Relative number of cells found in control and in different salt intake animals

Cells with:	Cilia	Short cilia	Microvilli only	Small protrusions	Large protrusions	Smooth surface	Others	Total No. of cells analyzed	No. of large protrusions per picture	Total No. of pictures
Control	3.6 ± 0.9*	2.3 ± 0.7	30.9 ± 5.8	23.4 ± 6.9	9.9 ± 2.7	28.1 ± 6.9	1.4 ± 0.2	1540	3.5 ± 0.7	77
High sodium	2.9 ± 0.8	2.9 ± 0.8	38.5 ± 4	13.7 ± 3.1	4.8 ± 3.1[a]	25.7 ± 4.3	0.9 ± 0.5	2540	1.93 ± 0.38[a]	127
Low sodium	2.1 ± 0.9	1.3 ± 0.2	59.4 ± 4.9[b]	10.5 ± 2.6	2.4 ± 0.8[a]	22.7 ± 3.9	1.1 ± 0.6	2160	1.17 ± 0.29[b]	108

* Means ± SEM of each type, per 100 cells; [a] $p < 0.05$ compared to control; [b] $p < 0.01$ compared to control.

SFO[3,4]. But some differences can be pointed out. The small protrusions found in all the cells[2] of zone 1 and 2 were present in our animals in about 25% of the cells, especially in zone 2. This may be explained by the different method of fixation used. This also may account for the bigger diameter fo our small protrusion. The short cilia found were shorter than the cilia described in rat[3]. They had the same length as the kinocilia described in rabbit[8] but they were devoid of any secretory covering material. Short cilia, although scarce, were found always in zone 2. Exceptionally 2 to 4 short cilia were found. We may also add to the description of cilia distribution that, although this was rare, only 2 or 3 long cilia were present in some cells. Microvilli have been described[9], though it may be possible that some of them represent membrane foldings with a length larger than the height, which increase the cell surface. This may be supported by the fact that SEM allow the complete view of this structure instead of a thin section as seen in TEM. The increase of cells with microvilli after submitting rats to a low sodium diet would increase the total area of interchange between SFO and cerebrospinal fluid. The large protrusions described could be considered as one type of structure, seen in different functional stages, or different structures which could take a similar shape. Their physiological function is unknown but we can speculate that they may have some secretory substance to be discharged into the cerebrospinal fluid or may have a receptor function. It seems improbable that a receptor of this magnitude can be atrophied after a stimulus of relatively short duration but if low and high sodium diets produce a common stimulus, the release of some substance by the SFO could explain the reduction in number of the large protrusions. The secretory function of the SFO has been considered[8]. Nevertheless, the possibility that some of them, the large protrusion with a long pedicle, are axons growing right through ependymal cells cannot be disregarded.

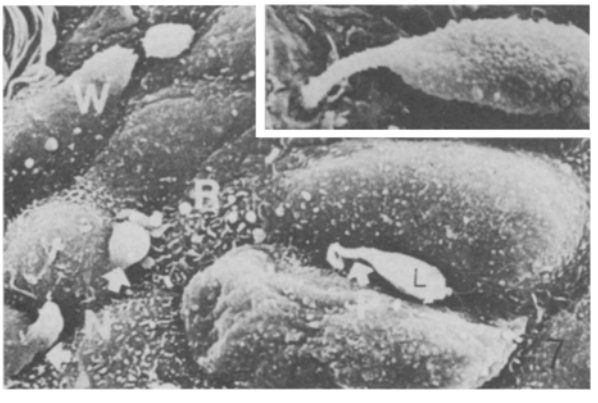


Figure 7. Large protrusions with narrow (N) or width (W) neck. Large protrusion (L) with a long pedicle (P). Small round protrusion (R). × 3000.

Figure 8. High magnification of a large protrusion covered by round excrescences. ×5000.

