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VITELLOGENIN AND VITELLIN IN INSECTS

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INTRODUCTION

Vitellogenin is the generic name for a unique group of proteins that are synthesized extraovarially and become the major egg yolk protein, vitellin. Similar proteins are found in all animals with yolky eggs. Because of their high concentration in the egg, and the relative ease with which they can be purified, these proteins are excellent material for the study of the regulation of their synthesis from the molecular, developmental, and physiological points of view. This review covers the synthesis, uptake, and physical and chemical properties of vitellogenins and vitellins in insects.

The term vitellogenin was derived from vitellogenesis (102), the period of rapid oocyte growth during which yolk is deposited. Once inside the oocyte these proteins are known as vitellins, because in some cases the molecules are changed during uptake. The definition of vitellogenins has traditionally included the following criteria: (*a*) They usually comprise 60–90% of the soluble egg yolk proteins (13, 69); (*b*) they are not made in the oocyte, the fat body being the site of synthesis (22, 69, 102); (*c*) they are present in large amounts only in females and thus have been called female-specific proteins (11, 102), although rigorous techniques have revealed small amounts in males (83, 119) and so this definition is not exclusive; and (*d*) they are selectively taken up by the oocyte during vitellogenesis (120). Because of the dynamics of the uptake mechanism (112,

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120), all hemolymph proteins are taken up, but only vitellogenin is selectively removed and concentrated in the oocyte 20 (119) to 100 (13) times the levels found in the hemolymph.

These criteria define the vitellogenins, but only for *Hyalophora cecropia* and *Periplaneta americana* have data been gathered on all four criteria. Most investigators have relied on one or two criteria to identify vitellogenin.

SYNTHESIS AND HORMONAL CONTROL

Although many studies have been made of vitellogenin (VG) as a component of the hemolymph, few studies have been made of the synthesis and endocrine control of VG with methods specific enough to be of value. To measure synthesis, titer determinations alone are inadequate; radioactive tracers should be used. Specificity is achieved only through the use of immunologic techniques. Separation techniques such as acrylamide gel electrophoresis are useful but not definitive, because VG can co-migrate with other proteins or change during synthesis and uptake. Immunologic techniques can be definitive if used correctly. Antisera produced against crude egg homogenates are useful only in techniques where precipitin bands are produced in agar [e.g. Ouchterlony (25, 113), Oudin (100, 119), immunoelectrophoresis (39), rocket immunoelectrophoresis (70, 113, 114)]. When immune precipitates are produced in solution [e.g. radioimmunoprecipitation[1] (50, 66, 70, 86, 102, 129), radioimmunoassay (26), hemagglutination (80)], highly specific antisera must be used. The best way to obtain a specific antiserum is to use only purified VG or vitellin as an antigen (23, 26, 69, 86) and to absorb the resulting antiserum with an extract from animals that do not contain VG [i.e. males (118)]. Without these precautions the precipitate may contain molecules other than VG. The immunoprecipitation of labeled VG requires considerable attention to controls because of the increased danger of detecting labeled non-VG in the precipitate (74).

Definitive work on endocrine control of VG synthesis demands an integrated in vivo and in vitro approach, but this has seldom been achieved. Researchers have often extirpated endocrine glands and applied hormones and have recorded the size of the oocytes or VG titers. Although such an approach is necessary and may be informative, it is not definitive, for three reasons. First, the growth of the oocyte is a complex process and is not necessarily directly related to VG synthesis. Second, since VG uptake often occurs concomitantly with synthesis, titers may not reflect the rate of

[1]The term radioimmunoassay is often applied incorrectly to radioimmunoprecipitation assays. The radioimmunoassay involves competition between labeled and unlabeled antigen, whereas radioimmunoprecipitation involves direct or indirect precipitation of labeled antigen.

synthesis. Third, the complexity of the in vivo situation precludes simple conclusions. For example the applied hormone could have its primary effects on other endocrine glands or tissues that only secondarily affect VG synthesis. Currently, in vitro documentation of the effect of endocrines on VG synthesis has been accomplished in only a few systems (17, 129). However, even here organ cultures were used that contain several cell types, including oenocytes. Optimally, cultures of fat body cells should be used.

In lieu of the totally in vitro approach, several workers have used isolated abdomens (22, 23, 39, 86), which, especially if they are ovariectomized, have the advantage of excluding the known endocrine glands.

This part of the review concentrates on those studies that have actually examined VG (as distinct from vitellogenesis). It is not an exhaustive review, but rather it focuses on those insects where definitive work has been done. More complete reviews can be found elsewhere (40, 46). The evidence for each species is examined separately in the hope that this will provide a better guide for future research. It is important that attention be paid to the techniques used, for the reasons discussed above. Therefore special note is made of instances where the techniques were not specific or definitive. Where available, data are given on the timing of VG synthesis relative to oocyte development. The reader should be aware that these data may be greatly affected by temperature.

Orthoptera

LOCUSTA MIGRATORIA VG synthesis (28) and appearance of VG in the hemolymph (27) both begin on day 7 after adult eclosion in female locusts kept in crowded conditions in the presence of mature males. The rate of synthesis continues to rise until, by day 13, 60% of the labeled protein secreted by the fat body is VG (28). Titers of VG reach a peak on day 8 and then fall (27), undoubtedly reflecting the uptake of VG. A study of the flow of VG from the fat body to the oocyte found that VG titers dropped abruptly when the chorion was laid down (7, 57).[2] Whether this was due simply to the termination of VG synthesis or is complicated by degradation or storage of unincorporated VG is not known. Hemolymph titers fluctuate widely during uptake by oocytes (27, 57).

Females allatectomized at eclosion fail to develop eggs or synthesize VG. Topical application of C_{18} juvenile hormone (JH I) caused VG synthesis (27), although repeated doses were necessary to obtain a dose response curve. A single dose of ZR 515 was sufficient, apparently because of the slower degradation of this JH analogue (28). About 2 days after application

[2]The specificity of the antiserum was not demonstrated.

of ZR 515 to allatectomized females, VG synthesis rapidly rose to a peak on day 3, and by day 7 synthesis had returned to a low level. After a second dose on day 13, synthesis began again, but this time the lag was much shorter (28).

Incubation of fat body from allactectomized females with JH in the incubation medium caused a three-fold increase in VG synthesis (129; R. Abu-Hakima and G. R. Wyatt, personal communication). This is the first time a JH effect on VG synthesis has been obtained in vitro.

RNA synthesis increases after eclosion in normal, but not in allatectomized females. Application of JH I stimulates RNA synthesis in these animals. The total DNA of the fat body doubles after eclosion, apparently reflecting an increase in ploidy. Allatectomy prevents the increase in DNA and JH restores it (28). Whether or not DNA synthesis is required for VG synthesis remains to be determined.

RNA from fat body of females maturing eggs will direct the synthesis of VG in a wheat germ cell-free system (27). mRNA obtained from males and allatectomized females did not do so, but the capacity was restored by application of JH I to the allatectomized females. Injection of total fat body RNA into frog oocytes resulted in the synthesis of peptides identifiable as VG on sodium dodecyl sulfate (SDS) acrylamide gels (T. T. Chen and G. R. Wyatt, personal communication).

Dictyoptera

PERIPLANETA AMERICANA Two VGs are seen in the hemolymph 5 days after adult emergence, at about the same time as the oocytes start to deposit yolk (13). They remain present throughout adult life, as do the vitellogenic oocytes (12). Allatectomy 12 hr after eclosion prevents appearance of the VG. They reappear after reimplantation of the corpora allata (CA) or topical application of farnesyl methyl ether (11). The suggestion that implantation of ovaries into males causes appearance of VG (108) was refuted (14). Fat body from egg-maturing females was found to synthesize and secrete labeled VG in vitro (102). Fat body from newly emerged females failed to do so.

