tive in forming pigment granules[16] (which are, however, present in the 2 other strains) it is assumed that there is some interdependence between the accumulation of 3-OH-kynurenine and the differentiation of pigment granules. Detection of 3-OH-kynurenine in isolated pigment granules is further evidence for this assumption[17].

Electron microscopical evidence shows that 3-OH-kynurenine does not only serve as a precursor for ommochrome synthesis but also has a function in the induction of the formation of pigment granules, and once formed, in their permittance[2,3]. From quantitative studies on *Ephestia,* a model has been proposed which is based on an induction step for the establishment of the biosynthetic apparatus[18].

Taking into account these results, 2 different explanations exist for the lack of 3-OH-kynurenine in the mutant *wa,* which is devoid of pigment granules. First, the accumulation of this tryptophan metabolite could be dependent on pigment granules or their precursors. Second, it could be possible that the mutant *wa* lacks a transport system which enables the entry of a sufficient amount of 3-OH-kynurenine into ommatidia cells. If this metabolite, as has been proposed[18], is necessary for the induction of pigment granules, such a transport defect would prevent the differentiation of these organelles as found in *wa.* In Drosophila melanogaster[19] and Lucilia cuprina[20] eye-color mutants have been traced to defects in tryptophan and/or its metabolites. Experiments with the ovary of *Ephestia* have revealed 2 systems of different affinity transporting 3-OH-kynurenine into the oocyte[21]. It should be tested whether the failure of the strain *wa* to accumulate 3-OH-kynurenine is due to a transport defect as well.

Thus ommochrome synthesis seems to be a rather complex event, starting with the accumulation of the precursor as a genetically distinct event controlled by the gene *wa.* The mutant *alb* is involved in one of the subsequent processes converting 3-OH-kynurenine to ommochrome. Up to now it was thought that in *Ephestia* compound eyes the ommochromes appear in a sequential order beginning with the less complex xanthommatin[22], suggestive of a precursor-product relationship. These previous results are perhaps due to the fact that ommin degrades relatively quickly under the solvent conditions used for extraction. From the results described in the present paper there are no indications for the successive appearance of the ommochrome components. In wild-type both xanthommatin and ommin are already present at an early stage during pigmentation in the retinula cells, a fact that can also be confirmed by thin-layer chromatography. Other evidence against a simple precursor-product relationship is given by the different developmental profiles of xanthommatin and ommin.

As pigment granules or one of their precursor structures seem to play a central role in the binding of 3-OH-kynurenine and in the synthesis of ommochromes, attempts should be made to characterize these organelles in detail.

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Distribution of free and conjugated ecdysteroids between follicle cell sheath and ooplasm in oocytes of the cockroach *Nauphoeta cinerea*[1]

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Summary. It is shown that both follicle cell sheath and ooplasm contain only small quantities of *Helix*-hydrolyzable ecdysteroid conjugates and that 20-hydroxy-ecdysone is the predominant free ecdysteroid. Its concentration is very high in the follicle cell sheath immediately before chorion formation (6825 ng/g) and much lower after chorion formation (150 ng/g), while it is 234 ng/g and 95 ng/g in the ooplasm at the same stages respectively.

It is known that ecdysteroids are present in both immature insects and reproducing adult females[3,4]. In larval and pupal stages ecdysone is synthesized by the prothoracic gland and then hydroxylated to 20-hydroxy-ecdysone, which is the moulting hormone in most insect species investigated[3]. In reproducing females, enormous differences between various species can be observed with regard to the quality, quantity and distribution of ecdysteroids, and their biological function is still very conjectural[4-6]. For *Nauphoeta cinerea,* the ovoviviparous cockroach investigated here, we know that 20-hydroxy-ecdysone is the predominant ovarian hormone and that its titre is highest (approximately 1 μM) at the beginning of chorion formation and drops towards ovulation[6]. Ecdysone is produced and converted to 20-hydroxy-ecdysone in the follicle epithelium and significant and variable quatities of this hormone are also found in the haemolymph[6,7]. For this insect we have suggested a dual role for the ovarian ecdysteroids, namely controlling chorion formation[6] and inactivating the corpora allata[7,8]. The possibility of storage of some conjugates for later use by the embryo, as happens in the case of *Locusta migratoria*[9], has not yet been clarified for *Nauphoeta cinerea,* since we detected only small quantities of *Helix*-hydrolyzable ecdysteroid conjugates in the ovaries of the latter[6].

