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Some aspects of the control of the gonotrophic cycle in the tick *Ornithodoros moubata* (Ixodoidea, Argasidae)

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8.1 INTRODUCTION

Ticks are obligate hematophagous ectoparasites which can transmit many diseases, and they have a high reproductive potential. They are of considerable economic importance and thus reproduction of numerous species has been studied extensively (see reviews of Diehl *et al.* 1982, Oliver 1974, Oliver Chapter 10 of the present book). However, in contrast with insects, very little is known about the hormonal physiology controlling oocyte maturation and oviposition. This fact is due in part to the difficulty in experimenting with this material. For instance, because of the 'sac-like-structure' of ticks, selective ablations of glandular tissues are very difficult and simpler experimental operations such as ligations induce high mortality. The anatomy and cytology of the glandular tissues associated with the brain have been investigated very recently, but in only a few species (see review of Binnington. Chapter 6).

The nature of the circulating hormones, the site of vitellogenin synthesis,

and the exact process of ovary development have not yet been described in most species of adult ticks. However, data obtained in our laboratory during the past 20 years with the argasid tick *Ornithodoros moubata* (Murray 1977, *sensu* Walton 1962) permit us to describe various interactions and succession of interdependent steps for the completion of a gonotrophic cycle in this species. We will briefly review these studies, and together with unpublished results and some data from other authors, we will provide a synthetic scheme for the possible control of the gonotrophic cycle in *O. moubata*. Of course, caution must be exercised in generalizing mechanisms described for one species to other species. Similar or different mechanisms controlling the gonotrophic cycle in other species are reviewed in Chapter 10 by Oliver.

8.2 THE GONOTROPHIC CYCLE OF *ORNITHODOROS MOUBATA*

As in other ticks, engorgement and mating are the usual prerequisites for a complete gonotrophic cycle in *O. moubata,* including vitellogenesis and oviposition. Descriptive aspects of oogenesis and cytological changes in the oocytes during vitellogenesis have been studied (Aeschlimann & Hecker 1969, 1970, Diehl 1970) and summarized diagrammatically in Diehl *et al.* (1982). In ixodids (with the exception of a few parthenogenetic species) mating is a requirement for completion of the blood meal (Aeschlimann & Grandjean 1973a, Graf 1974). In contrast, mating is not necessary for engorgement in argasids. This characteristic has allowed us to study separately the influence of the blood meal and mating on successful completion of the gonotrophic cycle in an argasid species.

8.2.1 Importance of the bloodmeal

O. moubata, like other tick species, is an obligate hematophagous ectoparasite. Each immature stage (except the larvae) requires a bloodmeal to complete a molting cycle (Hoogstraal 1956). Autogeny, however, does take place in a very low percentage of mated unfed females, and even two successive cycles have been observed (Aeschlimann & Grandjean 1973a).

In mated females, the size of the bloodmeal determines the number of eggs laid. Experiments using 82 fully engorged and 55 partially fed females demonstrated high linear correlations (correlation coefficient > 0.945) between the weight of the egg-batch, the number of eggs laid, and the weight of the bloodmeal. Such high correlations have also been observed with the weight of the engorged females after excretion of their coxal fluid, one day after feeding (Table 8.1). Later, for simplification, the correlation between the number of eggs laid and the weight of engorged females was used. Five partially fed females (experimentally detached) did not lay eggs. The percentage of the unfed weight represented by the bloodmeal was 52% (Table 8.2). Ingestion of a quantity of blood representing 65% of the unfed weight was sufficient to induce the oviposition of a reduced number of eggs.

In virgin females, as in mated females, feeding initiates the beginning of digestion. Vitellogenins are synthesized and found circulating in the hemo-

Table 8.1 — Linear correlations† between the Bloodmeal weight and the egg-yield (in mg or in number (No) of eggs) in ovipositing *O. moubata* females.

Equations	Correlation coefficients
Egg yield (mg) = 0.502 × Bloodmeal weight − 0.946	0.950
No. of eggs = 0.725 × Bloodmeal weight + 2.089	0.948
Egg yield (mg) = 0.460 × Engorged female weight − 17.34	0.948
No. of eggs = 0.663 × Engorged female weight − 21.422	0.945

†Correlations were established either with the estimated weight of the bloodmeal (difference between the weights of the engorged female 24 hrs after feeding and its unfed weight) or directly with the engorged female weight 24 hrs after feeding.

Table 8.2 — Number of eggs laid by mated female *O. moubata* in relation to the bloodmeal weight expressed as percentage of the unfed weight of the females.

Number of eggs	Number of females	Bloodmeal weight expressed as % of unfed weight†	
		$\bar{x}$	SD
0	5	52.0	32.7
$0 < N \leqslant 14$	7	65.0	15.2
$15 \leqslant N \leqslant 24$	11	97.4	34.8
$25 \leqslant N \leqslant 34$	10	121.9	82.6

†$\bar{x}$, SD: mean and standard deviation of the different estimated percentages respectively.

lymph. Spectrophotometric measurements at 400 nm (maximum absorbance of the dark hemoproteins which constitute vitellogenins) were not different in mated and virgin females at the same time after feeding. The concentration of the protein H8 increases in virgin females during the first 2 weeks post-feeding as in mated females, but, in contrast to mated females, its concentration does not decrease. However, the levels of proteins H6 and H7 in virgin females require several weeks to reach that attained within one

week in mated females (Diehl 1969, 1970). These vitellogenins are incorporated into the oocytes. However, the oocytes rarely reach the final stage, and the partially to fully developed eggs are finally resorbed. This 'abortive vitellogenesis' takes about 50 days. One hundred days after feeding, the ovaries of fed virgin females look like those of unfed females (Aeschlimann 1968, Germond & Aeschlimann 1977). Nevertheless, a few fed virgin females are able to oviposit a few non-viable eggs after a prolonged preoviposition period (Aeschlimann 1968). After fifteen years of research in the area we note that these females lay as many eggs as mated females of the same breeding colony (Fig. 8.1A). Generally, they represent a small percentage of the total female population, but surprisingly, this percentage of virgin females ovipositing is very high in some nutrition batches (Fig. 8.1B). The hormonal quality of pig blood on which the ticks fed could be a reason for such variation. Vertebrate steroids play a role in the synchronization of reproduction of certain fleas (Rothschild & Ford 1973). In addition, vertebrate steroids have been recently reported in insects (De Clerck *et al.* 1983, 1984). Thus, an interesting question centres on whether vertebrate hormones can sometimes induce oviposition in virgin female ticks.

To summarize, the bloodmeal constitutes a stimulus for the onset of vitellogenesis, but it is generally not enough to ensure completion of the gonotrophic cycle. In addition, the ingestion of a minimum quantity of blood (corresponding to approximately 60% of the weight of the unfed females) is necessary to induce oviposition in mated females.

8.2.2 Importance of mating

The dependency of oviposition upon when copulation takes place (Fig. 8.2) indicates the importance of mating for completion of a successful gonotrophic cycle. Mating alone, without a bloodmeal, except in certain rare cases of autogeny (Aeschlimann & Grandjean 1973a), does not induce oviposition.

If the female is mated before or soon after the bloodmeal, the isolated engorged female completes digestion of the bloodmeal and completes normal vitellogenesis. Vitellogenins are synthesized, secreted, and circulated in the hemolymph and are incorporated in the oocytes (Diehl 1969, 1970, Chinzei 1983, Chinzei *et al.* 1983). At 27 °C the preoviposition period varies between 10 and 20 days, with the maximum number of females ovipositing at 11–12 days. Disturbance of the female delays but does not reduce the number of eggs laid. Overcrowding also delays, and sometimes inhibits or reduces the number of eggs laid by the female. In *Argas arboreus* similar disturbances inhibit oviposition *via* a pheromone (Khalil 1984).

