eclosion were vitellogenic (fig. 4). These animals support development of fewer than one mature oocyte per animal on the average, with no development of early or intermediate stage oocytes (fig. 2B). Animals decapitated at five to ten minutes after eclosion exhibited early stage oocytes, as well as an increase in the number of late stage oocytes. After 72 hours, animals decapitated one hour after eclosion developed about half as many mature oocytes

TABLE 2

Induction of vitellogenesis in isolated abdomens

Preparation	Treatment	Number of preparations	% with vitellogenesis	Vitellogenic stage		
				Early	Intermediate	Late
Unoperated control	None	10	100	3.3 ¹	1.0 ¹	8.3 ¹
Isolated abdomen	None	14	0	0	0	0
Isolated abdomen	Ringer injection	12	0	0	0	0
Isolated abdomen	CA-CC complex implant	22	46	0.11	0.02	0.38
Isolated abdomen	Corpus allatum implant	14	50	0.57	0	0.32
Isolated abdomen	Corpus cardiacum implant	16	0	0	0	0
Isolated abdomen	Acetone	10	0	0	0	0
Isolated abdomen	0.15 µg ZR-515 in acetone	11	100	1.1	0.22	4.5

¹ Mean number of vitellogenic follicles per ovary.

as developed in unoperated animals (fig. 2), while the amount of early and intermediate stage oocytes was considerably lower (fig. 2B).

These results indicate that some type of cephalic factor is necessary near the time of eclosion for the initiation of vitellogenesis. Removal of the head after this time permits nearly normal continuation of this process. Egg maturation is limited somewhat in decapitated animals, presumably due to the inability of these animals to eat. A lack of nutrition after emergence, however, does not prohibit the formation of vitellogenic oocytes. In fact, decapitated animals displayed more vitellogenesis than animals deprived of only their labial palps (data not shown).

Vitellogenesis in isolated abdomens

Since egg maturation is able to continue without the head after eclosion, we considered whether factors located in the thorax were essential to vitellogenesis, or if the abdomen itself contained all the information necessary to carry on this process. The corpus allatum, which has been implicated as being necessary for egg maturation (Vogt, '40a,b, '43), resides in the thorax of *Drosophila*. Adjacent to the CA lies the corpus cardiacum, which has been shown to act as a storage and releasing organ for neurohormones in other insects (Golds-worthy and Mordue, '74). In order to determine whether ovarian maturation is able to proceed in the absence of these tissues, isolated abdomens were prepared at various times after eclosion. Egg maturation was assayed 72 hours after eclosion.

Figure 2A shows that none of the abdominal preparations ligated at any time up to 48 hours contained any early or intermediate stage oocytes when examined at 72 hours. Late stage oocytes appeared initially in abdomens isolated 16 hours after eclosion. An increasing number of mature oocytes appeared in abdomens isolated after this time. Figure 1 shows that mature oocytes were not present in unoperated animals at 16 or 20 hours after eclosion. Therefore the mature oocytes found in abdomens isolated at 16 and 20 hours after eclosion must have formed from early or intermediate stages. The average number of mature oocytes found in abdomens isolated later than 20 hours (fig. 2A) was higher than the number of mature oocytes present at the time of ligation (fig. 1). This indicates that a limited number of intermediate stage oocytes are able to continue their development to the mature stage without thoracic or cephalic information. Since the number of intermediate stage oocytes at the time of ligation (fig. 1) was greater than the increase in the number of mature oocytes after ligation (fig. 2A), some of the oocytes which were at an intermediate stage at the time of ligation do not continue development and presumably degenerate. At 72 hours, isolated abdomens contained no early or intermediate stage follicles, and so formation of early vitellogenic stages does not occur under these conditions.

