of neurosecretory granules from the hypothalamic nuclei to the median eminence and to the neurohypophysis, and block the release of vasopressin from the posterior lobe[12,13]. Neurons from the preoptic area produces LHRH which is transported to the MBH by an axonal route for storage and/or release into the hypophysial portal veins[14]. Ultrastructural studies have lent support to the participation of microtubules in hormone secretion from the AH[15]. Therefore it seems feasible that changes in tubulin levels of MBH and AH following castration and hormone replacement may be associated with modification of transport and/or secretions of various materials of neuroendocrine significance.

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Effect of α- and β-ecdysone on DNA synthesis in *Aeshna cyanea* (Insecta, Odonata) midgut

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Summary. In *Aeshna cyanea* larvae, α- and β-ecdysone stimulate DNA synthesis in the midgut regenerative cells. In last larval instar, the number of cells obtained after the imaginal epithelium genesis is greater after α- than after β-ecdysone supply. Such a result should be compared with the imaginal epithelium differentiation which occurs earlier after β-ecdysone injection.

It is well known that insect post-embryonic development is controlled by hormones. Numerous are the morphological and biochemical events attribuable to the action of ecdysones. Recent studies seem to indicate clearly the role of ecdysones in the stimulation of RNA synthesis[1]. On the other hand, the published data concerning the control of DNA synthesis do not appear to agree.

The following questions have not yet been answered completely: a) Is DNA synthesis under the control of ecdysones? Although some authors have shown a stimulatory in vivo or in vitro effect of α-[2-6], β-ecdysone[3,7,8] or inokosterone[9,10], the results often differ according to animal species, depending upon the developmental stage of the target organ[11] and the ecdysone level[6,12-15]. Moreover, Bullière and Bullière[10] have recently suggested that the inhibitory or triggering effect of the moulting hormone is function of the proliferative or differentiative nature of the epidermal divisions. b) Is α-ecdysone a true hormone having a specific role particulary on DNA synthesis prior to its conversion to β-ecdysone, as suggested by Oberlander[6]? Do α- and β-ecdysone effect DNA synthesis in the same way?

To answer these questions we have chosen as a model the midgut of a dragonfly, *Aeshna cyanea,* for several reasons: a) By its endodermal origin, the insect midgut is quite different from the organs usually studied in the control of DNA synthesis. b) This target organ contains principally a single cell type, the functional cells which, throughout the insect's life, are replaced from small embryonic cells, the regenerative cells, grouped in nidi, near the basement membrane. c) The last larval instar midgut of *A. cyanea* is characterized by the genesis of 2 tissues: the reticulate tissue and the imaginal epithelium which both result from the differentiation of the regenerative cells[16]. The first event of the midgut in this instar is therefore a proliferating activity of these cells. d) In controls, imaginal epithelium genesis occurs when the sclerification of the tarsal claws takes place. This tegument response, easily observable after α- or β-ecdysone injection led us to a first study[17] in which the experimental animals were killed some h after this event. At this time, we observed that there was always an increase in the number of regenerative cells. Furthermore, by means of an autoradiographic study we were able to demonstrate that β-ecdysone accounts for this increase in stimulating DNA synthesis.

It was also noted that identical amounts of α- and β-ecdysone give different results. Thus, as a general rule, the genesis of the imaginal epithelium occurs later when α- instead of β-ecdysone is supplied. In the same way, it may be asked whether α- and β-ecdysone stimulate DNA synthesis in a similar way.

Material and methods. We used antepenultimate, penultimate and last instar larvae of *Aeshna cyanea* which were injected on the 5th day of the instar with a 10% alcoholic solution of α- (Simes) or β-ecdysone (Sigma). Indices of cell proliferation were the number of mitosis (meta- and anaphase figures) and the number of regenerative cells per transversal section. 20 serial sections were studied to determine the number of mitotic figures per section.

Results. Figure 1 records the values of the mitotic index during the 10 days following the 20 µg/g α- or β-ecdysone supply to 55 last larval instar. These results demonstrate clearly that after α- or β-ecdysone injection there is always an increase of the mitotic index. This graph also shows that, at first, α- and β-ecdysone stimulate DNA synthesis in the same way. The time course of mitosis and the number of

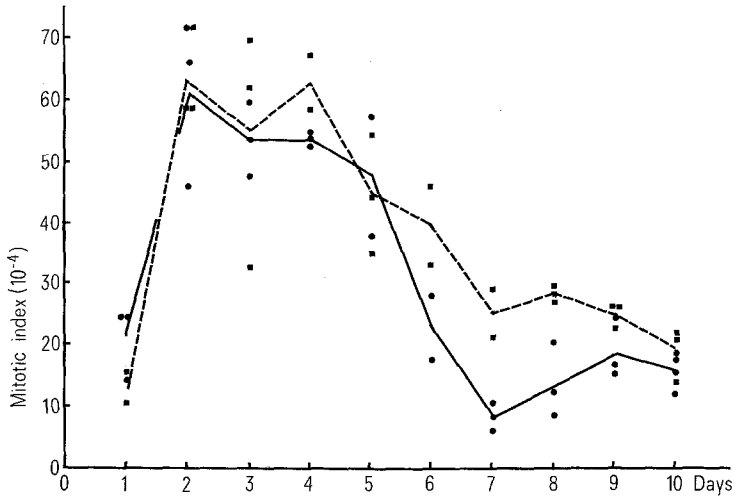

Fig. 1. Mitotic index variation during the 10 days following α-(- - -) or β-ecdysone (——) supply (circle: value obtained after β-ecdysone injection; square: value obtained after α-ecdysone injection).