LEUCOPHAEA MADERAE A considerable amount is known about VG synthesis in *Locusta maderae.* The fat body begins to secrete VG about 5 days after mating (23). Mating appears to stimulate or enhance VG synthesis in this species. VG synthesis remains high (5 mg/day) for at least 15 days and stops abruptly shortly before the ootheca is made (23, 86). Some VG remains unincorporated by the oocytes and apparently is stored in the fat body (41). VG reappears in the hemolymph 3–5 days after experimental

removal of the ootheca (39). It is not clear whether this represents new synthesis or release of the stored protein. After an injection of labeled leucine into an egg-maturing female, labeled VG in the hemolymph rapidly increases, reaching a plateau by 3–8 hr (possibly due to exhaustion of label) (50). Uptake of labeled VG by the oocyte continued for at least 24 hr (22, 50).

Removal of the CA by allatectomy, decapitation, or ligation of the abdomen results in cessation of VG synthesis. Topical application of JH or CA implantation restores VG synthesis (22, 23, 39, 49, 86, 115). A dose-response relationship was obtained to all three naturally occurring JHs with the greatest response to JH I and the least to C_{16}JH (JH III) (39). Following a single injection of JH into decapitated and ovariectomized females, VG synthesis begins after a lag of 18 hr, rising to a peak at 72 hr (86). Interestingly, ecdysone given concomitantly with JH inhibits VG synthesis (42).

Injection of actinomycin D concurrently with JH application to allatectomized females prevented VG synthesis. Delaying injection of the drug allowed increasingly greater amounts of VG synthesis (41). Injection of α-amanitin into normal egg maturing females depressed VG synthesis by 50% (44). α-Amanitin decreased leucine incorporation into VG associated with microsomes by 90%, and it also inhibited incorporation of orotic acid into RNA sedimenting between 4 and 18S (44). On the basis of these experiments, transcriptional control of VG synthesis was postulated. Owing to the difficulty of interpreting experiments using inhibitors in vivo, further evidence is required on this point.

Endoplasmic reticulum (microsomes) from fat body of reproductive females contained 25% more RNA and was significantly heavier on sucrose gradients than microsomes prepared from reproductively inactive females or from males (43). The microsomes were not sensitive to RNase but were decreased in S value by NaF (43) and puromycin (47). Microsomes from females injected with leucine contained VG that was not released by puromycin or high KCl (47).

Preliminary attempts to obtain VG synthesis in homologous (44) or heterologous (87) cell-free systems were more successful when microsomal membranes were included (44). Rigorous proof that authentic VG is, in fact, being synthesized in the cell-free system is lacking.

After several unsuccessful attempts, polysomes containing nacent VG have been obtained recently from fat body of egg-maturing females (45). This technical advance will permit a closer exmination of VG mRNA.

BYRSOTRIA FUMIGATA In *Byrsotria fumigata,* which is ovoviviparous, two VGs are found in the hemolymph and oocytes (8). Trace quantities appear in the hemolymph on days 4 and 5, concomitant with yolk deposi-

tion. VG titer increases until day 16, when it declines sharply, apparently because very rapid yolk deposition depletes the pool of hemolymph VG faster than it is synthesized. A second peak in VG titer on day 22 may represent an excess of VG synthesis over uptake, or VG from resorbed oocytes. No data on actual synthesis are available. VG disappears from the hemolymph at ovulation and is absent during pregnancy. In ovariectomized females VG titers are very high, but decline on schedule as if ovaries were still present. This suggests the interesting hypothesis that the VG titer regulation is independent of the ovary (8). Injection of various doses of farnesyl methyl ether caused a graded increase in VG titers in neck-ligatured females (15).

In two other ovoviviparous roaches, *Nauphoeta cinerea* and *Blattella germanica,* the titers of VG are high during egg development and decline during pregnancy (26, 117). A role for the ovary in regulating vitellogenesis in *B. germanica* was rejected by showing that ovariectomy three instars prior to the adult has no effect on VG synthesis (91).

Hemiptera

Despite the long history of physiological work on *Rhodnius prolixus,* few data are available on VG synthesis. The CA is presumed to have an effect (6, 32), but the only quantitative data avalable suggests that the effect of allatectomy only partially reduces VG titers (109). Neither the precise time of allatectomy nor the timing of the experiment were reported. In a related bug, *Triatoma protracta,* allatectomy prevents appearance of VG. Topical application of JH III stimulates a dose-related synthesis of VG. After a 50-μg dose of JH, 60% of the proteins being synthesized 4 days later were VG (96).

Coleoptera

TENEBRIO MOLITOR A protein can be detected in hemolymph and fat body of pupae and adults that appears to be accumulated in the oocytes and testes (93). No female-specific VG was found. "Allatectomy" is claimed to prevent the appearance of this protein in the hemolymph, although it appears to be still present in the fat body. Implantation of ovaries into males does not result in the deposition of yolk. The techniques used in these experiments were rather crude (cellulose acetate electrophoresis) or inadequately exploited (immunology), so that it is difficult to draw any firm conclusions. VG synthesis in this animal needs reexamination.

LEPTINOTARSA DECEMLINEATA With a combination of cellulose acetate electrophoresis and immunology, a presumed VG was found in females exposed to long days, but not in those exposed to short days (36). In

long-day females removal of the CA increased VG titers, suggesting an effect on VG uptake, while removal of both the CA and the corpora cardiaca (CC) at adult emergence prevented the appearance of VG. Short-day females, from which the CA and CC were removed, did not produce VG after either implantation of CC or JH application, but both treatments together (i.e. both CC and JH) resulted in the appearance of VG (36). Recent experiments show an effect of JH on synthesis, which may be indirect as synthesis can occur in the absence of JH, but the specificity of the response is then lost (J. T. Dortland, personal communication).

Lepidoptera

DANAUS PLEXIPPUS VG appears in the hemolymph 1–2 days after emergence of the adult. VG synthesis can be detected at about the same time. By day 3, 50–60% of the total incorporation into hemolymph proteins is VG (105). Ovariectomy in the last larval instar does not prevent VG synthesis in the adult. Thus the ovary is not involved in controlling VG synthesis (91). Ligation of adults shortly after emergence removes the source of JH and prevents the appearance of VG (104). JH I stimulates an increasing rate of VG synthesis from 10 to 30 hr after injection (105). A dose response to JH was found, with 1 μg giving a maximal response. Injection of actinomycin D concomitant with JH inhibited leucine incorporation into VG by 92%.

HYALOPHORA CECROPIA *Hyalophora cecropia* provided the earliest evidence that female insects contain a sex-limited protein that becomes part of the yolk (119), and that the fat body is the source of these proteins (102). VG first appears in the hemolymph shortly after the larva spins its cocoon. Titers remain high during diapause and drop only after yolk deposition begins in the pharate adult (119). VG synthesis rises 4–8 days after the larval-pupal apolysis and then declines to low levels during diapause (100, 102). Synthesis does not begin again until after the pupal-adult apolysis (102). As whole-body extracts failed to show an increase in the total amount of VG in the pharate adult, it was suggested that some VG is being used during metamorphosis.

Removal of the CA and CC had no effect on VG synthesis nor did injection of JH I (101). The conclusion that VG synthesis is not hormonally controlled may be premature since the initiation of synthesis may be linked with endocrine events during metamorphosis. This is discussed in more detail later.