Effects of CA and CC on egg maturation in isolated abdomens

In order to determine which tissue is responsible for continuation of egg maturation in iso-

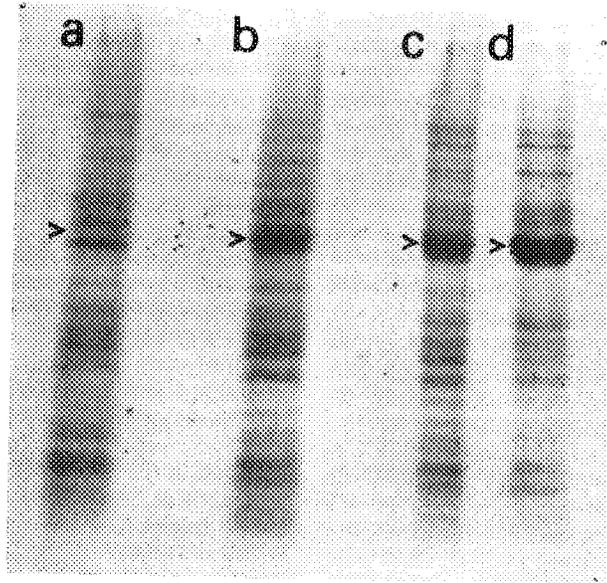


Fig. 5 SDS polyacrylamide gel electrophoresis of (a) immature ovaries, (b) mature ovaries from JHA treated isolated abdomens, (c) mature ovaries from untreated females, and (d) oviposited eggs. The arrow indicates a major protein band, lacking in immature ovaries.

lated abdomens, the CA and/or CC were transplanted into female abdomens isolated within ten minutes of eclosion. The previous experiment showed that abdomens isolated at this time do not normally undergo vitellogenesis. The initial experiment involved transplanting three CA-CC complexes from 60- to 72-hour-old females into host isolated abdomens. The data in table 2 show that 46% of the surviving host abdomens supported vitellogenesis. Oocytes of all stages were found. To more specifically discern whether vitellogenesis depends upon both the CA and CC glands, these tissues were transplanted separately into isolated abdomens. Corpora allata glands transplanted alone supported vitellogenesis in 50% of the surviving abdomens (table 2). Although early and late stage oocytes appeared, no intermediate stage oocytes were observed. In contrast, none of the surviving abdomens implanted with CC glands exhibited any vitellogenic oocytes. These results indicate that the CA gland is necessary for a continuation of egg maturation, and that neurosecretory hormones stored in the CC are not necessary later than ten minutes after eclosion.

Effects of JH analog treatments on egg maturation in isolated abdomens

Since the CA is sufficient to allow ovarian maturation in isolated abdomens, it is neces-

sary to see if JH, which is purportedly synthesized and secreted by the CA, is able to effect the same result. Abdomens isolated from newly emerged flies were treated with a topical application of 0.15 μg of the juvenile hormone analog, ZR-515. The data in table 2 indicate that 100% of the treated abdomens exhibited oocytes of early, intermediate, and late stages. These experiments show that a juvenile hormone analog can substitute for the effect of the CA.

Effect of JH analog treatments on animals decapitated prior to eclosion

Experiments described above (fig. 2B) in which egg maturation was not observed in animals decapitated before eclosion, indicated that cephalic information is needed near the time of eclosion. This information might provide a signal directly to the abdomen that allows the ovaries to mature, or alternatively, it might signal other events (for example, CA activation) that precede vitellogenesis. To test these possibilities, animals decapitated prior to eclosion were treated with JHA. Figure 4 indicates that ovaries within pharate adults decapitated as early as five hours before eclosion supported vitellogenesis when supplied with JHA. This included an average of 2.1 and 5.0 late stage follicles per ovary in pharate adults decapitated at five and zero hours

before the scheduled time of eclosion. Since the cephalic signal can be bypassed by JHA application, it must not directly affect the ovary's competence to develop. The development of ovaries in these animals also indicates that the lack of vitellogenesis in animals decapitated prior to eclosion is not due to surgically induced trauma.

