Concerning the rhythmic unitary activity induced in vestibular units by electrical trigeminal stimulation, the possibility that trigeminal inputs may influence the nystagmus rhythm-generating circuits must be suggested. In fact other authors have reported a modification in the intensity of vestibular nystagmus by stimulating the nasal mucosa[7], by evoking the corneal reflex[8] and after meningeal lesions[9]. In addition, ocular nystagmus following trigeminal stimulation or after trigeminal neurotomy can occur in compensated animals after hemilabyrinthectomy[2,10].

As regards the trigeminal tonic influence on the vestibular discharge, the hypothesis that there is a vestibular involvement in the actuation and coordination of trigeminal reflexes, through vestibulo-spinal pathways[11-13], can be put forward. Furthermore, the vestibular neurons exhibiting opposing sign responses to trigeminal stimulations of both sides could be involved in the avoiding and feeding reflexes, whose final effect is represented by moving the head away from or towards the stimulus[14]. The remaining vestibular neurons with equal sign responses could be implicated in the trigeminal reflexes which have as motor effects a dorsal extension or a ventral flexion of the head.

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Correlation between ovarian growth, vitellogenin titer, and yolk polypeptide pattern in the haemolymph of *Calliphora vicina*[1]

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Summary. During the first egg maturation cycle of *Calliphora vicina* changes in the vitellogenin titer and yolk polypeptide pattern of the haemolymph are correlated with the intensity of follicular growth, and the rate of yolk deposition.

Vitellogenesis, the process in which rapid oocyte growth and yolk deposition occurs, is one of the crucial periods in insect oogenesis. Formation of vitellogenins (egg yolk protein precursors) is a hormonally controlled process[2-5] involving vitellogenin production and secretion by the fat body[6-8], its transport via the haemolymph, and its sequestration by the cooperative action of the oocyte and the follicle cells[9, 10]. Vitellogenesis has been studied in several insect species, and essential differences in its biochemical course have been observed by different authors[11-13].

Previous work on the vitellogenin in *Calliphora vicina* has dealt with its ultrastructural localization in the fat body[8], and its purification and antigenic characterization[13].

This paper presents the results of the determination of the levels of vitellogenin in the haemolymph of *Calliphora vicina* during the 1st egg maturation cycle, its correlation with ovarian size and yolk deposition, and the yolk polypeptide pattern of the haemolymph.

Materials and methods. Haemolymph samples. Newly emerged female blowflies, *Calliphora vicina,* were kept on pig's heart, sucrose and water at 25 °C. The flies were anaesthetized with CO_2, the neck membrane punctured and two 1-µl haemolymph samples were collected, one for rocket immunoelectrophoresis, and one for SDS-polyacrylamide gel electrophoresis (SDS-PAGE). The length of the terminal follicle and the yolk-containing part of the oocyte was measured in each fly. Flies 2, 3, 4, 5 and 6 days old were used. If the ovaries of 3-, 4- or 5-day-old flies did not contain yolk, the haemolymph samples were discarded.

Rocket immunoelectrophoresis. Rocket immunoelectrophoresis[14] was used for determination of the vitellogenin levels in the haemolymph samples. Glass plates (10 × 10 cm, 1.1 mm thick) were covered half-and-half with 1% agarose containing 10 µl/cm^2 of antivitellogenin, and 1% agarose without antivitellogenin respectively. The agarose (HSB, Mr = 0.1, Litex, Glostrup, Denmark) was dissolved in 36 mM Tris-HCl, 12 mM sodium barbital buffer containing 0.3 mM calcium lactate and 1% Triton X-100 (pH 8.6.) The antivitellogenin (0.86%) was the same as used by Jensen et al.[13]. Haemolymph samples were diluted 3 times with 0.1 M Tris-HCl pH 7.0, and applied to holes in the antivitellogenin-free zone. They were allowed to diffuse 1 h at 4 °C before electrophoresis was run at 2 V/cm for 18 h at 16 °C. Standards: 1 µl (4), 2 µl (8), 5 µl (7), 10 µl (7), and 20 µl (6) of purified vitellogenin (0.72 mg/ml) were applied to the plates. The numbers in parentheses are the numbers of samples run. The immunoprecipitates were visualized by staining with Coomassie Brilliant Blue.

