JOURNAL OF MORPHOLOGY 173:313-324 (1982)

Sorbic Acid-Induced Differences in the Ultrastructural Development of Oocytes in the Microbially Ectosymbiotic Female of *Xyleborus ferrugineus* (Fabr.) (Coleoptera, Scolytidae)

H.M.CHU, D.M. NORRIS, AND K.D.P. RAO
Department of Entomology, University of Wisconsin, Madison, Wisconsin 53706

ABSTRACT The ovaries of the beetle *Xyleborus ferrugineus* reared on standard sawdust diet with and without 0.08% sorbic acid added were examined for differences in ultrastructural development of the oocytes. Indications of vigorous yolk deposition are an extensive rough-surfaced endoplasmic reticulum (RER), numerous electron-dense secretory vesicles and a prominent nucleus in associated follicle cells, and extremely electron-opaque material in the interfollicular cell spaces and the perioocytic area. After 6 days of feeding without added sorbic acid, a mature terminal oocyte is present in one of the two ovaries. This terminal oocyte at this mature stage contains yolk spheres and lipid bodies. However, the most mature oocyte in beetles reared on the standard sawdust diet to which 0.08% sorbic acid was added remained at a previtellogenic stage after 6 days of feeding. Titers of ecdysone in 6-day-old adult females reared on standard sawdust without and with 0.08% sorbic acid added were 534.64 ± 20.93 S.D. pg/mg and 39.94 ± 14.71 S.D. pg/mg body weight, respectively.

Oocyte maturation in insects is under nutritional and hormonal control. Nutritional deficiency may directly curtail the production of yolk materials for vitellogenesis or may affect the endocrine system and thus yolk formation (Engelmann '70, '79, '80; Doane, '73). The key hormone in the control of vitellogenesis in most insects appears to be juvenile hormone (JH). It may regulate vitellogenin synthesis by the fat body, secretion into the hemolymph, and uptake by oocytes (Engelmann and Ladduwahetty, '74). However, in *Aedes*, vitellogenin synthesis appears to be stimulated by ecdysone (Spielman et al., '71; Hagedorn, '74; Masler et al., '81), and in *Oncopeltus fasciatus* it involves both JH and ecdysone (Rankin and Jäckle, '80). After the intake of blood protein, the brain of an anautogenous mosquito releases the egg-developmental neuro-secretory hormone (EDNH) (Lea, '72) which stimulates the production of ecdysone (Hagedorn et al., '79). The ecdysone is then apparently hydroxylated to form 20-hydroxyecdysone (Fuchs et al., '79), which in turn induces the synthesis of vitellogenin in the fat body (Hagedorn et al., '75).

The coleopteran, *Xyleborus ferrugineus*, another anautogenous species, receives the protein equivalent to that provided to the mosquito by a blood meal through feeding on cultivated fungi (Kok and Norris, '72). If sorbic acid is added to the standard diet, food fungi do not grow (Norris and Baker, '67). We report here on the sorbic-acid-induced differences in the ultrastructural development of oocytes in *X. ferrugineus*, and their corresponding ecdysteroid levels determined by radioimmunoassay.

MATERIALS AND METHODS

Insect rearing

Xyleborus ferrugineus were reared routinely on a standard sawdust-agar-based diet (SSD) in our laboratory (Norris and Baker, '67; Saunders and Knoke, '67). Female pupae collected from these stock cultures were placed on 2% agar medium in petri dishes. Newly emerged adult females were then placed in tubes on either SSD or SSD plus 0.08% sorbic acid. All such tubes also contained a small inoculum plug of the food fungus, *Fusarium solani*, placed there 1 day prior to the transfer of the female beetle. Tubes were kept at 28°C

0362-2525/82/1733-0313$03.50

in darkness. The activities of the beetles were observed and recorded daily for 6 days of feeding on these two diets.

Microscopy

For light microscopy, whole beetles from each group were fixed in formol-alcohol (Lillie, '54) and double-embedded by the method of Briggs ('58). Sections of 7 μm were stained with alum haematoxylin and eosin. For transmission electron microscopy (TEM), ovaries were processed as previously described (Chu and Norris, '79). Ultrathin sections were stained by uranyl acetate and lead citrate, and viewed with a JEM-7 electron microscope at 60 kV.

Radioimmunoassay (RIA)

Quantification of ecdysteroids was by radioimmunoassay (Bollenbacher et al., '75), with α-[23,24-^{3}H(N)] ecdysone (63.5 Ci/mmole) as the radioligand. Ecdysone antiserum was a generous gift from Prof. L. I. Gilbert, Department of Zoology, University of North Carolina, Chapel Hill, NC.

RESULTS

Beetles reared on SSD with F. solani culture

By the second day on SSD the mycelium of *F. solani* started to grow out of the inoculum-bearing agar plug and began to spread over the surface of the diet. The mycelium penetrated as deep as 30 mm into the food by the end of the first week. The tunneling of the beetle in the diet provides avenues for rapid fungal penetration into the medium. The female usually makes a few accessory tunnels before she starts to make her main gallery. She lays eggs in cradles (i.e., very short tunnels) which are constructed off the sides of the main gallery, or in the end of some longer side tunnels. The average length of tunneling during 6 days of feeding was about 44 mm, but only 20% of the gallery was visible from outside the culture tube.

The paired ovaries of *X. ferrugineus* each contain two ovarioles, which are divided into three parts—terminal filament, germarium, and vitellarium (Fig. 1). The anterior portion of the germarium consists of trophocytes (nurse cells); the posterior portion houses oogonia and prefollicular tissues. The vitellarium contains previtellogenic and vitellogenic oocytes. The largest and most mature oocyte is positioned in the most proximal part of the ovariole (Figs. 1b, 2a).

Previtellogenic oocytes are sheathed by a simple follicular epithelium of tightly arranged cuboidal cells. From a tangential section they are hexagonal (Fig. 2b). They have a large central nucleus. There is intercellular space between adjacent follicular cells. This space may form a wide, funnel-shaped opening just beneath the ovarial sheath. There are enlarged areas within this space which usually contain electron-dense material(s) (Fig. 3). Extremely electron-dense material may occupy the apical part of this space. There is a prominent Golgi apparatus. Densely packed free ribosomes are widely spread throughout the cytoplasm. Some rough-surfaced endoplasmic reticulum (RER) appears in the apical portion of the follicular cells. Mitochondria vary in shape; most are spherical, but a few are long and rod-shaped. They all possess an electron-dense matrix. A zone of microvilli is formed at the interface between the oolemma and follicular cell membrane. This deeply folded surface has no preferred orientation; thus in a single section the microvilli are cut at many angles (Figs. 3, 8). Inside the oocyte, next to the zone of microvilli, there are some short RER and tubules. Mitochondria have a very electron-dense matrix (Fig. 3, compare with Fig. 7). There are some continuous circles of RER. The oocyte is densely packed with free ribosomes. Neither lipid nor yolk spheres have been found at this stage.

