itory effect of all the hormones tested by us[4]. This suggests that these C-21 steroids, which lack the 21 hydroxy group as a minimun requirement for a steroid to provoke lymphocytolysis[9], exhibit special structural characteristics allowing them, specifically at high concentrations, to inhibit RNA biosynthesis. Hence these steroids at these concentrations may act by a different mechanism, including a 'membrane effect'[8,10]. This alternate mechanism would not lead to cell lysis.

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Juvenile hormone levels, vitellogenin and ovarian development in *Acheta domesticus*

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Summary. Juvenile hormone hemolymph titres have been determined by radioimmunoassay at the beginning of imaginal life in the cricket *Acheta domesticus*. JH III levels increased during previtellogenesis and at the onset of vitellogenesis in the successive waves of ovocytes. Vitellogenin synthesis started between the 24th and 40th h of imaginal life after bursts of juvenile hormone production and before any ecdysteroïd had appeared in either the hemolymph or the ovaries.

Hormonal control of ovarian development and vitellogenin synthesis in insects have been studied by several authors[1-4]. In most species, reproduction is controlled by juvenile hormone which acts both on vitellogenin synthesis and ovocyte maturation. In numerous species, the stimulation of vitellogenin synthesis has been demonstrated in vivo, for example in *Leucophaea maderae*[5,6], *Leptinotarsa decemlineata*[7] and *Locusta migratoria*[8,9]. However, up to now only Wyatt and al.[10] were able to stimulate vitellogenin synthesis in vitro in *Locusta*. In *Aedes*, the concept that 20-hydroxyecdysone directly stimulated vitellogenin synthesis by fat body cells previously exposed to juvenile hormone has been supported by Fallon and al.[11] and Hagedorn and al.[12]; recent findings have, however, failed to confirm earlier observations[13]. In *Anopheles*[14], as in *Drosophila*[15,16] and in *Oncopeltus*[17], injections of ecdysterone did lead to the appearance of vitellogenin in the hemolymph. Induction of vitellogenin synthesis by ecdysone alone has been reported in *Sarcophaga bullata*[18,19]. Nevertheless, it seems that in Diptera both juvenile hormone and ecdysteroïds are involved in the hormonal regulation of yolk protein synthesis[20]. Juvenile hormone is the hormone controlling vitellogenesis, since it not only produces morphological changes in follicular cells of ovocytes[21-25], but also stimulates the production and uptake of vitellogenin by maturing ovocytes[26].

In the Orthoptera, *Locusta* has been the most widely used by insect endocrinologist, but in a few studies *Acheta domesticus* has been used. Yolk proteins have been studied in the house cricket by Kuntz and Petzelt[27] and Bradley and Edwards[28]. Female specific proteins have been identified both in the hemolymph of adult crickets and in the maturing ovocytes[29], and modifications of neurosecretory material in the course of ovarian maturation have been described[28]. But nothing was known about the changes in juvenile hormone during oogenesis in this species.

In a previous study[30], we showed that in *Acheta* some ecdysteroid production occurs in the ovary and variations

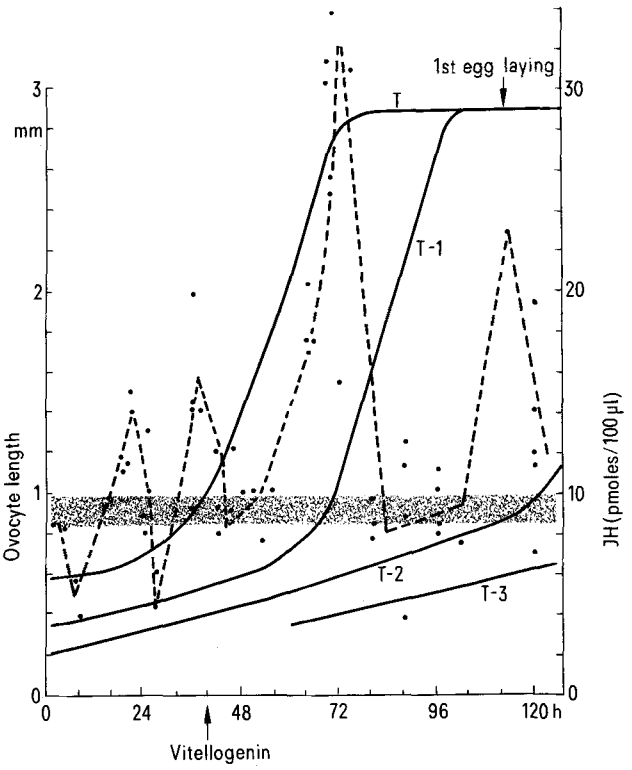

Figure 1. Hemolymph JH titre and ovocyte growth. Abscissae: age of female imagos. Ordinates: JH titres in pmoles/100 µl (---); ovocyte lengths in mm (—), T_1 lengths of terminal ovocyte; T-1, T-2, T-3, lengths of ovocytes from preceding ranks. Hatched area corresponds to the length at which the patency of the follicular spaces appears. Vitellogenin appears in the hemolymph at the time indicated. The first egg laying occurs at the time indicated by an arrow.

in the level of ecdysteroids in ovaries and in hemolymph were correlated with ovocyte maturation. In the present work, using a recently developed radioimmunoassay method[31] for juvenile hormone determination, we studied the titres of juvenile hormone and the appearance of vitellogenin in the hemolymph of adult females at the beginning of their imaginal life. Rearing conditions have been previously described[30]. In this study, females were reared with males and sand boxes for egg laying were provided.