If a virgin female engorges and is not mated, the 'abortive vitellogenesis' described above takes place. Such virgin females, possessing nutrient reserves in the midgut lumen, may be mated after several months. The

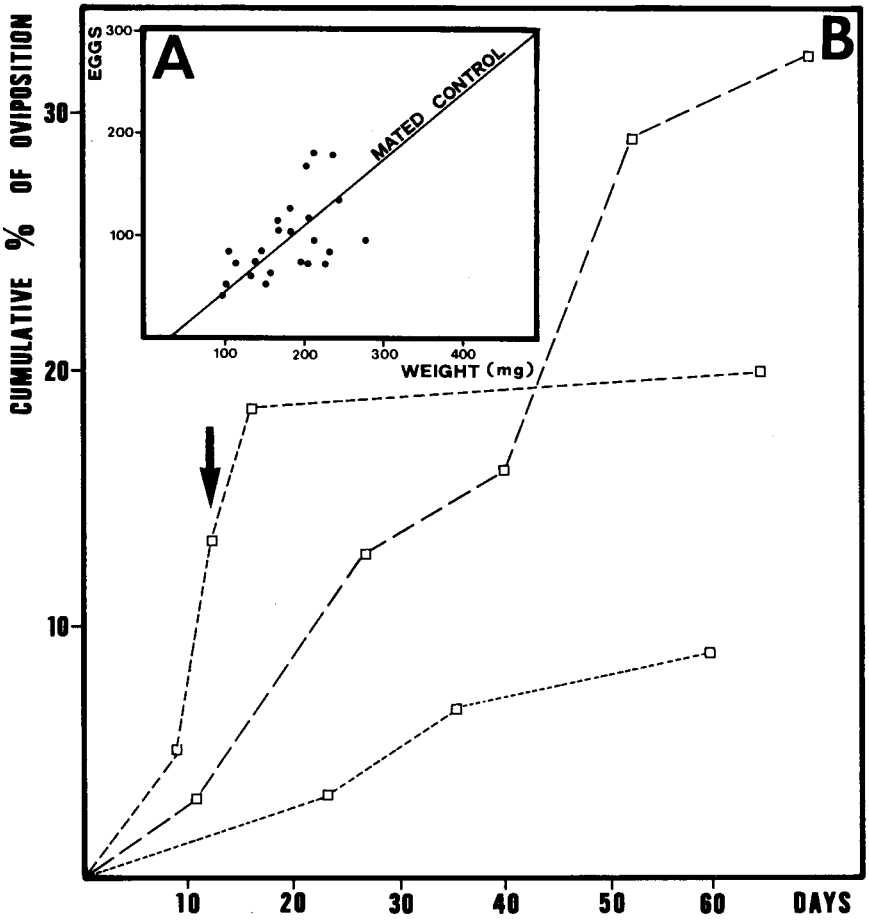

Fig. 8.1A — Occurrence of oviposition in fed virgin female *O. moubata*. The numbers of eggs laid by the ovipositing virgin females of our colony plotted in relation to the weight of the engorged females after excretion of their coxal fluid.

The numbers of eggs laid by these females are very close to those laid by mated females (regression line drawn with 137 mated females). Fig. 8.1B — Occurrence of oviposition in fed virgin female *O. moubata*. Cumulative percentage of ovipositing fed virgin females in 3 different nutrition batches. (The females were fed on defibrinated pig blood through a parafilm membrane). 60 females were used with blood A (----), 31 with blood B (——), and 35 with blood C (— — — —).

The arrow indicates the usual preoviposition period for fed mated females. Note that virgin females generally oviposit with a very prolonged preoviposition period.

delayed mating triggers digestive activity of the midgut cells, and the mobilization of food reserves stored in the midgut lumen (Aeschlimann 1968, Grandjean 1983). This resumption of digestion is followed by vitellogenesis leading to oviposition of viable eggs. However, the number of eggs laid is lower than if mating coincided with the bloodmeal. Only 14% of the

weight of the female tick is converted into eggs instead of about 30% if mating coincides with feeding (Grandjean 1983). Also the 'preoviposition' period (the time between mating and beginning of oviposition) is shorter (6–14 days instead of 10–20 days) (Aeschlimann 1968, Germond & Aeschlimann 1977).

O. moubata females can perform several gonotrophic cycles (up to 8), each cycle needing a bloodmeal. We have noted, however, that only one mating is necessary for the successive gonotrophic cycles, but in this case, the preoviposition periods become successively prolonged and egg viability decreases. Nevertheless, the number of matings or the number of endospermatophores does not influence the number of eggs laid (Aeschlimann & Grandjean 1973b).

8.3 CHARACTERIZATION OF THE STIMULI PRODUCED DURING MATING

8.3.1 Mechanical stimulation

We investigated the role of mechanical stimulation of the female genital ducts by introducing small metal beads into the vagina and the uterus of engorged virgin females more than 3 months after feeding (see details in Ducommun 1984). Upon dissection, vitellogenic oocytes were observed in these females. In addition, the dry weight of the ovaries increased within 7 days after treatment (Table 8.3). The weight of the ovaries of treated females was significantly higher ($P \leq 0.05$) than those of untreated females, but not significantly different ($P \leq 0.05$) from those of mated females. A high proportion of 'bead' treated females (73%) laid eggs within a slightly prolonged 'preoviposition' period (20–25 days). This percentage was not significantly different from those of mated controls of the same batch ($P =$ 43%, exact probability test of Fisher) (Table 8.4). However, the egg yield expressed as the number of eggs laid/weight of the female, was significantly lower in treated females (0.42 ± 0.19 instead of 0.59 ± 0.10).

Thus, these results clearly demonstrate that mechanical stimulation alone is enough to trigger vitellogenesis *and* oviposition in virgin females in which the ovary was in a resting stage after the 'abortive vitellogenesis'.

8.3.2 Chemical stimulation

Mature spermiophores, taken from the uteri of mated females, induce egg-laying or vitellogenesis when injected (either intact or as homogenate) into the hemocoel of engorged virgin females (Aeschlimann 1968). In addition, if the mature spermiophores are incubated for 20 h in a medium, injection of the washed spermiophores induced egg-laying in the virgins, indicating that the chemical stimulus was not in the seminal fluid but in the spermiophore. Furthermore, injection of homogenates of the accessory glands, in contrast to observations in *O. parkeri* by Oliver *et al.* 1984, did not induce vitellogenesis or oviposition, indicating further the inactivity of seminal fluid in *O. moubata*. Thus, in order to determine where this chemical stimulus origi-

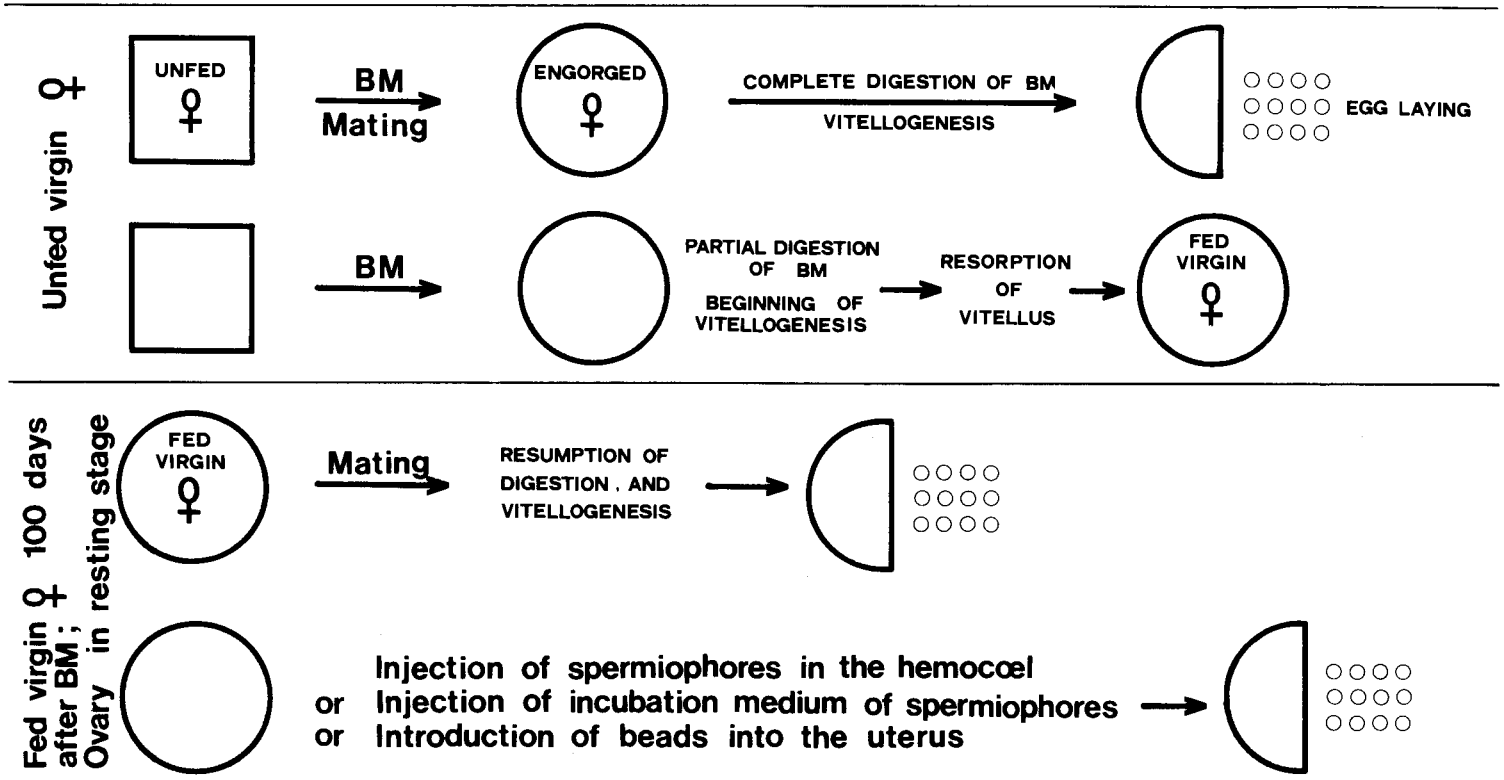

Fig. 8.2 — Diagrammatic summary of the possible inducers of vitellogenesis and/or oviposition in female *O. moubata* (BM = bloodmeal).

