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Endocrine effectors in insect vitellogenesis

Introduction: a formidable diversity

With almost one million species described, and some nine millions still to be named, insects are among the most successful creatures on earth. This formidable diversity is apparent not only at the morphological level, but also from a functional point of view. For example, insects have evolved a fascinating diversity of reproductive strategies to ensure the continuance of the species. Such strategies range from oviparity to viviparity, and from bisexual reproduction to functional hermaphroditism. This is further complicated with cases of parthenogenesis, paedogenesis and heterogony.

Reproduction and, in particular, vitellogenesis and egg maturation, are under hormonal control, and the diversity of reproductive strategies promises a parellel multiplicity of endocrine mechanisms of regulation. The present contribution deals with this multiplicity, and it has been written with the general purpose of discerning common themes, and to fit diverse observations into an orderly pattern.

The subject is too broad to be covered in a short review, therefore the bounds of the problem have been set in a rather restrictive manner. Firstly, only formally identified hormones that directly act on the vitellogenic tissues have been considered. This means that discussion of unidentified factors ('food factors', for example), or 'long-loop' mechanisms of regulation have been avoided. Secondly, the general idea has been to discuss only the essential facts and recent findings, and to display the information bearing in mind the phylogenetic position of the insect groups considered.

The above clarifications already suggest that this contribution is by no means an encyclopaedia, and makes no claim to completeness. Although objective considerations determined the problems to be treated, the choice and the differential emphasis was ultimately subjective. In any case, many comprehensive reviews (for examples, see Engelmann, 1983; Hagedorn, 1985; Koeppe *et al.*, 1985; Valle, 1993) provide the bulk of the information and the historical background that could not be included here.

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Vitellogenic proteins, tissues and hormones

In most insect species, vitellogenins are produced by the female fat body under the action of juvenile hormone, released into the haemolymph, and then incorporated into developing oocytes (Engelmann, 1983). However, not all insects synthesise the same type of vitellogenic protein; there are vitellogenic tissues other than the fat body; and there are hormones other than juvenile hormone involved in vitellogenesis.

Vitellogenins and yolk proteins

Vitellogenins are the precursors of yolk proteins in insects and other oviparous animals. In most insects, the primary vitellogenin gene product, with a molecular mass of more than 200 kDa, is processed into large (about 200 kDa) and small (about 50 kDa) units. This is the case in many orthopterans, heteropterans, lepidopterans and lower dipterans (Valle, 1993; Yano *et al.*, 1994a,b; Hiremath, Lehtoma & Nagarajan, 1994). In bees and wasps, the primary gene product, with a molecular mass of some 180 kDa, is secreted without processing (Valle, 1993; Kageyama *et al.*, 1994).

In contrast, the yolk proteins of higher Diptera are quite different from those of other insects. For example, in *Drosophila melanogaster* there are three major yolk proteins of around 40 kDa, each encoded by single-copy genes located on the X-chromosome (Bownes *et al.*, 1993). Similar examples are found in other higher Diptera such as the fruit fly *Ceratitis capitata* (Rina & Savakis, 1991) or the blowfly *Calliphora erythrocephala* (Martínez & Bownes, 1994).

In addition, the molecular analysis of vitellogenic proteins for which genomic or cDNA sequences are available (Table 1), has afforded new, more consistent information on the evolutionary relationships between different vitellogenic proteins. These analyses indicate that the yolk proteins of higher Diptera are not homologous to the vitellogenins of other insects (Romans *et al.*, 1995). Therefore, it seems that during their evolution flies shifted from the primitive type of vitellogenin to another protein to fulfil the role of nutrient reserve for the embryo.

Vitellogenic tissues

The fat body is the exclusive site of vitellogenin synthesis in the great majority of insects (Valle, 1993). During vitellogenesis fat body cells undergo dramatic changes and produce huge amounts of protein in a relatively short time (Keeley, 1985). However, in the Zygentoma (*Thermobia domestica*), in some Coleoptera (*Coccinella septempunctata, Leptinotarsa decemlineata*), and in most higher Diptera (*Dacus oleae, Musca domestica, Calliphora vicina, C. erythrocephala, Sarcophaga argyrostoma, D. melanogaster*) vitellogenesis occurs

Table 1 *Species where genomic or cDNA sequences of vitellogenin (Vg) or yolk proteins (YP) are available*

Species (Order)	Type of protein	Type of sequence available	References
Locusta migratoria (Orthoptera)	Vg	Genomic (partial)	Locke *et al.*, 1987
Anthonomus grandis (Coleoptera)	Vg	Genomic	Trewitt *et al.*, 1992
Aedes aegypti (Diptera)	Vg	Genomic	Romans *et al.*, 1995
		cDNA	Chen *et al.*, 1994
Drosophila melanogaster (Diptera)	YP	Genomic	Hung & Wensink, 1993; Garabedian *et al.*, 1987
Ceratitis capitata (Diptera)	YP	Genomic	Rina & Savakis, 1991
Calliphora erythrocephala (Diptera)	YP	Genomic	Martínez & Bownes, 1994
Bombyx mori (Lepidoptera)	Vg	Genomic	Yano *et al.*, 1994a
		cDNA	Yano *et al.*, 1994b
Lymantria dispar (Lepidoptera)	Vg	cDNA (partial)	Hiremath *et al.*, 1994
Athalia rosae (Hymenoptera)	Vg	cDNA (partial)	Kageyama *et al.*, 1994

both in the ovaries and in the fat body. An extreme case is represented by the stable fly *Stomoxys calcitrans*, where yolk protein synthesis occurs exclusively in the ovary (Valle, 1993; Rousset & Bitsch, 1993; Martínez & Bownes, 1994). The fact that in the primitive insect *T. domestica* vitellogenins are produced by the ovary suggests this is a primitive feature that has been lost in more modified insect groups that have panoistic ovaries (like most orthopteroids). Conversely, the production of vitellogenin by the ovary in beetles and flies would have been a later development in meroistic ovaries.