regenerative cells stimulated are therefore the same after α- and β-ecdysone injection. After day + 5, the DNA synthesis stimulation is quite different after α- or β-ecdysone injection, although in both cases the mitotic index decreases. It should be emphasized that it is higher after α-ecdysone injection.

Such a result should be compared with the genesis of the imaginal epithelium which happens at different moments after α- or β-ecdysone supply (figure 2). The 1st qualitative changes of the regenerative cells during their imaginal differentiation occur respectively 7 or 10 days after α- or β-ecdysone injection. After β-ecdysone supply, on day + 7, the regenerative cells, instead of being spherical become columnar. Then, in the following days, they increase in size. On day + 10, the β-ecdysone induced imaginal epithelium genesis is completed as microvilli have appeared at the cells apex whereas only the first stages of differentiation happen after α-ecdysone supply.

The differentiation of the imaginal epithelium after a single injection of α- or β-ecdysone occurs, therefore, earlier after β-injection. When the majority of the regenerative cells undergo differentiation, few of them are able to DNA synthesis. This means that the mitotic index decreases sooner and more abruptly after β-ecdysone injection than after α-ecdysone supply. Besides, the time relations between DNA synthesis and cellular differentiation in regenerative cells of experimental larvae are the same as those observed in controls: once imaginal epithelium differentiation begins, the number of DNA synthesizing regenerative cells falls (compare figure 1 to figure 3).

From these results it appears that the number of cells constituted in the regenerative nidi will always be smaller after β- than after α-ecdysone injection. Since in *A. cyanea* the number of regenerative and epithelial cells varies all along the midgut (it is higher in the posterior end)[18], and since their ratio seems constant, we have chosen to demonstrate the intensive proliferation occurring in the regenerative nidi by the variation of this ratio (figure 4).

We observe that this ratio increases until day + 4 at a very low rate and at a high rate afterwards. It seems that the slight progression of the ratio, with nevertheless a high mitotic index, should be compared with the differentiation of some regenerative cells in the larval way as shown by the simultaneous injection of 3H-thymidine and β-ecdysone[17]. We can also note that on day + 8, whatever the ecdysone supply may be, the ratio is the same. Afterwards it increases in a large way, only, after α-ecdysone injection. In this case, the increase concerns both imaginal and tissue reticulated cells.

In controls, the genesis of the reticulated tissue happens a few days before the differentiation of the imaginal epithelium (figure 3). In experimental larvae, whatever the ecdysone supply may be, the 1st cells of the reticulated tissue can be observed on day + 7 but in β-ecdysone injected larvae, the stage of the reticulated tissue is more or less completely short-circuited, whereas after α-ecdysone supply, as the imaginal epithelium differentiation occurs later, more regenerative cells form part of the reticulated tissue.

From these results we may conclude that in *A. cyanea* last larval instar a) α- and β-ecdysone stimulate DNA synthesis in regenerative cells, b) β-ecdysone induces the differentia-

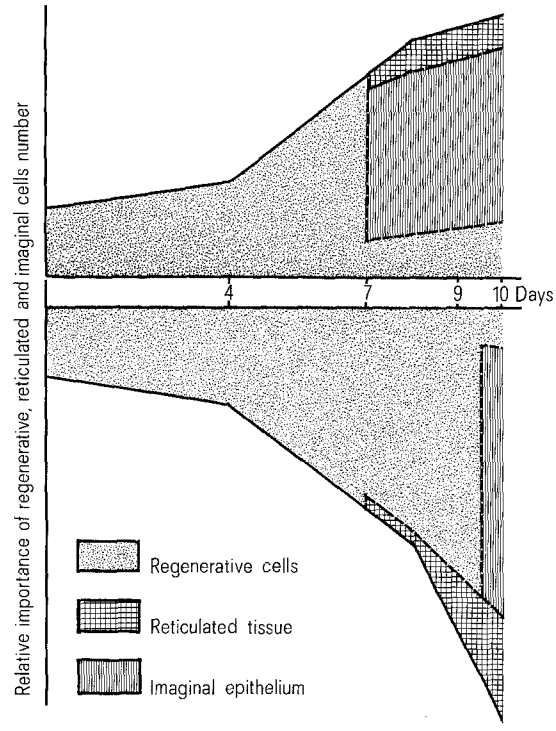

Fig. 2. Midgut response to α- (lower graph) or β-ecdysone (upper graph) injection. The relative importance of the regenerative, reticulated tissue and imaginal epithelium cells number indicated in Y-axis is a function of the delay following injection time (in X-axis). The genesis of the reticulate tissue occurs on day + 7, whereas the imaginal epithelium begins to differentiate on day + 7 (β-ecdysone injection) or on day + 9 or + 10 (α-ecdysone supply). After the imaginal epithelium genesis, there are still some regenerative cells left which continue to divide actively.