BOMBYX MORI VG appears in the hemolymph after the larval-pupal ecdysis (82), although exactly when is not clear, as the proper (immu-

nologic) experiments have not been reported. The occurrence of VG has been demonstrated unequivocally in hemolymph of the pharate adult (99). Incubation of fat body with ecdysterone resulted in a twofold stimulation of synthesis of VG and non-VG proteins (99). A double peak in ecdysone titer has been shown to occur during pupal development (73) (see also 18). The second peak, on day 6, represents ovarian synthesis of ecdysone that remains in the ovary and occurs late in oocyte development. The first peak, on day 2, is more closely related to the vitellogenic stages of oocyte development, and therefore, if VG synthesis is regulated by ecdysone in this species, it is probably the initial peak that is involved. The fact that ecdysone stimulates both VG and non-VG protein synthesis in vitro is not very surprising since these events occur during metamorphosis when ecdysone would be expected to have a multitude of effects on protein synthesis. The concept of ecdysone or JH stimulating VG synthesis preferentially has been developed in adults where these hormones are not causing large-scale developmental changes. Although these data are provocative, further experiments are needed to investigate a possible relationship between ecdysone and vitellogenin synthesis in vivo.

By means of a wheat germ cell-free system and a VG-specific antiserum, the amount of VG mRNA was found to increase after the pupal ecdysis (77).

Diptera

AEDES AEGYPTI In the mosquito *Aedes aegypti,* VG is made only after a blood meal (69). [Recent criticisms of the radioimmunoprecipitation assay used in these experiments (20) have been found to be unwarranted (70).] The rate of synthesis increases rapidly after feeding, reaching a peak at 28–30 hr (68). Ovariectomy abolishes this response (67). The effect of the ovary can be mimicked by ecdysterone (55), and in fact the ovary secretes ecdysone after the blood meal (71). The ovarian ecdysone is found in the hemolymph (H. H. Hagedorn, unpublished observations) in contrast to the situation in other insects where it never leaves the ovary and appears after vitellogenin uptake is completed (18, 19, 61, 73, 92). The fat body produces VG in response to ecdysterone both in vivo (55) and in vitro (17, 55, 71). The response of the fat body to ecdysterone appears to be internally programmed to last about 30 hr (17), after which VG synthesis declines (12, 68, 71) and the cellular machinery needed for protein synthesis is broken down (10, 17).

A sharp peak in RNA synthesis occurs in the fat body 3 hr after a blood meal. Total RNA also increases threefold; most of this RNA is ribosomal. By 6 hr after a blood meal a transient fivefold increase in DNA synthesis

occurs, with little change in total DNA (W. J. Kaczor and H. H. Hagedorn, manuscript in preparation). The relationship of these events to VG synthesis is an intriguing question. Actinomycin D inhibits VG synthesis in vivo (60, 68), and cordycepin and α-amanitin do not in vivo (60) but do in vitro (W. J. Kaczor and H. H. Hagedorn, manuscript in preparation). Given the complexity of the endocrine control of VG synthesis in mosquitoes, it is difficult to interpret the in vivo effects of inhibitors, as they could act in numerous ways.

Unphysiological doses of ecdysterone (5 μg/female) are required for stimulation of VG synthesis in vivo (55). However, when used in vitro, the required dose is almost identical to the amount actually found in the female (71). Large doses may be needed in vivo because of rapid degradation; however, the presence of other negative factors has not been ruled out. Also, although ecdysterone appears to be sufficient for VG synthesis, other factors may control the timing of snythesis. Evidence for such factors include the fact that some ecdysterone may exist in the non-blood-fed female without any detectable VG synthesis (68, 71). Also, implantation of ecdysone-secreting ovaries into non-blood-fed females does not result in VG synthesis (H. H. Hagedorn, unpublished results). It is evident that much remains to be learned.

The timing of VG synthesis after a single injection of ecdysterone (55) is remarkably similar to that seen in the fed animal, even including the 5- to 6-hr lag in response (68). The timing is also similar after exposure to ecdysterone in vitro (17), but the magnitude of the response is smaller, perhaps because of nonperfect conditions in the medium.

The fat body must be exposed to JH before it can respond to ecdysterone (58). This normally occurs within the first 2 days after adult eclosion. In these experiments the JH was topically applied in vivo. Therefore the target tissue of JH may not be the fat body.

How these hormones control VG synthesis is an interesting problem. The use of JH and ecdysone in sequence may allow a more rapid response after a blood meal (58). JH could be stimulating commitment to a new cellular program by causing cell replication, or it could be stimulating the appearance of ecdysterone receptors, or it could be causing the synthesis of VG mRNA that is translated only in response to ecdysterone (58). Regarding the first possibility, we have recently found no evidence for DNA synthesis in the fat body preparation during the 3 days after adult eclosion when JH is known to have its effect (W. J. Kaczor and H. H. Hagedorn, unpublished observations). Thus cell replication is ruled out. Distinguishing between the two other possibilities will require a more direct approach than is now technically feasible.

DROSOPHILA MELANOGASTER Technical problems have delayed research on *Drosophila* VG and vitellin. The native protein appears to be inadequately separated on acrylamide gels. Therefore most investigators have resorted to using denaturing SDS acrylamide gels. The number of vitellin subunits present was disputed (63, 65, 80), but there now appears to be a general consensus that there are three subunits with molecular weights ranging from 44,000 to 47,000 (21). The relationship between the native molecule and the three subunits is not clear. Some non-VG proteins co-migrate with the subunits (21, 72). Several purification schemes for native vitellin have been described (63, 80), but antisera raised against the purified native protein apparently are not monospecific, as they cross-react with non-VG proteins in males (63, 80). An antiserum raised against vitellin subunits purified by SDS electrophoresis apparently is specific, and no cross-reaction with male proteins was found (72).

Implantation of ovaries into males results in the appearance of VG in the hemolymph (80). The suggestion that this is due to a positive effect of an ovarian factor on VG synthesis (80) was negated by the recent discovery that both the ovaries and fat body synthesize two of the three subunits in vitro (M. Bownes, personal communication). Furthermore, RNA from both fat body and ovary can direct synthesis of two of the three subunits in a cell-free system (M. Bownes, personal communication). These results raise a number of questions concerning the adequacy of the demonstration that the fat body alone makes VG in other insects. However, several questions need to be resolved about the *Drosophila* data. These concern the specificity of the antiserum, the absence of fat body from the ovary preparation, the reason why only two of the three subunits are made, what part of the ovary is making VG, and whether or not the ovary VG mRNA actually is active in vivo. The question about the antiserum is especially relevant, since SDS acrylamide gels are used to purify the antigen (72), check the specificity of the antiserum (72), and determine the results of the cell-free system. Since some non-VG proteins co-migrate with the three subunits (21, 72), it is important that some alternative way of preparing the antigen be developed to avoid an otherwise circular argument.

With rocket immunoelectrophoresis VG was first seen in the hemolymph 24 hr after eclosion (63). A more sensitive method (hemagglutination, detection limit 2 ng) showed that VG was present within 15 min after eclosion (80). By 48 hr VG represented one third of the total hemolymph protein. α-Amanitin depressed the appearance of VG in the hemolymph when injected 1 hr after eclosion.

Studies on the mutant ap^4 have yielded interesting results. This mutant has a defective CA and does not develop eggs (107), but the normal titer of VG appears in the absence of JH (62). Thus, although synthesis of VG

is apparently not under JH control, uptake of VG by oocytes is, as JH treatment causes the appearance of yolk in the oocytes (62, 81). Recent work indicates that both JH and ecdysterone can induce synthesis of VG in isolated abdomens. Perhaps one acts on the fat body and the other acts on the ovary (J. H. Postlethwait and A. M. Handler, personal communication).