In order to learn more about the fate and the function of free

and conjugated ovarian ecdysteroids in *Nauphoeta cinerea* we investigated their localization within the oocytes by measuring the quantities of free ecdysteroids and of *Helix*-hydrolyzable ecdysteroid conjugates in follicle epithelium and ooplasm separately.

Materials and methods. Insects were kept at 26 °C and 60% r.h. on flakes of dog food and water at a photoperiod of 12 h. Under these conditions the oocyte maturation cycle lasts 12–13 days and chorion formation occurs on day 10–11. Terminal oocytes were carefully dissected into follicle cell sheath and ooplasm and separately extracted for ecdysteroid determinations using 65% aqueous methanol. In the subsequent thin layer chromatography (TLC) step 5 different zones were eluted with ethanol: zone 1 with the highly polar products at the origin, zone 2 between the origin and the 20-hydroxy-ecdysone zone, zone 3 corresponding to 20-hydroxy-ecdysone, zone 4 corresponding to ecdysone and zone 5 containing the low polarity products[6]. Ecdysteroids were quantified by a radioimmunoassay (RIA)[10,11]. Ecdysone antibodies were a generous gift from J.D. O'Connor (Los Angeles) and 23,24-[^{3}H]-ecdysone from J. Koolman (Marburg). Enzymatic hydrolysis of the highly polar products (zone 1) was carried out using 1.5 ml of 50 mM acetate buffer at pH 5.0 for 18 h at 37 °C with 4200 Fishman Units of β-D-glucuronide glucuronhydrolase and arylsulphatase (Sigma, purified powder from *Helix pomatia* type H-L).

Results and discussion. The distribution of free ecdysteroids between follicle cell sheath and ooplasm in oocytes immediately before and after chorion formation is shown in table 1. The data reveal the presence of small quantities of RIA positive material in the zones 1 (highly polar products), 4 (ecdysone) and 5 (low polarity products) and significantly higher quantities in the 20-hydroxy-ecdysone zone in both follicle cell sheath and ooplasm. Before chorion formation the amount of RIA positive material is much higher in the ooplasm than in the follicle cell sheath in all zones, except the 20-hydroxy-ecdysone zone, where approximately ¼ is recovered from the follicle cell sheath. After chorion formation only traces of RIA positive material are found in the follicle cell sheath ($^1/_{100}$ of the 20-hydroxy-ecdysone) and also the ooplasm contains much less except for the highly polar products zone. Taking into account the fact that the entire follicle cell sheath of one ovary weighs approximately 0.8 mg while the ooplasm totals approximately 60 mg in weight, the concentration of 20-hydroxy-ecdysone is 6825 ng/g for the follicle cell sheath and 234 ng/g for the ooplasm at the stage immediately before chorion formation, and approximately 150 ng/g for the follicle cell sheath and 95 ng/g for the ooplasm after chorion formation. Obviously the quantity of ecdysteroids detected by our RIA is lower after than before chorion formation in both ooplasm and follicle cell sheath and the decrease in the concentration of 20-hydroxy-ecdysone is more pronounced for the follicle cell sheath than for the ooplasm. Even smaller quantities have been observed in newly ovulated egg cases[12]. Since we know that the 20-hydroxy-ecdysone titre fluctuates in a similar way in ovary and hemolymph, being 10 times lower in the latter[6,7], and since the follicle cell sheath has been shown to produce 20-hydroxy-ecdysone[6], the above findings could be interpreted to mean that some of the 20-hydroxy-ecdysone produced by the follicle cell sheath is released into the hemolymph and that some of it is deposited in the ooplasm.

