intracellular receptor molecules, or by an active transport of ecdysteroids into distinct compartments.

Since the titer of ecdysteroids in the hemolymph of male flies is low, the ecdysteroids must be concentrated in some other tissues. By radioimmunoassay technique, we found one-third of the total of ecdysteroids in the testes (table). Since these make up only a minor fraction of the biomass of the insect, the local concentration of ecdysteroids in testes is extraordinarily high. In female flies, on the other hand, most of the ecdysteroids can be detected in the hemolymph which has a high titer similar to that in the testes. In comparison to the remainder of the insect, the ovaries also contain a relatively high concentration of ecdysteroids.

What type of ecdysteroid is detected in the adult fly? At present we can give only a preliminary answer. In TLC (silica gel, Merck F60, solvent: chloroform/methanol 80/20) as well as in HPLC (Absorbent: Poragel PN, Waters, or Lichrosorb RP8, Merck, elution with methanol/water) the ecdysteroid from male as well as female adult flies behaves like ecdysterone (=20-hydroxy-ecdysone). An HPLC-analysis, which we owe to E.S. Chang (University of California, Los Angeles), shows that in methanolic extracts of blowflies more than 85% of the ecdysteroids detected by radioimmunoassay with antiserum M-20, is ecdysterone in males as well as in females. Ecdysone is found only in minor amounts. Although only mass spectral analysis of the ecdysteroid from adult blowflies will give a clear proof for its molecular identity, the analyses with different chromatographic systems (TLC and HPLC) in combination with ecdysteroid specific antisera (H21B and M-20[4,8]) make ecdysterone the most likely candidate.

Our results demonstrate that moulting hormones (=ecdysteroids) occur in male blowflies, as they do in male fruitflies[7], and that the hormone detected is mainly ecdysterone. This is in contrast to locusts, in which ecdysteroids exclusively occur in adult females and ecdysone is the most prominent ecdysteroid[9]. Parallel to these findings, a high concentration of ecdysterone was detected in the testes of other arthropods, the heteropterian bug *Dysdercus intermedius* (unpublished observations) and of the brachyurian crustacean *Pachygrapsus crassipes*[10].

What is the biological function of ecdysterone in male adult blowflies? From the high concentration in the testes it can be assumed that ecdysterone plays a role in the morphogenesis of the reproductive system and in reproduction itself. This is in agreement with the observation that the imaginal differentiation of the testes and the spermatogenesis in insects require ecdysteroids[11,12].

Is the ecdysteroid part of the seminal fluid, as can be speculated on the basis of results of Hadorn and Bellido[13], who found that imaginal discs grow better in fertilized than in virgin female flies? We have tried to answer this question by injection of radiolabeled ecdysone (5.7 ng, 0.85 nCi per insect) into male adults, which were kept together with the same number of female flies. 6 h later the flies were separated according to their sex and were analysed for radioactivity. Both male and female flies were radioactive, containing 0.9% and 9% respectively, of the amount injected. However, in a control experiment where female flies were injected analogously, radioactivity could be detected in male flies as well, after a 6-h contact of both sexes. Thus, the transfer of radioactivity from male to female flies could be unspecific due to the uptake of excreta, and the question of whether ecdysteroids occur in the seminal fluid of the blowfly must be left to further experiments.

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Does the insect brain count larval instars?

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Summary. It has been presumed on the basis of transplantations of the neuroendocrine complex that the 'counting of instars' mechanism lies within the brain. The brain is programmed already in the 3rd instar to inactivate the corpus allatum. The inactivation sets in in the late penultimate (4th) instar.

Insect metamorphosis takes place when the corpus allatum (CA) ceases to secrete juvenile hormone (JH)[2]. Evidence has been provided that the CA is inactivated by the brain prior to metamorphosis[3-8]. The physiological nature of the signal causing the brain to inactivate CA is still unknown. This function has been ascribed to a certain critical threshold rate of growth[9], or a threshold weight[10,11] reached in the last instar. Various signals that the brain receives in the last instar may be important for the inactivation of the CA, but changes in the neuroendocrine system in earlier instars might be of the same importance. The idea of the 'counting of instars' by the central nervous system[12] fits this presumption.

In *Pyrrhocoris apterus*, the CA is inactivated in the late penultimate (4th) instar[13]. I have investigated whether changes in the neuroendocrine system in earlier instars might also affect the process of CA inactivation.

The larvae of *P. apterus* L. (Hemiptera) were reared on linden-seed at 25±1 °C and daylengths of 18 h. Recipient larvae were deprived of food within a few h after ecdysis

and operated upon the next day. The glands were implanted through an incision in the abdomen. Each animal received the glands from 1 donor. The endocrine activity of the implanted glands was measured by their ability to induce development into adultoids instead of adults. 5th (last) and 4th instar recipients were evaluated after their adult ecdysis.