During vitellogenesis, the apical portion of the follicular cells becomes very densely packed with parallel-oriented RER. Many electron-dense bodies and Golgi with vesicles are closely associated with the RER. The apical portion of the interfollicular cell space appears free of the previously described electron-dense material. The basal portion of this space, close to the oocyte, is filled with electron-dense material. At this stage, there are many large secretion vesicles (Figs. 4-6) in the cytoplasm of the follicular cells. They are especially abundant in the apical portion of the cells. Their length ranges from 430 nm to 1.04 μm with a mean of 565 nm. They are membrane-bounded with a fiberlike electron-dense matrix and a few peripheral needlelike crystals (Figs. 4-6). The zone of microvilli is filled with electron-dense material at this stage. Inside the oocyte, there are many small vesicles, filled with electron-dense material, located near the zone of microvilli. Large numbers of lipid and yolk spheres appear first in the cortex of the oocyte and subsequently spread throughout the oocyte (Fig. 7). There are many mitochondria with an electron-dense matrix, and some short RER. Densely packed ribosomes are

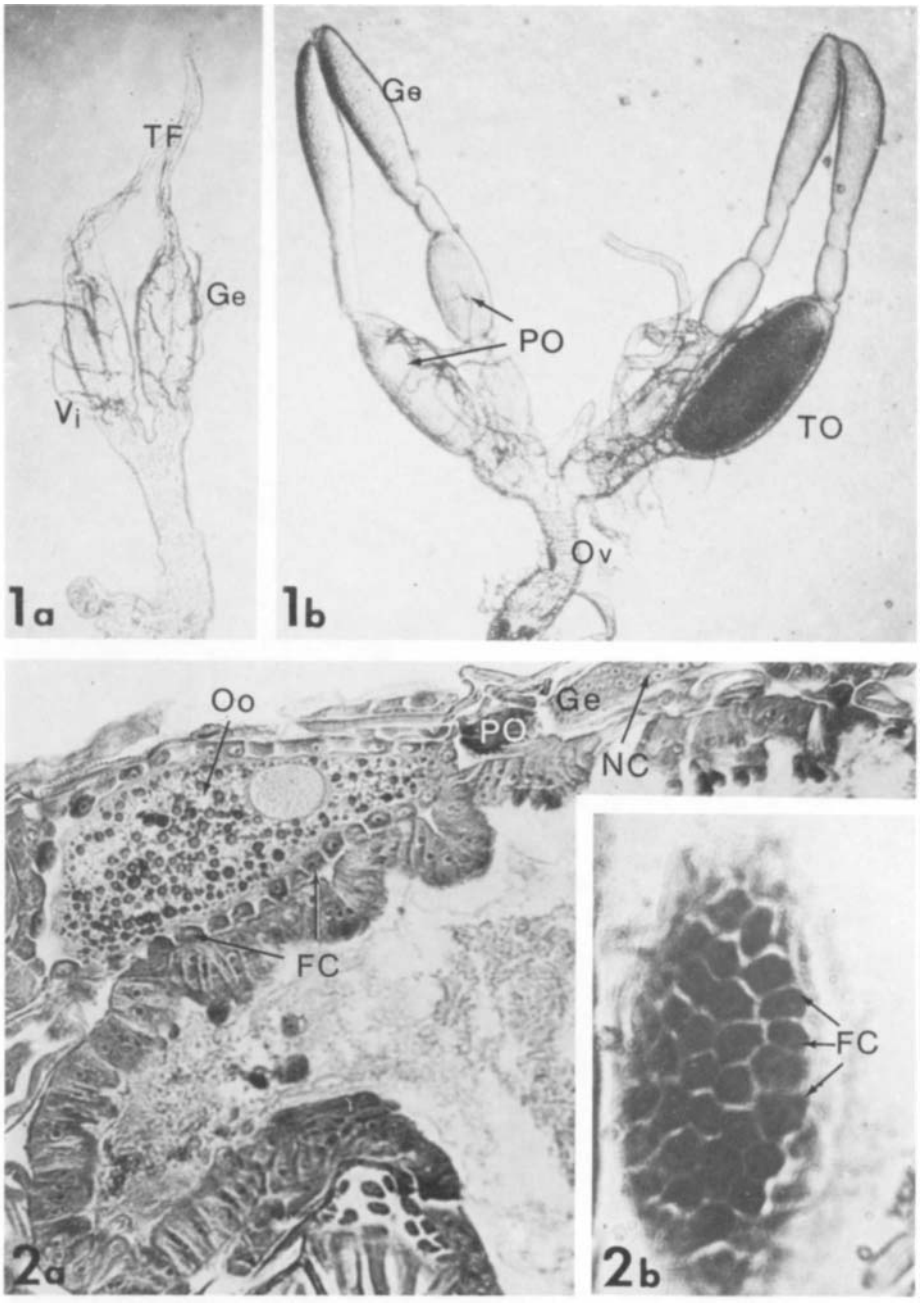


Fig. 1.a. Whole mount of ovaries of female beetle after 6 days of feeding on sorbic-acid-containing diet, showing a very long filament (TF), germarium (Ge), and vitellarium (Vi). × 53. b. Whole mount of ovaries of female beetle after 6 days of feeding on SSD diet, showing the one well-developed terminal oocyte (TO), penultimate oocytes (PO), germarium (Ge), and oviduct (Ov). × 53.

Fig. 2.a. Longitudinal section of the ovary of a female beetle after 6 days of feeding on SSD diet, with vitellogenic oocyte (Oo) surrounded by a layer of follicle cells (FC), penultimate oocyte (PO), germarium (Ge), and well-developed nurse cells (NC) × 117. b. A tangential section through follicular epithelium showing the hexagonally shaped follicle cells (FC) of an ovary. × 480.