1 Acknowledgments. We thank Dr A.C. Taquini for his helpful discussions and Servicio de Microscopía Electrónica de Barrido, Conicet, and Facultad de Odontología for technical assistance.
2 Akert, K., Potter, H.D., and Anderson, J.W., J. comp. Neurol. *116* (1961) 1.
3 Phillips, M.I., Balhorn, L., Leavitt, M., and Hoffman, W., Brain Res. *80* (1974) 95.
4 Phillips, M.I., Deshmukh, P.P., Larsen, W., Scanning Electron Microsc. *2* (1978) 349.
5 Dellmann, H.D., and Simpson, J.B., Int. Rev. Cytol. *58* (1979) 333.
6 Phillips, M.I., Leavitt, M., and Hoffman, W., Fedn Proc. *33* (1974) 563.
7 Thornborough, J.R., Passo, S.S., and Rothballer, A.B., Brain Res. *58* (1973) 355.
8 Leonhardt, H., and Lindemann, B., Z. Zellforsch. *146* (1973) 243.
9 Dellmann, H.D., and Simpson, J.B., Brain Res. *116* (1976) 389.

0014-4754/84/050492-03$1.50 + 0.20/0

3-OH-Kynurenine content and ommochrome formation in the developing compound eye of *Ephestia kühniella*

K. Cölln and E. Hedemann

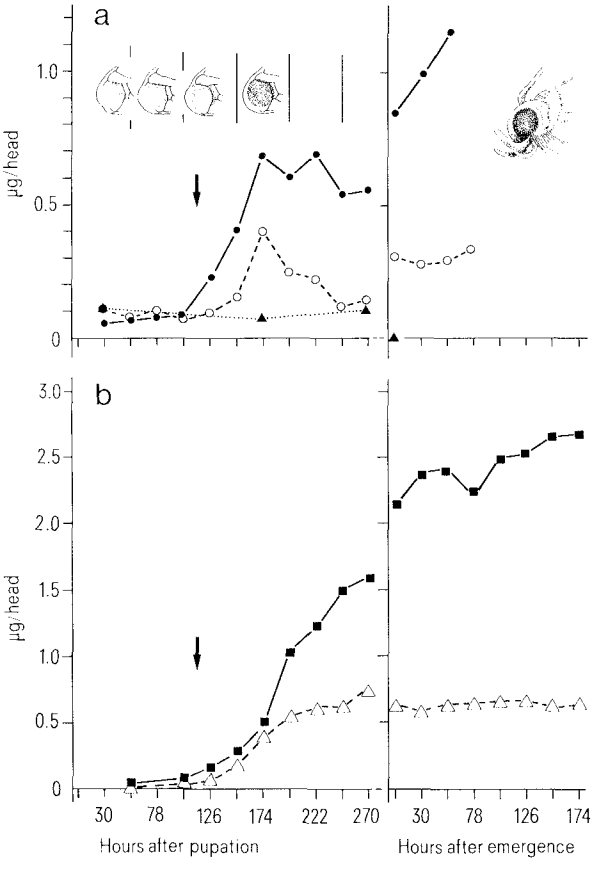
Zoologisches Institut der Universität zu Köln, Lehrstuhl für experimentelle Morphologie, Weyertal 119, D-5000 Köln 41 (Federal Republic of Germany), 5 April 1983

Summary. The eye color of *Ephestia kühniella* is primarily determined by the ommochromes xanthommatin and ommin. The pigmentation, an important part of eye differentiation, occurs mainly during the pupal stage. Comparative studies on eye colour mutants indicate that a first step in ommochrome synthesis is the binding of the precursor 3-OH-kynurenine to developing pigment granules. Both xanthommatin and ommin are present from the early beginning of eye differentiation, and exhibit different developmental profiles.

