Ecdysteroids in the adult male blowfly *Calliphora vicina*

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Summary. Male adult blowflies contain ecdysteroids. A considerable amount of ecdysteroid is concentrated in the testes and gives rise to a high local concentration. Analysis by high performance liquid chromatography and thin-layer chromatography indicate that ecdysterone is the most prominent ecdysteroid in male flies. The results can explain several hormone-dependant processes.

It is well known that growth and differentiation of imaginal discs during metamorphosis of insects are under hormonal control[1]. Bodenstein transplanted eye and leg discs into adult female fruit flies *(Drosophila melanogaster)* and observed that they grew and underwent metamorphosis[2]. When these discs were transplanted into adult male *Drosophila,* they metamorphosed only if larval ring glands which secrete moulting hormones were simultaneously transplanted into the hosts. Similar experiments were reported by Nöthiger and Oberlander with genital discs[3]. These authors suggested that different concentrations of the moulting hormones (ecdysone and/or ecdysterone) in the male and female hosts are responsible for the differentiation of the transplanted discs. Accordingly, female *Drosophila* flies should contain significantly more moulting hormones than male flies.

We are now in a position to analyse the titer of ecdysteroids in the blowfly *(Calliphora vicina)* with a very sensitive method, the radioimmunoassay[4]. Hence we repeated Bodenstein's experiments with *Calliphora* in order to correlate growth and differentiation of imaginal discs with the concentration of ecdysteroids.

Leg discs of *Calliphora* larvae were transplanted into 1-day-old adults of both sexes; 9 days later, the growth of the transplanted discs in male and female hosts was compared. The larval leg discs were dissected in insect Ringer solution[5] and their diameter was measured with an ocular micrometer. 4 discs were transplanted into the abdomen of each imago. In 1 series of experiments, 4 ring glands were transplanted simultaneously with the discs. 9 days after transplantation the discs were dissected from the host animals and measured.

The results are depicted in the figure. A significant difference between the transplants in ♂♂ and ♀♀ was detected. The discs in ♀♀ had doubled their diameters and reached an average size of 23 units. At this size they began to evaginate in vivo. The discs in ♂♂ had grown very little, 3 units at the maximum. But when ring glands were transplanted simultaneously, the discs in ♂♂ grew to a size comparable to the size in ♀♀.

From these results we conclude that the growth rate of the discs depends upon the concentration of one or more substances secreted by the ring gland. Ecdysteroids, which have now definitely been shown to be secreted by ring glands[6] might be the active principle. Our results could be explained by assuming a significant amount of moulting hormones in adult female blowflies. Therefore we wished to provide evidence that the ecdysteroid concentration is different in male and female *Calliphora* flies.

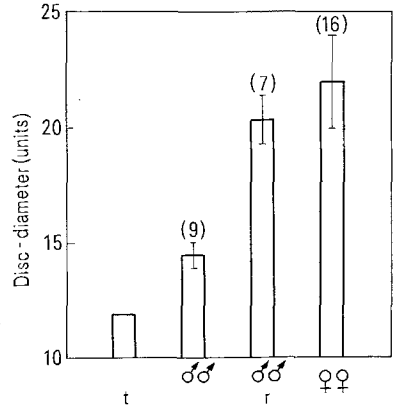
The amount of ecdysteroids in homogenates of male and female adults of the blowfly (*Calliphora vicina* R.D.) was analysed by radioimmunoassay with antiserum H21B (gift of J.D. O'Connor, University of California, Los Angeles). Mature male flies (8 days after emergence) contain 150 ng and female flies 138 ng ecdysone equivalents per g liveweight. These data are in contrast with those of Hodgetts et al.[7], who found more RIA-positive material (ecdysteroids) in female adult *Drosophila* than in males. The question is why imaginal discs cannot grow in male blowflies inspite of the high ecdysteroid content.

The medium in which the transplanted discs grow is the hemolymph of the host insect. We therefore analysed the titer of ecdysteroids in hemolymph of adult blowflies (table). The titer in the hemolymph of the female blowflies is 7 times higher than in the hemolymph of male flies. This result is in agreement with the assumption that the growth of imaginal discs is induced by ecdysteroids (ecdysone or one of its metabolites). Moreover it demonstrates that the hemolymph titer of ecdysteroids may differ from the average content in the whole insects. The differences reflect an uneven distribution of hormone, which may be caused by

Ecdysteroids in adult blowflies

Tissue	Male Concentration (ng/g)	Amount relative to total (%)	Female Concentration (ng/g)	Amount relative to total (%)
Testes	28.6	35	–	–
Ovaria	–	–	7.3	14
Hemolymph	4.4	14	30.8	57
Rest of insect	1.7	51	2.3	30

10 days after eclosion mature male and female blowflies (*Calliphora vicina* R.-D.) were dissected into gonads, hemolymph and carcass (rest of the insect). The hemolymph was separated from hemocytes by centrifugation. The tissues were homogenized in methanol and analyzed by radioimmunoassay with antiserum H21B as described elsewhere[4]. The concentrations of ecdysteroids detected by radioimmunoassay were expressed as equivalents of ecdysone and were related to g weight of the tissues.



Comparison of growth of transplanted equal leg discs in ♂ and ♀ hosts. Donor discs from 5-day-old larvae, hosts: 1-day-old imagines at the time of transplantation (t). ♂♂ r represents the experiment when 4 ring glands from 6-day-old larvae were transplanted together with the discs. The numbers in parentheses indicate the number of cases, the vertical bars SD. 1 unit = 25 µm.

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.

1 H. Ursprung and R. Nöthiger, The biology of imaginal discs. Results and Problems in Cell Differentiation, vol. 5. Springer, Berlin, Heidelberg, New York 1972.
2 D. Bodenstein, J. exp. Zool. *84*, 23 (1940).
3 R. Nöthiger and H. Oberlander, J. exp. Zool. *164*, 61 (1967).
4 L. Reum and J. Koolman, Insect Biochem., in press (1978).
5 V.J.A. Novak, in: Insect hormones, p. 20. Methuen Co. Ltd, London 1966.
6 W.E. Bollenbacher, W. Goodman, W.V. Vedeckis and L.I. Gilbert, Steroids *27*, 309 (1976).
7 R.B. Hodgetts, B. Sage and J.D. O'Connor, Dev. Biol. *60*, 310 (1977).
8 D.H.S. Horn, J.S. Wilkie, B.A. Sage and J.D. O'Connor, J. Insect Physiol. *22*, 901 (1976).
9 M. Lagueux, M. Hirn and J.A. Hoffmann, J. Insect Physiol. *23*, 109 (1977).
10 E.S. Chang, B.A. Sage and J.D. O'Connor, Gen. Comp. Endocr. *30*, 21 (1976).
11 J. Nowock, J. Insect Physiol. *18*, 1699 (1972).
12 M.P. Kambysellis and C.M. Williams, Biol. Bull. *141*, 541 (1971).
13 E. Hadorn and A. Garcia-Bellido, Revue suisse Zool. *71*, 576 (1964).

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