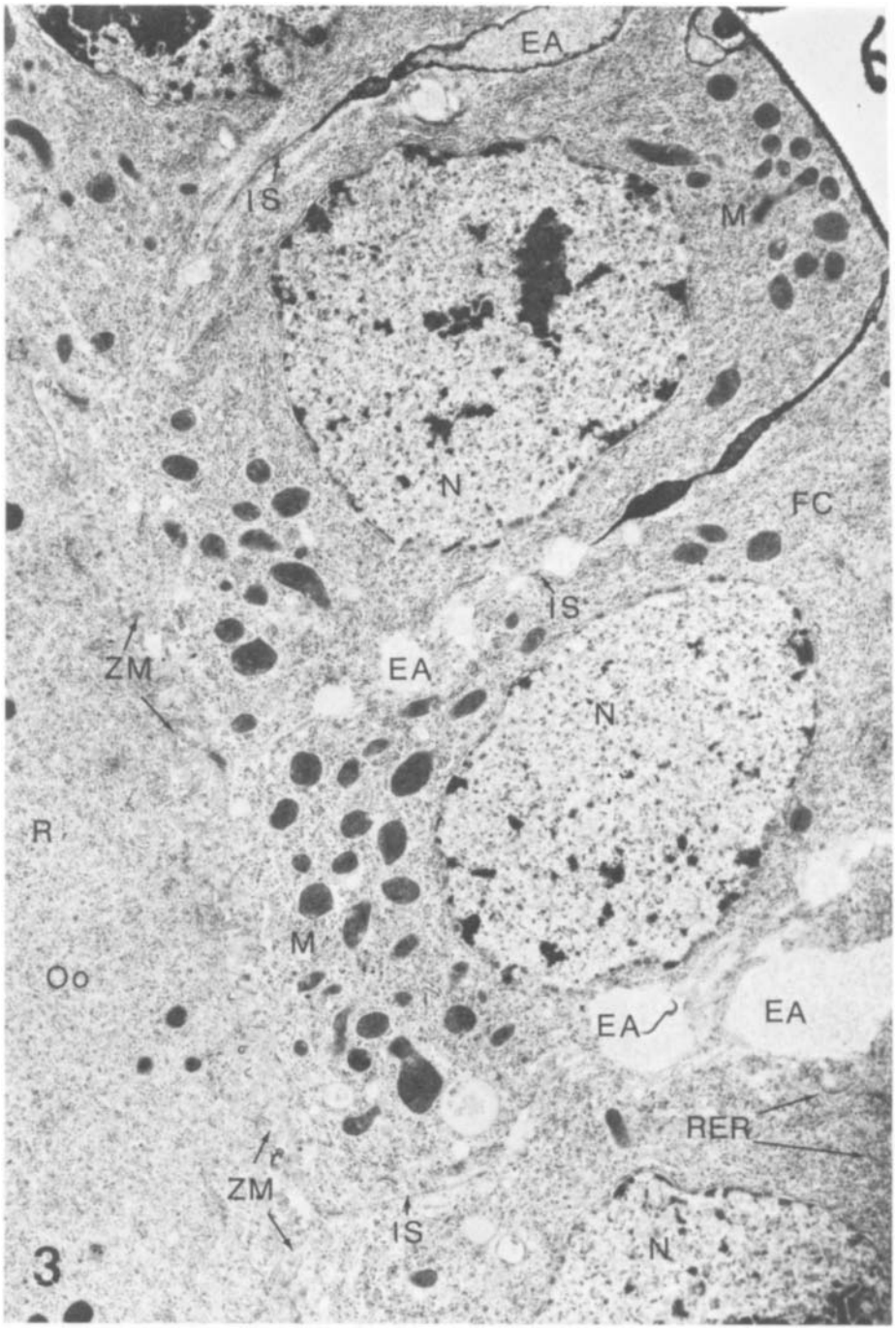


Fig. 3. Previtellogenic oocyte, showing compactly arranged follicle cells (FC) with a large nucleus (N), mitochondria (M), rough-surfaced endoplasmic reticular (RER), and numerous ribosomes (R). Apical portion (away from the oolemma) of the interfollicular cell space (IS) is filled with extremely electron-dense material even within the expanded areas (EA). Between the follicle cells and oocyte (Oo) there is a zone of microvilli (ZM). Densely packed free ribosomes, some rough-surfaced endoplasmic reticulum, and mitochondria appear in the cortex of oocyte. × 7,399.

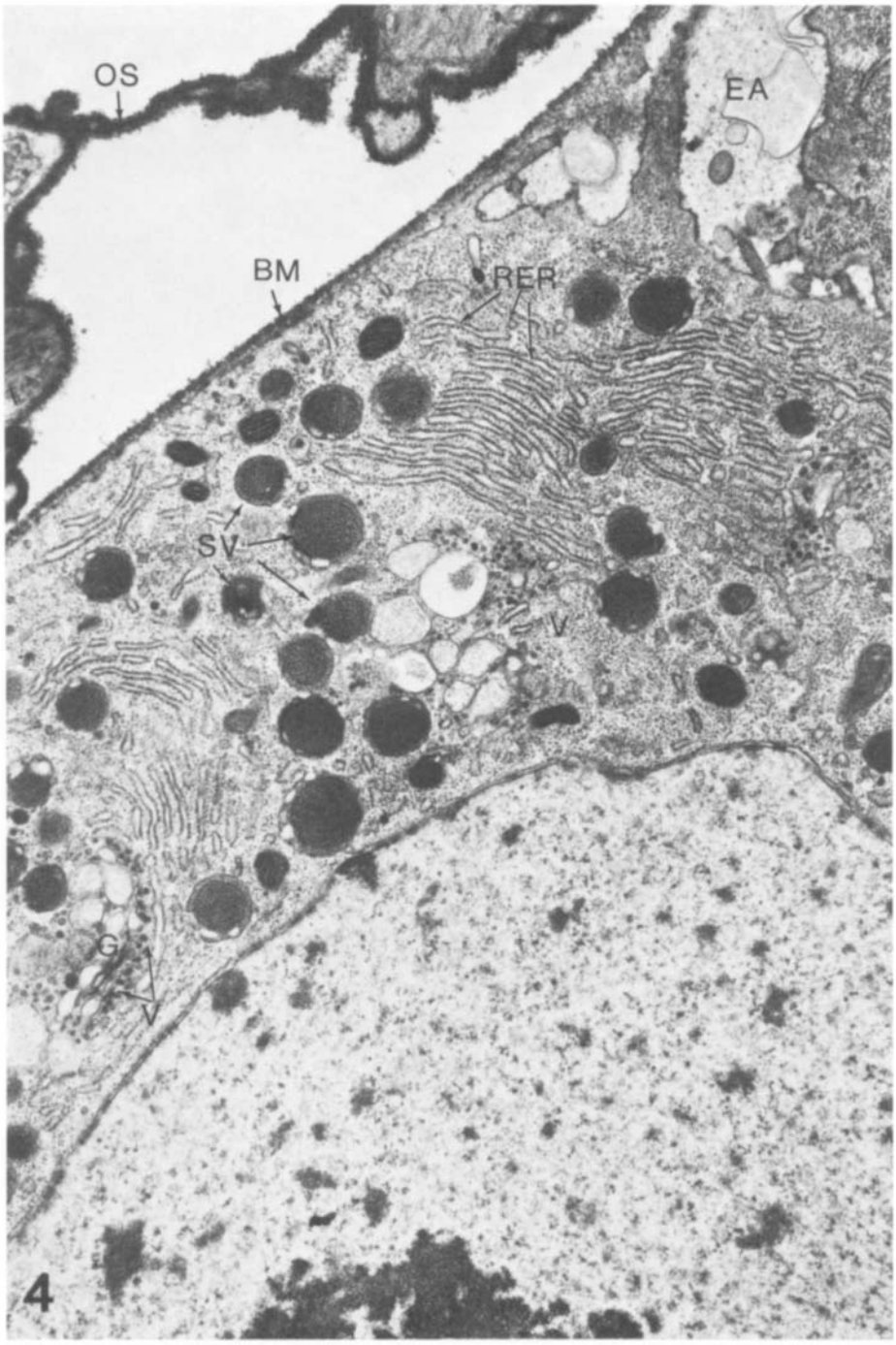
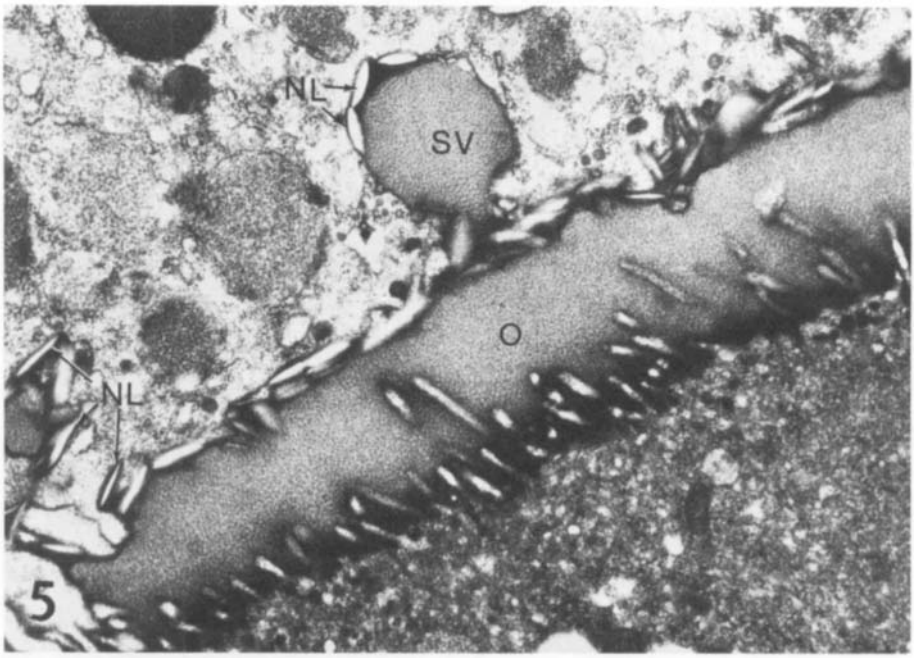


