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Stimulation of RNA Synthesis by Juvenile Hormone in isolated Fat Body Nuclei from Calliphora Erythrocephala

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The foregoing paper by Carroll Williams has already outlined our modern concept on the mechanism of action of insect hormones. It is based mainly on the observation of Clever and Karlson (1960) that ecdysone can induce puffing in salivary gland chromosomes. This was interpreted as induction of gene activity, or in biochemical terms as derepression of certain genes with consequent RNA and protein synthesis.

Later work by Sekeris and Karlson (1966) has shown that this is indeed true for the action of ecdysone in Calliphora. Stimulation of RNA synthesis was demonstrated in the whole animal (Karlson and Peters 1965) as well as in isolated nuclei of epidermal cells (Sekeris, Lang and Karlson 1965, Sekeris, Dukes and Schmid 1965). This system of isolated cell nuclei eliminates a number of variables and underlines the fact that ecdysone is indeed acting at the level of the cell nucleus, not at the level of membranes as proposed by Kroeger (for a detailed discussion, see Karlson and Sekeris 1966).

Since epidermal cell nuclei are somewhat difficult to obtain, we have used fat body nuclei for the experiments to be described. The isolation of fat body nuclei turned out to be more difficult than expectedi In epidermal cells, the difficulty lies in the isolation of the epiderma. tissue ; in fat body cells, the difficulty lies in the isolation of nuclel because they are large, lobulated and very fragile. Intensive homogenization of the tissue must be avoided ; moreover, attempts to isolate the nuclei by differential centrifugation yielded fractions which were heavily contaminated with cytoplasmatic bodies. We have therefore developed a new method for the isolation with the use of millipore filters.

Fat body tissue was gently homogenized, filtered through cheesecloth and a nylon net with 50 μ pores and centrifuged. The sediment was resuspended and filtered through a millipore filter of about 8 μ pore width. The nuclei were collected on the filter, while

contaminating cytoplastic bodies and fragments of endoplasmatic reticulum went through. The experiments to be described were all carried out with these nuclei on millipore filters.

First, the optimal incubation conditions where worked out. RNA synthesis, i.e. the incorporation of labelled UTP into TCA-precipitable material, was stimulated by Mg^{2+}-Ions (5–7 mM) and to lesser extent by Mn^{2+}. In the complete system, all four nucleoside triphosphates have to be present, together with an energy generating system, i.e. creatinphosphate + creatinphosphokinase. The influence of other inorganic ions was tested; Na^{+} was stimulating in concentrations of 50–150 mM, while K^{+} had no significant effect on uridine incorporation (Congote, Sekeris and Karlson 1969). This is just the opposite alkali ion dependence as that found by Kroeger (1966) and Lezzi (this symposium).

Both ecdysone and juvenile hormone have a significant stimulating effect on the RNA synthesis in these isolated fat body nuclei as shown in Figure 1.

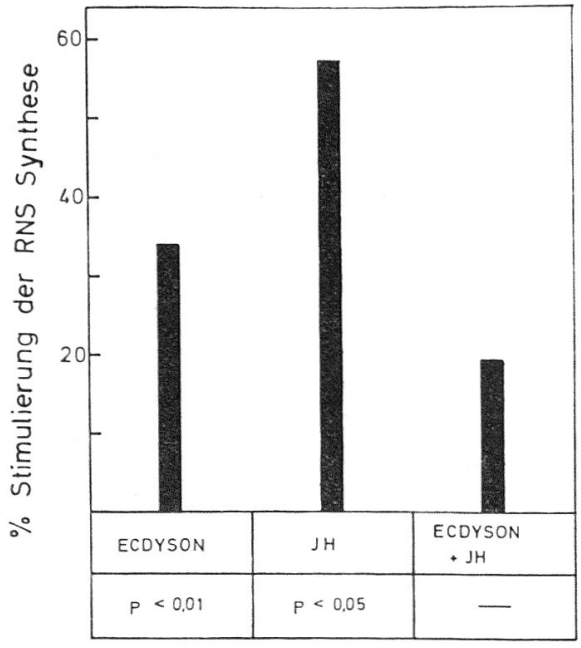

Fig. 1. — Effects of ecdysone and juvenile hormone on RNA synthesis of isolated fat body nuclei from five days old larvae.
The nuclei were incubated for 10 min in the presence of the hormones alone and then for a further fifteen minutes in the presence of the RNA synthesizing mixture. The concentrations of the hormones in the medium were 1 µg/ml ecdysone and 0.1 µg/ml of a juvenile hormone preparation containing 24% of the trans, trans, cis isomer (JH). The final medium contained 0.5 µMol each of ATP, CTP and GTP, 0.2 µC ^{14}C-UTP (sp. act. 54 mC/mM), 3.0 µMol creatine phosphate, 10 µg creatine phosphokinase, 3.0 µMol mercaptoethanol, 6 µMol $MgSO_4$, 60 IU penicilline G and 20 µMol tris-HCl pH 7.9, end volume 0.3 ml.