Ovarian proteins and JH analog treatments

The previous experiments showed that either the CA, or a juvenile hormone analog, can cause morphologically normal mature oocytes to be formed in isolated abdomens. But since developing *Drosophila* oocytes can sequester extraneous proteins such as ferritin from the hemolymph (Mahowald, '72), it was necessary to evaluate the effect of JHA on the presence of major ovarian proteins in the maturing oocyte. The electrophoretic mobility of proteins obtained from ovaries that were artificially matured in isolated abdomens treated with JHA were compared to proteins found in immature ovaries, naturally maturing ovaries and oviposited eggs. The results (fig. 5) show a major band that was present both in ovaries containing mature oocytes and in oviposited eggs, but which was lacking in immature ovaries. The morphologically mature ovaries from isolated abdomens treated with JHA also displayed this band. In all important features, the ovaries from JHA treated isolated abdomens contained the same electrophoretic bands as mature ovaries from normal flies. The juvenile hormone analog is apparently inducing a normal developmental process.

DISCUSSION

Initiation of vitellogenesis

Morphological evidence for early stage vitellogenic oocytes was not observed in *D. melanogaster* ovaries until nine hours after eclosion, while mature oocytes were first evident at 24 hours (fig. 1). The initiation of the processes which result in vitellogenic oocytes must therefore occur sometime beforehand. Egg maturation in many flies is dependent upon feeding, which either fulfills nutritional requirements or triggers the release of an essential hormone (Lea, '72). In *D. melanogaster*, feeding after eclosion is not vital for the production of the initial group of eggs (Bodenstein, '47), and therefore the stimulus for vitellogenesis must be independent of feeding.

Flies eclosing at the same time proceeded

with vitellogenesis at approximately equal rates (table 1). This suggests that an event around the time of emergence might trigger the processes leading to vitellogenesis. In *D. melanogaster*, the completion of adult development and the act of eclosion were shown to be independent events (fig. 3). Experiments with carefully staged animals showed that it is the event of eclosion rather than the completion of adult development which is synchronized with vitellogenesis (table 1). Since the time of emergence is controlled by a circadian clock, this clock must control, either directly or indirectly, the onset of vitellogenesis. Initiation at the time of emergence is supported by the findings of Kambysellis and Craddock ('76), who report that yolk proteins are first detected in the hemolymph of female flies just before, or at the time of emergence.

The precise location of the circadian clock in *D. melanogaster* is unknown, but evidence from genetic mosaics suggests it is in the brain (Konopka and Benzer, '71). To test the effect of the brain on vitellogenesis, animals were decapitated before and after the time of eclosion. Animals decapitated shortly before emergence were unable to support normal egg production, but those decapitated after eclosion did undergo normal vitellogenesis, including the production of new early and intermediate stage oocytes (fig. 2B). Therefore neither the cephalic influence which triggers vitellogenesis, nor any other cephalic information is necessary for ovarian maturation after eclosion.

The nature of the cephalic factor which triggers vitellogenesis, whether nervous or neurohormonal, is unknown. In *Antherea pernyi*, a circadian clock controls the release of an eclosion hormone, produced in the brain, which results in eclosion behavior (Truman, '71a). Since the circadian clock which controls eclosion in *A. pernyi* and *D. pseudoobscura* are both of the "hour-glass" type (Truman, '71b), it is interesting to consider whether an eclosion hormone is present in *D. melanogaster*, and whether it is related to the onset of egg maturation.

The precise function of the cephalic factor is unknown, but neither the triggering of hormonal activity at emergence, nor the temporary necessity of a cephalic factor for oogenesis is unique to *Drosophila*. In *Aedes aegypti*, emergence triggers the release of JH from the corpus allatum, which allows the development of the ovaries up to the pre-vitellogenic rest-

ing stage (Gwadz and Spielman, '73). It is also found in *Aedes* that an egg development neurohormone, whose release is triggered by a blood meal, is necessary only during the initial phase of egg maturation. Hagedorn ('74) suggests that the ovary is the target of the neurohormone in *Aedes*. This interaction seems unlikely in *D. melanogaster*. In animals deprived of the cephalic signal by decapitation, the production of morphologically normal stage 14 oocytes could be induced by applications of JHA (fig. 4). This argues against a direct effect of neurohormones on the ovary, and shows that the effect of the brain in inducing morphologically normal oocytes can be bypassed by JHA.