The activity of the neuroendocrine complex of the brain + corpora cardiaca + CA (BR + CC + CA) from larvae of different ages was tested by transplanting the complex into the 4th instar larvae. Figure A shows that more than 2/3 of the complexes taken from early 3rd instars induced development into adultoids. This could be due to their own secretion of JH or to activation of the host's glands. In contrast, the complexes were almost inactive when taken from the late 3rd or early 4th instars (figure B–D). This indicates that the endocrine functions of the complex were changed during its passage through the 3rd instar.

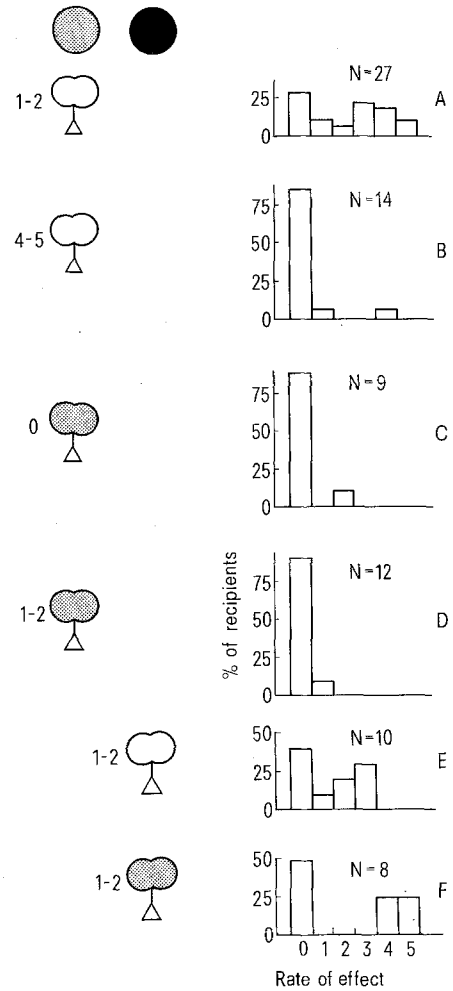
When 5th instar larvae served as recipients, the complexes from both the early 3rd and early 4th instars induced development into adultoids in at least 50% of recipients (figure E and F). Thus, in this case, the complex taken from the early 4th instar was still able to produce the JH-effect, but its activity was subsequently lost within the 4th instar. The change which the complex underwent in the 3rd instar is hereafter referred to as potentiation.

In contrast to the transplantation of the whole neuroendocrine complex, no difference was observed when the CA without the brain was transplanted into 4th instar larvae. The CA from the early 3rd as well as early 4th instars had no effect on the development of the recipients. Therefore, the JH-effect produced by the complex of BR + CC + CA from the early 3rd instars (figure A) may have been caused by stimulation of the CA function from the brain. Conversely, the failure of the complexes from the late 3rd or early 4th instars to produce the JH-effect (figure B–D) might be due to the loss of the stimulatory action of the brain. According to these presumptions, potentiation of the neuroendocrine complex probably originates within the brain.

It still remains to be investigated whether the chain of events leading to the arrest of CA activity after potentiation is irreversibly time-dependent, or whether the internal milieu of the 4th instar recipients plays a substantial role. The 2nd possibility is quite feasible, because inactivation of the CA in situ is development-dependent, but not dependent on absolute time. For example, the CA of 4th instar larvae retained its ability to produce JH even when development was interrupted by long periods of starvation (unpublished observation).

The results obtained in *P. apterus* suggest that the mechanisms engaged in the 'counting of instars' reside in the neurocrine system of the brain. Apparently they are already programmed in the 3rd larval instar, being finally realized in the late penultimate (4th) instar.

Due to a lack of comparative data, it is not clear whether the mechanism assumed for *P. apterus* would also apply to other insects. It is claimed that in *Galleria mellonella* the brain can recognize the maximum rate of body growth, to which it responds by inactivating the CA[9]. It cannot be decided here whether some earlier developmental changes in the brain are important. Unlike in *P. apterus*, the brains taken from any larval instar of *G. mellonella* stimulate the CA in the same recipients where the brain in situ has an inhibitory function[7]. In another lepidopteran species, *Manduca sexta*, the 'counting of instars' mechanism has also been ignored on account of a threshold weight stimulus[11]. A substance released at the critical 5 g b.wt and inhibiting the CA via the brain has been found in this species[14]. However, the effectiveness of this substance has not been tested for possible inhibition of the neuroendocrine complexes in early larval instars.



Effects of the complex of BR + CC + CA on the development of 4th and 5th instar recipients. Circles symbolize the body of recipient, double spheres with triangles symbolize the complex of BR + CC + CA from donors. Instars of donors and recipients are indicated, as follows: open = 3rd instar, dotted = 4th instar, and solid = 5th instar. Numbers left of the symbols indicate days after ecdysis of donors. The classification of adultoids is based on a published scoring system[15]. Juvenilization increases from 1 to 5.

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