The panoïstic ovary of *Acheta* consists of 160 ovarioles whose development is not exactly synchronous. In contrast with *Locusta,* where massive egg laying occurs at regular intervals of 4–5 days, the female of *Acheta* lays from 60 to 100 eggs a day from the 112th h after imaginal molt.

The data summarized in figure 1 concerns the mean length of the 6 largest ovocytes from the different rows: terminal (T), penultimate (T-1), antepenultimate (T-2)... As seen in figure 1, terminal ovocytes reached their maximal size 72 hours after emergence, chorionation then ensued and the first egg laying began at approximately 112 h. Patency was monitored by means of Evans blue[16] which revealed the appearance of spaces between follicular cells as early as the 36th h, at which time ovocyte length was between 0.8 and 1 mm. It was also at this time that vitellogenesis suddenly started and the ovocyte growth sped up greatly. T-1 ovocytes developed in a similar manner except that their development began only after T ovocytes were fully grown and had been ovulated in the oviduct. Thus, patency appeared in T-1 ovocytes at some time between 64 and 72 h. A similar delay occurred in T-2 ovocytes which first showed patency between 112 and 120 h, i.e. at the time of the first egg laying.

Hemolymph juvenile hormone titres were determined using radioimmunoassay[31]. Determinations were performed on 40-µl samples. Synchronization of samples collected from 2 females was done on the basis of ovocyte length correlated with age; before assaying, the ovaries of each female were examined to be sure that their length corresponded to the supposed age. If this was not the case, the animal was discarded.

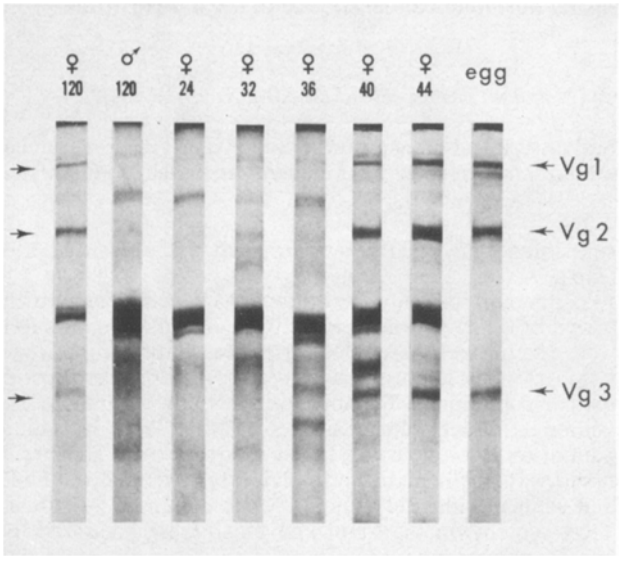

Figure 2. Separation of *Acheta* hemolymph and yolk proteins on 6.7% SDS gels. Age in hours and sex of animals are indicated above Arrows indicate the 3 major vitellogenic proteins Vg1, Vg2, Vg3.

The presence of only JH III both in corpora allata cultures and in hemolymph of *Acheta*[32] was prechecked by means of High Pressure Liquid Chromatography studies. Results are summarized in figure 1. Four main peaks could be detected after emergence. The first came early in imaginal life while the others could be correlated with the onset of vitellogenesis as evidenced by patency and rapid growth in the 3 ovocyte rows.

SDS gel electrophoresis was performed according to the method described by Bradley and Edwards[28]. Samples containing 50 µg of protein were electrophoresed and stained with Coomassie bright blue. The separations obtained are displayed in figure 2. Both in eggs and in female hemolymph, 3 main proteins were detected; they probably represented subunits of larger vitellogenin molecules. These proteins clearly appeared 40 h after emergence; in fact, densitometric scans (not shown here) revealed that on the SDS gels, light bands of proteins can be detected in the same position in younger females and even in male hemolymph.

Our results shed light on the rate of ovarian development in *Acheta domesticus.* The data obtained are in agreement with other findings on terminal ovocyte growth in Orthoptera[33]. Moreover, for T-1, T-2... ovocyte development, the present results strictly coincide with the observations made by Tobe and Pratt[34] during ovarian maturation in *Schistocerca gregaria*; they state that an ovocyte cannot enter vitellogenesis until the vitellogenic distal one has completed its growth.