Fig. 4. A portion of a follicle cell with vitellogenic oocyte showing many electron-dense secretory vesicles (SV), Golgi complex (G), and groups of packed rough-surfaced endoplasmic reticulum (RER) distributed distally. Flocculent material and invaginations are present in the expanded area (EA) of interfollicular cell space (upper right corner). Basement membrane (BM) and ovarian sheath (OS) are seen in the upper left. × 16,275.



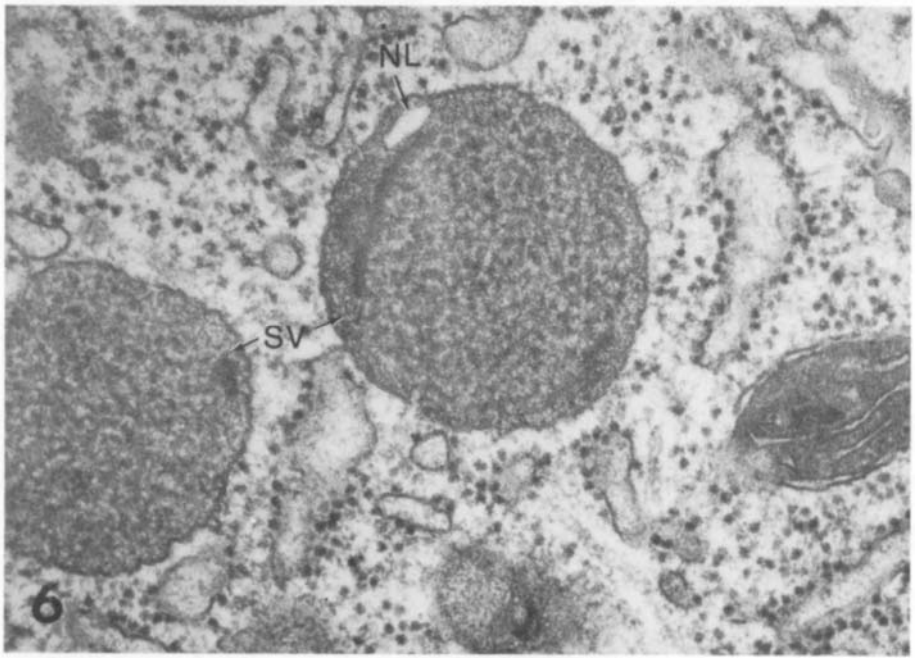


Fig. 5. Needlelike crystal structures (NL) at the periphery of a secretory vesicle (SV) on the outer surface of oolemma. This particular secretory vesicle appears in contact with the oolemma (O). × 17,360.

Fig. 6. Secretory vesicle (SV) is membrane bound; note the shape of a forming needlelike crystal structure (NL) × 59,896.

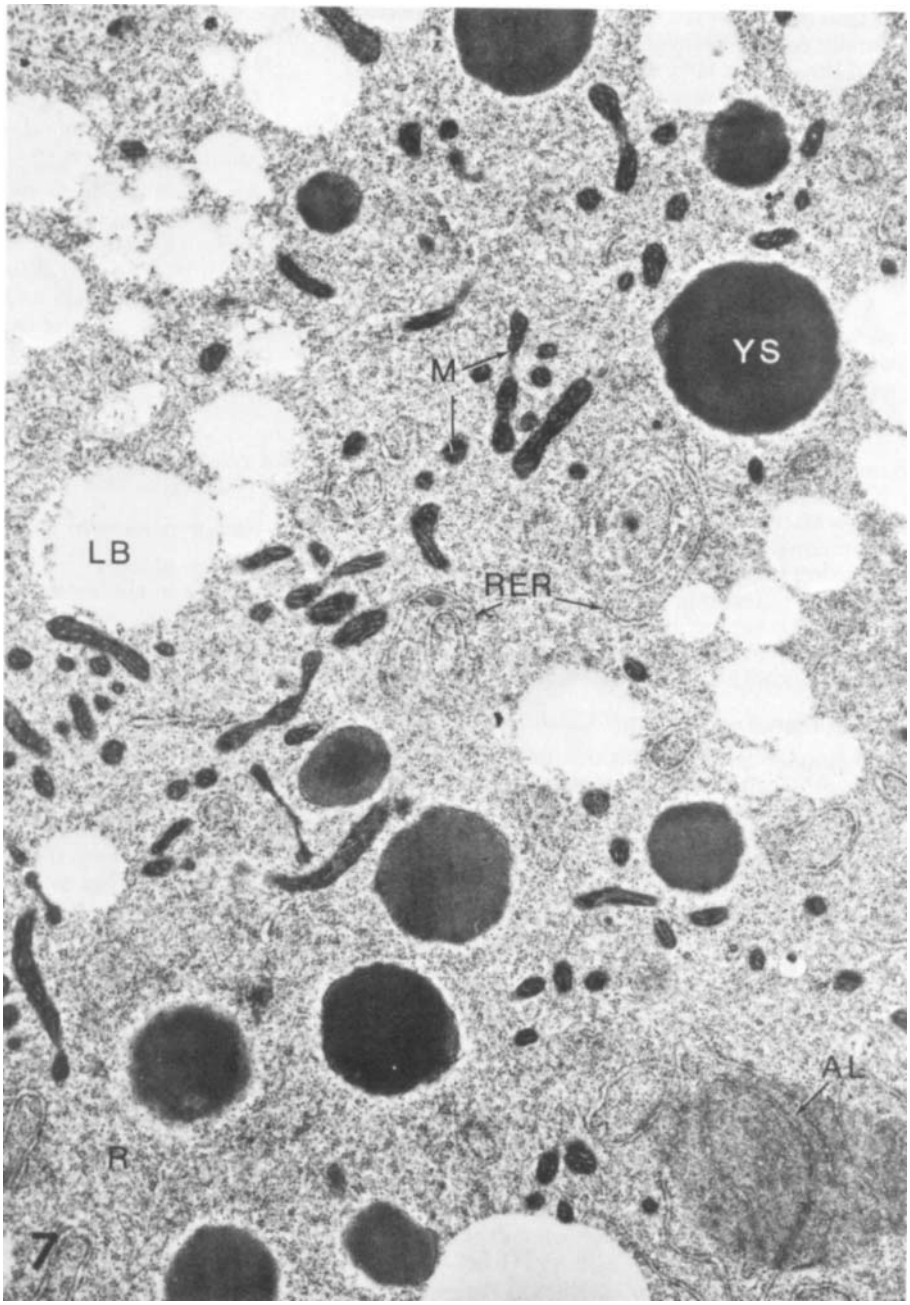


Fig. 7. Vitellogenic oocyte possesses variously sized electron-dense yolk spheres (YS), lipid bodies (LB), mitochondria (M), numerous ribosomes (R), rough-surfaced endoplasmic reticulum (RER), and stacks of annulate lamellae (AL). × 16,909.