Hormones

Identified hormones involved in vitellogenesis belong to the three classic families of insect endocrines: juvenile hormones, ecdysteroids, and peptides and proteins.

The best-known hormones regulating vitellogenesis are juvenile hormones (Engelmann, 1983). These are sesquiterpenoids that are produced mainly in the corpora allata, part of the retrocerebral complex. Juvenile hormones were initially identified from their juvenilising action, prolonging the larval stage. Juvenile hormone III (JH III), methyl (2*E*,6*E*)-10,11-epoxy-3,7,11-trimethyl-2,6-dodecadienoate, is the JH homologue most frequently involved in vitellogenesis, and it is present in the great majority of insect orders. In adult females of higher Diptera, the bisepoxy homologue, methyl (2*E*)-6,7;10,11-bis-epoxy-3,7,11-trimethyl-(2*E*)-dodecenoate also seems to play a role in vitellogenesis (Kelly, 1994).

Ecdysteroids stimulate vitellogenesis in several insect species (Hagedorn, 1985), although they can also inhibit this process in others (see below). They were first known as moulting hormones, and in juvenile stages they are produced mainly by the prothoracic glands. In the adult, the principal ecdysteroidogenic organ is the ovary, and the ecdysteroid which acts most frequently in vitellogenesis is 20-hydroxyecdysone.

With regard to peptides, adipokinetic hormones and allatostatins may be involved in the regulation of vitellogenesis. The most typical action of adipokinetic hormones is to regulate energy metabolism by mobilising fat body nutrient reserves. Locust adipokinetic hormone I (AKH I) (pGlu-Leu-Asn-Phe-Thr-Pro-Asn-Trp-Gly-Thr-NH_2) has been shown to inhibit vitellogenin production in *Locusta migratoria*. Finally, allatostatins were initially described as inhibitors of juvenile hormone biosynthesis (Stay, Tobe & Bendena, 1994). However, it has recently been shown that these compounds can participate in the termination of vitellogenesis in cockroaches (Martín, Piulachs & Bellés, 1996b).

Hormonal control of initiation and maintenance of vitellogenesis

The fact that there is a formidable bulk of data on the initiation and maintenance of vitellogenesis (Engelmann, 1983; Hagedorn, 1985; Koeppe *et al.*, 1985; Valle, 1993) invites a systematic approach. A classification of the different cases into four groups has therefore been attempted. Only those hormones truly sustaining vitellogenesis have been considered here; that is, juvenile hormones and ecdysteroids.

The co-ordination of moulting and reproduction in the Zygentoma

Within the Apterygota, the order Zygentoma deserves special attention not only because it is one of the most primitive among insects, but also because

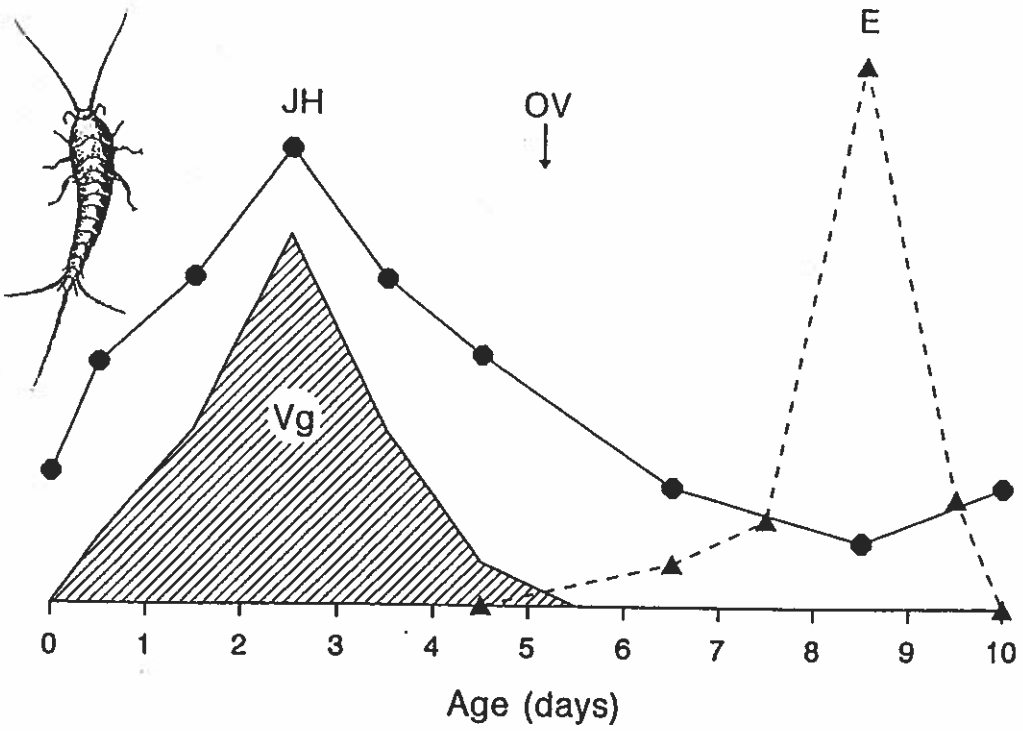

Fig. 1. Patterns of juvenile hormone (JH), ecdysteroids (E) and vitellogenin (Vg, shaded area) during the first days of adult life in the firebrat *Thermobia domestica*. OV, oviposition. Data from Rousset & Bitsch (1993) and references therein.

its representatives continue to moult during adult life. Most of the data available have been obtained with the firebrat, *T. domestica*. In this species, allatectomy prevents the vitellogenic phase of oocyte development, and treatment with allatotoxins (precocenes) also inhibits vitellogenesis. This indicates that juvenile hormone stimulates vitellogenesis (Rousset & Bitsch, 1993, and references therein).