Table 8.3 — The role of mechanical stimulation of mating in *O. moubata*: The dry weight of ovaries of virgin females 100 days after feeding was estimated 7 days after implantation of small metal beads into the uterus.

	Number of females	Mean weight of a dry ovary ± SD (μg)
Mated control	5	1500 ± 1360
Virgin control	10	270 ± 170
Beads in the uterus of virgins	10	1060 ± 680

Table 8.4 — The role of mechanical stimulation of mating in *O. moubata*: Number of ovipositing virgin females 100 days after feeding in which beads were introduced into the uterus. The females oviposited approximately 25 days after treatment.

	Number of females	Number of ovipositing females
Mated control	8	7 (88%)
Virgin control	15	0 (0%)
Beads in the uterus of virgins	15	11 (73%)

nates, we investigated the effects of injecting homogenates of testes. Again, in contrast with *O. parkeri* (Oliver *et al.* 1984), vitellogenesis was not observed. Only the injection of homogenates of seminal vesicles distended with immature spermiophores was effective; 71% of the females injected with crude extracts and 50% injected with the extract diluted 5 times produced vitellogenic ovaries. Thus, the chemical responsible for inducing oogenesis was already present in prospermium. Aeschlimann (1968) noted that the medium in which mature spermiophores were incubated was also effective in inducing vitellogenesis when injected into fed virgin females. Recently, Sahli *et al.* (1985) demonstrated, *in vitro,* that the incubation medium of immature, non-devaginated spermiophores did not induce vitellogenesis or oviposition. The medium becomes active only after the spermiophores are almost fully devaginated and the contents of the apical cisternae are liberated. The activity of the *in vitro* incubation medium

decreases rapidly (after about 12 h of incubation), but the incubation medium of spermiophores removed from females 48 h after mating still shows high activity. This suggests that, *in vivo,* the chemical stimulus begins to be released by the spermiophore after devagination, that is, soon after mating, and continues to be released for several days thereafter.

Preliminary experiments (Germond & Aeschlimann 1977) showed that the compound inducing oogenesis was heat sensitive; it was eluted in the void volume when passed through a Sephadex G-100 column, suggesting a high molecular weight. Results of Sahli *et al.* (1985) indicated that it could be a protein. The molecular weight of this compound has been estimated after chromatographing an active spermiophore incubation medium on a Bio Gel A5M column and injecting aliquots of eluted fractions into virgin females 3 months after feeding (Fig. 8.3). This bioassay showed a peak of activity corresponding to an apparent molecular weight of 1 600 000 to 1 900 000 daltons.

Analysis of the denatured protein from different incubation media of spermiophores by SDS polyacrylamide electrophoresis demonstrated the presence of two proteins of high molecular weight (between 100 000 and 200 000 daltons) in the bioassay positive supernatants (Sahli *et al.* 1985). The differences between the molecular weights found by gel filtration and electrophoresis may be due to the formation of a proteinaceous complex between different subunits. The fact that treating with urea inactivates the compound and that the activity is recovered only after slow but not after rapid dilution of urea (Sahli personal communication), suggests that the active compound consists of several subunits that can be denatured and then reconstituted. If the active substance is not itself a protein, it must be linked to a protein when active. However, because of the difficulties of working with proteins, investigations on the molecular weight of this compound and the possible existence of subunits perhaps should be carefully repeated.

Finally, Germond & Aeschlimann (1977) demonstrated that this chemical stimulus was not species specific, since injections of spermiophores of *O. tholozani* or *O. tartakowsky* induce oviposition in virgin females of *O. moubata.*

8.4 POSSIBLE PATHWAYS VIA WHICH THE ACTIVE SUBSTANCE ACTS

Although we do not yet know the exact nature of the chemical compound inducing vitellogenesis and oviposition in *O. moubata,* we are interested in knowing how it exerts its effect. We note that this compound is produced by an individual and transferred to another individual of the same species (in the present case, from a male to a female). In addition, it seems to act at very low concentrations. Therefore, it corresponds to what is usually called a *pheromone.* This pheromone is normally introduced into the vagina during mating, and we hypothesize that receptors for this product exist in this organ or in the genital ducts. However, it also exerts its effects when introduced into the hemocoel, suggesting the presence of other receptors. Two possibi-