distributed throughout the ooplasm. Continuously circled RER often are found in the central portion of the oocyte. At a later stage, there are many coated vesicles oriented along the cortex of the oocyte; they are accompanied by much RER and some uncoated vesicles. The number of large secretion vesicles in follicular cells also is greatly reduced as compared to earlier stages. A lamina has replaced the zone of microvilli. Needlelike structures have appeared at the follicular cell side on this lamina (Fig. 5). They resemble the needlelike crystals found in the large electron-dense secretion vesicles. At this stage, there are still many coated vesicles and much RER distributed along the oolemma. Ribosomes, smaller lipid bodies, annulate lamellae, and different-sized yolk spheres occur inside the oocyte cortex (Figs. 5, 7).

Based on radioimmunoassay (RIA), the titer of free (i.e. nonconjugated) ecdysteroids in adult females after feeding 1 to 5 days on the SSD diet remained more or less constant (i.e., about 170 pg/mg body weight). However, during the sixth day of feeding, the titer increased to 634 ± 20 S.D. pg/mg body weight. This marked increase was correlated with the ovarian cycle and initial oocyte maturation.

Beetles reared on sorbic acid diet

The food fungus *F. solani* did not grow in this diet. Beetles ultimately tunneled extensively in this diet. The mean length of tunneling increased more than five times from 21.1 mm after 2 days of feeding to 108.2 mm after 12 days. After 6 days of feeding, a few empty egg cradles were found along some of the main galleries; however, progenies were not observed.

After 6 days of beetle feeding on sorbic acid diet, the ovariole only possesses previtellogenic oocytes (Figs. 1a, 3). The follicle cells are compact with large nuclei. The interfollicular cell space may be expanded in some areas, but it does not contain electron-dense material. Large lysosome-like bodies containing one or more concentric whorls of membrane (Fig. 8) are the most striking characteristic of the oocyte at this stage. A few mitochondria, short rods and single whorls of RER, and Golgi which may be associated with small electron-dense vesicles are sparsely distributed in the ooplasm.

The titer of free ecdysteroids in adult females through 6 days of feeding in diet containing sorbic acid averaged 39.9± 14.7 S.D. pg/mg body weight. Thus, the normal ovarian cycle did not occur, and the marked increase in the titer of free ecdysteroids which accompanies the maturation of oocytes also was absent.

DISCUSSION

Diet, ectosymbiotic microbial growth, and progeny

X. ferrugineus has been routinely reared on standard sawdust diet (SSD) in our laboratory since 1967. Hundreds of generations have been completed since then (Norris and Baker, '67; Saunders and Knoke, '67). Female adults are responsible for constructing galleries in the medium, inoculating their ectosymbiotic microbes in the diet, and tending progeny which feed on the microbial growth on the walls of the tunnels. Addition of sorbic acid to the diet inhibits growth of the food fungi, and progeny are not produced.

Ovaries, and oocyte maturation

Because of the lack of nutritive cords between the trophocytes in the germarium and the young oocyte, the ovaries of *X. ferrugineus* may be classified as pseudotelotrophic, the same as reported for the Colorado potato beetle, *Leptinotarsa decemlineata* (de Loof et al., '72). Bridges ('75) distinguished seven stages of oocyte development in *X. ferrugineus*, but because there is no need for such detailed stages here, we have simply divided oocyte development into previtellogenic and vitellogenic stages. In *X. ferrugineus*, there is only one mature oocyte in one of the ovarioles at a time. Yolk deposition will not start in the penultimate oocyte until the terminal oocyte has almost completed development. Tobe ('77) also reported that growth of the penultimate oocyte in the desert locust is normally inhibited until the terminal one has been ovulated.

In *Aedes aegypti* (Anderson and Spielman, '71), the basement lamina was reported to serve as a coarse mechanical filter, freely permeable to particles with molecular weights ranging from 12,000 to 500,000 daltons and dimensions less than 110 Å. de Loof and coworkers ('72) found that ferritin particles can pass through the ovariole wall without being packed in vesicles, and concluded that there is probably no specialized mechanism for the absorption of hemolymph proteins in the Colorado potato beetle. If in *X. ferrugineus* the basement lamina of follicle cells possesses