In the firebrat, there are two large vitellogenins processed from large precursors and produced by both the fat body and the ovaries (Rousset & Bitsch, 1993). Interestingly, in this species moulting in the female (regulated by ecdysteroids) is interspersed with gonadotropic cycles (regulated by juvenile hormone) (Fig. 1), which suggests that moult and reproductive cycles are regulated in a co-ordinated manner.

Vitellogenesis directed by juvenile hormone

The most thorough studies on the vitellogenic action of juvenile hormones, including data on molecular action, have been carried out on the orthopteran *L. migratoria*. In the female of this species, juvenile hormone

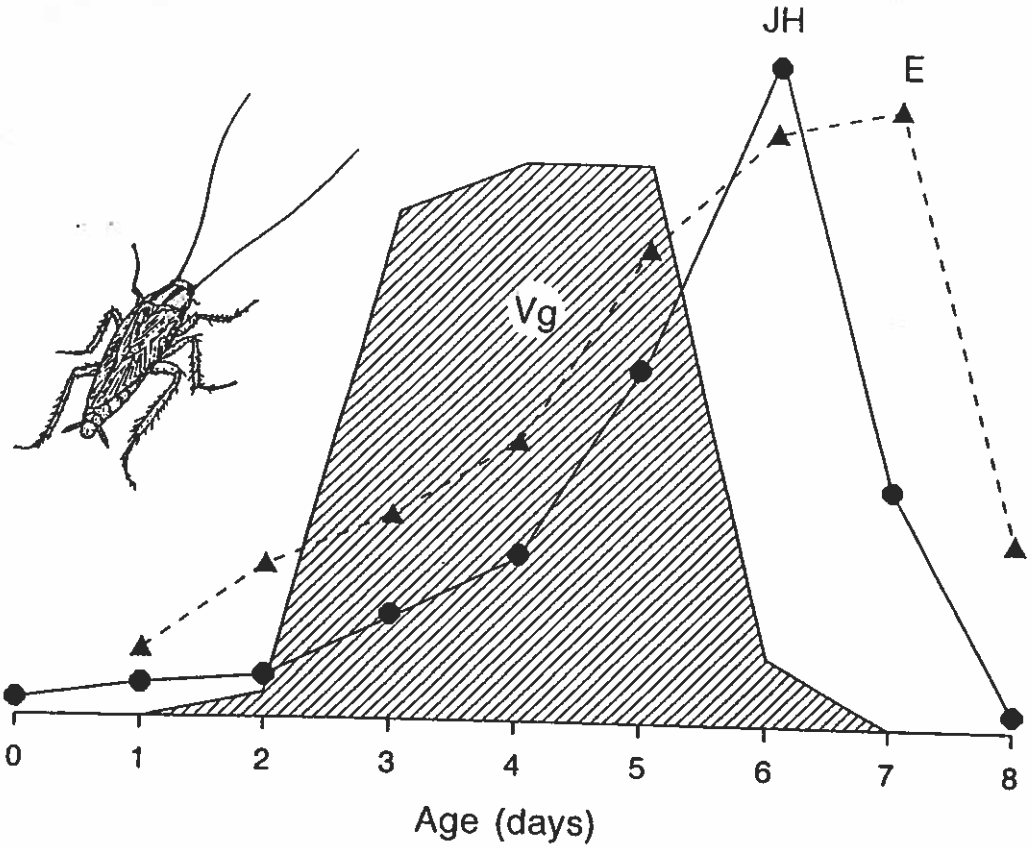

Fig. 2. Patterns of juvenile hormone (JH), ecdysteroids (E) and vitellogenin (Vg, shaded area) during the first days of adult life in the German cockroach, *Blattella germanica*. Data from Bellés *et al.*, 1987 (JH); Romañá *et al.*, 1995 (E); and Martín *et al.*, 1995 (Vg).

regulates vitellogenin synthesis in the fat body, vitellogenin mRNAs have been detected in this organ, and it has been shown that juvenile hormone influences their levels (Zhang, McCracken & Wyatt, 1993, and references therein). More recently, Glinka & Wyatt (1996) have shown that a juvenile hormone analogue activates vitellogenin gene transcription in the fat body.

As in other orthopteroids, it is also juvenile hormone that directs the synthesis of vitellogenin in the fat body of cockroaches. Most of the information has been obtained in the oviparous *Periplaneta americana*, the ovoviviparous *Blattella germanica*, the pseudoviviparous *Leucophaea maderae* and *Nauphoeta cinerea*, and the viviparous *Diploptera punctata* (Engelmann, 1983). In viviparous, pseudoviviparous or ovoviviparous species, in which vitellogenesis follows discrete cycles (separated by periods of egg transport when the endocrine system is quiescent), juvenile hormone production rates and circulating vitellogenin levels show cyclic patterns (Fig. 2). Conversely, in oviparous species, where vitellogenesis is sustained throughout successive

phases of oocyte maturation, juvenile hormone production and haemolymph levels of vitellogenin are rather constant (Martín *et al.*, 1995, and references therein).

Within Heteroptera most data have been obtained from the bloodsucking bug *Rhodnius prolixus* (Davey, 1993), in which juvenile hormone stimulates vitellogenin synthesis in the fat body. Similar data have been reported for other heteropteran species, both haematophagous, such as *Triatoma protracta*, and phytophagous, for example *Oncopeltus fasciatus* or *Pyrrhocoris apterus* (Engelmann, 1983).

In Coleoptera, the influence of the corpora allata and juvenile hormone on vitellogenin synthesis has been thoroughly studied in *L. decemlineata*. Juvenile hormone also directs vitellogenesis in *Tenebrio molitor*, *Dendroctonus pseudotsugae* or *Pterostichus nigrita* (Engelmann, 1983).