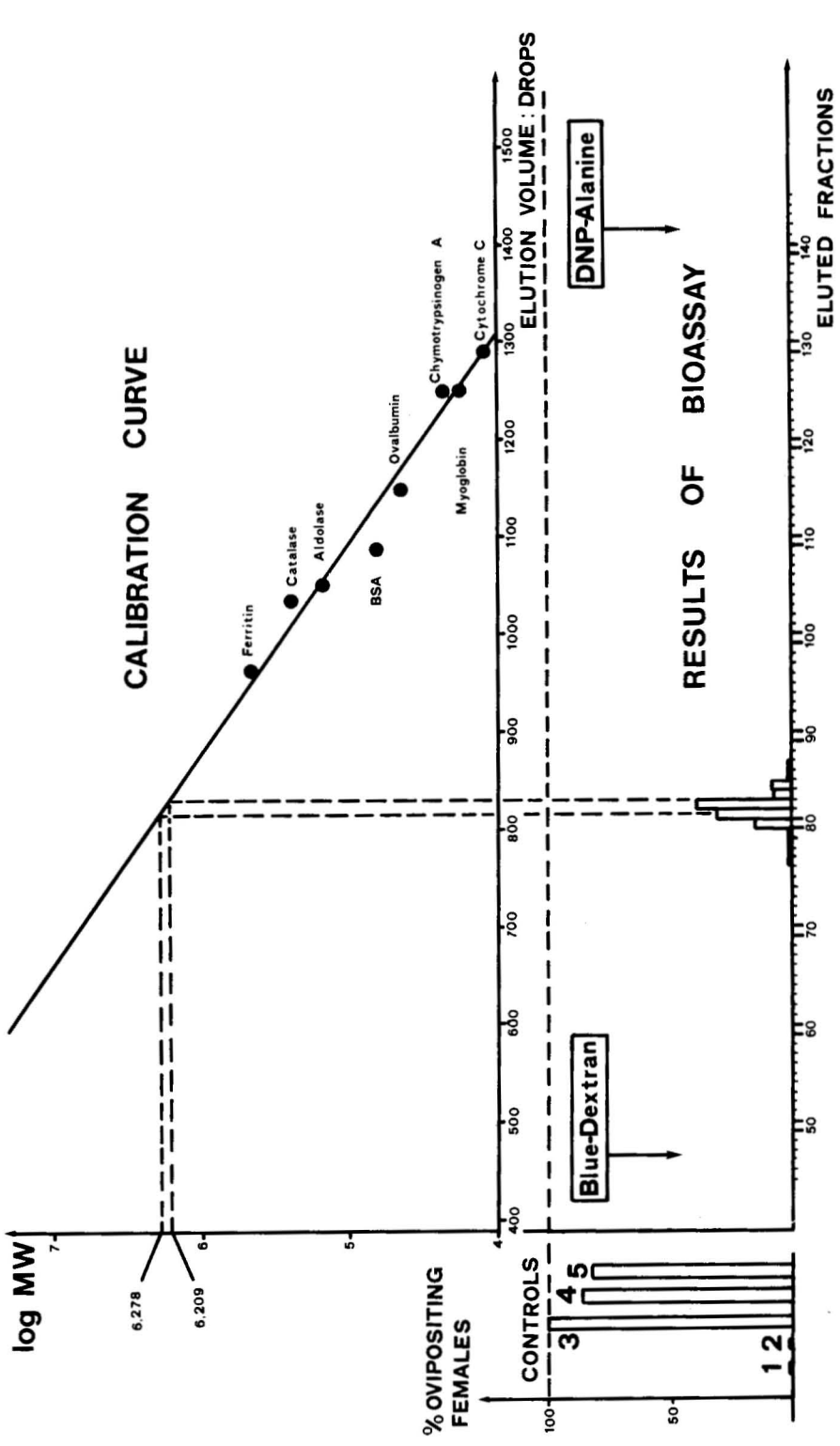

Fig. 8.3 — Estimation of the molecular weight (MW) of the vitellogenesis inducing factor present in the supernatant of incubated spermiophores. The supernatant was separated into fractions of 10 drops (600 μl) on a Bio-Gel A5M column, (length 60 cm, ø 15 mm). A bioassay was performed by injecting aliquots of these fractions into the hemocoel of 11 to 18 virgin females 100 days after feeding. Percentage of ovipositing females are recorded. Controls were completed by injecting the elution solvent (NaCl 2%) into fed virgin females (1) and fed mated females (3). Similar controls were done with Arachnid Ringer, the solution in which the sample was deposited onto the column (2: virgin females; 4: mated females). Control no. 5 corresponded to the injection of an aliquot of the supernatant before chromatography in fed virgin females.

lities exist: either a direct action of the substance on the target organs (i.e. midgut cells for digestion, and cells which synthesize vitellogenins), or an indirect action on endocrine tissues, whose secretions are necessary for vitellogenesis and oviposition.

8.4.1 Neurosecretions

The anatomy of the brain of *O. moubata* has been well described (Eichenberger 1970, see Binnington, Chapter 6 of the present book). It contains numerous neurosecretory cells. In closely related species (*O. tholozani* and *Argas persicus*) neurosecretory activity has been related to feeding, mating, and oogenesis (Gabbay & Warburg 1976, Eisen *et al.* 1973). In addition, we know that insect neurohormones play an important role in the control of reproduction in two different ways: 1) directly by acting upon the target organ itself, or 2) indirectly by acting upon the neuroendocrine glands which, in turn, regulate various reproductive steps (see reviews of Raabe 1982, 1984). Aeschlimann (1968) injected whole brain homogenates into the hemocoels of virgins 3 months after feeding. Five of 12 virgin females injected with homogenates from mated fed females laid eggs within a normal preoviposition period (11–15 days). None of the virgins injected with homogenates from virgin unfed females and 1 of 12 with homogenates from fed virgin females laid eggs (preoviposition period = 17 days). Similar experiments were repeated without success (Ducommun 1984). However, implantations of brains dissected from mated females of various physiological states induced vitellogenesis (Table 8.5). The ovaries of up to 56% of

Table 8.5 — Effect of implantation of brains dissected from fed mated females at different times after their bloodmeal on the induction of vitellogenesis in virgin females 100 days after feeding. Brains, in physiological medium, were injected with a microsyringe through a hole punctured at the level of the 4th leg pair coxa.

		Number of females	Number and percentage of females, the ovary of which indicated vitellogenesis after 14 days	
Untreated control		38	4	(10.5%)
Sham transplanted control		37	4	(11%)
Transplantation of brains from fed mated females	unfed	36	20	(55.5%)
	0 days	34	15	(44%)
	2 days	32	18	(56%)
	3 days	11	9	(82%)
	4 days	24	10	(42%)
	7 days	10	5	(50%)
	12 days	24	9	(38%)
	25 days	22	10	(45%)

transplanted females contained large brown oocytes. Thus, transplantation has a stimulating effect on vitellogenesis but not on oviposition. Unfortunately, however, this effect does not seem to be specific. In fact, implantation of brains from either virgin females of corresponding ages, or males, induced vitellogenesis in about the same proportions (40 to 52% of females oviposited). Other experiments on brain implantation in which the retrocerebral complex has been carefully preserved did not significantly modify the results ($P \leq 0.05$). This complex is believed to have a structure very close to that of the corpora allata — corpora cardiaca complex of insects. Abortive vitellogenesis was induced in 44 to 67% of the virgin females (fed 3 months before) with transplanted brains from either virgin females, mated females, or males.

8.4.2 Production of juvenile hormone-like substances

Mating may also act as a stimulus for the production of hormones related to juvenile hormones (JHs) that play an important role in the control of vitellogenesis in insects (see review of Engelmann 1984).

It is possible to induce egg-laying in virgin females (either immediately after feeding or 3 months after feeding) by topical application of JH analogues or JH stereoisomers (Connat *et al.* 1983b). In recently fed virgin females, these compounds are able to maintain the vitellogenic process and cause a high percentage of ovipositing females (up to 70% instead of less than 13% in virgin females). The length of the preoviposition period is inversely related to the dose applied, but the number of eggs laid — though nonviable — is always as high as that expected for mated females of the same weight. Surprisingly, 10 μg of farnesyl methyl ether per female induced hyperstimulation of vitellogenesis leading to a greater egg yield than in mated females.

Topical applications of the same compounds to virgins with a resting stage ovary 3 months after feeding were less potent. A high dose (500 μg) of a 1:1 ratio of JH1:JH3 induced only 58% of the females to oviposit. However, in this case the action of the hormone induces both a resumption of digestion and vitellogenesis. Injecting the hormone into the ticks yielded higher percentages of ovipositing females. Fifteen to 60 μg of a JH1:JH3 mixture (1:1) induced oviposition in about 60% of the females. The preoviposition period was also longer than in the control females. Injections of the natural isomers of insect juvenile hormone were much less effective than stereoisomeric mixtures or JH analogues.

Our results, with large doses of JHs or JH analogues do not necessarily show a direct effect, but suggest, as did the results of Pound & Oliver (1979) with *O. parkeri*, that a hormone structurally related to insect JH may exist in these animals. We have thus attempted in collaboration with Dr B. Mauchamp, CNRA, Lab. of Phytopharmacy, Versailles, France to analyze samples with gas chromatography coupled to mass fragmentometry after extraction and purification. The technique is similar to that used in analyzing insect JHs (Bergot *et al.*, 1981), but without derivative formation since we used a chemical ionization for detection (see Mauchamp *et al.* 1984).

Analysis of 3 different samples of 300 μl haemolymph each from 3 different periods during vitellogenesis were performed. Several products exhibited retention times close to those of the insect juvenile hormone JH3 on gas chromatography. However, analysis of the spectra, corresponding to these products, showed that they did not correspond to insect JHs. At present, it is not yet possible to say if these products correspond to JH-like substances or to lipidic compounds without hormonal properties. A bioassay should be used to answer this question.

Thus, the products detected with the retention time of insect JHs and having some identical fragments are not the insect JHs themselves. This emphasizes the importance of doing precise analysis with reliable methods, to determine the exact nature of possible JH-like hormones in ticks.

8.4.3 Possible role of ecdysteroids

Another hormonal factor which is at present being studied is the role of ecdysteroids during vitellogenesis. In insects, these hormones have various roles in different species. For example, in certain diptera they induce the fat body to synthesize vitellogenins, in *Rhodnius* they trigger oviposition *via* a neurohormone, and in cockroaches oocyte development ceases during a 'pregnancy' stage because ecdysteroids inhibit JH production (see review of Hagedorn 1983). When *O. moubata* was fed with blood containing ecdysteroids, the gonotrophic cycle was inhibited during the induction of a supermolting cycle (Connat *et al.* 1983a). When ecdysone, 20-OH-ecdysone, makisterone A, or ponasterone A, are ingested, quite large doses (more than 10 μg/ml blood) are required for induction of the supermolt, probably owing to an efficient mechanism for detoxifying ingested ecdysteroids by the conjugation of fatty acids to the 22-OH position (Diehl *et al.* 1985, Connat *et al.* 1986). In contrast, ingestion of minute quantities (20–30 ng) of the synthetic 22,25-dideoxyecdysone induced supermolting in all the females and delayed the onset of vitellogenesis (Connat *et al.* 1983a). However, doses of 5 μg or less of ecdysone or 20-OH-ecdysone per ml blood, or doses of 22,25-dideoxyecdysone which did not induce supermolting influenced neither the preoviposition period nor the number of eggs laid.