We tried to characterize the RNA synthesized under the influence of ecdysone and juvenile hormone. The sedimentation characteristics do not differ among control, juvenile hormone — and ecdysone-treated nuclei. However, by the method of DNA-RNA-hybridization it was shown that ecdysone and juvenile hormone gave rise to new RNA species ; the amount of hybridizable RNA under conditions of saturation with DNA was significantly higher in ecdysone — and juvenile hormone-treated nuclei as in controls (Fig. 2a). The same conclusion

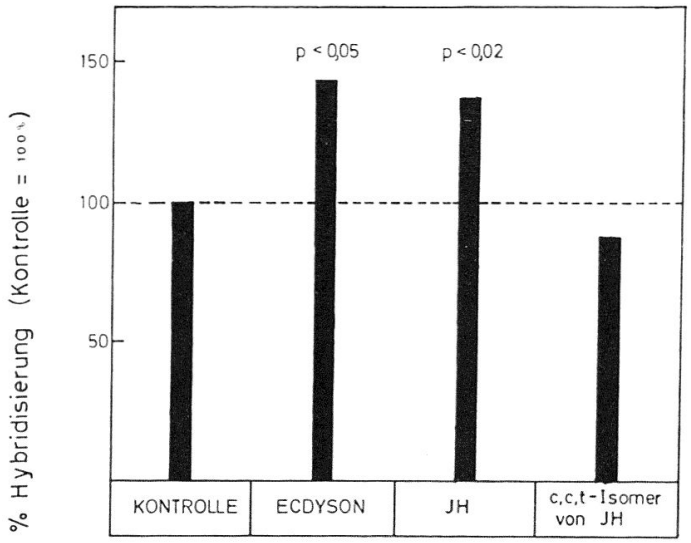

Fig. 2a. — Hybridization of RNA isolated from hormone treated nuclei.
The nuclei were stimulated in vitro as described in legends to Figure 1. RNA was extracted by the hot phenol method. Hybridization was performed in principal according to Nygaard and Hall under conditions of DNA saturation (DNA : RNA = = 5 : 1). The hybridization values of the control RNA has been taken as 100%. p-values from 10 experiments.

can be drawn from experiments with competitive DNA-RNA-hybridization as shown in Figure 2 b.

As shown in Figure 1, ecdysone and juvenile hormone when given together, give less stimulation of RNA synthesis than one hormone alone ; the stimulation is not even statistically significant. This effect was further investigated. One possibility was that one hormone could possibly set the stage for the action of the second one. Therefore, nuclei were preincubated with ecdysone and then juvenile hormone added ; in another experiment, both hormones were added simultaneously. There was no difference between these experiments, i.e. a preconditioning of the nuclei could not explain our results. However, we got at least some evidence from experiments with competitive hybridization between RNA from ecdysone and juvenile hormone treated nuclei (Tab. 1).

TABLE 1

Cross-reaction of RNA synthesized in vitro under influence of Ecdysone or J.H. in competitive hybridization

Labelled RNA synthesized in	Competitive RNA synthesized in		
	control nuclei	ecdysone-treated nuclei	J.H.-treated nuclei
Ecdysone-treated nuclei	91% 64%	— —	66% 59%
J.H.-treated nuclei	54% 59%	42% 55%	— —

Values without competitive RNA = 100% ; total RNA : DNA = 12 : 1 ; labelled RNA : competitive RNA = 1 : 1

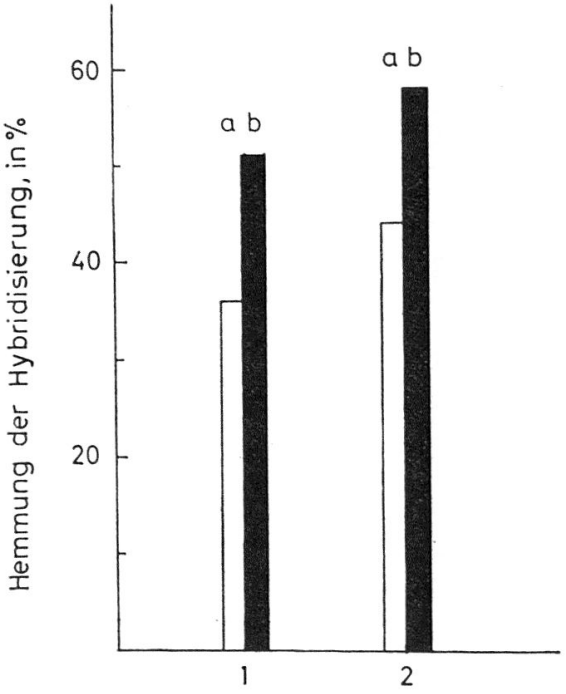

Fig. 2b. — Competitive DNA-RNA hybridization of RNA from hormone treated nuclei.

