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mapping of it by the method of Cleveland et al.[20] gives a pattern identical to that obtained from YP 3 from ovaries (unpublished results).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

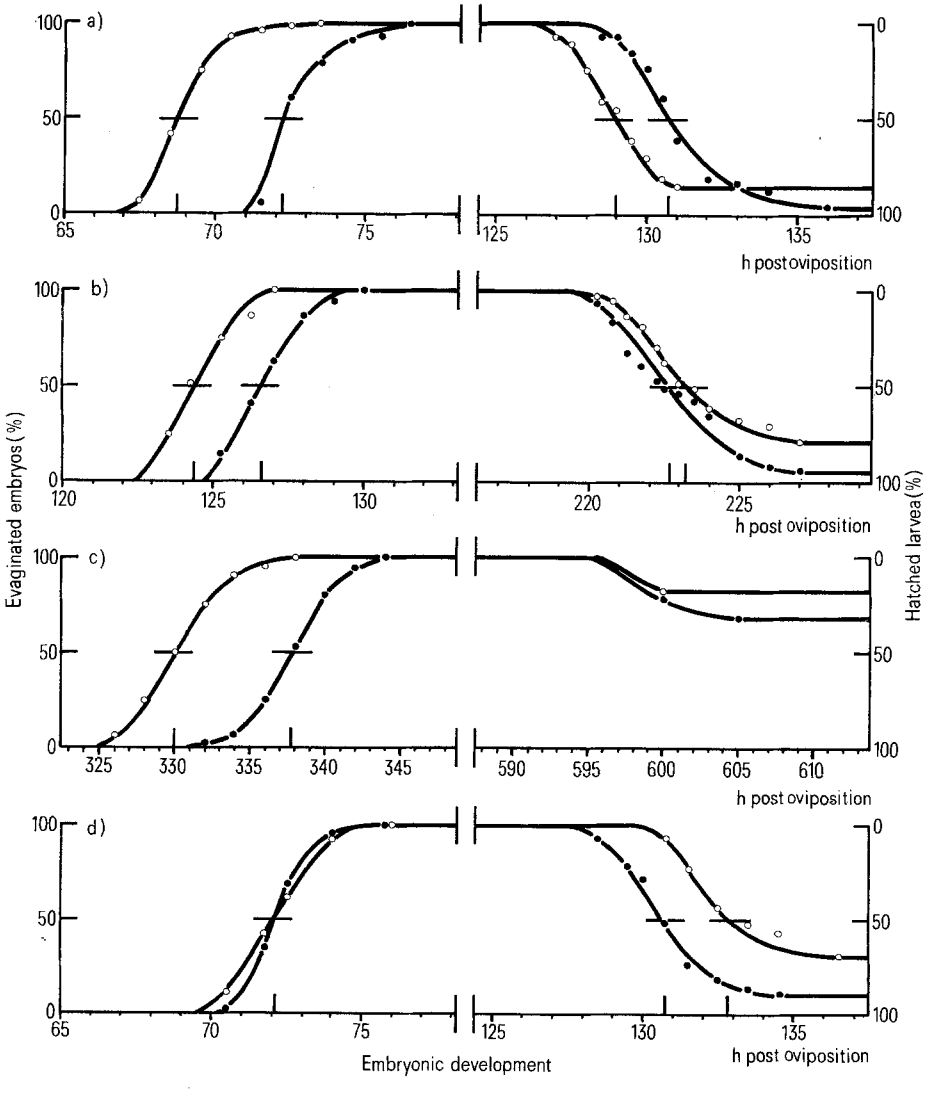
24 h after adult moult and was continued until the 1st egg batch was deposited, i.e. between 7th and 10th day after adult moult. Only the 1st egg batch was used. The amount of ecdysteroids present in newly-deposited eggs was determined by a radioimmunoassay (RIA) developed by Spindler et al.[10]. Eggs from females treated with the solvents only served as controls.

In order to find out which developmental processes were accelerated, we tested the termination of early embryonic development. This could be easily determined by stereo microscopical observation of evagination (katatrepsis). We defined early development as completed, when the head of the evaginating embryo had reached half way to the anterior egg pole.

Results and discussion. Low doses of exogenous ecdysteroids applied to females affected reproduction only moderately, whereas higher doses (500 ng makisterone A and 5000 ng 20-hydroxyecdysone per day and female) had a sterilizing effect[9]. The percentage of eggs that hatched from females treated with 50 ng/day 20-hydroxyecdysone was the same as in controls. About 20% fewer eggs hatched when the mother was treated with 50 ng/day makisterone A. Surprisingly, the eggs from makisterone A treated females hatched significantly earlier than nontreated eggs and controls. (Nontreated eggs and controls hatched at the same time.) Higher, substerilizing doses triggered the same effect. Low doses of 20-hydroxyecdysone had no impact on the timing of embryogenesis. Higher doses (2000 ng/day) prolonged embryogenesis. But, as shown in figure d, the prolongation concerned only late embryonic development, whereas the timing of early embryogenesis was the same as in the controls.

In the experiments shown in the figure, the females were treated with 250 ng/day makisterone A and 2000 ng/day 20-hydroxyecdysone respectively. Newly-deposited eggs from these females had a considerably higher content of RIA active ecdysteroids than controls, where 15 pg (equivalents of makisterone A) per egg were found. Eggs from makisterone A treated females contained about 900 pg RIA active ecdysteroids (equivalents of makisterone A) per egg, and eggs from 20-hydroxyecdysone-treated females contained about 2300 pg RIA active ecdysteroids (equivalents of 20-hydroxyecdysone) per egg. It should be mentioned that ecdysteroids present in excessive amounts had an ovicidal activity which is reported elsewhere[9]. However, the acceleration of early embryogenesis induced by exogenous makisterone A, which is described here, could be also observed at doses that hardly disturbed embryogenesis or subsequent larval development otherwise, (i.e. 50 ng/day makisterone A which led to about 400 pg RIA active ecdysteroids per egg).