Immunoprecipitation of fat body vitellogenin. Fat body pieces from 5 to 10 flies were prepared as described by

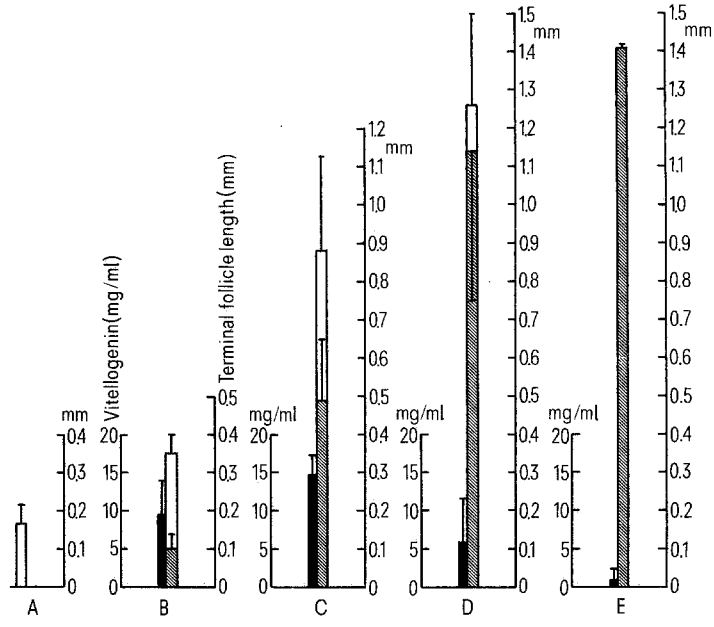

Figure 1. Haemolymph vitellogenin concentration (black columns), length of terminal follicles (white columns), and length of the yolk-containing part of the oocyte (gray columns) of *A* 2-day-old flies (17); *B* 3-day-old flies (12); *C* 4-day-old flies (18); *D* 5-day-old flies (8); and *E* 6-day-old flies (2). Numbers in parentheses: numbers of flies investigated.

Jensen et al.[13] with the exception that 1% Triton X-100 was added to the homogenization medium.

SDS-polyacrylamide gel electrophoresis (SDS-PAGE). Incubation of samples, gel preparation and running was performed as described by Laemmli[15]. Fixation and staining was in 25% (v/v) methanol, 7.5% (v/v) acetic acid with 1% (w/v) TCA containing 250 mg Coomassie Brilliant Blue/l. Destaining was in 5% (v/v) methanol in 7.5% (v/v) acetic acid.

Results. The 1st egg maturation cycle of *Calliphora vicina* fed meat, sucrose and water takes 5–6 days. Yolk deposition in the oocytes starts when the terminal follicles reach a length of about 0.3 mm, the length of ripe eggs varied between 1.26 and 1.54 mm.

Haemolymph vitellogenin levels were determined by comparison with a standard curve obtained from the heights of the precipitation arcs of the standard vitellogenin samples. These gave the following values: 1 µl: 0.9±0.4 mm, 2 µl: 3.7±1.3 mm, 5 µl: 10.3±1.3 mm, 10 µl: 17.9±1.1 mm and 20 µl: 30.4±2.2 mm.

Haemolymph from 2-day-old flies did not contain vitellogenin detectable by immunoelectrophoresis, figure 1 A, but SDS-PAGE of haemolymph samples show that the yolk polypeptide 3 (YP 3, 46 kdal) is present, figure 2 A. Fat body homogenates from 2-day-old flies did not form antivitellogenin-vitellogenin precipitates when they were treated with antivitellogenin. During the 3rd day the length of the terminal follicle is doubled and immunoelectrophoresis shows vitellogenin to be present in the haemolymph in an average concentration of 9.6 mg/ml, figure 1 B. In addition to YP 3 already present, YP 1 (52 kdal) and YP 2 (48.5 kdal) can be seen after SDS-PAGE, figure 2 B. The appearance of YP 1, YP 2 and YP 3 in the haemolymph coincides with a positive response for vitellogenin in the immunoelectrophoresis, and the appearance of yolk in the terminal follicles, see figure 1 B. Follicles of the 4-day-old flies are already half filled with yolk, and the vitellogenin concentration in the haemolymph reaches its maximal average value of 14.8 mg/ml, figure 1 C. All 3 yolk polypeptide bands are present after SDS-PAGE, figure 2 C. SDS-PAGE analysis of antivitellogenin precipitates of fat body homogenates from 3-day- or 4-day-old flies reveals yolk polypeptides of apparently identical molecular weight to those found in the haemolymph, figure 2 D. The most pronounced individual variation in ovarian size and in the

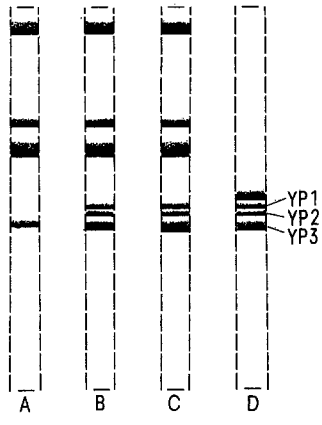

Figure 2. Major polypeptide bands from SDS-PAGE analysis (10% gels) of haemolymph from *A* 2-day-old flies; *B* 3-day-old flies; *C* 4-day-old flies. *D* SDS-PAGE analysis of immunoprecipitates from 3- or 4-day-old fat bodies. The broad, slower moving band represents an immunoglobulin fraction.

degree of yolk deposition was observed in the 5-day-old flies, figure 1 D. At this time some of the flies already have mature eggs and the concentration of vitellogenin has decreased markedly. In 1 of the 5-day-old flies with fully mature eggs vitellogenin was undetectable, and haemolymph from this fly contained only YP 3. Other 5-day-old flies still had the YP 1+2+3 haemolymph pattern. The 6-day-old flies had fully mature eggs and none or negligible amounts of vitellogenin in the haemolymph, figure 1 E. However, YP 3 was found to persist in these flies, and was even detected in 3 of the 6-day-old flies which were in the 2nd egg maturation cycle (not shown in the figure).