1. Competition of ^{3}H-RNA from ecdyson treated nuclei with non-radioactive RNA form *a)* control and *b)* ecdysone treated nuclei. (Non-radioactive to radioactive RNA = 1 : 1).

2. Competition of ^{3}H-RNA from juvenile hormone treated nuclei with non-radioactive RNA from *a)* control and *b)* juvenile hormone treated nuclei.

Stimulation of the nuclei with the hormones as described in legend to Figure 1.

From the data presented it can be concluded that at least a part of those genes which are activated by ecdysone, are also activated by juvenile hormone. On the other hand, the fact that simultaneous action of both hormones leads to less stimulation of RNA synthesis can be tentatively explained by the hypothesis that the repressor(s) of some genes can combine simultaneously with juvenile hormone and ecdysone ; in this case no derepression occurs, i.e. transcription of this locus is not initiated. This would result in lower RNA synthesis rates. However, more experiments are needed to clarify the interaction of juvenile hormone and ecdysone at the level of the cell nucleus.

Another experiment might be mentioned in relation to these observations. There is a significant difference among nuclei isolated from larvae of different ages in the response to ecdysone and juvenile hormone. The nuclei from about five days old larva (larvae weight 60 mg) respond quite well to the action of juvenile hormone ; the effect is lower with 6 days old larvae and practically nil with seven and eight days old larvae with a larvae weight of 90–100 mg (Fig. 3). In the terms of developmental physiology, one might say that the nuclei of younger larvae are competent to respond to juvenile hormone, while the older nuclei are not.

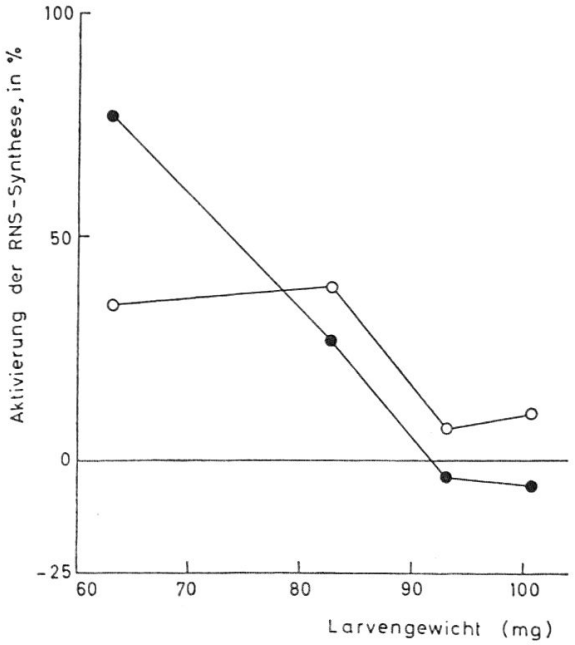

Fig. 3. — The dependence of hormonal stimulation of RNA synthesis of isolated fat body nuclei from Calliphora on the developmental stage of the animal.
Fat body nuclei isolated from four groups of larvae of different weight were incubated in the presence of ecdysone or juvenile hormone as described in legend to Figure 1. RNA synthesis was measured by the incorporation of ^{14}C-UTP into acid precipitable material.
Abscissa : weight of animals, ordinate : % activation or inhibition over the controls.
o———o by ecdysone and •———• by juvenile hormone

To my knowledge, this is the first time that "competence" has been observed at the subcellular level in an in vitro system. It should be quite interested to investigate this system further.

In summary, the experiments described demonstrate that juvenile hormone as well as ecdysone can stimulate RNA synthesis in isolated nuclei. The scheme for the mechanism of hormone action first introduced by Karlson (1961, 1963) for ecdysone has therefore been substantiated for juvenile hormone in *Calliphora*.

The interaction of ecdysone and juvenile hormone seems to be rather complicated in that both hormones are apparently competitive ; the mechanism of this competition awaits further study. Also, our observations about the competence of cell nuclei to respond to ecdysone and juvenile hormone may lead to further interesting observations about the nature of cellular competence.

We do not doubt that our hormones which act at the level of the cell nuclei react directly with the chromatin material, presumably with the regulatory proteins of the chromosome.

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