In order to investigate the presence and distribution of *Helix*-hydrolyzable conjugates the highly polar products (TLC zone 1) from ooplasm and follicle cell sheath of oocytes immediately before chorion formation we re-incubated with *Helix pomatia* enzymes and then analyzed for the presence of ecdysteroids (table 2). The data show that both follicle cell sheath and ooplasm contain small quantities of *Helix*-hydrolyzable conjugates, 20-hydroxy-ecdysone being predominant. Approximately $^1/_5$ of the *Helix*-hydrolyzable 20-hydroxy-ecdysone conjugates of an oocyte is found in the follicle cell sheath. Taking into account relative weights, we find that ooplasm releases 18 ng 20-hydroxy-ecdysone equ./g and follicle sheath 381 ng upon hydrolysis with *Helix pomatia* enzymes. A comparison with table 1, where quantities of free ecdysteroids are given for intact oocytes and for follicle cell sheath and ooplasm separately, reveals that the quantity of free 20-hydroxy-ecdysone is about 10 times greater than that of *Helix*-hydrolyzable 20-hydroxy-ecdysone conjugates in the ooplasm and about 20 times greater in the follicle cell sheath. Since the values measured for 20-hydroxy-ecdysone conjugates in ooplasm were very variable (2.16 ng ± 0.81) it is difficult to interpret unequivocally the data obtained, but they suggest that some of the 20-hydroxy-ecdysone produced by the follicle cell sheath forms a *Helix*-hydrolyzable conjugate which goes into the ooplasm. In the ooplasm some RIA positive material can be detected even after hydrolysis (table 2), indicating the presence of ecdysteroid-like highly polar products not susceptible to the *Helix pomatia* enzymes. The fact that the highly polar products zone of the follicle cell sheath did not display RIA positive material before hydrolyzation (table 1) but nonetheless contains *Helix*-hydrolyzable con-

Table 2. Analysis of highly polar products in follicle cell sheath and ooplasm immediately before chorion formation, using hydrolyzation with *Helix pomatia* enzymes

Tissue	Ecdysteroid content (ng ecd. equ./tissue)			
	HPP	20-hydroxy-ecdysone	Ecdysone	LPP
Follicle cell sheath	0	0.61 ± 0.04	0.07 ± 0.04	0.02 ± 0.02
Ooplasm	0.25 ± 0.12	2.16 ± 0.81	0.19 ± 0.05	0.18 ± 0.09

In each experiment the terminal oocytes from both ovaries were dissected and the follicle cell sheath was separated from the ooplasm. The highly polar products from these 2 parts were isolated by TLC and then hydrolyzed with *Helix pomatia* enzymes. The ecdysteroid content was then measured using TLC combined with RIA and expressed for the highly polar products (HPP) zone, the ecdysone zone and the low polarity products (LPP) zone in ng ecdysone equivalents and for the 20-hydroxy-ecdysone zone in ng 20-hydroxy-ecdysone equivalents. Data are the mean ± SEM of 5 replicates.

Table 1. Quantities of free ecdysteroids in intact terminal oocytes or in follicle cell sheath and ooplasm measured separately at stages immediately before and after chorion formation

Developmental stage	Tissue	Ecdysteroid content (ng ecd. equ./tissue)			
		HPP	20-hydroxy-ecdysone	Ecdysone	LPP
Before chorion formation	Intact oocytes	0.33 ± 0.13	16.75 ± 4.57	1.86 ± 0.52	0.17 ± 0.10
	Follicle cell sheath	0	5.46 ± 1.07	0.23 ± 0.06	0.03 ± 0.03
	Ooplasm	0.71 ± 0.57	14.08 ± 1.21	1.18 ± 0.22	0.19 ± 0.06
After chorion formation	Intact oocytes	0.33 ± 0.11	4.93 ± 1.92	0.87 ± 0.39	0.07 ± 0.04
	Follicle cell sheath	0.06 ± 0.03	0.12 ± 0.09	0.09 ± 0.06	0.03 ± 0.03
	Ooplasm	0.40 ± 0.30	6.32 ± 1.46	0.90 ± 0.17	0.07 ± 0.04

In each experiment the right ovary was used for measuring the ecdysteroid content of the intact terminal oocytes; the left ovary was used for measuring the ecdysteroid content of the follicle cell sheath and the ooplasm separately. Ecdysteroid content was quantified by TLC combined with RIA and expressed for the highly polar products (HPP) zone, ecdysone zone and the low polarity products (LPP) zone in ng of ecdysone equivalents and for the 20-hydroxy-ecdysone zone in ng of 20-hydroxy-ecdysone equivalents. Data are the mean ± SEM of 4 replicates for each stage.

jugates (table 2) demonstrates that such conjugates are not or only weakly detected with our RIA.