Suprisingly, 2 compounds closely related to 22,25-dideoxyecdysone: 2,22,25-trideoxyecdysone or 2,22,25-trideoxy,5βOH-ecdysone were unable to induce a supermolt, even when ingested at 5 μg/ml blood (which is the lethal dose for 22,25-dideoxyecdysone). Females treated in this manner oviposit a normal quantity of eggs, but a high proportion of the eggs dessicate, suggesting a possible action of these compounds on the function of Géné's organ. Topical application of 5 μg of these ecdysteroids in 5 μl methanol did not induce the same effects.

When a supermolt cycle is induced by 22,25-dideoxyecdysone, vitellogenesis is completely inhibited. Egg laying occurs 10 days after ecdysis, corresponding to the usual preoviposition period, suggesting that vitellogenesis starts shortly after ecdysis. Nevertheless, the number of eggs laid per supermolting female was lower than in control mated females. The higher the dose of 22,25-dideoxyecdysone ingested, the lower the number of eggs

laid (Connat *et al.* 1983a). We investigated, with the use of radioimmunoassay (RIA) (De Reggi *et al.* 1975), the hemolymph ecdysteroid level during the supermolting cycle of these females (Fig. 8.4), and we correlated

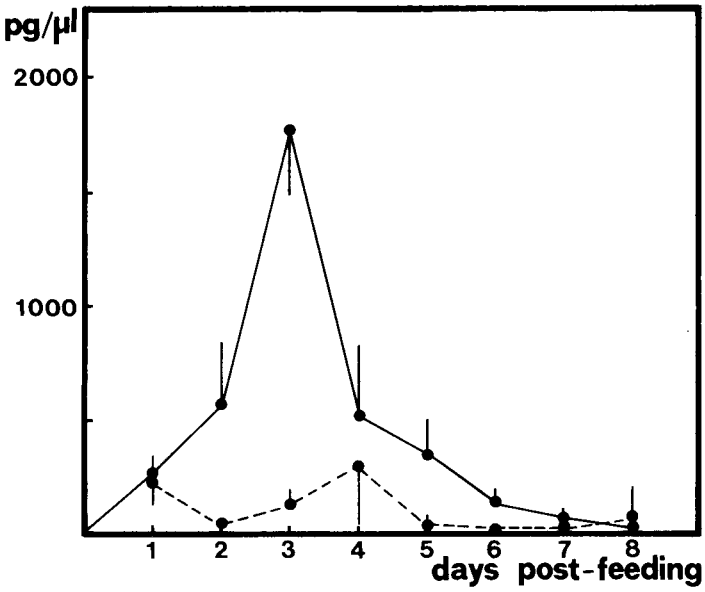

Fig. 8.4 — Hemolymph ecdysteroid levels expressed as pg 20-hydroxyecdysone equivalent/µl hemolymph assayed by RIA (antiserum provided by Drs De Reggi, Hirn and Delaage) in female *O. moubata* either during a supermolting cycle induced by ingestion of 0.04 ng 22,25-dideoxyecdysone per ml blood (●—●) or during vitellogenic cycle (● - - -●). 10 to 30 µl hemolymph were withdrawn, put into 500 µl methanol, vortexed, sonicated and centrifuged. The supernatant was evaporated to dryness and then replaced by 175 µl citrate buffer and 175 µl of 3H-antigen (ecdysone) solution in order to perform the assay in duplicate. Each point corresponds to the mean value obtained with 3 or 4 females. Vertical bars correspond to ±SD.

those results with cytological observations of the integument. Observations of semi-thin sections demonstrated a sequence of cuticular events similar to that observed in fifth stage nymphs (Germond *et al.* 1982). Ecdysteroid levels increased during the first 3 days and reached a peak of 1788 pg 20-OH-ecdysone equivalent per µl hemolymph at the beginning of epicuticle synthesis. It is surprising that this peak, induced by 22,25-dideoxyecdysone ingestion, is about 3 times higher than that in fifth stage nymphs. Then, as in fifth stage nymphs, the ecdysteroid levels gradually decrease during procuticle and cement deposition. RIA coupled with HPLC analysis of the 3rd day hemolymph showed 2 peaks of immunoreactive material comigrating with 20-OH-ecdysone and ecdysone (Fig. 8.5). Surprisingly, however, metabolic

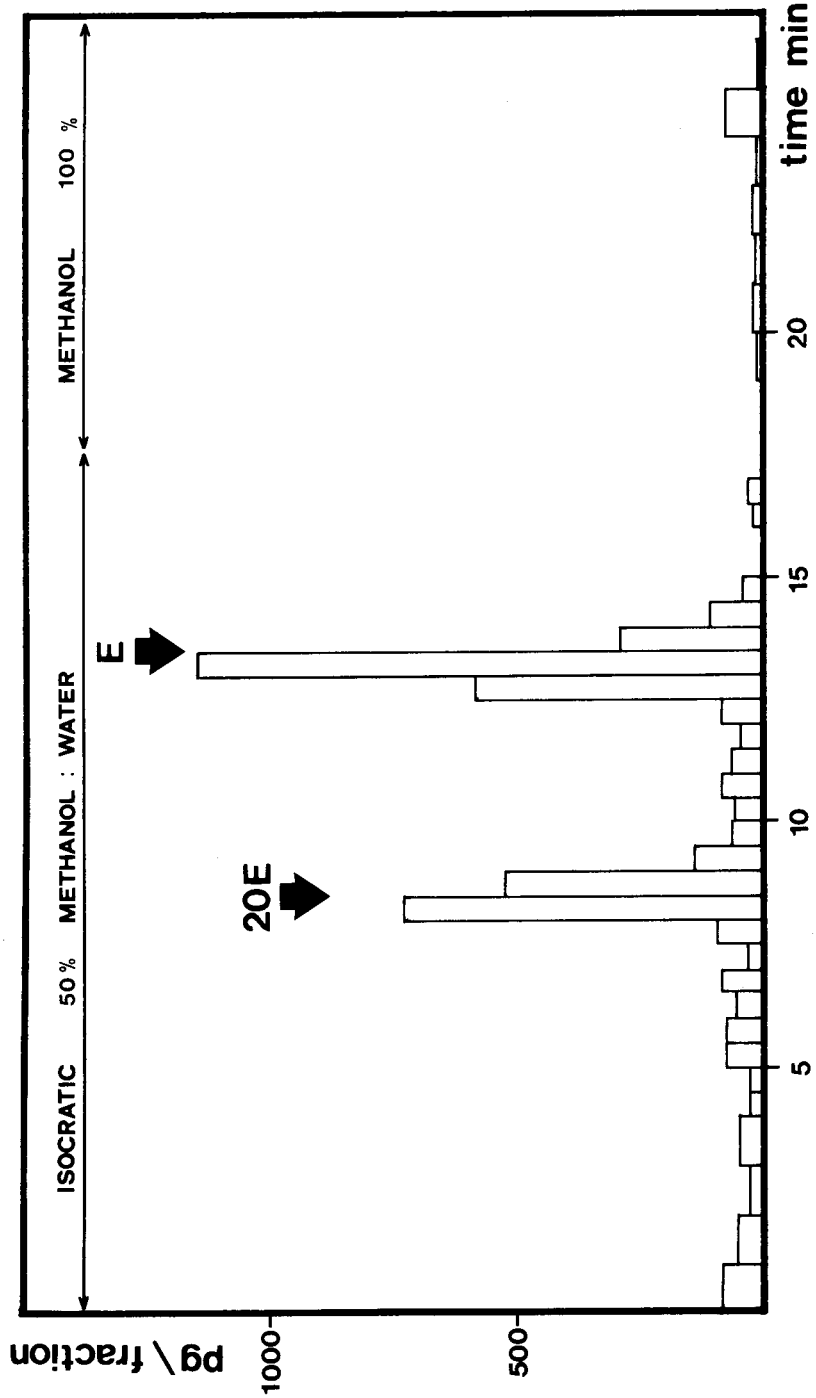

Fig. 8.5 — HPLC coupled RIA analysis of hemolymph from supermolting females *O. moubata* 3 days after the ingestion of 0.04 µg 22,25-dideoxyecdysone per ml blood. 20E and E indicate the retention times of 20-hydroxyecdysone and ecdysone respectively, under the same chromatographic conditions. Results are expressed in pg ecdysone equivalent per fractions. Merck RP-18 reverse phase column was used with a flow of 0.8 ml/min. Fractions were collected every min. or every 30 sec.