The flexibility of Lepidoptera

Within the Lepidoptera it is important to distinguish between species that start vitellogenesis after adult emergence, and those in which vitellogenin production begins before adult emergence (Fig. 3).

In species that begin vitellogenesis after adult emergence, the influence of corpora allata and juvenile hormone on oocyte growth has been reported in many cases (Engelmann, 1983), and direct evidence that juvenile hormone (or juvenile hormone analogues) induce vitellogenin production has been found in *Danaus plexippus*, *Nymphalis antiopa*, *Helicoverpa zea* and *Pseudaletia unipuncta* (Cusson *et al.*, 1994, and references therein). In *P. unipuncta*, a correlation has been observed between ovarian growth, juvenile hormone release rates *in vitro*, and vitellogenin synthesis (Cusson *et al.*, 1994).

Species in which vitellogenin production begins in the late pharate adult stage, such as *Plodia interpunctella* and *Manduca sexta*, constitute a special case. In *P. interpunctella*, vitellogenesis coincides with the decline in ecdysteroid titre which occurs at the pharate adult stage, and treatment with 20-hydroxyecdysone inhibits vitellogenin production (Shirk, Bean & Brookes, 1990). In *M. sexta*, vitellogenesis starts three to four days before adult emergence and proceeds in the absence of the pupal corpora allata. Nevertheless, juvenile hormone is necessary to complete oocyte growth, although it does not seem to be essential for vitellogenin production (Imboden & Law, 1983, and references therein). In summary, vitellogenin production by the fat body in *M. sexta* appears to be stimulated by juvenile hormone and ecdysteroids.

In *Hyalophora cecropia*, vitellogenin production begins in the prepupal stage, whereas in *Bombyx mori* it starts during early pupal development. In these two species, juvenile hormone does not appear to be involved in

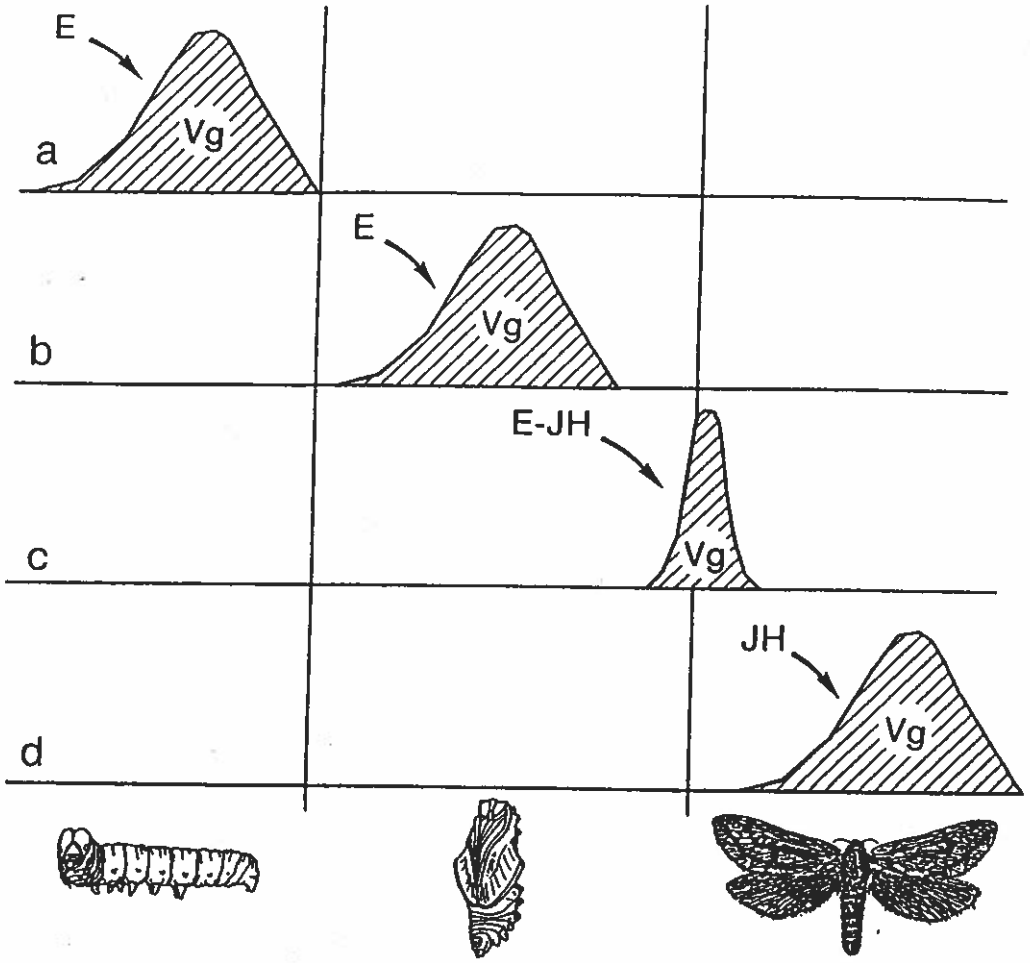

Fig. 3. In Lepidoptera, vitellogenesis (Vg, represented schematically by a shaded area) is sustained by ecdysteroids (E) and/or juvenile hormone (JH) depending upon whether it takes place in last larval stage (a), in the pupae (b), in late pharate adult stage (c) or after adult emergence (d). See the text for references.

ovarian development, whereas ecdysteroids have been shown to stimulate this process. The beginning of vitellogenin synthesis and uptake in *B. mori* coincides with a peak in ecdysteroid titre, and administration of 20-hydroxyecdysone to brainless pupae stimulates vitellogenin production (Tsuchida, Nagata & Suzuki, 1987).

A possibly similar case occurs in *Lymantria dispar*, in which vitellogenin production begins during the last larval instar. The treatment of larvae of this species with a juvenile hormone analogue suppresses vitellogenin production (Fescemyer *et al.*, 1992) and vitellogenin mRNA in the fat body (Hiremath & Jones, 1992). This suggests that a low or declining titre of juvenile hormone is necessary for vitellogenin accumulation in *L. dispar*.