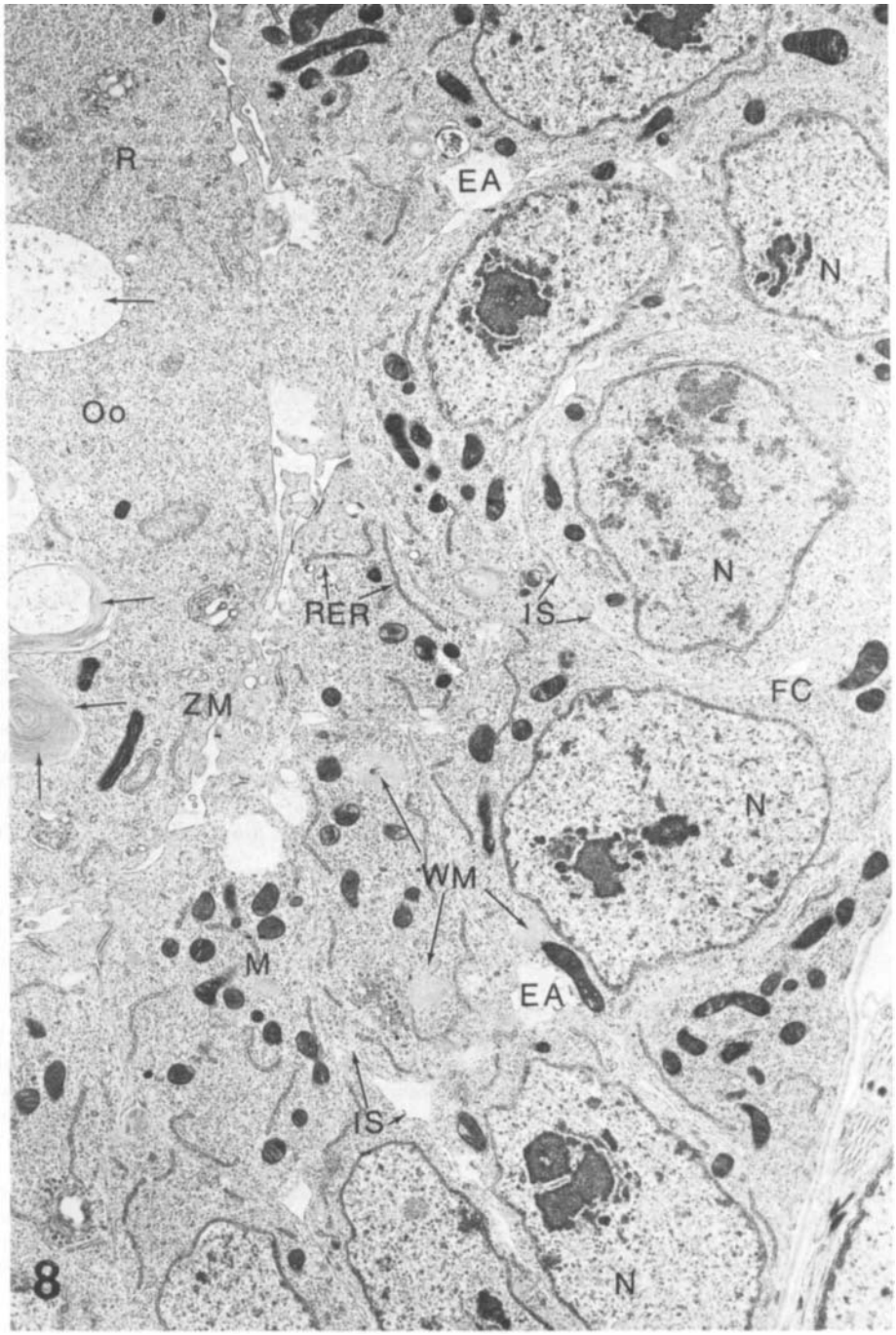


Fig. 8. Ovary from a female beetle that has been fed on sorbic-acid-containing diet for 6 days. Compact follicle cells (FC) with large nucleus (N). Interfollicular cell spaces (IS) with expanded areas (EA), mitochondria (M), ribosomes (R), and small vesicles with whorls of membrane (WM) distributed in the cytoplasm of the follicle cells. The zone of microvilli (ZM) is very simple with only a few invaginations. In the oocyte (Oo), besides ribosomes (R), mitochondria (M), and rough-surfaced endoplasmic reticulum (RER), the most striking characteristic is the presence of large-sized vesicles with whorls of concentric membrane (arrows). × 7,482.

properties similar to those in *A. aegypti* and the Colorado potato beetle, then similar functions could be expected.

Since the first demonstration of extraovarian proteins in the oocyte of *Rhodnius prolixus* and other blood-sucking arthropods by Wigglesworth ('43), it has been confirmed in other insects and has been reviewed by several authors (King and Aggarwal, '65; Hagedorn and Kunkel, '79; Engelmann, '70, '79, '80; Telfer and Smith, '70). The route of the uptake of vitellogenins was demonstrated histochemically and immunologically in interfollicular cell spaces, brush border (peri-oocytic space), and finally the yolk spheres (Telfer and Melius, '63; Telfer, '60, '61, '65, '79). The interfollicular cell spaces were shown to enlarge during vitellogenesis in *Hyalophora cecropia* (King and Aggarwal, '65). These enlarged spaces serve as channels for vitellogenins to reach the surface of the oocyte. Besides the vitellogenin, endogenous substances (e.g., yolk components) synthesized within the follicle cells also are present in these spaces (Anderson and Telfer, '69; Bast and Telfer, '76). In *X. ferrugineus*, before and during vitellogenesis, the interfollicular cell spaces are filled with extremely electron-dense material. Expanded interfollicular cell spaces persist after the electron-dense material disappears. However a less electron-dense flocculent substance remains in these expanded spaces (Figs. 3, 4).

There are mitochondria in the cortex of the previtellogenic oocyte but neither yolk spheres nor lipid granules are evident. Microvilli are present in the peri-oocytic space before yolk deposition (Fig. 3). The follicle cells lack an extensive RER system and secretion vesicles (Fig. 3).

Golgi complexes with small electron-opaque vesicles (Fig. 4) are present in the follicle cell of vitellogenic oocytes. The numerous large secretion vesicles may have originated through the aggregation of the small vesicles in these complexes. The large secretion vesicles probably contain the precursor material(s) for the dense yolk spheres in oocytes. An extensively developed RER system, large nucleus, Golgi complexes, and small secretion vesicles in follicle cells of *X. ferrugineus* during vitellogenesis provide a system for the synthesis of the involved protein. De Loof ('71) has provided evidence of such a system in the Colorado potato beetle. In his work ^{3}H-leucine incorporation occurred first in association with RER, and subsequently in secretion vesicles during yolk deposition. When this deposition ended, many secretion vesicles were still present in the basal portion of the follicle cells. De Loof thus hypothesized that the same secretion vesicles are involved in proteid yolk deposition and vitelline membrane formation. Many secretion vesicles located peripherally in the basal portion of the follicle cells of *X. ferrugineus* possess needlelike crystals which are similar to those reported in the Colorado potato beetle (de Loof, '71). Secretion vesicles with needlelike crystals of a different morphology occur in the apical portion of these follicle cells (Figs. 4, 5). Because the needlelike crystals embedded in the vitelline membrane are similar to those in the secretion vesicles in the basal portion of the follicle cells (Fig. 5), these vesicles are probably formed in the apical portion of the follicle cells and are transported to the basal portion for involvement in proteid yolk deposition and vitelline membrane formation.

The numerous large secretion vesicles found in the follicle cells associated with the vitellogenic oocyte were not present in those of the previtellogenic oocyte associated with 6-day-old beetles fed on sorbic-acid-containing diet. In addition, the absence of dense yolk spheres in the previtellogenic oocyte indicates that there is no uptake of hemolymph proteins and there is no synthesis of protein by the follicle cells. The lysosomelike bodies in the previtellogenic oocyte of *X. ferrugineus* on sorbic acid diet are similar to those reported in the Colorado beetle (de Loof and Lagasse, '70).

Blockage of oocyte maturation

In *X. ferrugineus*, females do not reproduce if they are reared on sorbic-acid-containing diet. The ovaries do not develop beyond the previtellogenic stage. The sorbic-acid-containing diet, however, supports the nutritional requirements of the adult female beetle except for reproduction. Sorbic acid inhibits the growth of the mutualistic fungus, *F. solani*, in the diet medium, and the lack of nutrients required specifically for oocyte maturation, which are normally provided by this mutualistic fungus, arrests reproduction. The fungus-produced nutrients that are required for oocyte maturation include sterol (Chu et al., '70) and essential amino acids (Kok and Norris, '72; Bridges and Norris, '77). These nutrients are required for the marked increase in ecdysteroid titer which normally occurs during the ovarian cycle in female beetles. Thus, although reproductive arrest may be a direct result of nutritional deficiency (Sams, '75; Takken, '80; Tobe and Langley, '78; Trepte, '80), an inability to produce mature eggs also

may be the indirect result of a nutritional deficiency, and consequent suppression of the neuroendocrine system (Rankin and Riddiford, '77; Tobe and Chapman, '79).