studies with ingested ^{14}C-22,25-dideoxyecdysone indicated that this latter compound is not the direct precursor of ecdysone and 20-OH-ecdysone (Connat *et al.* 1986). We can speculate that this ingested hormone may act on tissues which synthesize the molting hormones. Nevertheless, our results indicate that vitellogenesis does not occur when high ecdysteroid levels are present. We have compared, by the same method, the hemolymph ecdysteroid levels in females performing a normal vitellogenic cycle (Fig. 8.4). When they were assayed by RIA after a methanolic extraction, the levels never reached values as high as those obtained during the supermolting cycle. The levels were generally low (less than 100 pg 20-hydroxyecdysone eq./μl). However, on days 1 and 4 higher levels were obtained (301 pg/μl on day 4). Nevertheless, we have noted discrepancies in hemolymph ecdysteroid levels obtained by different extraction or separation procedures. For example, assays of the 3 different fractions (25% methanol, 60% methanol, 100% methanol) obtained after the elution of hemolymph extracts with a C18-SEP-PAK® cartridge (Waters) demonstrated that 2 peaks of immunoreactive material of about 700 pg 20-OH-ecdysone eq./μl hemolymph each were present on days 3 and 9 after feeding. This is different from methanolic extracts where only one smaller peak was obtained on day 4. The poor synchronization of the onset of vitellogeneses in female *O. moubata* could partially account for this difference. New experiments using more females and estimating the stage of vitellogenesis by observing the ovary instead of the time elapsed after the bloodmeal would give more accurate results. Another example of difficulties met with female *O. moubata* was the apparent greater quantity of immunoreactive material (up to 40-fold increase) found when assaying with the use of Silica TLC. These differences could be due to hydrolysis of unstable conjugates which cross-react poorly with our RIA or to unspecific lipid interference (Rash *et al.* 1980). No such artefacts have been observed with nymphal hemolymph or with hemolymph from female ixodids e.g. *Amblyomma hebraeum* or *Boophilus microplus* (see Diehl *et al.* Chapter 7 of the present book). Thus, we do not as yet have a precise idea of the real nature or even the quantities of ecdysteroids present in females during vitellogenesis. Similar difficulties seem to occur in *O. porcinus* where a discrepancy between data obtained by RIA and the *Musca* bioassay were noted (Solomon *et al.* 1982). However, in this closely related species, the hemolymphatic ecdysteroid titers were determined by the *Musca* bioassay during the post-engorgment period of oogenesis and oviposition and compared to the pattern observed in supermolting females where vitellogenesis is stopped (Mango 1979). 'In normally fed and mated females the ecdysterone-equivalent titers fluctuate rhythmically around mean background levels throughout the period of oogenesis'. This suggests that no peak of activity was found during this period with the bioassay. In contrast, 'hemolymph ecdysteroid titers of supermolting females rose abruptly on days 10–12 post-feeding and these increased titers were correlated with the termination of vitellogenesis and the onset of oocyte resorption' (Solomon *et al.* 1982). The authors' conclusions agree with our observations on *O. moubata* i.e. 'Peak titers of ecdysterone activity in

supermolting *O. porcinus* females appear to be incompatible with the maintenance of vitellogenesis and oocyte maturation'.

To confirm the hypothesis that high ecdysteroid titers are incompatible with vitellogenesis, we investigated the effect of injecting ecdysteroids into the hemocoel of mated females. Doses up to 10 ng of ecdysone or 20-OH-ecdysone that were injected 2, 4, 6, or 8 days after feeding were totally ineffective, the preoviposition period and egg-yield were absolutely normal. Injection of 50 ng of ecdysone injected 8 days after feeding inhibited oviposition in 8 of 10 females. Later, four died imprisoned in a second synthesized cuticle. Upon dissection, all of the ovaries of these females showed signs of vitellus resorption. However, the 2 females which laid eggs displayed a slightly delayed preoviposition period but a normal egg-yield, indicating an 'all or none' effect. Doses of 100 ng injected at 2, 4, 6, or 8 days after feeding induced supermolting in all the females. However, the animals were unable to ecdyse and died imprisoned in a second cuticle. The injection of 250 ng caused most females to die before synthesizing a second cuticle, and doses of 500 ng rapidly induced mortality. We have reinvestigated the effects of injecting 100 ng of ecdysone into mated females 8 days after feeding to observe the ovocytes cytologically. At the moment of injection, the females had an ovary with fully developed ovocytes. Ovaries were dissected in physiological medium each day for 4 days and then thin-sectioned for observation. After one day, the injection triggered the appearance of numerous lysosomes in the cytoplasm of the oocytes. Two days after injection, the dissected ovaries displayed signs of vitellus resorption identical to that seen during an abortive vitellogenic cycle. The oocytes showed a disorganized cytoplasm with lysed mitochondria, and numerous lysosomes (Fig. 8.6). The egg-shell was also lysed. It was thus possible to induce an abortive vitellogenesis by injection of ecdysone in mated females progressing through a normal gonotrophic cycle. Some preliminary results with RIA indicate that, in contrast to fed mated females, much higher levels of ecdysteroids could be found in the hemolymph of virgin females going through an abortive vitellogenic cycle. Thus, secretion of high levels of ecdysteroids may be the natural way by which the females induce the resorption of vitellus. However, the reason why these females, in contrast to the females in our experiments, do not supermolt remains to be determined. Circulation of these ecdysteroids bound to a protein could be an element of the response.

8.5 CONCLUSIONS

In this last part, we want to present our working hypothesis integrating the above results (Fig. 8.7). We hypothesize that at least two categories of hormones play a role in the control of the gonotrophic cycle of *O. moubata:* Juvenile hormone-like substances and ecdysteroids. The bloodmeal may act on the brain *via*, for example, stretch receptors, to induce the production of a small burst of JH-like substances. This hormone could be responsible for the initiation of digestion and vitellogenesis. However, in the absence of