Other possible functions for the cephalic factor include induction of yolk protein synthesis by the fat body (Hill, '62), its uptake by the ovary (Wilkins, '69), or the stimulation of the corpus allatum (Thomsen, '52; Engelmann, '60). Influences on vitellogenesis which are not continuously necessary, are not likely to have an effect on each maturing follicle, such as to induce the uptake of vitellogenins. It is more likely that influences which are needed only temporarily trigger the activity of tissues whose presence is continuously necessary. The fat body, which secretes vitellogenin (Gelti-Douka et al., '74), and the corpus allatum hormone, which possibly induces vitellogenin uptake (Gavin and Williamson, '76b), are both continuously necessary for normal vitellogenesis and hence are candidates for the target(s) of the cephalic factor.

Continuation of vitellogenesis

The experiments described above show that cephalic information is needed for the initiation of vitellogenesis, but that vitellogenesis can proceed normally without such information one hour after emergence. In contrast, abdomens isolated as late as 12 hours after emergence could not support vitellogenesis (fig. 2A). Some process that succeeds the action of the cephalic factor is lacking in isolated abdomens. This factor is not only required later than the cephalic factor, but it also has a different function. After ligation, isolated abdomens were unable to support the development of early or intermediate stage oocytes, although a few follicles which are at intermediate stages at the time of ligation were able to become mature. Therefore, this presumably thoracic factor is required to cause non-vitellogenic stage 7 follicles to be-

come vitellogenic, and it promotes the advancement of early to intermediate, and intermediate to late stage follicles.

The tissue responsible for the thoracic factor was shown to be the corpus allatum by transplants of various tissues into isolated abdomens (table 2). Topical treatment of isolated abdomens with JHA showed that a substance with juvenile hormone activity in flies (Postlethwait, '74) could replace the effects of the CA. Transplants also ruled out the possibility that residual neurosecretory material in the CC might allow vitellogenesis to proceed in the absence of the brain.

This work concerning the endocrine control of vitellogenesis was based on morphological evidence for maturation. Since oocytes in *D. melanogaster* (Mahowald, '72) as well as in *Cecropia* (Telfer, '60) may sequester some hemolymph proteins in addition to vitellogenin, morphologically mature oocytes do not guarantee that a normal process has been effected. Using electrophoresis to analyze the proteins sequestered by ovaries in JHA treated abdomens, we have found that the major ovarian proteins found in these ovaries, and in ovaries from untreated females, are the same (fig. 5). This shows that the JHA is inducing both morphologically and biochemically normal development.

The experiments reported here lead us to conclude that a function of juvenile hormone in vitellogenesis is to permit events which lead to yolk deposition in each follicle. This conclusion for wild type flies is supported by work with female sterile mutants. Vitellogenesis occurs in some mutants only when they are treated with JHA (Postlethwait and Weiser, '73; Postlethwait et al., '76), and yet some of these mutants have vitellogenin in their hemolymph (Kambysellis and Craddock, '76; Gavin and Williams, '76b). Since these mutants have defective adult corpora allata (Postlethwait et al., '76), yolk protein synthesis and secretion is apparently independent of JH, although yolk protein uptake requires JH.

Previous experiments by Vogt ('43) and Bodenstern ('47) implicated the necessity of the CA during vitellogenesis, but neither of these studies directly tested the adult CA, nor did they eliminate the possible influences of cephalic information. Much of their work was done in species other than *D. melanogaster*. Vogt ('40a,b, '41) also performed interspecific transplants of ovaries and larval ring glands

in different species of *Drosophila*. The results indicated that the gonadotropic function of the CA is not necessarily conserved among the different species, and therefore evidence from other species cannot necessarily be extended to *D. melanogaster*.

Our evidence demonstrating the continued requirement of the CA to cause stage 7 follicles to become vitellogenic does not agree with Bouletreau-Merle's ('73) conclusion that brain hormones probably have a more important role in oogenesis than the CA. Appearance of new stage 8 follicles can proceed without the brain, but not without the CA. Cephalic influences, in conjunction with paragonial substances, may regulate the rate of ovarian maturation and especially oviposition, but brain hormones are not necessary to continue vitellogenesis.