SARCOPHAGA BULLATA Two studies have shown that allatectomy at adult emergence prevents the appearance of VG in the hemolymph in 30–50% of the animals (49, 128). Allatectomy in the pupal stage has no effect (128). Application of JH (juvenate) to allatectomized flies induced the appearance of VG in the hemolymph of those animals affected by allatectomy (49). Cauterization of the medial neurosecretory cells within 12 hr of eclosion had no effect on presence of VG in the hemolymph (49, 128). However, the CC were not removed. A recent report stated that ecdysterone injected into males induces the appearance of VG in the hemolymph (75), whereas implanted ovaries had no effect. Unfortunately, sufficient data to support these interesting claims were not presented. It is difficult to interpret these data. We hope work on this animal will continue.

Hymenoptera

APIS MELLIFERA VG first appears in queen hemolymph shortly after eclosion and represents 70% of the total hemolymph proteins by day 4 (52). During active oviposition titers fall to 55% of the total, but rise again during winter when oviposition ceases (52). The rate of synthesis of VG is three times greater than that of other hemolymph proteins during oviposition. Of the labeled proteins, 85% are VG. In the non-laying queen, although the titer of VG is high, the rate of synthesis drops to levels similar to other hemolymph proteins (51). Treatment of virgin queens with CO_2 has long been known to induce egg development and oviposition normally seen only in mated queens. VG synthesis remains low in the unmated queen, but it is stimulated by two exposures to CO_2 if the queen is placed in a colony after CO_2 treatment. If she is kept in the laboratory with workers, the CO_2 does not have any effect (53). Within 1 day after the second CO_2 treatment, VG synthesis increases. The specific activity of the hemolymph VG remains relatively constant while the first batch of eggs is produced, but suddenly increases after oviposition begins.

VG is also found in worker hemolymph (51), and it is immunologically identical to that found in the queen (113). Titers are highest in the 12-day-old nurse bees and lowest in the foraging bees (52, 113). The titers found in egg-laying workers are comparable to those found in laying queens (52, 113). No evidence for VG transfer from workers to queen could be found

(113). The fact that nurse bees have high titers of VG suggests that the queen pheromone may prevent VG uptake by the oocytes.

Low titers of endogenous JH seem to be correlated with high VG titers in the worker (59, 114). Injections of low doses of JH III induce increased amounts of VG in the hemolymph; higher doses inhibit this response. Allatectomy of workers prevents appearance of VG, whereas injection of low doses of JH into the operated animals induces an earlier recovery than high doses (76). However, higher titers of VG were eventually reached in animals injected with higher doses.

In contrast, allatectomy of 7-day-old egg-laying queens had little effect on hemolymph VG titers or on VG synthesis measured 15 days later (111). Repeated applications of JH I had a slight stimulatory effect on VG titers and synthesis. Also, when unmated queens were allatectomized 2–4 days after emergence and then treated with two doses of CO_2, they showed normal titers of VG and normal rates of VG synthesis 30 days later (54).

These data are difficult to interpret. Either the hormonal control of VG synthesis is different in queens and workers, or, perhaps, JH is not in control of VG synthesis at all but somehow stimulates a factor that is. The correlation between JH and the presence of VG in bees is so far no more than a correlation. No direct evidence of an effect of JH on VG synthesis by the fat body has been presented. Engelmann has suggested that the queen might obtain JH from workers as she is fed by them (52). If so, the JH would have to survive gut enzymes, which seems unlikely. However, special mechanisms for JH transfer (e.g. topical application by workers during grooming) may exist.

Engels and co-workers (51–54, 111) obtained most of his results by electrophoresis of protein samples on cellulose acetate strips. The reliability of the results has been checked on acrylamide gels (51) and immunologically (W. Engels, personal communication). However, the data of Luscher and co-workers (59, 76, 113, 114) using immunologic techniques have substantiated the results of Engels, except that Luscher has not worked with queens.

Molecular Aspects

DNA synthesis is known to occur prior to VG synthesis in two cases (*Leucophae migratoria, Aedes aegypti*). There are important differences between these cases, and it is not clear what relationship the DNA synthesis bears to VG synthesis. Future work in this area could have profound implications for our understanding of the control of cellular differentiation.

All of the available evidence demonstrates that considerable RNA synthesis occurs concomitantly with VG synthesis, most of which is concerned with the elaboration of the rough endoplasmic reticulum needed for large-

scale synthesis and export of protein. This has been shown biochemically (43) and ultrastructurally (10, 27, 38, 124). In only one case (29) has it been shown that mRNA for VG is made after experimental hormone stimulation, although there is evidence for an increase in presumed VG mRNA in microsomes prepared from fat body of *Leucophaea maderae* during vitellogenesis (44, 47). Other evidence for transcriptional control is from in vivo use of various inhibitors of RNA synthesis (41, 60, 68, 80, 105). Using evidence of this type, Fong & Fuchs (60) have proposed that ecdysterone controls VG synthesis at the translational level in the mosquito. The recent evidence that the fat body must be exposed to JH before it can respond to ecdysterone (58) also raises the distinct possibility that JH is acting at the transcriptional level, leaving a post-transcriptional role for ecdysterone as Fong and Fuchs suggested. If this is true it would be a unique and remarkable situation. However, drawing conclusions from the in vivo use of inhibitors can be very misleading because of the complexity of the in vivo system. A direct effect of these drugs on the fat body is implied, but many other tissues (including endocrine glands) may be affected. In fact we have confirmed that α-amanitin has no effect on VG synthesis in vivo under the conditions used by Fong & Fuchs (60); however in vitro this same compound inhibits VG synthesis (W. J. Kaczor and H. H. Hagedorn, manuscript in preparation). More direct evidence is needed to resolve this interesting problem.

Studies on VG synthesis have only begun to exploit the power of the available techniques. The synthesis of VG in *L. maderae* (86), *L. migratoria* (28), and *A. aegypti* (68) appears to fit the message-accumulation model of Kafatos (78), but the data available are few. Much can be learned about molecular mechanisms by a detailed study of VG synthesis, as has been done for synthesis of cocoonase by galea (79) and chorion by follicle cells (78).

VG synthesis is cyclical in several insects (e.g. *L. migratoria,* ovivivíparous cockroaches, *A. aegypti*), and here the regulation of the decline in VG synthesis merits attention. Recent studies have indicated that the decline is somehow internally programmed (8, 17) and involves the breakdown of the protein synthetic machinery without the destruction of the fat body cells (17). This phenomenon should be examined more closely in ovoviviparous cockroaches such as *Byrsotria fumigata.*

JH Control

It is apparent from this survey that VG synthesis is not always under the direct control of JH, as has been claimed (40). Indeed, the data show that considerable diversity exists within the Insecta. Definitive evidence is lacking in most cases, but it is possible to draw some tentative conclusions. Evidence for the direct control of VG synthesis by JH is strongest in

Orthoptera, where JH has been shown to have a direct effect in vitro on locust fat body in organ culture (129).

Topical application of JH to isolated *Leucophaea* abdomens (22, 23) or decapitated, ovariectomized females (86) induced VG synthesis. Evidence for a response to JH by cockroach fat body in organ culture has not yet been reported.