The pigmentation of plants and animals is a favored subject in developmental biology and genetics. A classical example is the eye color of insects. The screening pigment in the compound eyes of these animals consists mainly of ommochromes and only to a very small extent of pteridines. The direct precursor of ommochromes is 3-OH-kynurenine[1]. The synthesis of this metabolite, which is derived from tryptophane through a well-characterized series of enzymatically catalyzed oxidation steps, is well understood. In contrast, studies of the processes governing the formation of ommochromes are still at best preliminary

in character[2,3]. But just these events are of particular interest because they occur concurrently with the formation of specific cell organelles, the pigment granules. We have started to investigate the characteristics and the development of these granules in *Ephestia kühniella*[4]. As a basis for our studies, we followed the quantitative dependence between the formation of ommochromes and the level of their precursor 3-OH-kynurenine. Comparative studies on wild-type animals and 2 eye-color mutants indicate that a first step in ommochrome synthesis is the binding of the precursor 3-OH-kynurenine to granular material.

Material and method. Wild type and mutant strains *alb* and *wa* of *Ephestia kühniella Z.* from the stock center of the Zoological Institute of the University of Cologne were used as experimental animals[5]. The characteristics of these strains and the rearing conditions (23 °C) have been summarized by Caspari and Gottlieb[6]. Animals were staged by collecting freshly molted pupae or emerged adults at 12 h intervals[7]. Heads or eyes were cut off immediately after collection and stored either at − 18 °C or in 96% ethanol when 3-OH-kynurenine and ommochromes, respectively, were to be examined. Most determinations were done on whole heads, because 60% of the dry weight and 90% of ommochromes of the imaginal head arise from the 2 complex eyes. With the exception of L-3-OH-kynurenine (Calbiochem, Giessen), all chemicals were purchased from Merck (Darmstadt). Substances and solvents were of the highest purity available. The analytical procedures are described in detail elsewhere[8]. 3-OH-kynurenine was determined fluorometrically after isolation by thin layer chromatography and ommochromes were evaluated photometrically after separation by column chromatography[9] with Sephadex SP-C-25.



3-Hydroxy-kynurenine and ommochromes during differentiation of the complex eye. (The arrows indicate the onset of pigmentation in secondary pigment cells.) *a* 3-Hydroxy-kynurenine: wild (○——○); *alb* (●——●); *wa* (▲·····▲); values are the mean of 3 separate experiments with 5 heads per sample. The highest concentrations per head are in wild 0.41 ± 0.03 µg, in *alb* 1.14 ± 0.07 µg and in *wa* 0.11 ± 0.03 µg. *b* Ommochromes in wild type: xanthommatin (△——△); ommin (■——■); values are the mean of 2–4 experiments with 25–160 heads. The highest concentrations per head are for xanthommatin 0.76 ± 0.02 µg and for ommin 2.63 ± 0.20 µg.

Results. In wild type *Ephestia,* the pigmentation in the compound eyes occurs mainly during pupal development. This can be directly observed through the cuticle. Starting from the retinula cells of the dorsoposterior part of the eye anlage, the coloration spreads out, reaching the margins of the eye field 84 h after pupation. About 30 h later pigment formation begins simultaneously in all secondary pigment cells. The bulk of the ommochrome of the eye is synthesized by these cells during subsequent development. Primary pigment cells, which are the last to become pigmented, synthesize only pteridines as screening pigments[10].

The wild-type and the mutant strains analyzed show identical developmental patterns of the 3-OH-kynurenine content of the heads during the first 102 h after pupation (fig., a). During subsequent development the 3 strains diverge dramatically. In wild-type heads the amount of 3-OH-kynurenine increases until 174 h after pupation, and becomes continuously reduced during the last third of the pupal stage. After eclosion an enhanced but constant level is observed. Both eye-color mutants, the faintly pigmented *alb* and the colorless *wa* strain, are able to synthesize 3-OH-kynurenine and to store it in the unpigmented ovary – a situation identical to that in wild-type animals[8]. However, in the heads of both mutants the 3-OH-kynurenine levels show different alterations of the normal developmental profile. In the mutant *alb* this compound accumulates beyond wild-type levels. The strain *wa* is characterized by a constant low level of 3-OH-kynurenine throughout pupal life. 174 h after pupation in the mutant *wa* the total 3-OH-kynurenine content of the head is localized in the developing compound eyes. Immediately after eclosion 3-OH-kynurenine can no longer be detected in the heads.