Endocrine interactions in Drosophila vitellogenesis

The results as discussed above permit us to delineate in part the interactions of endocrine factors in vitellogenesis. After the development of the pharate adult is complete, the circadian clock controlling eclosion becomes the prime mover in the series of events leading to vitellogenesis. The clock stimulates the activity of a nervous or neuroendocrine cephalic factor, which then acts to trigger an event near eclosion in the thorax or abdomen of the fly. After the cephalic event has occurred, the corpus allatum becomes active and it secretes a substance with juvenile hormone activity. This hormone is continuously necessary for each follicle to become vitellogenic. In the absence of the brain factor, JH alone can cause morphologically normal mature oocytes to develop.

A major link in the control of vitellogenesis which has not been resolved is the control of yolk protein synthesis in the fat body. It is unknown whether the effect of the brain, or juvenile hormone, are involved in this process. In another dipteran, *Aedes aegypti*, yolk protein synthesis is stimulated by ecdysone, secreted by the ovary (Hagedorn, '74). Although such a function of the ovaries in *Drosophila* has not been reported, ecdysone has recently been detected in the ovarian tissues of *D. melanogaster* adults (De Reggi et al., unpublished, cited in Legay et al., '76). Doane ('61) suggests that the ovaries in *D. melanogaster* have a regulatory effect on lipid metabolism, as well as inhibitory effects on the CA. Evidence sug-

gesting a more direct role for the ovaries in vitellogenin synthesis is presently being pursued.

ACKNOWLEDGMENTS

We would like to thank Christine Kirby for helping us with the electrophoresis procedure. Appreciation is also extended to Ron Sederoff, Harry Teitelbaum, Rick Johns, and Chris Kirby for helpful suggestions during preparation of the manuscript.

This work was supported by Grant GM 21548 from the National Institutes of Health. J. H. Postlethwait was supported by a Research Career Development Award from NIH and A. M. Handler was supported by a Behavioral Biology Training Grant, PHS MH 14281.

LITERATURE CITED

- Bodenstein, D. 1947 Investigations on the reproductive system of *Drosophila*. *J. Exp. Zool.*, 104: 101-152.
- Bouletreau-Merle, J. 1974 Stimulation de l'ovogenèse par la copulation chez les femelles de *Drosophila melanogaster* privées de leur complexe endocrine rétro-cérébral. *J. Insect Physiol.*, 20: 2035-2041.
- 1976 Destruction de la pars intercérébrale, chez *Drosophila melanogaster*: Effet sur la fécondité et sur sa stimulation par l'accouplement. *J. Insect Physiol.*, 22: 933-940.
- Brett, W. J. 1955 Persistent diurnal rhythmicity in *Drosophila* emergence. *Ann. ent. Soc. Am.*, 48: 119-131.
- Chan, L., and W. Gehring 1971 Determination of blastoderm cells in *Drosophila melanogaster*. *Proc. Nat. Acad. Sci.*, (U.S.A.), 68: 2217-2221.
- Davis, B. J. 1964 Disc electrophoresis. II. Method and application to human serum proteins. *Ann. N. Y. Acad. Sci.* 121: 404-427.
- Doane, W. W. 1961 Developmental physiology of the mutant female sterile (2) adipose of *Drosophila melanogaster*. III. Corpus allatum-complex and ovarian transplantations. *J. Exp. Zool.*, 146: 275-298.
- Engelmann, F. 1960 Mechanisms controlling reproduction in two viviparous cockroaches (Blattaria). *Ann. N. Y. Acad. Sci.*, 89: 516-536.
- 1970 *Physiology of Insect Reproduction*. Pergamon Press, Oxford.
- Ephrussi, B., and G. Beadle 1936 A technique for transplantation for *Drosophila*. *Amer. Natur.*, 70: 218-225.
- Gavin, J. A., and J. H. Williamson 1976a Synthesis and deposition of yolk protein in adult *Drosophila melanogaster*. *J. Insect Physiol.*, 22: 1457-1464.
- 1976b Juvenile hormone-induced vitellogenesis in apterous⁴, a non-vitellogenic mutant in *Drosophila melanogaster*. *J. Insect Physiol.*, 22: 1737-1742.
- Gelti-Douka, H., T. R. Gingeras and M. P. Kambyellis 1974 Yolk proteins in *Drosophila*: identification and site of synthesis. *J. Exp. Zool.*, 187: 167-172.
- Goldsworthy, G. J., and W. Mordue 1974 Neurosecretory hormones in insects. *J. Endocr.*, 60: 529-558.
- Gwadz, R. W., and A. Spielman 1973 Corpus allatum control of ovarian development in *Aedes aegypti*. *J. Insect Physiol.*, 19: 1441-1448.
- Hagedorn, H. H. 1974 The control of vitellogenesis in the mosquito, *Aedes aegypti*. *Amer. Zool.*, 14: 1207-1217.
- Harker, J. E. 1965 The effect of a biological clock on the