The evidence for Hemiptera is surprisingly meager and inconclusive. The best work suggests that in *Triatoma* VG synthesis is regulated by JH (96). However, the experiments were conducted with topical applications of JH onto allatectomized females, and as pointed out before (66), the target tissue of the JH in such an experiment is unknown. Hemolymph of females ovariectomized at emergence contained VG, but rates of synthesis were not examined.

The situation in Coleoptera is confusing and needs reexamination with more definitive techniques.

The Lepidoptera show an interesting dichotomy in the timing and apparent regulation of VG synthesis. In two Saturniid moths, *Bombyx mori* and *Hyalophora cecropia,* VG synthesis occurs during metamorphosis. A role for JH in *H. cecropia* has been convincingly ruled out (101). However, the conclusion that VG synthesis in these moths is not under endocrine control seems premature, especially given the evidence from *B. mori* that ecdysone stimulates VG synthesis in vitro (99). It is possible, even likely, that endocrine events during metamorphosis trigger VG synthesis in these cases. The most likely candidate for such a role is ecdysone. This situation needs to be looked into. It should be noted that the development of eggs in the pupal or pharate adult stage is not a phenomenon unique to Saturniid moths (e.g. certain Diptera), so that similar mechanisms may occur outside of the Lepidoptera.

In the monarch butterfly, *Danaus plexippus,* VG synthesis appears to be under the control of JH (104, 105), although the target tissue is once again not proven. A role for the ovary has been ruled out (91).

Definitive evidence indicates that ecdysterone controls VG synthesis in the primitive dipteran, *Aedes aegypti* (71). The evidence for the higher Diptera is not as clear. In *Drosophila melanogaster* some evidence exists for regulation of VG synthesis by both ecdysone and JH, whereas JH alone appears to regulate uptake. In *Sarcophaga bullata* the role of JH is unclear (49) and injection of ecdysterone into males apparently induces VG synthesis (75). Taken at face value, these data suggest a link between the mosquito and the higher Diptera. However, the data are not conclusive, as pointed out earlier. It is clear, at any rate, that the control of VG synthesis in the Diptera is considerably different than in the more primitive insects.

The honey bee offers the most interesting puzzle. The difference in the effect of allatectomy and JH applications between queens and workers is not easy to interpret at this point, but it is reminiscent of the confusing effects of the same operation in *S. bullata.* In both cases it is possible that JH does not have a direct effect on VG synthesis.

The complete divergence of the mosquito system from the accepted hypothesis of JH control has been disturbing. However, it now appears that several facts help to make the uniqueness of the mosquito more apparent than real. First, it is now evident that the ovaries of many insects produce ecdysone, although in most insects the ecdysone is apparently retained in the ovary, in contrast to the mosquito. Second, JH is involved in the control of mosquito egg development, but here its role is confined to previtellogenic events, including an effect upon the fat body. Third, this review has shown that insects regulate VG synthesis in diverse ways. A unifying scheme is impossible at this point, but some speculations are permissible. How can one rationalize the evidence for JH regulation of VG synthesis in Orthoptera and Dictyoptera with ecdysone regulation in the mosquito? The Lepidoptera may provide the key; in the saturniid moths VG synthesis is closely correlated with metamorphosis. There is some evidence that in *B. mori* ecdysterone stimulates VG synthesis. If this turns out to be generally valid, the utilization of ecdysterone by the adult mosquito may be a specialized adaptation of a relationship first established in insects that produce VG during metamorphosis. Alternatively, the model might have developed much earlier in such primitive insects as *Thysanura,* where molting and reproduction are cyclical events in the adult (127).

If these speculations have any validity then what the mosquito has accomplished is interesting in that not only has a novel method of controlling VG synthesis been established (or reestablished), but also the source of the ecdysone has been changed from the prothoracic gland to the ovary. It is now clear that the ovaries of a number of insects produce ecdysone, but so far only the mosquito uses this source of ecdysone to control VG synthesis. In contrast, in *B. mori* (in which both the prothoracic glands and the ovaries produce ecdysone at different times during pharate adult development), if ecdysone does control synthesis of VG, it is the ecdysone from the prothoracic glands that is involved.

PHYSICAL AND CHEMICAL PROPERTIES

Substantial information is being accumulated on the composition and physical properties of VG and vitellins (Table 1). It is appropriate to take a critical look at these data to decide what generalities are emerging and in which areas new data are needed. Since these data are primarily quantita-

Table 1 V and VG: their physical properties

Insect	V, VG or proVG	Acronym	+/− amino acid analysis	% CHO	Molar ratio Man: GlcN[a]	% Lipid	Native (MW × 10^{-3})	Subunit (MW range × 10^{-3})	S	Ref.[b]
Orthoptera										
Locusta migratoria	V	*LOV*$_1$	+	13.6	10:1	9.6	585	52, 140	17	29
L. migratoria	V	*LOV*$_2$	+	11.0			530	55, 130		64
L. migratoria	V	*LOV*$_3$	+				570			95
L. migratoria	V	*LOV*$_4$	−		7:1					130
L. migratoria	proVG	*LO*PVG	−					250, 265		29
Dictyoptera										
Leucophaea maderae	V	*LMV*$_{14}$	+				559	70, —	14	35
L. maderae	V	*LMV*$_{28}$	+				1590	70, —	28	35
L. maderae	V	*LMV*$_1$	−	8.3	4:1	6.9			14 + 28	35
L. maderae	V	*LMV*$_2$	+							48
L. maderae	VG	*LMVG*$_1$	+							48
L. maderae	VG	*LMVG*$_2$	−					57, 118		86
L. maderae	proVG	*LM*PVG	−					179, 260		86
Blattella germanica	V	*BGV*$_1$	−	8.0		7.6				98
B. germanica	V	*BGV*$_2$	−	4.5	5:1			52, 100	17 + 31	88, K
B. germanica	V	*BGV*$_{17}$	+					52, 100	17	K
B. germanica	V	*BGV*$_{31}$	+					52, 100	31	K
B. germanica	VG	*BG*VG	+	4.5		15.7	659	52, 100	17	90
Lepidoptera										
Hyalophora cecropia	VG	*HC*VG	+	1.0		9.4	516	43, 230	16	90, 103
Philosamia cynthia	V	*PC*V	+	2.5	1:0	7.8				31
P. cynthia	VG	*PC*VG	+	2.5	1:0	10.4	500	55, 120		31
Manduca sexta	VG	*MS*VG	+	3.0	8:1	12.0	260	50, 180		97, ML
Coleoptera										
Leptinotarsa decemlineata	VG	*LD*VG	+							37
Diptera										
Aedes aegypti	V	*AA*V	−	10.5			270			69
Drosophila virilis	V	*DV*V	+	1.2				49		80
Drosophila melanogaster	V	*DMV*$_1$	+	2.7				46		80
D. melanogaster	V	*DMV*$_2$	+							M

[a] Moles of mannose vs glucosamine.
[b] K, J. G. Kunkel, unpublished data; ML, E. Mundall and J. H. Law, manuscript in preparation. M, A. P. Mahowald, manuscript in preparation.

tive, there is also some need to establish standards by which to judge the current data and to guide future data collection.

The question of what constitutes a VG or a vitellin (V) is not just a semantic problem. Although it is often clear which molecules should be called V and which VG in an individual species, it is not at all clear how to establish homologies between the V of different families, orders, or classes of arthropods. From the inception of its study, V (123) and its hemolymph precursor, VG (102), have been identified within a given species by immunologic techniques. This is partly because no known enzymatic activity of V exists and partly because the immune reactions allow measurement of the antigens in extremely small quantities (110, 126). Although in closely related species Vs cross-react, allowing testing of their homologies, in some groups vitellin cross-reaction does not occur outside the genus (89) or subfamily level (123) of the relationship. Without enzymatic or immunologic criteria to test, we must resort to compiling physical and biological evidence that may allow inferences of homology.