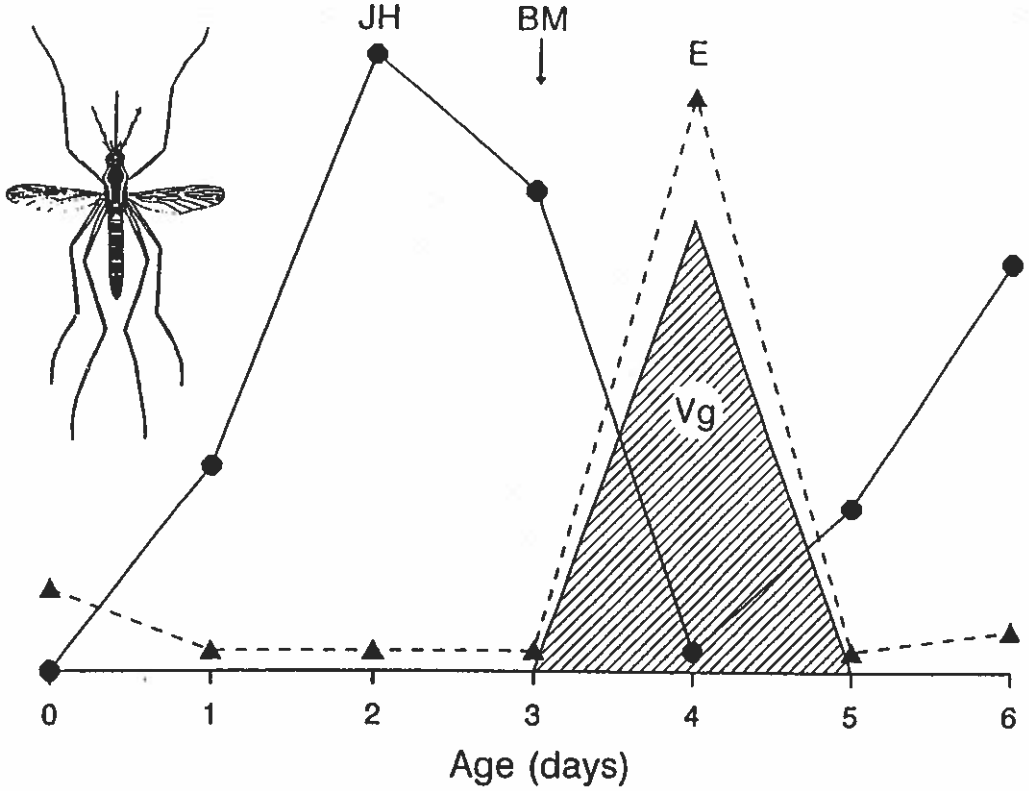

Fig. 4. Patterns of juvenile hormone (JH), ecdysteroids (E) and vitellogenin (Vg, shaded area) during the first days of adult life in the yellow fever mosquito, *Aedes aegypti*. BM, blood meal. Data are from diverse sources (e.g. Hagedorn, 1985; Raikhel, 1992).

The shift from juvenile hormone to ecdysteroids in Diptera

Within the Diptera, a distinction should be made between orthorraphid (lower) and cyclorrhaphid (higher) species, because there are substantial differences between these two groups.

With regard to the Orthorrhapha, most of the data available come from research on the mosquito *Aedes aegypti* (Hagedorn, 1985; Raikhel, 1992; Dhadialla & Raikhel, 1994). In this species, Hagedorn and co-workers proposed 20–hydroxyecdysone as the factor directing vitellogenin synthesis in the fat body, and that the ovary was the source of ecdysteroids. Data supporting this view have also been reported for other mosquitoes: *Culex pipiens, Aedes atropalpus* or *Anopheles stephensi* (Hagedorn, 1985).

In *A. aegypti*, it has also been shown that juvenile hormone potentiates the action of 20-hydroxyecdysone, and that juvenile hormone may induce fat body capacitation in previtellogenesis, whereas ecdysteroids would direct the vitellogenic phase (Fig. 4). In addition, the importance of a blood meal and

of midgut factors to the full development of vitellogenesis in response to physiological doses of 20-hydroxyecdysone has also been stressed (Dhadialla & Raikhel, 1994).

A number of groups have described the action of ecdysteroids at a molecular level on vitellogenin production in *A. aegypti* (Dhadialla & Raikhel, 1994, and references therein). The production and, particularly, the accumulation of a high-molecular weight vitellogenin precursor (provitellogenin) is stimulated by 20-hydroxyecdysone *in vitro*, which suggests that the hormone triggers the transcription of vitellogenin genes. More recent studies have shown that cycloheximide inhibits the vitellogenic response induced by 20-hydroxyecdysone (Deitsch *et al.*, 1995), which suggests that the action of this hormone is indirect, and is mediated by transcriptional factors.