These findings are very different from those in *Locusta migratoria* where the majority of ecdysteroids in the ovary are present in the form of *Helix*-hydrolyzable conjugates of ecdysone (50 µM) and of 2-deoxy-ecdysone (100 µM) bound to vitellin and outnumbering the corresponding free compounds 50 to 100-fold[9,13]. Only traces of ecdysteroids are observed in the hemolymph[14] and in this insect hydrolysis of the ecdysteroid conjugates in the eggs appears to be the source of the free ecdysteroids observed in early embryonic development[9]. In embryos of the cockroach *Nauphoeta cinerea* we have also observed a peak of 20-hydroxy-ecdysone before dorsal closure[12], when the prothoracic glands are not yet differentiated. For this species experiments using *Helix pomatia* enzymes reveal that the increase in 20-hydroxy-ecdysone before dorsal closure cannot be due to hydrolysis of *Helix*-hydrolyzable conjugates only[6,12]. We assume that ecdysteroid conjugates not susceptible to hydrolysis by *Helix* enzymes are formed in *Nauphoeta,* or that the free 20-hydroxy-ecdysone observed before dorsal closure does not originate from conjugate hydrolysis. On the basis of earlier investigations[6,7,12] and of the data shown here, we suppose for the cockroach *Nauphoeta cinerea* that the follicle epithelium of near-mature oocytes produces 20-hydroxy-ecdysone which plays a role in inducing chorion formation and which to some extent is released into the hemolymph, possibly to inactivate the corpora allata[7,8]. In addition part of the 20-hydroxy-ecdysone seems to enter the ooplasm in free form and as a conjugate of unknown identity, resistant to hydrolysis by *Helix pomatia* enzymes, which might give rise to the free 20-hydroxy-ecdysone observed before dorsal closure.

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Somatomedin-C in zinc deficiency

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Summary. A significant decrease in the activity of somatomedin-C (SM-C) was observed due to zinc deficiency. SM-C activity correlated significantly with b.wt gain and with the status of zinc in rats. The present findings provide an insight into the mechanism by which zinc promotes growth and development.

Recently, it has become apparent that nutritional status may be an important factor that affects the activity of somatomedin. Low serum somatomedin with associated growth failure despite elevated growth hormone (GH) was first noted in children with Laron-type dwarfism[1]. Similar findings have been reported in children with marasmus[2] and kwashiorkertype malnutrition[3-4]. When these children were refed, somatomedin levels increased toward normal[5]. An inverse relationship between dietary fat and plasma somatomedin was observed in rats[6]. Moreover, the levels of somatomedin increased with increasing levels of protein[6-7] even though no change occurred in the levels (conc.) of GH. Increasing levels of protein in the diet may also mean additional micronutrients such as zinc. Poor growth in male villagers, subsisting on diet low in animal protein, has been related to zinc deficiency[8]. The present study was conducted to investigate the effect of zinc deficiency and supplementation on the activity of plasma somatomedin-C (SM-C) and to correlate these levels with the status of zinc in 4 different groups of rats: zinc deficient, pairfed control, continuously pairfed control, and ad lib-fed control group.

Materials and methods. White male rats of the Holtzman strain (Holtzman Company, Madison, Wis.) were housed individually in stainless steel cages. Initial body weight of rats ranged from 95 to 100 g. The rats were fed a standard diet (Purina rat chow, St. Louis, MO) for 4 days after which they were randomly allotted into 4 dietary treatment groups: zinc deficient (ZD), pair-fed (PF) control, continuously pair-fed (CPF) control, and ad lib-fed (ad lib) control. ZD group received (ad libitum) a semi-purified diet based on egg white solids[9] containing 1.2 ppm of zinc and 18% crude protein. The PF group received the basal diet which was supplemented with $ZnSO_4$ to provide 100 ppm of zinc. The amount of daily food given to the PF group matched the intake of their counterparts of the ZD group. The CPF group received the same amount of food daily from the zinc supplemented diet (100 ppm). The feeding pattern in this group simulated that of the ZD group. For this purpose, food was placed in a grooved auto-rotating feeders. A complete cycle of the feeder required 24 h. The ad lib-fed group received the zinc supplemented diet ad libitum. Distilled deionized water was offered ad libitum. Daily food intake and