The immunologic criteria that distinguish VG and V from other proteins are their absence from larvae and adult males and their selective uptake (see Introduction). Although these criteria have sufficed to establish the rightful VG for numerous species, they may be too restrictive in the face of other serum protein molecules that may serve as VG in some insects. For instance, lipoprotein I of *Philosamia* is neither female specific nor the major protein in the egg, yet it occurs in one sixth the abundance of V in the egg (30). Though this protein has an established role as a diglyceride carrier in pupal and adult bloodstreams, it is also known to accumulate in substantial amounts in the oocyte, but whether or not it retains its diglyceride carrier function in the embryo is not known. Such an active role in the embryo's development or physiology would depart from the passive role V is thought to play as a nutritive source of amino acids, lipids, carbohydrate, and phosphorus. It would be best to keep an open mind as to what is and is not a VG until more is known about the functions of the minor protein constituents that enter the oocyte, whether actively, or passively by solvent drag.

Is V Different From VG?

We might first ask whether distinguishing features exist, aside from source, by which we might tell VG and V apart. No immunologic differences have been published between the V and VG of any insect species. This has usually been demonstrated by an Ouchterlony double diffusion identity reaction (83, 117). Such tests, however, can allow up to a 4% difference in amino acid sequence difference and still give an identity reaction. Indeed, an electrophoretic difference in the absence of an Ouchterlony spur has been demonstrated in *Oncopeltus* (83). No comparisons of the more sensitive

measures of antigen differences, e.g. microcomplement fixation, have been published. The lack of obvious differences between V and VG has tempted workers to abandon the distinction between them (30, 95, 98, 118). If, with more sensitive methods, no differences were found to exist between V and VG, we might consider using Occam's razor and simplify the terminology. The following discussion speaks to this point.

Table 1 lists the gross compositional and physical data accumulated for the best-known V and VG. The data are not complete enough to make a comprehensive statement, but some differences between V and VG suggest themselves. For instance, the lipid content of *Blattella germanica* vitellin (*BGV*) (98) differs from vitellogenin (*BG*VG) (90). This difference may be due to differences in technique, strain of animals, diet, or physiological state of the animal in different laboratories, or even in the same laboratory. For example, the analysis of carbohydrate in BGV_2 (88) differs from BGV_1 (98) but agrees with *BG*VG (90). Differences in extraction technique are more likely to affect lipid content than covalently bound substances such as amino acids and carbohydrates. Some *Hyalophora cecropia* V and VG lipid binds tightly to ion exchange columns and some is left behind when the VG is eluted (M. L. Pan, unpublished observation). Of particular concern for comparative purposes is the custom of obtaining VG from ovariectomized females. The lipid metabolism of these females is drastically altered and VG accumulates to high concentration in the hemolymph (48, 90). Comparing VG from an ovariectomized female to V from a normal female can hardly be considered a well-controlled comparison.

One approach to testing the existence of differences between V and VG is to look in those groups in which a substantial concentration of VG exists in the hemolymph prior to yolk deposition, e.g. giant silk moths (31, 100). The only data that conform to these criteria are the *Philosamia cynthia* data of Table 1. In this instance there is both a quantitative and qualitative difference in the lipid from the two proteins; the VG, *PC*VG, is richer in lipids than the vitellin, *PCV*, particularly with respect to diacylglycerol and sphingomyelin (31). This may be associated with a similar but more extensive lipid loss by the diacylglycerol-carrying lipoprotein I as it enters the oocyte (30). The VG lipid may be being used to construct oocyte membranes or as an energy source during oocyte development.

Another approach that has yielded qualitative differences between VG and V is the analysis of protein subunit structure during the synthesis, secretion, and uptake of VG. In two species, *Lucosta migratoria* (27, 29) and *Leucophaea maderae* (85), a sequential processing has been reported, with a high-molecular-weight peptide(s) being synthesized in the fat body and rapidly processed into smaller peptides before secretion into the

hemolymph. These proteins might be called "provitellogenins." Other changes in peptide molecular weights (85) and aggregation of subunits (24, 98) occur after uptake into the oocyte. Unlike the lipid composition differences discussed above, a general function for the subunit processing has not been found. Two laboratories have reported that all the carbohydrate of VG is on the largest subunit (88, 97), but it is not known whether or not this has anything to do with the processing of the peptides. The smaller peptide of native *Manduca sexta* VG is protected from tryptic digestion (E. Mundal and J. H. Law, personal communication). This is consistent with its lack of carbohydrate and its possible core location in the native protein.

Current evidence indicates a change in subunit composition concomitant with trimerisation of *Leucophaea maderae* V (24, 86). Though vitellin trimerisation is found in another cockroach, *Blattella germanica* (98, J. G. Kunkel, unpublished observations), it is not a general phenomenon even in cockroaches (J. G. Kunkel, unpublished observations). Therefore the subunit changes associated with this process are not of compelling interest.

Therefore it seems that the distinction between VG and V must be retained and used properly (102, 122). Investigators studying the yolk-derived protein should not refer to it as VG even if it appears to be grossly similar to VG and retains its ability to be taken up selectively by oocytes (30, 95, 98, 118). We may soon have to contend with more finely divided terminology, such as provitellogenin and preprovitellogenin (16) to describe presecretory stages and provitellin for early uptake stages of this varied molecule.

Composition

The failure to discover differences in amino acid and carbohydrate composition in VG and V is due to limited sensitivity of the technique (i.e. amino acid analysis), to inadequate attention to the problem (i.e. carbohydrate analyses), or to an absence of differences. The following is a critical view of the data available on composition.

AMINO ACID ANALYSES One particular problem currently is to decide whether or not to proceed and how to proceed with the amino acid analyses of VG and V. Multiple independent amino acid analyses are available for VG or V of five insect species, as shown in Table 1. *Locusta migratoria* vitellin has been analyzed by three laboratories, and *Leucophaea maderae* vitellin has been analyzed by two laboratories; one of these laboratories has also analyzed *L. maderae* VG. *Blattella germanica* VG and V have been analyzed by one laboratory, *Philosamia cynthia* VG and V have been analyzed by one laboratory, and *Drosophila melanogaster* V has been analyzed

by two laboratories. These data can serve as a basis for deciding whether or not further total amino acid analyses can provide any useful insights into comparisons of V and VG within or between species.

Before attempting to analyze these data, appropriate quantitative methods must be chosen. Two types of metric that can be used for comparing amino acid compositions have been described and compared (34, 94). The SΔQ and the Manhattan distance metric have contrasting usefulness. The SΔQ metric (94) compares protein X and Y,

$$\mathrm{S\Delta Q} = \sum_{i=1}^{16} (X_i - Y_i)^2,$$

where X_i and Y_i are the mole percent content of amino acid i in proteins X and Y, respectively. The mole percent must be based only on the amino acids for which analyses are available for all proteins to be compared. This excludes cysteine and tryptophan, which are commonly not analyzed, and lumps aspartate with asparagine and glutamate with glutamine. These are co-reported as aspartate and glutamate, respectively. The Manhattan distance (MD) metric uses the same values in a different way,

$$\mathrm{MD} = \sum_{i=1}^{16} |X_i - Y_i|.$$

The SΔQ method emphasizes those amino acids that differ the most between two proteins since it squares each difference; it is appropriate for searching for differences between proteins. MD, which simply adds differences, is more appropriate for comparing the degree of difference between proteins and has been described as useful for building phylogenies based on difference relationships (56).