- developmental rate of *Drosophila* pupae. *J. Exp. Biol.*, **42**: 323-337.
- Highnam, K. C., O. Lusia and L. Hill 1963 The role of the corpora allata during oocyte growth in the desert locust, *Schistocerca gregaria* Forsk. *J. Insect Physiol.*, **9**: 587-596.
- Hill, L. 1962 Neurosecretory control of hemolymph protein concentration during ovarian development in the desert locust. *J. Insect Physiol.*, **8**: 609-619.
- Kambysellis, M. P., and E. M. Craddock 1976 Genetic analysis of vitellogenesis in *Drosophila*. *Genetics*, **183**: s38 (Abstract).
- King, R. C. 1970 Ovarian Development in *Drosophila melanogaster*. Academic Press, New York.
- Konopka, R. J., and S. Benzer 1971 Clock mutants of *Drosophila melanogaster*. *Proc. Nat. Acad. Sci. (U.S.A.)*, **68**: 2112-2116.
- Lea, A. O. 1969 Egg maturation in mosquitoes not regulated by the corpora allata. *J. Insect Physiol.*, **15**: 537-541.
- 1972 Regulation of egg maturation in the mosquito by the neurosecretory system: the role of the corpus cardiacum. *Gen. Comp. Endocrinol. Suppl.*, **3**: 602-608.
- Legay, J. M., B. Calvez, M. Hirn, and M. L. De Reggi 1976 Ecdysone and oocyte morphogenesis in *Bombyx mori*. *Nature*, **262**: 489-490.
- Lumme, J., and P. Lankinen 1973 An apparatus for recording the eclosion rhythm of *Drosophila*. *Dros. Inf. Serv.*, **50**: 190.
- Mahowald, A. 1972 Ultrastructural observations on oogenesis in *Drosophila*. *J. Morph.*, **137**: 29-48.
- Nijhout, M. M., and L. M. Riddiford 1974 The control of egg maturation by juvenile hormone in the tobacco hornworm moth, *Manduca sexta*. *Biol. Bull.*, **146**: 377-392.
- Pan, M. L., W. J. Bell and W. H. Telfer 1969 Vitellogenic blood protein synthesis by insect fat body. *Science*, **165**: 393-394.
- Pan, M. L., and G. R. Wyatt 1971 Juvenile hormone induces vitellogenin synthesis in the Monarch butterfly. *Science*, **174**: 503-505.
- Pittendrigh, C. S. 1954 On temperature independence in the clock system controlling emergence time in *Drosophila*. *Proc. Nat. Acad. Sci. (U.S.A.)*, **40**: 1018-1029.
- Pittendrigh, C. S., and S. D. Skopik 1970 Circadian systems. V. The driving oscillation and the temporal sequence of development. *Proc. Nat. Acad. Sci. (U.S.A.)*, **65**: 500-507.
- Postlethwait, J. H. 1974 Juvenile hormone and the adult development of *Drosophila*. *Biol. Bull.*, **147**: 119-135.
- Postlethwait, J. H., and P. Gray 1975 Regulation of acid phosphatase activity in the ovary of *Drosophila melanogaster*. *Devel. Biol.*, **47**: 196-205.
- Postlethwait, J. H., A. M. Handler and P. W. Gray 1976 A genetic approach to the study of juvenile hormone control of vitellogenesis in *Drosophila melanogaster*. In: *The Juvenile Hormones*. L. Gilbert, ed. Plenum Press, New York, pp. 449-469.