Figure a shows the time of evagination and hatching of



Diagrammatic representation of evagination (termination of early embryonic development) and hatching. Only eggs with proper evagination are considered. The time of development is given in h post oviposition. The time when 50% of the embryos are evaginated or hatched is indicated at the abscissa. O----O test eggs, ●----● control eggs (test and control eggs were collected at the same time and incubated together). Curves a - d are representatives chosen from several repeats (see text). *a* Eggs from makisterone A-treated (250 ng/day) and nontreated females incubated at 26.5°C. *b* Eggs from makisterone A-treated (250 ng/day) and nontreated females incubated at room temperature. *c* Eggs from makisterone A-treated (250 ng/day) and nontreated females incubated at 17°C. Most of the evaginated embryos failed to hatch, although they were normally developed. *d* Eggs from 20-hydroxyecdysone-treated (2000 ng/day) and nontreated females incubated at 26.5°C.

eggs derived from makisterone A treated females and controls. At the given temperature (26.5 °C), makisterone A accelerated embryonic development for 3.1±0.4 h compared with controls. (Number of tests: n=6; total of tests eggs: t_t=514; total of control eggs: t_c=420.) Test eggs also hatched earlier than control eggs, but the difference was not as pronounced as at evagination, namely 1.7±0.4 h. This means that eggs with a high content of exogenous makisterone A (respectively RIA active metabolites) develop faster during early embryogenesis but more slowly during late embryogenesis as compared with nontreated eggs.

These observations were confirmed when the eggs were incubated at room temperature (fig. b), and at low (17°C) temperature (fig. c). The experiment in figure b was repeated twice; t_t=172; t_c=200. The experiment in figure c was also repeated twice; t_t=216; t_c=342. In all repeats, there was no great variation from figures b and c.

Maternal treatment with exogenous 20-hydroxyecdysone had no influence on the timing of early embryonic development (fig. d), although the content of RIA active ecdysteroids was 2.5 times higher than after makisterone A treatment. Late embryonic development, however, was prolonged for about 2 h compared with controls. (The experiment of figure d was repeated twice with identical results; t_t=190 and t_c=300.)

It is shown (fig. a) that maternal makisterone A treatment accelerates the early development during 3.5 h but that the embryo hatches only 1.3 h earlier than controls. Hence, late embryonic development must have been slowed down in comparison to controls. In this respect - prolongation of late embryonic development - makisterone A and 20-hydroxyecdysone had a similar effect, but only makisterone A accelerated early embryonic development. The slowdown of late embryonic development is not yet understood. Kaplanis et al.[5] suggested that makisterone A might be the biologically active ecdysteroid in the embryo of *Oncopeltus fasciatus*. Evidence that makisterone A might also be the active ecdysteroid in reproducing females was found previously[9,11]. It is shown here that only exogenous makisterone A applied to the mother can stimulate early development. This effect may indicate a role of maternal ecdysteroids which are regularly found in normal eggs (see Hoffmann et al.[12] for review). There have been only a few indications for such a function of ecdysteroids[13]. Future studies will have to reveal whether makisterone A stimulates specific processes of the early differentiation, or whether growth and/or differentiation in general are influenced.

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Stimulation of H^+ ion secretion from the isolated mouse stomach by sodium fluoride

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Summary. The effect of sodium fluoride on H^+ ion secretion was investigated in the isolated distended mouse stomach. It was found that sodium fluoride on its own caused dose-related stimulation of H^+ ion secretion. Sodium fluoride did not inhibit H^+ ion secretion induced by histamine. The possible mechanisms involved are discussed. It is considered that sodium fluoride might stimulate H^+ ion secretion by causing histamine release and by increasing cyclic AMP formation in the intact gastric mucosa.

It has been shown that instillation of sodium fluoride (NaF) into the cat gastric lumen produced marked reduction in the output of H^+ ions secreted in response to histamine[2] and to gastrin[3]. Most recently, Reed and Smy[4] studied the effects of NaF on gastric acid and electrolyte output in the anaesthetized cat. They found that in the histamine-stimulated stomachs, the NaF-induced inhibition of H^+ ion secretion was accompanied by reduction of blood flow. To eliminate possible complicating factors involved in in vivo experiments, the isolated distended mouse stomach known to be a useful preparation for quantitative studies[5] was used in the present work to investigate the mode of action of NaF on in vitro gastric acid secretion.

Materials and methods. Gastric acid secretion was studied according to the method described previously[5]. Fed mice (Charles River) of either sex, about 20 g b.wt, were used. While the animal was anaesthetized with ether, the stomach was exposed, polythene cannulae were tied into the cardiac and the pyloric region and the oesophagus was ligated. The stomach was then isolated and placed immediately in an organ bath containing 30 ml of serosal solution maintained at 37 °C and gassed vigorously with 95% O_2+5% CO_2. The pyloric cannula was connected to a perfusion pump and the cardiac cannula was connected to a pH electrode unit adjusted to raise the intragastric pressure to 18 cm H_2O to achieve the distension effect. The stomach lumen was continuously perfused with warm oxygenated mucosal solution at 1 ml/min and the perfusate was passed over the pH electrode (a micro, dual electrode) and then collected into a vessel at 15 min intervals and titrated to pH 7.0 with 10^{-2} M NaOH. The pH readings were continuously noted as a function of time with a pen recorder and the volumes of titrant used were recorded on a titrigraph every 15 min.