Among the Cyclorrhapha, the species that has been studied most thoroughly is *D. melanogaster*. As stated above, yolk proteins of *D. melanogaster*, and presumably of higher Diptera in general, are peculiar and different from vitellogenins of other insects. In addition, yolk proteins are produced not only by the fat body but also by the ovary. The sex of the adults is the primary factor for correct expression of yolk proteins genes in *D. melanogaster* (Bownes *et al.*, 1993). Once the genes are active in the female, the level of expression is modulated by 20-hydroxyecdysone, juvenile hormone and neurosecretory factors. The endocrine regulation of yolk proteins in *D. melanogaster* has recently been reviewed by Kelly (1994). Both juvenile hormone and ecdysteroids stimulate yolk protein synthesis in the fat body and in the ovary and juvenile hormone induces yolk protein uptake in the ovary. Significant data are also available in the housefly, *M. domestica*. In the adult female, 20-hydroxyecdysone and juvenile hormone can induce yolk protein synthesis, and the two hormones have an additive effect (Adams & Filipi, 1988). Both the fat body and the ovary are stimulated by 20-hydroxyecdysone, whereas juvenile hormone induces a greater response in the ovary than in the fat body (Agui *et al.*, 1991). 20-Hydroxyecdysone induces lower production of yolk proteins in houseflies allatectomised before juvenile hormone was released (Adams & Filipi, 1988), and in specimens from which both corpora allata and ovaries had been removed (Adams & Gerst, 1992). Taken together, the results indicate that both ecdysteroids and juvenile hormone are necessary for vitellogenesis, and the data of Agui *et al.* (1991) suggest that juvenile hormone could direct the synthesis of yolk proteins in the ovary whereas 20-hydroxyecdysone could do this in the fat body.

Ecdysteroids also induce vitellogenesis in *C. erythrocephala*, *C. vicina*, *Neobellieria* (= *Sarcophaga*) *bullata*, *Protophormia terraenovae*, *Phormia regina* and *Lucilia caesar* (Yin & Stoffolano, 1994).

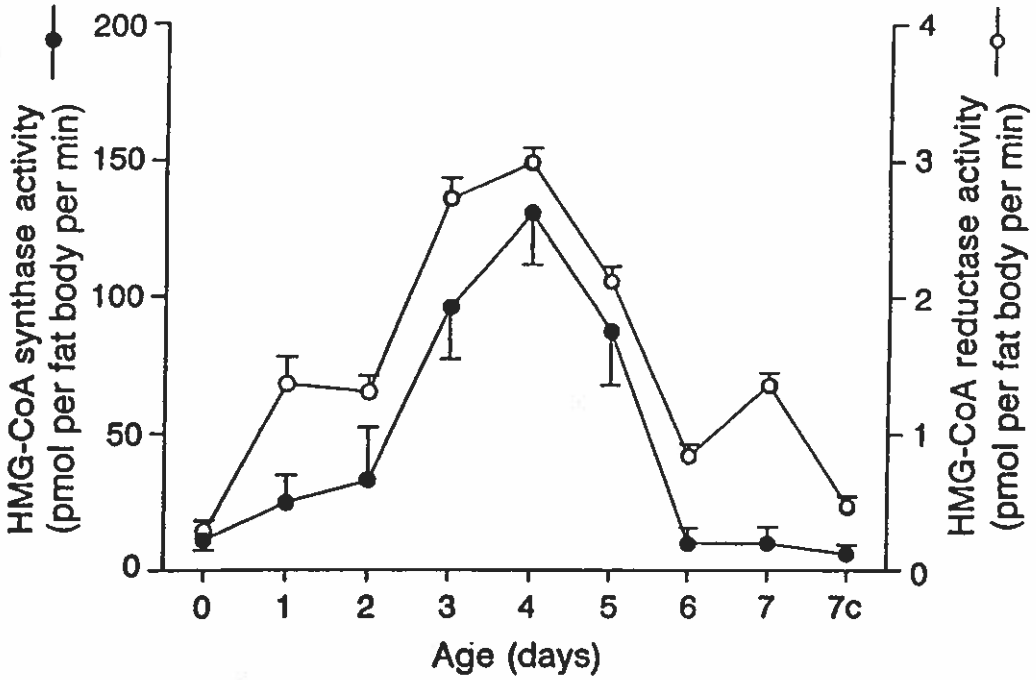

Fig. 5. Pattern of activity of the enzymes 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) synthase and HMG-CoA reductase in the fat body of *Blattella germanica*. Data from Casals *et al.* (1996).

The problems of cyclicity

The brief outline of vitellogenesis in different insect groups presented above shows that cyclicity may be related to physiological constraints (vitellogenesis co-ordinated with moulting in Zygentoma, Fig. 1, or in some Lepidoptera, Fig. 3) critically connected to trophic factors. For example, vitellogenic cycles are associated with the timing of blood meals in most haematophagous insects (Fig. 4), or may be interspersed with discrete oviposition pulses as in locusts.

In addition to being a by-product, so to speak, of trophic or physiological constraints, cyclicity might also be a genuine evolutionary step towards viviparity. In this context, cockroaches offer a paradigm of a continuum from oviparous species, with almost constant production of vitellogenin (*P. americana*), to ovoviviparous or viviparous species, with cycles of vitellogenesis separated by periods of ootheca or embryo transport equivalent to pregnancy (*D. punctata, N. cinerea* or *B. germanica* (Martín *et al.*, 1995, and references therein) (Fig. 2).

Furthermore, cyclicity is observed not only in vitellogenin production, but also in other parameters related to vitellogenesis. A recent example is the cyclic pattern of expression and activity of the enzymes 3-hydroxy-3-methylgutaryl-CoA (HMG-CoA) synthase and HMG-CoA reductase observed in the fat

body of *B. germanica* (Casals *et al.*, 1996). These enzymes regulate the mevalonic acid pathway, which leads, among other end-products, to dolichol, a donor of oligosaccharide residues in the process of glycosylation of proteins. In the fat body of *B. germanica*, the cyclic expression of these enzymes (Fig. 5) is related to the glycosylation of vitellogenin.

These cases suggest not only that vitellogenesis is hormonally induced but also that it is terminated in a highly integrated manner.

Regulation by a short-loop feedback

The simplest mechanism to regulate vitellogenesis would be a short inhibitory loop resulting from the increasing levels of circulating vitellogenin. Indeed, this mechanism has been proposed for mosquito vitellogenesis (Raikhel, 1992). In connection with this, it has been shown that lysosomal activity in the fat body contributes to the termination of vitellogenesis in *A. aegypti*, and also that a high concentration of circulating vitellogenin in ovariectomized vitellogenic mosquitoes provides feedback regulation of this lysosomal activity (Raikhel, 1992).