- Postlethwait, J. H., and K. Weiser 1973 Vitellogenesis induced by juvenile hormone in the female sterile mutant *apterous-four* in *Drosophila melanogaster*. *Nature New Biol.*, **244**: 284-285.
- Schaffner, W., and C. Weissman 1973 A rapid, sensitive and specific method for the determination of protein in dilute solution. *Anal. Biochem.*, **56**: 502-514.
- Skopik, S. D., and C. S. Pittendrigh 1967 Circadian systems. II. The oscillation in the individual *Drosophila* pupa; its independence of developmental stage. *Proc. Nat. Acad. Sci. (U.S.A.)*, **58**: 1862-1869.
- Telfer, W. H. 1960 The selective accumulation of blood proteins by the oocytes of Saturniid moths. *Biol. Bull.*, **118**: 338-351.
- Thomsen, E. 1952 Functional significance of the neurosecretory brain cells and the corpus cardiacum in the female blow-fly, *Calliphora erythrocephala* Meig. *J. Exp. Biol.*, **29**: 137-172.
- Thomsen, E., and A. O. Lea 1968 Control of the medial neurosecretory cells by the corpus allatum in *Calliphora erythrocephala*. *Gen. Comp. Endocrinol.*, **12**: 51-57.
- Truman, J. W. 1971a Physiology of insect ecdysis. I. The eclosion behavior of saturniid moths and its hormonal release. *J. Exp. Biol.*, **54**: 805-814.
- 1971b Hour-glass behavior of the circadian clock controlling eclosion of the silkworm *Antheraea pernyi*. *Proc. Nat. Acad. Sci. (U.S.A.)*, **68**: 595-599.
- Ursprung, H. 1967 *In vivo* culture of *Drosophila* imaginal discs. In: *Methods in Developmental Biology*. F. Wilt and N. Wessels, eds. Crowell, New York, pp. 485-492.
- Vogt, M. 1940a Die Forderung der Eireifung innerhalb heteroplastisch transplantierte Ovarien von *Drosophila* durch die gleichzeitig Implantation der arteigenen Ringdrüse. *Biol. Zentr.*, **60**: 479-484.
- 1940b Zur Ursache der Unterschied zwischen gonadotropen Wirkung der Ringdrüse von *Drosophila funebris* und *D. melanogaster*. *Wilhelm Roux' Arch. Entwicklungsmech. Organ.*, **140**: 525-546.
- 1941 Zur Artpezifität der Ringdrüsenwirkung auf die Dotterbildung und die imaginale Differenzierung bei *Drosophila*arten. *Biol. Zentr.*, **61**: 242-252.
- 1943 Zur Produktion gonadotropen Hormones durch Ringdrüsen des ersten Larvenstadiums bei *Drosophila*. *Biol. Zentr.*, **63**: 467-470.
- 1946 Inhibitory effects of the corpora cardiaca and of the corpus allatum in *Drosophila*. *Nature*, **157**: 512.
- Wallace, R. A., and E. W. Bergink 1974 Amphibian vitellogenin: properties, hormonal regulation of hepatic synthesis and ovarian uptake, and conversion to yolk proteins. *Amer. Zool.*, **14**: 1159-1175.
- Wilkens, J. L. 1968 The endocrine and nutritional control of egg maturation in the fleshfly *Sarcophaga bullata*. *J. Insect Physiol.*, **14**: 927-943.
- 1969 The endocrine control of protein metabolism as related to reproduction in the fleshly *Sarcophaga bullata*. *J. Insect Physiol.*, **15**: 1015-1024.