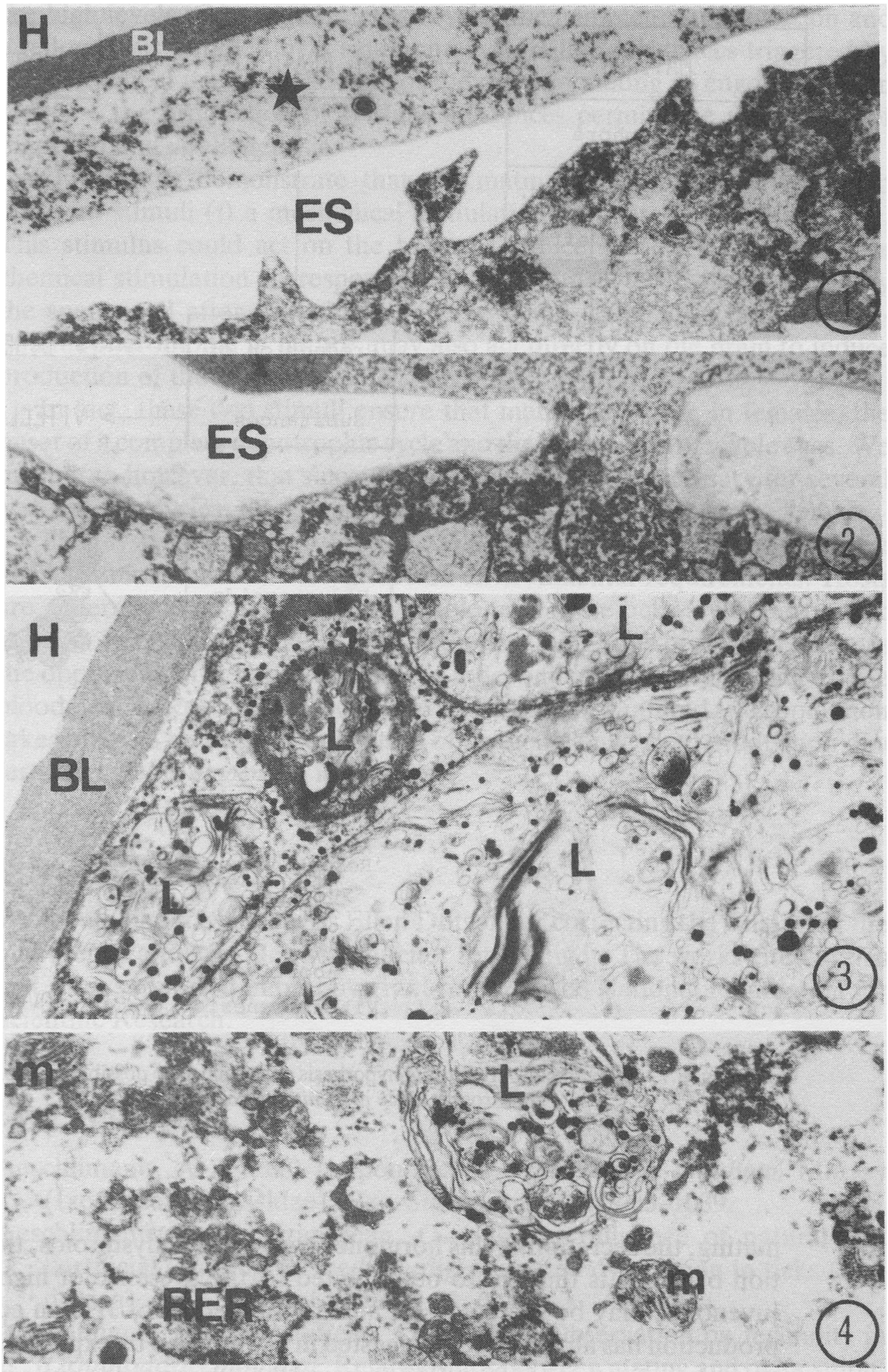

Fig. 8.6 — Cytology of the oocytes in which resorption of vitellus is induced after injection of 100 ng of 20-OH-ecdysone into mated females 8 days after feeding. Observation of resorbing oocytes of fed virgin females showed a similar pattern. ① The inner side of the eggshell (ES) is being lysed. H: Hemocoel, BL: Basal lamina, ★: artifactual space. (× 7900) ② Parts of the eggshell (ES) are completely lysed and oocyte cytoplasm is leaking out. (× 7900) ③ and ④ Advanced disorganisation of the oocyte cytoplasm, with numerous lysosomes (L), partially lysed mitochondria (m) and altered rough endoplasmic reticulum (?) (RER). H: Hemocoel, BL: Basal lamina (× 16100).

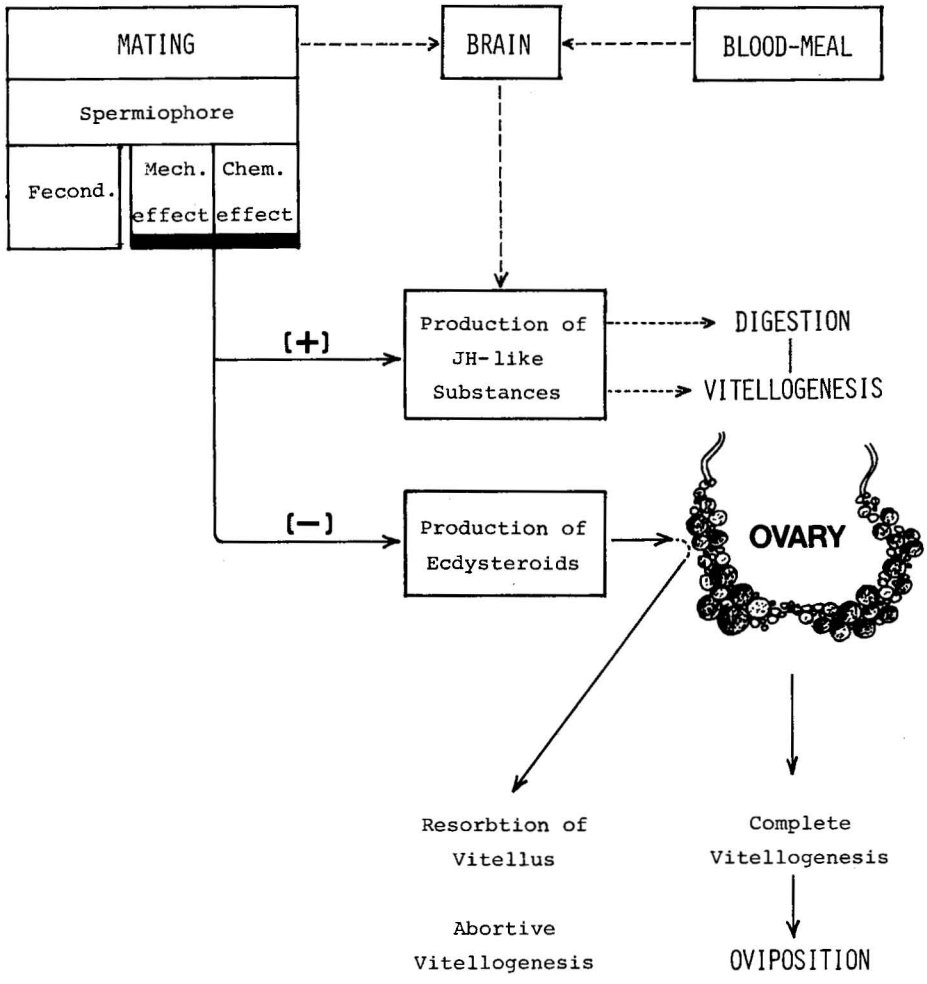

Fig. 8.7 — Scheme of our working hypothesis concerning the possible control of the gonotrophic cycle in *Ornithodoros moubata*.

mating, the secretion of this hormone ceases, and ecdysteroids, the production of which is thought to be inhibited by the presence of high levels of juvenoids, may be secreted. An antagonistic effect of JHs on ecdysteroid production has already been suggested in *O. porcinus* by experiments where topical application of 10 μg JH3 reversed the supermolting cycle induced by 20-OH-ecdysone (Obenchain & Mango 1980). The ecdysteroids could act at the level of the ovary to induce the resorption of the vitellus. Thus, the typical 'abortive vitellogenesis' of virgin females occurs. Mating probably acts as a stimulus, allowing a continuous secretion of JH-like substances. If mating occurs at approximately the same time as engorgement, it maintains

the high levels of juvenoids necessary for the completion of digestion and vitellogenesis initiated by a small burst of JH-like substances triggered by the bloodmeal itself. In the case of the delayed mating of engorged virgin females, the production of JH-like substances permits the restoration of digestion and vitellogenesis.

Our results demonstrate that the mating acts through at least two different stimuli (i) a mechanical stimulation by stretching of the vagina. This stimulus could act on the brain to induce JH-like secretion; (ii) a chemical stimulation corresponding to a proteinaceous factor released by the sperm cell after devagination in the female genital ducts. This compound, pheromonal in nature, may also act directly on the brain to induce production of the vitellogenic hormone.

In fact, these two stimuli ensure that mating provides, in females, the onset of a complete gonotrophic cycle and the oviposition of viable eggs. We must note however, that since only a single mating is necessary for several gonotrophic cycles, the mating information is 'memorized' by the female.

This system would provide *O. moubata* with a very good adaptive mechanism to meet its biological and environmental requirements. Hosts are generally not continuously available, and these ticks are opportunistic feeders. Females are able to feed without mating; this property facilitates the opportunity for both sexes to feed independently. In virgin females, the bloodmeal is stored in the midgut until a male is encountered and copulation takes place. This ensures utilization of nutrients only when production of a fertilized viable egg-batch is possible.

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REFERENCES

Aeschlimann, A., (1968) La ponte chez *Ornithodoros moubata,* Murray (Ixodoidea, Argasidae), *Rev. Suisse Zool.* **75:** 1033–1039.

Aeschlimann, A. & Grandjean, O., (1973a) Influence of natural and "artificial" mating on feeding, digestion and oviposition in ticks (Ixodoidea), *Fol. Parasitol. (Praha)* **20:** 67–74.

Aeschlimann, A. & Grandjean, O., (1973b) Observation on fecundity in *Ornithodorus moubata.* Relationships between mating and oviposition, *Acarologia* **15** (2): 206–217.

Aeschlimann, A. & Hecker, H., (1969) Vitellogenèse et formation cuticulaire chez l'oeuf d'*Ornithodoros moubata.* Etude au microscope électronique., *Acarologia* **11** (2): 180–192.

Aeschlimann, A. & Hecker, H., (1970) Présence de membranes annelées

dans les ovocytes d'*Ornithodoros moubata,* Murray (Ixodoidea, Argasidae). Etude au microscope électronique. *Acarologia* **11:** 180–192.

Bergot, B. J., Ratcliff, M., & Schooley, D. A., (1981) A method for quantitative determination of the four known Juvenile Hormones in insect tissue using gas chromatography — mass spectroscopy, *J. Chromatogr.* **204:** 231–244.

Chinzei, Y., (1983) Quantitative changes of vitellogenin and vitellin in adult female ticks, *Ornithodoros moubata,* during vitellogenesis, *Mie Medical Journal* **23** (3): 117–127.