Less than 2% of comparisons of unrelated proteins have been found to result in an SΔQ less than 100 (94). Related proteins have SΔQs that are linearly related to the degree of amino acid sequence differences they share (34). Past experience suggests that an SΔQ value of 4 is to be expected for comparisons of analyses of identical proteins in different laboratories (94). Therefore, a low SΔQ of about 4 can be considered to be a test for whether or not accurate estimates of amino acid composition have been made of a given protein for which multiple analyses are available, and also it is the lower resolution limit for testing whether or not any differences exist between the various V and VG within a species.

Within *L. maderae* the SΔQ statistic finds no differences greater than 3 for the six possible comparisons of *LM* VG, *LM* V_1, *LM* V_{14}, and *LM* V_{28}. For *P. cynthia* the SΔQ comparing *PC*VG with *PC*V is 0.12. Within *B.*

germanica the SΔQs comparing *BG*VG with *BG*V_{17} and *BG*V_{31} (which are similar to *LM*V_{14} and *LM*V_{28}; J. G. Kunkel, unpublished data) are 2, 3, and 6. In these species, therefore, no substantive proof shows any difference between the amino acid composition of V and VG.

In *L. migratoria,* three analyses are available on V. Two of the analyses agree somewhat (SΔQ of *LO*V_1 and *LO*V_2 is 13), but the third analysis disagrees with the first two (SΔQs of *LO*V_3 with *LO*V_1 and *LO*V_2 are 175 and 164, respectively). The major departure of *LO*V_3 is in glycine mole percent. If the aberrant analysis (*LO*V_3) is rejected, the best estimate for *L. migratoria* V might be the average of the two agreeable analyses. However, some criterion must be used for such a course of action.

In a related dilemma the *D. melanogaster* vitellin analyses also depart dramatically from one another (SΔQ of *DM*V_1 and *DM*V_2 is 146). Again a high glycine content of *DM*V_1 (80) is suggestive of contamination, possibly due to a glycine buffer used during purification. Does an intellectual basis, other than technical, exist for rejecting one or the other analysis?

One theoretical criterion for rejecting amino acid analyses of V is that Vs are a rapidly evolving group of proteins (88–90), primarily because of physiologically lax structural requirements. The only evolutionary restrictions for VG are that they must remain large to avoid being filtered by the pericardial cells (33, 90) and they must retain a recognition site for uptake into oocytes. There may be other conservative features of VG such as carbohydrate attachment sites or subunit cleavage sites, but the strength of selection for these features is debatable. The only other requirement for VG is that it maintain a nutritive amino acid composition. This nutritive purpose could be served by passively accepting mutations as they occur in most of the molecule. In this way V would approach the mutational equilibrial amino acid composition suggested by King & Jukes (84). Such an equilibrium would give all typical Vs a similar amino acid composition even if they diverged from each other very rapidly in absolute sequence (89, 90). Of the 18 available amino acid analyses of insect VG and V, all but three have compositions that fall close to the observed average protein (OAA) (84), having SΔQs compared to OAA ranging between 28 and 62. The three that depart from the OAA are *LO*V_3 and the two *Drosophila* spp. analyses, *DM*V_1 and *DV*V, which have SΔQs compared to OAA of 123, 156, and 670, respectively. All three of these reported analyses have extraordinarily high glycine contents. On the basis of these criteria, the validity of *DM*V_1 and *LO*V_3 can be summarily rejected, since acceptable analyses are available for them from other laboratories (Table 1). The *DV*V analysis is also suspect as atypical of insect V compositions, but is the only analysis available.

Since no significant differences were found by the SΔQ metric in the amino acid compositions of V or VG within a species for *L. maderae, B. germanica,* and *P. cynthia,* it would be appropriate to average their compositions to obtain a best estimate. For *L. migratoria,* the average of LOV_1 and LOV_2 (eliminating LOV_3) should give the best estimate. Table 2 gives the amino acid compositions of these best estimates and the SΔQ and MD metrics comparing them to each other and to the other proteins for which only single analyses are available. Of particular note is that the cockroach analyses, *LM*AV and *BG*AV, resemble one another more closely than they do species of other orders; the same is true for the Lepidopterans. If aberrant analyses are excluded, it would seem that V tends to cluster about an amino acid composition that is close to the average protein composition. The *DV*V remains as a suspect analysis and its high SΔQs with other Vs and the average protein, OAA, in Table 2 bears this out. It should also be noted that even if the obviously aberrant glycine content is removed from the DMV_1 and *DV*V analyses and mole percents are recalculated, these analyses still dramatically depart from similarly modified analyses of the other proteins in Table 2 (J. G. Kunkel, unpublished observation; A. P. Mahowald, personal communication).

Although a low SΔQ metric comparing amino acid compositions of Vs to each other or to an average amino acid composition may be a necessary condition to consider a protein a vitellin, it certainly is not a sufficient condition. Many proteins, especially large proteins, might be expected to approach the average protein composition. It has been noted that *L. migratoria* V is similar in amino acid composition to the serum diglyceride carrier protein of the same animal (64). It is probably more appropriate to say that both the diglyceride carrier protein and V closely approach the average amino acid composition. Critical data on this point are not abundant, but three species exist, *L. migratoria, P. cynthia,* and *B. germanica,* for which both vitellin and the diglyceride carrier protein have been analyzed (31, 90, 106). In these three species the diglyceride carrier protein (SΔQs vs OAA = 28, 30, 34) and the vitellin (SΔQ vs OAA = 37, 54, 42) both show similarly small SΔQ distances from the average amino acid composition. The diglyceride carrier proteins seem to be a more tightly similar group among themselves (SΔQs vs each other = 25, 32, 24) than are the vitellins of these same three species (SΔQs vs each other = 48, 54, 40). Moreover the diglyceride carrier proteins compared to the Vs give generally higher SΔQ values (SΔQs = 60, 48, 46, 66, 43, 65, 76, 65, 63), suggesting a systematic departure of the diglyceride carrier protein from the V. One such systematic difference that has been pointed out previously (90) is the lack (or low percentage) of methionine in the diglyceride carrier proteins, which would make them poor nutritive proteins.

Rather than give the impression that all large serum proteins in insects would yield small SΔQs when compared to the average amino acid composition, it should be noted that the class of large serum proteins in insects that have been characterized as blood storage proteins (125, 130) have relatively high SΔQs when compared to the average protein composition (J. G. Kunkel, unpublished observations).