Juvenile hormones have often been identified as the key molecules which control vitellogenin synthesis, and thus vitellogenesis (Engelmann, '71, '74, '79, '80; Pan and Wyatt, '76; Chen et al., '76, '79). Juvenile hormone not only induces fat body to synthesize vitellogenin (Wyatt, '80), but also acts on the ovary by inducing large intercellular spaces between follicle cells which allow the passage of the vitellogenin from the hemolymph into the oocyte (Pratt and Davey, '72; Davey and Huebner, '74; Abu-Hakima and Davey, '75, '77; Koeppe and Wellman, '80). In contrast to this, ecdysone is reported to induce vitellogenin synthesis in *Drosophila* (Handler and Postlethwait, '78). A positive correlation between oocyte production and ecdysteroid levels in *Aedes* is also reported (Masler et al., '81). However, in houseflies and some beetles (Robbins et al., '68), vitellogenesis is curtailed after the application of ecdysone. In *R. prolixus* (Garcia et al., '79) oogenesis and oviposition can be inhibited by ecdysone and reestablished by the application of a juvenile hormone analogue. Rankin and Jäckle ('80) reported a different set of controls of vitellogenesis in *Oncopeltus fasciatus* which involves both juvenile hormone and ecdysone. They suggested that synthesis of the vitellogenin precursor seems to be ecdysteroid mediated, while uptake, and perhaps the processing, of the precursor appear to be under juvenile hormone control.

In *X. ferrugineus*, the ovaries from 6-day-old female beetles reared on SSD medium have a very low titer of free ecdysteroids in comparison with that of the whole beetle (10.9 pg/ovary vs. 534.64 pg/mg of whole beetle). Thus, if ecdysteroids are synthesized largely in the ovaries of this beetle, then they must be rapidly released into the hemolymph or conjugated in the ovary. Otherwise, unlike *Galleria mellonella* (Hsiao and Hsiao, '79) and *Locusta migratoria* (Lagueux et al., '77), the ovary of *X. ferrugineus* must not be a major site of ecdysteroid synthesis. The free ecdysteroid titer of the whole female beetle reared on sorbic-acid-containing diet does not increase on the sixth day as it does in those reared on SSD. Thus, there is a positive correlation between the free ecdysteroid titer and ovarian development in *X. ferrugineus*, but this does not indicate with certainty what role(s) ecdysteroids play in the regulation of vitellogenesis. However, our demonstrated highly reversible chemical (nutritional) inhibition of both ecdysteroid synthesis and oocyte maturation apparently offers us a unique animal model to study both the regulatory mechanisms of vitellogenesis and what specific roles juvenile hormones and free ecdysteroids play in this system.

ACKNOWLEDGMENTS

This research was supported by the College of Agricultural and Life Sciences, University of Wisconsin, Madison; and in part by research grants No. RR-00779, Division of Research Resources, and No. AG-01271, Institute on Aging, of the National Institutes of Health; and No. PFR 77-08279 from the National Science Foundation to D.M.N. We thank Jon Gunderson and Steven Schnurrer for technical assistance and Roxanne Moermond for typing the manuscript.

LITERATURE CITED

Abu-Hakima, R. and K.G. Davey (1975) Two actions of juvenile hormone on the follicle cell of *Rhodinius prolixus* Stäl. Can. J. Zool. *53:*1187-1188.

Abu-Hakima, R., and K.G. Davey (1977) Effects of hormones and inhibitors of macromolecular synthesis on the follicle cell of *Rhodnius*. J. Insect Physiol. *23:*913-917.

Anderson, W.A., and A. Spielman (1971) Permeability of the ovarian follicle of *Aedes aegypti* mosquitos. J. Cell Biol. *50:*201-221.

Anderson, L.M., and W.H. Telfer (1969) A follicle cell contribution to the yolk spheres of moth oöcytes. Tissue Cell *1:*633-644.

Bast, R.E., and W.H. Telfer (1976) Follicle cell protein synthesis and its contribution to the yolk of *Cecropia* moth oöcyte. Dev. Biol. *52:*83-97.

Bollenbacher, W.E., W.V. Vedeckis, L.I. Gilbert, and J.D. O'Connor (1975) Ecdysone titers and prothoracic gland activity during the larval-pupal development of *Manduca sexta*. Dev. Biol. *44:*46-53.

Bridges, J.R. (1975) Some Aspects of the Reproduction of the Ambrosia Beetle, *Xyleborus ferrugineus* (F.): Inhibition of Reproduction by Ascorbic Acid and Related Chemicals, and Reproduction Under Gnotobiotic Conditions. Ph.D thesis, Univ. Wisc., Madison.

Bridges, J.R., and D.M. Norris (1977) Inhibition of reproduction of *Xyleborus ferrugineus* by ascorbic acid and related chemicals. J. Insect Physiol. *23:*497-501.

Briggs, N.L. (1958) A double embedding technic for fibrous connective tissue. Stain Technol. *33:*299.

Chen, T.T., P. Couble, F.L. de Lucca, and G.R. Wyatt (1976) Juvenile hormone control of vitellogenin synthesis in *Locusta migratoria*. In L.I. Gilbert (ed): The Juvenile Hormones. New York, London. Plenum Press, pp. 505-529.

Chen, T.T., P. Couble, R. Abu-Hakima, and G.R. Wyatt (1979) Juvenile hormone-controlled vitellogenin synthesis in *Locusta migratoria* fat body. Dev. Biol. *69:*59-72.

Chu, H.M., D.M. Norris, and L.T. Kok (1970) Pupation requirement of the beetle, *Xyleborus ferrugineus*: Sterols other than cholesterol. J. Insect Physiol. *16:*1379-1387.

Chu, H.M., and D.M. Norris (1979) Comparative morphology and ultrastructure of the corpora allata in newly emerged and sexually mature female *Xyleborus ferrugineus* (Fabr.) (Coleoptera: Scolytidae). Int. J. Insect