Discussion. Titer of vitellogenin. Vitellogenesis involves massive synthesis of specific proteins. We have followed the haemolymph titer of vitellogenin during the 1st egg maturation cycle and compared it with the haemolymph yolk polypeptide pattern. During follicle development changes in the amount and composition of vitellogenin were observed. The concentration was found to increase until the 4th day after eclosion. High vitellogenin concen-

tration in the 4-day-old flies coincides with the beginning of rapid follicle growth and yolk deposition. Marked individual variations in vitellogenin titer have been found in the 5-day-old flies, where in some flies with mature eggs vitellogenin was undetectable. These changes in the vitellogenin level of *Calliphora* are comparable to those of Locusts[16, 17] and *Drosophila*[18] where the vitellogenin level also declined temporary as the oocytes approached maturation. The changes in the vitellogenin level in *Calliphora* correlate well with the secretory activity of the fat body as judged from its ultrastructure[19].

The yolk polypeptide pattern of the haemolymph. During the 1st egg maturation cycle we observed a reproducible pattern of yolk polypeptides in the haemolymph. We now consider the haemolymph polypeptide called YP 3 in the 2-day-old flies (identical with the mol. wt 46,000 polypeptide of Jensen et al.[13]) to be a yolk polypeptide. This polypeptide has the same position after SDS-PAGE as YP 3 from fat body, haemolymph or ovaries from 3- or 4-day-old flies, and peptide mapping of it by the method of Cleveland et al.[20] gives a pattern identical to that obtained from YP 3 from ovaries (unpublished results). Thus YP 3 appears in the haemolymph before YP 1 and YP 2 in contrast to *Drosophila*[21] where all 3 yolk polypeptides are detected at the same time. In 2-day-old *Calliphora* YP 3 could not be detected by immunoelectrophoresis showing that this polypeptide alone will not precipitate the antibodies (which were raised against YP 1+2+3 from ovaries[13]). Identical results were obtained for flies with mature chorionated follicles, and YP 3 persists in the haemolymph into the 2nd egg maturation cycle. SDS-PAGE analysis of immunoprecipitates from fat bodies correlates with these results being negative for 2-day-old flies as already demonstrated immunocytochemically by Thomsen et al.[8], and shows all 3 polypeptides to be present in 3- or 4-day-old flies. The period of yolk accumulation is always found to correlate closely with the period where YP 1+2+3 are present in the haemolymph, but we are still in doubt regarding the origin of YP 3 in the 2-day-old flies. Investigation on the synthesis and release of this polypeptide in relation to the hormonal milieu is therefore under progress.

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Exogenous makisterone A accelerates early embryonic development in the milkweed bug *Oncopeltus fasciatus*[1]

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Summary. Reproducing females of *Oncopeltus fasciatus* which were treated with exogenous makisterone A and 20-hydroxyecdysone laid eggs with considerably elevated ecdysteroid contents. The early embryonic development was markedly accelerated when the mother was treated with makisterone A, whereas 20-hydroxyecdysone had no influence.

Newly deposited eggs of the milkweed bug *Oncopeltus fasciatus* (Insecta, Heteroptera) contain considerable amounts of moulting hormone[3]. The titre fluctuates only slightly during early development, i.e. from egg deposition until katatrepsis, but increases dramatically during late embryonic development[4]. Kaplanis and coworkers[5] found that in embryos at a more advanced stage the predominant ecdysteroid is makisterone A, the function of which is not yet konwn. During late embryonic development, where distinct peaks occur, ecdysteroids may control embryonic «moults»[4,6–8]. However, a possible function in early development appears to be even more interesting, because this would add a new facet to the diversity of roles of this group of hormones.

It has been found[9] that exogenous ecdysteroids applied to reproducing females of *Oncopeltus fasciatus* are transferred to the eggs and can affect embryogenesis. One of the most interesting effects is the acceleration of early embryonic development by makisterone A but not by 20-hydroxyecdysone. This finding is described in detail here, since it might give an indication as to the function of makisterone A in normal eggs.

Materials and methods. Females of *Oncopeltus fasciatus* were injected daily with 2.5 µl of a saline or aqueous solution of 20-hydroxyecdysone or makisterone A (Simes, Milan). The doses applied per day and per female ranged from 50 ng to 5000 ng 20-hydroxyecdysone and from 50 ng to 500 ng makisterone A. The treatment was started within