Chinzei, Y., Chino, H., & Takahashi, K., (1983) Purification and properties of vitellogenin and vitellin from a tick, *Ornithodoros moubata, J. Comp. Physiol.* **152:** 13–21.

Connat, J. L., Diehl, P. A., Dumont, N., Carminati, S., & Thompson, M. J., (1983a) Effects of exogenous ecdysteroids on the female tick *Ornithodoros moubata:* Induction of supermolting and influence on oogenesis, *Z. ang. Ent.* **96** (5): 520–530.

Connat, J. L., Ducommun, J., & Diehl, P. A., (1983b) Juvenile hormone-like substances can induce vitellogenesis in the tick *Ornithodoros moubata* (Acarina: Argasidae), *Int. J. of Invertebrate Reprod.* **6** (5, 6): 285–294.

Connat, J. L., Diehl, P. A., & Thompson, M. J., (1986) Inactivation of ingested ecdysteroids by conjugation with fatty acids in 22-OH position in the female tick *Ornithodoros moubata* (Acarina, Argasidae), *Arch. Insect Biochem.* (in press).

De Clerck, D., Eechaute, W., Leusen, I., Diederik, H., & De Loof, A., (1983) Identification of testosterone and progesterone in hemolymph of larvae of the fleshfly *Sarcophaga bullata, Gen. Comp. Endocrinol.* **52** (3): 368–678.

De Clerck, D., Diederik, H., & De Loof, A., (1984) Identification by capillary gas chromatography — mass spectrometry of eleven non-ecdysteroid steroids in the hemolymph of larvae of *Sarcophaga bullata, Insect Biochem.* **14** (2): 199–208.

De Reggi, M. L., Hirn, M. H. & Delaage, M. A., (1975) Radio-immunoassay of ecdysone. An application to *Drosophila* larvae and pupae, *Biochem. Biophys. Res. Commun.* **66:** 1307–1315.

Diehl, P. A., (1969) Hämolymphenproteine und Vitellogenese bei *Ornithodorus moubata,* Murray (Ixodoidea, Argasidae), *Bull. Soc. ent. suisse* **42:** 117–125.

Diehl, P. A., (1970) Zur Oogenese bei *Ornithodorus moubata* Murray (Ixodoidea: Argasidae) unter besonderer Berücksichtigung der Vitellogenese, *Acta Tropica* **27** (4): 302–355.

Diehl, P. A., Aeschlimann, A., & Obenschain, F. D., (1982) Tick Reproduction: Oogenesis and Oviposition, In *Physiology of ticks* (Obenchain F. D. & Galun R. Eds) Pergamon Press, p. 277–350.

Diehl, P. A., Connat, J. L., Girault, J. P., & Lafont, R., (1985) A new class of apolar ecdysteroid conjugates: esters of 20-hydroxyecdysone with

long-chain fatty acids in ticks, *Int. J. Invertebrate Reprod. Develop.*, **8**: 1–13 .

Ducommun, J., (1984) Contribution à la connaissance de la reproduction chez la tique *Ornithodoros moubata,* Murray, 1877; *sensu* Walton, 1962 (Ixodoidea: Argasidae), Thesis Univ. Neuchâtel 138 pp.

Eichenberger, G., (1970) Das Zentralnervensystem von *Ornithodorus moubata* Murray und seine postembryonale Entwicklung, *Acta Trop.* **27:** 15–53.

Eisen, A., Warburg, M. R., and Galun, R., (1973) Neurosecretory activity and oogenesis in the fowl-tick *Argas persicus* (Oken), *Gen. Comp. Endocrinol.* **21:** 331–340.

Engelmann, F., (1984) Regulation of vitellogenesis in insects: The pleiotropic role of juvenile hormones. In *Biosynthesis, metabolism and mode of action of invertebrate hormones* (Hoffmann J. & Porchet M. eds.) Springer-Verlag Berlin Heidelberg p. 444–453.

Gabbay, S., & Warburg, M. R., (1976) Neurosecretory activity as related to feeding, mating and oogenesis in the female tick, *Ornithodoros tholozani., J. Insect Physiol.* **22:** 1291–1301.

Germond, J. E. & Aeschlimann, A., (1977) Influence of copulation on vitellogenesis and egg-laying in *Ornithodoros moubata* (Ixodoidea: Argasidae), In *Advances in invertebrate reproduction* (Adiyodi K. G. & Adiyodi R. G. Eds.) p. 308–318.

Germond, J. E., Diehl, P. A., & Morici, M., (1982) Correlation between integument structure and ecdysteroid titers in fifth-stage nymphs of the tick, *Ornithodoros moubata* (Murray, 1977; *sensu* Walton, 1962), *Gen. Comp. Endocrinol.* **46:** 255–266.

Graf, J. F., (1974) Ecologie et éthologie d'*Ixodes ricinus* L. en Suisse (Ixodoidea, Ixodidae). III. Copulation, nutrition et ponte, *Acarologia* **16** (4): 636–642.

Grandjean, O., (1983) Blood digestion in *Ornithodorus moubata* Murray *sensu stricto* Walton females (Ixodoidea: Argasidae). Modifications of midgut cells related to the digestive cycle and to the triggering action of mating, *Ann. Parasitol. Hum. Comp.* **58:** 493–514.

Hagedorn, H. H., (1983) The Role of Ecdysteroids in the Adult Insect, In *Endocrinology of insects* (Downer R. G. H. & Laufer H. Eds.), Alan R. Liss, Inc., New York p. 271–304.

Hoogstraal, H., (1956) African Ixodoidea. *I. Ticks of the Sudan.,* Dep. Navy, Bur. Med. Surg., Washington, 1101 pp.

Khalil, G. M., (1984) Fecundity-reducing pheromone in *Argas (Persicargas) arboreus* (Ixodoidea: Argasidae), *Parasitology* **88:** 395–402.

Mango, C. K. A., (1979) The effects of beta-ecdysone and ponasterone A on the soft tick *Ornithodoros porcinus porcinus.* Walton, 1962., PhD thesis Univ. Nairobi.

Mauchamp, B., Zander, M., & Wolff, R., (1984) The qualitative and quantitative determination of juvenile hormones by mass spectrometry. In *Biosynthesis, metabolism and mode of action of invertebrate*

hormones, (Hoffmann J. A. & Porchet M. Eds.) Springer-Verlag p. 363–372.

Obenchain, F. D. & Mango, C. K. A., (1980) Effects of exogenous ecdysteroids and juvenile hormones on female reproductive development in *Ornithodoros p. porcinus, Am. Zool.* **20** (4): 936.

Oliver, J. H. Jr., (1974) Symposium on reproduction of arthropods of medical and veterinary importance. IV. Reproduction in ticks (Ixodoidea)., *J. Med. Ent.* **11:** 26–34.

Oliver, J. H., Pound, J. M., & Andrews, R. H., (1984) Induction of egg maturation and oviposition in the tick *Ornithodoros parkeri* (Acari: Argasidae), *J. Parasit.* **70** (3): 337–342.

Pound, J. M. & Oliver, J. H., (1979) Juvenile Hormone: evidence of its role in the reproduction of ticks, *Science* **206:** 355–357.

Raabe, M., (1982) Contrôle neuroendocrine de la reproduction des insectes, *J. Physiol.* **78:** 553–557.

Raabe, M., (1984) Insect neurohormones and female reproduction. In *Advances in invertebrate reproduction* (Engels W. *et al.* Eds.) Elsevier p. 163–176.

Rash, J. M., Jerkunica, I., & Sgoutas, D. S., (1980) Lipid interference in steroid radioimmunoassay, *Clin. Chem.* **26** (1): 84–88.

Rothschild, M. & Ford, B., (1973) Factors influencing the breeding of the rabbit flea *(Spilopsyllus cuniculi):* A spring-time accelerator and a kairomone in nestling rabbit urine, *J. Zool. Lond.* **700:** 87–137.

Sahli, R., Germond, J. E., & Diehl, P. A., (1985) Spermateleosis and secretory activity of the sperm cell of the tick *Ornithodoros moubata. Exp. Parasitol.***60:** 383–395

Solomon, K. R., Mango, C. K. A., & Obenchain, F. D., (1982) Endocrine Mechanisms in Ticks: Effects of Insect Hormones and their Mimics on Development and Reproduction. In *Physiology of ticks* (Obenchain F. D. & Galun R. Eds.) Pergamon Press p. 399–438.