Morphol. Embryol. *8*:359-374.
Davey, K.G., and E. Huebner (1974) The response of the follicle cells of *Rhodnius prolixus* to juvenile hormone and antigonadotropin in vitro. Can. J. Zool. *52*:1407-1412.
Doane, W.W. (1973) Role of hormones in insect development In S.J. Counce and C.H. Waddington (eds): Developmental Systems: Insects. Vol 2. New York: Academic Press, p. 725.
Engelmann, F. (1970) The Physiology of Insect Reproduction. Oxford: Pergamon.
Engelmann, F. (1971) Juvenile hormone-controlled synthesis of female-specific protein in the cockroach *Leucophaea maderae*. Arch. Biochem. Biophys. *145*:439-447.
Engelmann, F. (1974) Juvenile hormone induction of the insect yolk protein precursor. Am. Zool. *14*:1195-1206.
Engelmann, F. (1979) Insect vitellogenin: Identification, biosynthesis and role in vitellogenesis. Adv. Insect Physiol. *14*:49-108.
Engelmann, F. (1980) Endocrine control of vitellogenin synthesis. pp. 311-324. In M. Locke and D.S. Smith (eds): Insect Biology in the Future. New York, London: Academic Press, pp. 311-324.
Engelmann, F. and M. Ladduwahetty (1974) The female specific protein, an extraovarian protein essential for egg maturation in insects. Zool. Jahrb. Physiol. *78*:289-300.
Fuchs, M.S., D.A. Schlaeger, and D. Shroyer (1979) α-Ecdysone does not induce ovarian development in adult *Aedes aegypti*. J. Exp. Zool. *207*:153-159.
Garcia, M.L., R.P. Mello, and E.S. Garcia (1979) Ecdysone, juvenile hormone and oögenesis in *Rhodnius prolixus*. J. Insect Physiol. *25*:695-700.
Hagedorn, H.H. (1974) The control of vitellogenesis in the mosquito, *Aedes aegypti*. Am. Zool. *14*:1207-1217.
Hagedorn, H.H., and J.G. Kunkel (1979) Vitellogenin and vitellin in insects. Ann. Rev. Entomol. *24*:475-505.
Hagedorn, H.H., J.D. O'Connor, M.S. Fuchs, B. Sage, D.A. Schlaeger, and M.K. Bohm (1975) The ovary as a source of α-ecdysone in adult mosquitoes. Proc. Nat'l. Acad. Sci. U.S.A. *72*:3255-3259.
Hagedorn, H.H., J.P. Shapiro and K. Hanaoka (1979) Ovarian ecdysone secretion is controlled by a brain hormone in an adult mosquito. Nature *282*:92-93.
Handler, A.M., and J.H. Postlethwait (1978) Regulation of vitellogenin synthesis in *Drosophila* by ecdysterone and juvenile hormone. J. Exp. Zool. *206*:247-254.
Hsiao, T.H., and C. Hsiao (1979) Ecdysteroids in the ovary and the egg of the greater wax moth. J. Insect Physiol. *25*:45-52.
King, R.C., and S.K. Aggarwal (1965) Oögenesis in *Hyalophora cecropia*. Growth *29*:17-83.
Koeppe, J.K., and S.E. Wellman (1980) Ovarian maturation in *Leucophaea maderae*: Juvenile hormone regulation of thymidine uptake into follicle cell DNA. J. Insect Physiol. *26*:219-228.
Kok, L.T., and D.M. Norris (1972) Symbiotic interrelationships between microbes and ambrosia beetles. VI. Amino acid composition of ectosymbiotic fungi of *Xyleborus ferrugineus*. Ann. Entomol. Soc. Am. *65*:598-602.
Lagueux, M., M. Hirn, and J.A. Hoffman (1977) Ecdysone during ovarian development in *Locusta migratoria*. J. Insect Physiol. *23*:109-119.
Lea, A.O. (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.
Lillie, R.D. (1954) Histopathologic Technic and Practical Histochemistry. New York: The Blakiston Co.
de Loof, A. (1971) Synthesis and deposition of egg envelopes in the Colorado beetle, *Leptinotarsa decemlineata*. Z. Zellforsch. *115*:351-361.
de Loof, A., and A. Lagasse (1970) Resorption of the terminal oöcyte in the allatectomized Colorado beetle, *Leptinotarsa decemlineata* Say. Proc. Konigkl. Ned. Akad. Wet. Ser. C *73*:284-307.
de Loof, A., A. Lagasse, and W. Bohyn (1972) Proteid yolk formation in Colorado beetle with special reference to the mechanism of the selective uptake of haemolymph protein. Proc. Konigkl. Ned. Akad. Wet. Ser. C *75*:125-143.
Masler, E.P., M.S. Fuchs, B. Sage, and J.D. O'Connor (1981) A positive correlation between oöcyte production and ecdysteroid levels in adult *Aedes*. Physiol. Entomol. *6*:45-49.
Norris, D.M., and J.K. Baker (1967) Symbiosis: Effects of a mutualistic fungus upon the growth and reproduction of *Xyleborus ferrugineus*. Science *156*:1120-1122.
Pan, M.L., and G.R. Wyatt (1976) Control of vitellogenin synthesis in the monarch butterfly by juvenile hormone. Dev. Biol. *54*:127-134.
Pratt, G.E., and K.G. Davey (1972) The corpus allatum and oögenesis in *Rhodnius prolixus*. 1. The effects of allatectomy. J. Exp. Biol. *56*:201-214.
Rankin, M.A., and L.M. Riddiford (1977) Hormonal control of migratory flight in *Oncopeltus fasciatus*: The effects of the corpus cardiacum, corpus allatum, and starvation on migration and reproduction. Gen. Comp. Endocrinol. *33*:309-321.
Rankin, M.A., and H. Jäckle (1980) Hormonal control of vitellogenin synthesis in *Oncopeltus fasciatus*. J. Insect. Physiol. *26*:671-684.
Robbins, W.E., J.N. Kaplanis, M.J. Thompson, T.J. Shortino, C.F. Cohen, and S.C. Joyner (1968) Ecdysones and analogs: Effects on development and reproduction of insects. Science *161*:1158-1159.
Sams, G.R. (1975) Vitellogenic arrest in the cockroach, *Blatta orientalis*. J. Insect Physiol. *21*:1203-1210.
Saunders, J.L., and J.K. Knoke (1967) Diets for rearing the ambrosia beetle, *Xyleborus ferrugineus* (Fabr.) in vitro. Science *157*:460-463.
Spielman, A., R.W. Gwadz, and W.A. Anderson (1971) Ecdysone-initiated ovarian development in mosquitoes. J. Insect Physiol. *17*:1807-1814.
Takken, W. (1980) The effect of an albumin deficient diet on the reproductive performance of *Glossina palpalis palpalis* females (Diptera: Glossinidae). Proc. Konigkl. Ned. Akad. Wet. Ser. C *83*:387-397.
Telfer, W.H. (1960) The selective accumulation of blood proteins by the oöcytes of saturniid moths. Biol. Bull. *118*:338-351.
Telfer, W.H. (1961) The route of entry and location of blood proteins in the oöcytes of saturniid moths. J. Biophys. Biochem. Cytol. *9*:747-759.
Telfer, W.H. (1965) The mechanism and control of yolk formation. Ann. Rev. Entomol. *10*:161-184.
Telfer, W.H. (1979) Sulfate and glucosamine labeling of the intercellular matrix in vitellogenic follicles of a moth. Wilhelm Roux's Arch. *185*:347-362.
Telfer, W.H., and M.E. Melius, Jr. (1963) The mechanism of blood protein uptake by insect oöcytes. Am. Zool. *3*:185-191.
Telfer, W.H., and D.S. Smith (1970) Aspects of egg formation. R. Entomol. Soc. Symp. *5*:117-134.
Tobe, S.S. (1977) Inhibition of growth in developing oöcytes of the desert locust. Experientia *33*:343-345.
Tobe, S.S., and C.S. Chapman (1979) The effects of starvation and subsequent feeding on juvenile hormone synthesis and oöcyte growth in *Schistocerca americana gregaria*. J. Insect Physiol. *25*:701-708.
Tobe, S.S., and P.A. Langley (1978) Reproductive physiology of *Glossina*. Ann. Rev. Entomol. *23*:283-307.
Trepte, H.H. (1980) Delayed vitellogenesis due to larval food deficiency in the house fly. J. Insect Physiol. *26*:801-806.
Wigglesworth, V.B. (1943) The fate of haemoglobin in *Rhodnius prolixus* (Hemiptera) and other blood-sucking arthropods. Proc. R. Soc. Lond. [Biol.] *131*:313-339.
Wyatt, G.R. (1980) Fat body as a protein factory. In M. Locke and D.S. Smith (eds): Insect Biology in the Future. New York, London: Academic Press, pp. 201-225.