In orthopteroids, the huge amounts of vitellogenin found in the haemolymph of ovariectomized females seems to suggest that the possible inhibitory action of vitellogenin upon its own synthesis would not operate under physiological conditions. Instead, these cases point to the ovarian ecdysteroids as candidates for terminating vitellogenesis.

Ecdysteroids as candidates for terminating vitellogenesis

Experiments involving ovariectomy have been useful in elucidating homeostatic relationships between the ovary and the fat body during vitellogenesis (Maestro *et al.*, 1994, and references therein). A frequent consequence of ovariectomy is the continuous synthesis of vitellogenin and its marked accumulation in the haemolymph. This has been well described in primitive insects such as locusts (*L. migratoria*) and cockroaches (*Byrsotria fumigata, N. cinerea, D. punctata, B. germanica*) (Martín *et al.*, 1996a, and references therein), and suggests that the ovary is involved in the termination of the vitellogenic cycle.

It has also been shown that the ovaries of these species produce considerable amounts of ecdysteroids, and these hormones have been detected in the haemolymph. In both ovary and haemolymph, ecdysteroids reach maximal levels towards the end of vitellogenesis, and haemolymph ecdysteroid levels decrease after ovariectomy (*Blaberus craniifer, N. cinerea, B. germanica, P. americana*) (Romañá, Pascual & Bellés, 1995, and references therein). It seems, therefore, that some of the ecdysteroids produced by the ovary are

released to the haemolymph, and they could eventually act on the fat body. Experiments with the fat body of *B. germanica* incubated *in vitro* (D. Martín, N. Pascual, M. D. Piulachs & X. Bellés, unpublished observations) showed that physiological doses of 20-hydroxyecdysone added to the incubation medium inhibited the production of vitellogenin, which supports the above hypothesis.

This inhibitory action of ecdysteroids could be extended to other insects in which vitellogenesis is directed by juvenile hormone. In the case of Zygentoma, the increase in ecdysteroid levels at the end of the reproductive cycle induces a subsequent moult, but could also be related to the termination of vitellogenesis. In Heteroptera, Davey (1993) has suggested that ovarian ecdysteroids in *R. prolixus* might inhibit vitellogenin production. With regard to beetles, ecdysteroids have been detected in the haemolymph and ovaries of *L. decemlineata* (Hagedorn, 1985), although the possible role of ecdysteroids in vitellogenin synthesis has not been investigated.

The contribution of peptides

The first clue to the involvement of an identified peptide in the negative control of vitellogenesis came from the experiments of Carlisle and Loughton in the late 1970s (cited in Kodrík & Goldsworthy, 1995), demonstrating that locust adipokinetic hormone I (AKH I) (pGlu-Leu-Asn-Phe-Thr-Pro-Asn-Trp-Gly-Thr-NH_2) inhibited the synthesis of proteins in the fat body of *L. migratoria*. Furthermore, Moshitzky & Applebaum (1990) stated that vitellogenesis by the fat body of *L. migratoria* incubated *in vitro* is inhibited when locust adipokinetic hormone I is added to the incubation medium. This, together with the observation that titres of locust AKH I markedly increase in the haemolymph at the end of egg maturation (Moshitzky & Applebaum, 1990), seemingly implicate AKHs in the negative control of vitellogenesis of *L. migratoria*. More recently, Kodrík & Goldsworthy (1995) have shown that locust AKHs inhibit RNA synthesis *in vitro* in the fat body of *L. migratoria*.

The inhibitory effect of locust AKH I on protein production in *L. migratoria* fat body contrasts with reports describing the stimulatory effect of hypertrehalosaemic hormone (HTH), which belongs to the same peptide family as locust adipokinetic hormone, on protein synthesis in the fat body of the cockroach *Blaberus discoidalis* under certain experimental conditions (Keeley *et al.*, 1991). This suggests that hormonal regulation may be different in locusts and cockroaches.

Allatostatins belonging to the Tyr-Xaa-Phe-Gly-Lys-NH_2 family were identified on the basis of their inhibitory action on insect juvenile hormone synthesis (Stay *et al.*, 1994) (see Weaver *et al.*, this volume), but their ubiquity

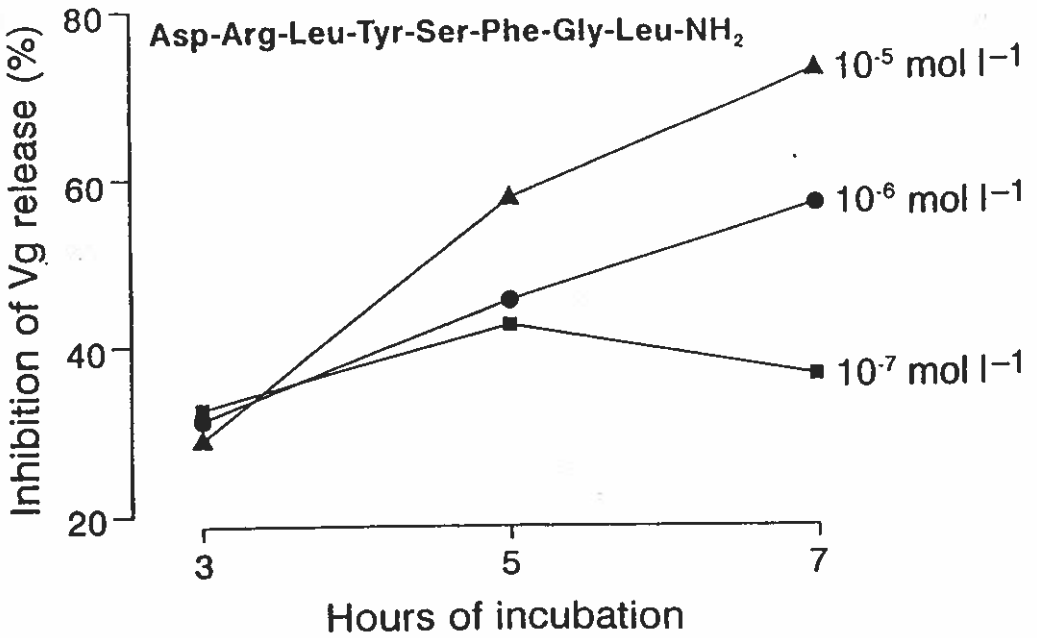

Fig. 6. The inhibition of vitellogenin (Vg) release from fat bodies incubated *in vitro* produced by allatostatin 2 of *Blattella germanica*. Periovaric fat bodies from 4-day-old females of *B. germanica* were used. The inhibition was dose- and incubation time-dependent. Data from Martín *et al.* (1996b).