Values of the mitotic index in antepenultimate and penultimate larvae injected since 24 or 48 h with β-ecdysone

Larval instar	Amount of β-ecdysone injected (µg/g net weight)	Mitotic index 24 h after injection	48 h after injection	Controls
Antepenultimate	10	$14.7 \cdot 10^{-4}$	$33.5 \cdot 10^{-4}$	
Penultimate	30	$29.5 \cdot 10^{-4}$	$35.9 \cdot 10^{-4}$	$15.6 \cdot 10^{-4}$

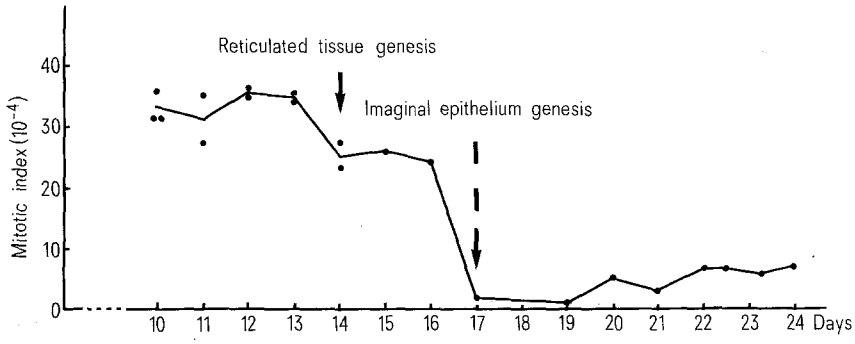

Fig. 3. Mitotic index variation in controls during the 2nd half of the last larval instar. The reticulated tissue genesis occurs on day 14, the imaginal epithelium differentiation happens on day 17.

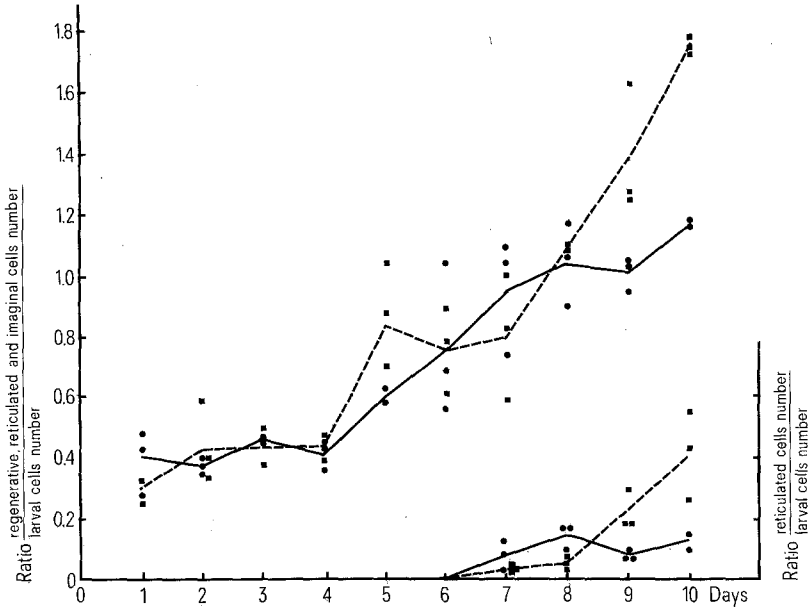

Fig. 4. Variation of the ratio: regenerative + reticulated tissue + imaginal cells/larval cells during the 10 days following α-(– – –) or β-ecdysone (——) supply. The graph situated in the righthand corner concerns only the ratio: reticulated tissue cells/larval cells (square: value obtained after α-ecdysone injection; circle: value obtained after β-ecdysone injection).

tion of the imaginal epithelium sooner. Consequently it is made up of fewer cells than in controls or in α-ecdysone injected larvae. For the same reason, the reticulated tissue is very much reduced, whereas it is more important after α-ecdysone supply.

As the entire renewal of the epithelium occurs only at the last larval instar, it may be asked whether α- or β-ecdysone stimulate DNA synthesis outside this time. To answer this question, β-ecdysone was supplied to 29 fasting antepenultimate or penultimate larval instar which were killed 24 or 48 h afterwards. From the results, given in the table, we can say that the value of the mitotic index differs significantly between controls and 48-h β-ecdysone injected antepenultimate or penultimate larvae.

The midgut of Insecta is an original model whose regenerative cells always respond to ecdysones by stimulation of their DNA synthesis. In last larval instar, cell multiplication is followed by cell differentiation when α- or β-ecdysone supply is adequate. The time course of mitosis and the number of regenerative cells triggered to synthesize DNA are the same after α- or β-ecdysone supply. What is different is the number of mitotic cycles that regenerative cells undergo before their differentiation.

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