In wild-type animals both ommochrome components, xanthommatin and ommin, can be observed as early as 54 h after pupation (fig.) at a time when less than half of the differentiating retinula cells synthesize pigment. With the onset of secondary pigment cell coloration both ommochrome components increase markedly. Xanthommatin reaches its maximum level at the end of the pupal stage, whereas ommin continues to increase during imaginal life. The final concentration of ommin is about 4 times higher than that of xanthommatin.

Discussion. The present study shows that in the heads of wild-type animals the concentration of 3-OH-kynurenine exhibits a characteristic fluctuation during metamorphosis. When ommochrome synthesis decreases in the imago its precursor is still present in appreciable amounts. The origin of 3-OH-kynurenine in the cells is not quite clear. It has been demonstrated that pupal heads can metabolize tryptophan to kynurenine[11]. In the wild-type the appearance of this metabolite precedes that of 3-OH-kynurenine, which suggests a precursor-product relationship. However, the maximal level of kynurenine only amounts to 10% of the concentration of 3-OH-kynurenine[12] and the converting enzyme 3-hydroxylase of *Ephestia* has only a low affinity in comparison to the substrate pool. Furthermore there is only very little activity of 3-hydroxylase in the eye[13]. Thus external 3-OH-kynurenine, which is for example released in large amounts to the hemolymph by the histolyzing fat body just prior to the main onset of ommochrome synthesis in the eye[7,14], might be the main source of precursor. It has already been proved that the eye can sequester 3-OH-kynurenine[15].

The accumulation of 3-OH-kynurenine starts at a stage where the eye field is already differentiated into preommatidia. It occurs both in wild type animals and in the strain *alb,* but it is not to be found in the mutant *wa.* Since this mutant is defec-

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.

1 Ziegler, I., Adv. Genet. *10* (1961) 349.
2 Linzen, B., Adv. Insect Physiol. *10* (1974) 117.
3 Linzen, B., in: Biochemical and Medical Aspects of Tryptophan Metabolism. Ed. O. Hayaishi. Elsevier, Amsterdam 1980.
4 Cölln, K., Hedemann, R., and Ojijo, E., Experientia *37* (1981) 44.
5 We thank Mrs A.M. Gramlich from the Ephestia Stock Center for supplying us with experimental animals.
6 Caspari, E.W., and Gottlieb, F.J., in: Handbook of Genetics, vol. 3, p. 125. Ed. R.C. King. Plenum Press, London 1976.
7 Cölln, K., Wilhelm Roux Arch. *172* (1973) 231.
8 Cölln, K., and Klett, G., Wilhelm Roux Arch. *185* (1978) 127.
9 Stratakis, E., Insect Biochem. *6* (1976) 29.
10 Cölln, K., Verh. dt. zool. Ges. (1977) 288.
11 Egelhaaf, A., Z. Vererblehre *97* (1963) 348.
12 Cölln, K., unpublished results.
13 Stratakis, E., Insect Biochem. *12* (1982) 419.
14 Cölln, K., and Hedemann, E., Experientia *38* (1982) 1327.
15 Muth, F.W., Wilhelm Roux Arch. *161* (1968) 336.
16 Shoup, J.R., J. Cell Biol. *29* (1966) 223.
17 Cölln, K., Verh. dt. zool. Ges. (1976) 292.
18 Muth, F.W., Wilhelm Roux Arch. *162* (1969) 56.
19 Sullivan, D.T., Biochem. Genet. *18* (1980) 1109.
20 Summers, K.M., and Howells, A.J., Biochem. Genet. *18* (1980) 643.
21 Mayer, B., and Cölln, K., Verh. dt. zool. Ges. (1981) 322.
22 Kühn, A., Z. Naturforsch. *18b* (1963) 252.

0014-4754/84/050494-03$1.50 + 0.20/0

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

X.X. Zhu and B. Lanzrein[2]

Division of Animal Physiology, Zoological Institute, University of Bern, Erlachstrasse 9a, CH-3012 Bern (Switzerland),
9 June 1983

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