Our studies on vitellogenins further confirm previous results obtained in *Acheta.* Using the same SDS-gel electrophoresis technique as Bradley and Edwards[28] we detected 3 main fractions related to vitellogenesis. These 3 fractions corresponded to the 4 fractions found by the above authors, since densitometric scans of our gels revealed that VG3 was a double peak, which actually corresponds to the Vg3 and Vg4 fractions reported by Bradley and Edwards. In this work, our intention was to determine accurately the time of the appearance of vitellogenins. Accordingly we found that the vitellogenins are present in the hemolymph of vitellogenic females at a very early time, i.e. only 36 h after the imaginal molt. By 40 h the increase in the vitellogenic fractions is such that it can be clearly revealed.

Recent development of radioimmunoassay methods[35,36,31] offers the possibility of quantifiying juvenile hormone titres in vivo. The data presented here indicate that hemolymph JH titre fluctuates; the peaks observed seemed to be related to the different events of ovocyte growth. In *Acheta,* the rise in the level of juvenile hormone in the hemolymph during the 1st day of imaginal life can probably be associated with an activation of corpora allata and/or with previtellogenesis. In newly emerged *Leptinotarsa* females a rapid activation of corpora allata occured in the first 4 h after emergence[37]. Similarly small peaks of juvenile hormone biosynthesis have also been observed during previtellogenesis[34,38,42]. Peaks of juvenile hormone production during vitellogenesis have been reported elsewhere[34,38–42]. In *Acheta,* juvenile hormone titre increases seemed to correspond to the beginning of vitellogenesis in the different waves of ovocytes. Peaks of juvenile hormone synthesis preceding the onset of vitellogenesis in T-1 and T-2 ovocytes have been observed in *Schistocerca gregaria*[34].

Although it is tempting to speculate on the relationship between juvenile hormone titre and ovary maturation, prudence demands that we restrict ourselves to emphasizing the synchronization revealed by our study. Juvenile hormone is not exclusively associated with ovarian growth and in most species the hormone triggers the synthesis of vitellogenin. In *Acheta* vitellogenins make their massive appearance in the hemolymph after the 2nd burst of

juvenile hormone, but the onset of vitellogenin synthesis is probably related to the 1st burst of juvenile hormone. Additional experiments such as juvenile hormone applications and allatectomies will be performed at various times; they should provide further information on the role of the peaks of juvenile hormone. In any case, the synthesis of yolk proteins cannot be initiated by ecdysteroids in *Acheta*, since previous work[30] has shown that ecdysteroid titres begin to rise in hemolymph after the appearance of vitellogenins in the blood.

Thus, we can conclude that there are strong temporal relations between ovarian physiological features and juvenile hormone levels which would appear to play a prominent role in the onset of vitellogenin synthesis and in initiating yolk deposition in the oocytes.

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Influence of the route of administration on thyrotropin-releasing hormone concentration in the mouse brain[1]

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Summary. TRH penetrates the mouse brain after i.v., i.p., i.m., oral or rectal administration. Except when TRH is administered orally, the penetration rate is about 0.2% of the total dose administered. Higher TRH contents are reached after rectal or i.m. administration.

Thyrotropin-releasing hormone (TRH) is administered in treatment of several neurological and psychiatric disorders and is usually given i.v[2–5]. By this method of administration, low concentrations in the brain are achieved, but these decrease rapidly[6–8]. Until now, the influence of different routes of administration on the brain penetration rate of TRH in the mouse, with possible consequences for therapeutic use in humans, has not been investigated. We examine in this paper, therefore, the influence of the route of administration on TRH brain concentrations in mice.

Material and methods. Animals. Male (dd strain) mice weighing 20 g were used. They were housed in temperature (22 °C)- and humidity (60%)-controlled quarters and fed a diet of laboratory chow and water ad libitum.

Drugs. Synthetic TRH was obtained from Takeda Co., Ltd (Japan). For rectal administration of TRH, a suppository containing 5 mg of TRH was provided by Takeda Co., Ltd (Japan)[1].

Experimental procedure. 1 mg of TRH dissolved with 0.2 ml of saline was injected i.v., i.p., or i.m. 2 mg of TRH were administered through gastric tubes. Suppositories containing 5 mg TRH were administered. Saline was used in the control animals. The mice were serially decapitated by guillotine under ether anesthesia, after TRH or saline administration and trunk blood was collected. The brain tissue was obtained by the previously described method[9] and washed with cold saline (4 °C) to eliminate the blood. TRH contents in the brain and blood were measured by specific radioimmunoassay[10].

Statistics. Mean and SE of samples were calculated for each group. Student's t-test was used to evaluate the differences between control and experimental groups.