in the central and peripheral nervous system and gut suggests that they have a broad spectrum of functions (see Duve *et al.*, this volume). In a search for new functions for this peptide family, the effect of one of the allatostatins of *B. germanica* (BLAST-2: Asp-Arg-Leu-Tyr-Ser-Phe-Gly-Leu-NH_2, Bellés *et al.*, 1994) on vitellogenin production by fat body incubated *in vitro* (Martín *et al.*, 1996b) was investigated. The results (Fig. 6) showed that the allatostatin inhibited vitellogenin production *in vitro* by fat bodies from four-day-old females, an age which precedes the decline of vitellogenin production (see Fig. 2). Furthermore, results from enhanced chemiluminescence labelling (ECL) western blot analysis suggested that the allatostatin had inhibited the glycosylation of vitellogenin, which would account for the reduction in release of vitellogenin previously observed. Martín *et al.* (1996b) suggested that the allatostatin might have inhibited the mevalonic acid pathway and the synthesis of dolichol, an intermediate necessary to vitellogenin glycosylation. In line with this hypothesis, it was shown that mevalonolactone, used as precursor of the mevalonic acid pathway, was able to restore the normal levels of vitellogenin release in allatostatin-treated fat bodies. Taken together, these results suggest that allatostatins contribute to the termination of the vitellogenic cycle in the cockroach *B. germanica*, by inhibiting the glycosylation, and thus interrupting the release, of vitellogenin.

Epilogue: tailored solutions

The first conclusions that can be drawn from this overview is that hormonal systems regulating vitellogenesis can vary greatly from group to group. However, this is not surprising given the great diversity at all levels (morphological, physiological, ecological and behavioural) not only within the class Insecta, but also within a single order or even a family.

Results from the Zygentoma, the least modified group studied so far, suggest that the primitive trophic hormone in insect vitellogenesis is juvenile hormone, and that ecdysteroids would 'frame' each reproductive cycle. The fact that the most primitive groups within the Pterygota (all the orthopteroids, for example) also use juvenile hormone to direct vitellogenesis supports this hypothesis. It also appears that in these primitive groups, where vitellogenesis is directed by juvenile hormone, ecdysteroids may be involved in terminating the process, occasionally in conjunction with peptide hormones.

The Lepidoptera are a paradigm of plasticity. Juvenile hormone directs vitellogenin synthesis in those species in which oocyte growth is strictly a post-emergence phenomenon. Conversely, in those which develop oocytes in late larval or in pupal stages, stimulation by juvenile hormone would be in conflict with the regulation of metamorphosis, which requires low titres of this hormone. In these lepidopterans, ecdysteroids seem to play the role of gonadotrophic hormone, in the context of the hormonal events of moulting and metamorphosis, which require a high titre of this hormone. Species in which vitellogenin production is initiated a few days before adult eclosion would represent an intermediate case, where both juvenile hormone (perhaps as a primary, capacitatory, factor acting on the fat body and/or the ovary) and ecdysteroids (acting as gonadotrophic hormone to induce the synthesis of vitellogenin in the fat body) may be required for vitellogenesis and oocyte development.

In Diptera, two important developments are found. One, accomplished by the higher dipterans (Cyclorrhapha), is the change from the primitive type of vitellogenin to another protein to fulfil the role of nutrient reserve for the embryo. The other is the shift from juvenile hormone to ecdysteroids to direct vitellogenesis. In dipterans, juvenile hormone continues to participate in the gonadotropic process, but ecdysteroids are the main factor directing the production of vitellogenic proteins.

The shift from juvenile hormone to ecdysteroids to direct vitellogenesis is an intriguing issue. Although the nature of the juvenile hormone receptor remains elusive, it is plausible that belongs to the nuclear receptor superfamily (for a review, see Mangelsdorf *et al.*, 1995), as does the ecdysone receptor. If this were true, then it would open many possibilities of 'interchangeability', so to speak, of both juvenile hormone and ecdysteroids to induce or repress vitellogenin transcription, with discrete changes, either at

the ligand- or DNA-binding sites in the receptor, at the site of regulatory elements in vitellogenic protein genes, or even through different combinations with the heterodimer partner (see also Thummel, 1995). In addition, evidence on the induction of transcription of vitellogenic proteins suggests that the mode of action of these hormones is indirect, and is mediated by transcriptional factors (Wyatt, 1991; Deitsch *et al.*, 1995), which opens further possibilities to explain the interchangeability of both kinds of hormone.

Taken together, the evidence suggests that endocrine strategies do not follow strict phylogenetic lines, nor do the hormones have rigid actions along these lines. Rather it seems that the evolution and constraints of the reproductive strategies have modelled hormonal systems of regulation *ad hoc*, and that this has been achieved by using (co-opting, if you wish) the arsenal of hormonal molecules available in a quite flexible manner. Therefore, the most certain conclusion is that stated in the first sentence of this epilogue, and we are just seeing the tip of the iceberg. Every new species studied brings new surprises, and there are more than nine million of them yet to be investigated!

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