Although these comments and criticisms are meant to establish a more critical basis for viewing amino acid data, it is hoped that it will not inhibit

Table 2 *V/VG* mole percent for averaged[a] or unique[b] amino acid compositions compared to each other and the OAA of King & Jukes (84) by the SΔQ (upper right) and Manhattan (lower left) distance metrics

	Metrics									
Metrics	*LO*AV	*LM*AV	*BG*AV	*HC*VG	*PC*AV	*MS*VG	*LD*VG	*DMV*$_2$	*DV*V	OAA
*LO*AV		49	48	51	40	31	38	47	812	37
*LM*AV	22		23	63	71	57	47	68	962	56
*BG*AV	25	14		59	54	43	23	77	951	42
*HC*VG	22	23	24		21	22	24	67	843	57
*PC*AV	19	24	24	12		6	23	67	847	54
*MS*VG	17	23	20	14	8		25	59	867	46
*LD*VG	21	19	16	16	15	16		54	795	28
DMV$_2$	22	23	25	25	23	20	22		635	15
*DV*V	63	73	73	62	65	66	64	62		670
OAA	20	22	19	22	20	19	16	12	61	
Mol% compositions										
ASX	10.8	15.9	12.2	9.7	10.2	10.7	10.0	11.2	8.1	10.8
THR	4.9	5.4	5.7	5.1	5.2	4.5	5.4	5.8	4.7	6.5
SER	8.2	7.6	9.1	9.2	7.7	8.0	9.7	6.2	13.5	8.5
GLX	12.8	11.2	10.9	14.3	15.7	14.4	12.8	10.1	11.9	10.0
PRO	7.0	4.8	4.3	4.4	5.4	5.2	5.2	(5.2)[c]	2.6	5.2
GLY	5.1	3.3	3.0	4.4	4.6	4.1	5.2	9.2	31.3	7.8
ALA	8.2	5.4	4.9	7.5	7.2	8.5	5.5	8.5	7.5	7.8
VAL	7.2	6.8	8.5	5.6	6.0	6.3	6.8	6.4	3.7	7.1
MET	1.5	2.0	2.8	2.0	2.1	2.1	2.5	1.9	0.5	1.9
ILE	4.5	4.9	5.2	4.5	4.7	4.7	5.7	5.0	2.1	4.0
LEU	9.8	9.2	8.6	6.0	6.5	6.9	7.0	8.0	4.9	8.0
TYR	5.8	4.5	4.4	5.2	4.8	4.7	3.8	3.9	2.0	3.5
PHE	2.8	4.2	4.4	3.5	3.2	3.0	4.7	2.8	1.7	4.2
LYS	5.6	6.1	7.3	7.5	8.9	8.1	7.8	7.5	3.0	7.5
HIS	1.8	3.2	4.3	3.1	3.4	4.4	2.9	2.2	0.4	3.0
ARG	4.0	6.5	4.3	7.9	4.2	4.3	5.0	5.9	2.2	4.4

[a] $LOAV = (LOV_1 + LOV_2)/2$; $LMAV = (LMVG + LMV + LMV_{14} + LMV_{28})/4$; $BGAV = (BGVG + BGV_{17} + BGV_{31})/3$; $PCAV = (PCVG + PCV)/2$.

[b] Proteins for which a single reported analysis was available.

[c] PRO was not analysed for DMV_2 but was estimated to equal the proline content of the average protein, OAA, for the sake of computation.

reports of divergent analyses. It might be expected, for instance, that a VG derived from follicle cells (4, 9) might dramatically depart from the typical V profile. The methods available are not stable or sensitive enough to detect small differences in amino acid composition in such large proteins as V. Therefore we cannot expect to derive much useful information from simply comparing the amino acid composition of V and VG within a species. More time might be profitably spent on analyses of the individual peptides from the different stages of vitellogenesis to see if the amino acid analyses of cleavage products add up to the precursor (97). In any event, the question of identity between V and VG will only be unequivocably answered at the levels of peptide mapping and sequencing, which are formidable tasks for such large proteins but might be practiced with the subunits. Since the Vs in general have the predictably low methionine content of the average protein, cyanogen bromide cleavage is an obvious first step. Even this technique would produce about eight large peptides from the smallest subunit of V, which generally weighs about 50,000 daltons.

CARBOHYDRATE ANALYSES Carbohydrate has been found covalently linked to purified V or VG in every instance in which it has been sought (29, 31, 35, 64, 90, 97, 98, 131). This is not surprising since many secretory proteins are glycoproteins. As more is learned about the carbohydrate moiety, it will be interesting to discover whether or not any distinct functional role(s) exists for this carbohydrate, i.e. maintaining the protein structure, taking part in the oocyte recognition, or providing additional nutritional value for the developing embryos.

The only well-controlled test of whether or not the carbohydrate content of V and VG differ is that for *P. cynthia;* no difference was found (Table 1). More detailed analyses of carbohydrate have been reported for either the V or VG (Table 1: *MS*VG, *LO*V_1, *LO*V_4, *LM* V_{28}, *BG*V). In each of these cases mannose and glucosamine, in ratios from 4:1 to 9.5:1, were the only identified sugars involved (29, 35, 88, 97, 131). The mannose is in part α-linked since two Vs are reported to be precipitated by concanavalin A (64, 89), and α-mannosidase removes 30% of the mannose residues from the *BG*V glycopeptide (88). Endo-β-N-acetylglucosaminidase H releases all mannose from the *BG*V glycopeptide, indicating a proximal chitobiosyl linkage of the carbohydrate to the peptide; the released oligosaccharide has 10 to 11 mannoses by gel-permeation sizing (88). Methylation analysis of the oligosaccharide indicates a branched structure (88). The small size of the oligosaccharide compared to the carbohydrate content of *BG*V would suggest multiple chains of oligosaccharide on the large subunit of the V and, as a result, multiple attachment sites. This leads to the question of whether or not all the oligosaccharide chains are identical within a molecule, and

to the more general question of whether or not these chains are the same or different from the chains found on other serum proteins in the same animal (30, 64, 89).

Uptake

The recognition and uptake of VG by the oocyte and its transformation into V have been studied from many perspectives, but the complete story is not yet available for any insect. The following mechanics are generally agreed upon, and details have been extensively reviewed (2, 46, 112, 122). The vitellogenic protein makes its way from the circulating hemolymph to the oocyte surface by way of channels between the follicle cells. At the oocyte surface a selective process takes place in which VG is preferentially taken up by the oocyte. The uptake into the oocyte is via micropinocytosis. The initially coated vesicles aggregate and coalesce into larger and larger vesicles to form the mature yolk granules.

CYTOLOGICAL MECHANISM Although micropinocytosis is agreed to be the mode of entry of VG into the oocyte, there is controversy over the exact cytological machinery involved. Whether a single type of coated vesicle is involved in all uptake into the oocyte (5, 116) or whether two (1) or more functional classes of vesicles exist (one for VG and another for nonspecific or "other" molecules) is undecided. The resolution of the problem will require comparison of simultaneous uptake of a VG and a non-VG, differentially labeled, so that they can be discriminated by light (118) or electron microscopy (5). This process is an important biological model for specific pinocytosis, since there are few other systems where single proteins are transported into a cell at a comparable rate (112).

MOLECULAR MECHANISM The molecular basis of uptake has been dealt with only briefly. High specificity has been demonstrated for the uptake process using purified radioactively labeled VG (90) or V (112). The suitability of using V instead of VG in uptake experiments has been questioned (90); no difference in the rate of uptake of *BG*VG vs *BG*V_{17} into *Blattella germanica* oocytes was found, but the *BG*V_{31} uptake rate is negligible compared to the rate for the VG, (J. G. Kunkel, manuscript in preparation). This would argue against using the crude mixture of 17S and 31S vitellins of *B. germanica* for uptake experiments. The active site for uptake on the VG or on the oocyte has not yet been found. However a vitellogenic protein derived from the ovarial follicle cells has been identified (4) and characterized (9) and is hypothesized to play an active role in VG-oocyte recognition. The importance of the follicle cells (if not its product) was elegantly demonstrated in the lack of vitellogenesis by naked

oocytes (3). Such a product, if it does play an adaptor role, does not simplify the situation but adds an additional recognition step to the vitellogenic process. The subunit size of the follicle cell product, 55,000 daltons (9), is close to the small subunit size of most VG. It will be interesting to see if the follicle cell product is a typical VG, or if its suggested role in uptake puts evolutionary constraints on it that make it depart from the average protein composition. It would also be useful to know if it is more immunologically conservative than the typical V (120).

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CONTENTS