surements were performed in the laboratory of Dr E. Polig; Kernforschungszentrum, Karlsruhe). 2 sections of 2 animals were analysed per bone fragment. The density of colony-forming stem cells was highest in the femoral diaphysis, 50% less in the lumbar vertebrae and the distal femoral end, and more than 5 times less in the sternum (table 1). With the ^{3}H-Tdr suicide technique the mitotic activity of the CFUs in several bone marrow sites may be compared. Corroborating earlier findings[8], about 40% of CFUs were in S-phase in the peripheral part of the femoral shaft, whereas in the axial femoral marrow only a few of them were mitotically active. In the distal end of the femur, about 40% of the CFUs were in S-phase. It is an open question why the proportion of mitotically active cells is important in the peripheral diaphysis and the distal epiphysis of the femur and why it is not in the lumbar vertebrae, the sternum and the axial femoral marrow. No relationship with bone structure exists since both the distal epiphysis and the lumbar vertebrae are characterized by a large ratio of endosteal surfaces per unit volume of marrow.

Variation in CFUs concentrations between individual mice (table 2) was of the same magnitude for the different marrow sites, and about the same in SPF mice (between 0.4 and 6.6) and in conventional mice (between 0.7 and 6.7). Individual variability was small ($p < 0.05$) compared with other experimental errors (between 9.1 and 33.7 for SPF mice and between 9.7 and 25.4 for conventional mice). In the pooled experiments more recipient animals were used (10 instead of 6) but the variance was not influenced. Pooling cell suspensions instead of working with marrow suspensions of individual mice did not influence the total variance (table 2).

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Tissue distribution of juvenile hormone hydrolytic activity in *Galleria mellonella* larvae

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Summary. Juvenile hormone (JH) hydrolytic activity was determined in different tissues of day-4 last instar larva of *Galleria mellonella*. Midgut, gonad, imaginal wing discs and fat body contain higher JH hydrolytic activity than hemolymph, while silk gland and body wall have lower activity. JH esterase activity in imaginal wing discs exhibits a pattern of age-related changes different from that of the hemolymph.

Juvenile hormone (JH) inhibits metamorphosis of juvenile insects and differentiation of adult characteristics[3]. In several lepidopteran, coleopteran and orthopteran insects endogenous JH is inactivated by hemolymph JH esterase (JHE) just before initiation of a metamorphic molt[4-8]. Although it was shown that in *Manduca*[9] and *Galleria*[10] fat body is the source of hemolymph JH esterase, distribution of JH hydrolytic activity in other tissues of insect larvae has not been studied extensively. Furthermore, inactivation of JH prior to metamorphosis is critical for normal post-embryonic development of insects[3,11], and it has been shown that hemolymph JHE may play an important role in regulation of differentiation of tissues in larval insects.[6]. Hence it is important to know the tissue distribution of this enzyme activity in last instar larvae. In the present report data on JHE activity in extracts of fat body, gonad, midgut, body wall (containing cuticle, some muscle tissue, and the chitogenous epithelium) and imaginal wing discs from last instar larva of *Galleria* and age-related changes in JHE activity in the wing discs are presented.

Wax moths, *Galleria mellonella*, were reared in the laboratory according to procedures described earlier[12]. Last instar larvae were collected within 8 h after ecdysis into the instar and were aged for use in these studies. Larvae less than 8 h after ecdysis into the last stadium are designated as day-0 larvae. Larvae aged for 24 h after collection are designated as day-1 larvae and so on. When raised under optimum conditions of density the rate of growth of different larvae is nearly uniform, all pupating in 8 days $\pm$ 12 h.

Specific tissues were dissected from larvae and cleaned of attached tissues. They were rinsed first in Ephrussi-Beadle Ringers and then in 0.05 M phosphate buffer pH 7. The tissue was homogenized in the buffer and centrifuged at $10{,}000 \times g$ for 10 min, and the supernatant was used for all assays. JHE activity in the supernatant was determined by procedures described in the literature[4,6]. In brief, this procedure consists of incubations of an aliquot of the supernatant (50 - 500 µg protein) with labelled JH for 10 to 30 min at 30 °C. The time was varied depending on the total amount of JH hydrolyzed. All incubations were controlled such that only 20 to 30% of JH in the incubation mixture was hydrolyzed by the end of the incubation period. At the end of the incubation the unhydrolyzed JH, JH acid, JH diol and JH acid-diol were separated by TLC and were quantitated by scintillation counting. At least 1 sample of each series was measured in the presence of DFP which inactivated general carboxylesterases. In the studies using gonads, penultimate larval midgut and fat body, JHE

activity was measured by determining the amount of radioactive methanol released from JH labeled in the methoxy group[13]. Protein content of supernatant solutions was determined according to the method of Lowry et al.[14] using bovine serum albumin as a standard. All enzyme assays were run in triplicate and the data reported show the average amount of JH acid formed per g of protein per min. The total amount of radioactivity in JH diol and JH acid-diol in these studies was less than 25% of the acid formed and only the data pertaining to the JH acid is presented as nmoles/g of protein in the extract and the total wet weight of the tissue.

The data pertaining to JHE activity of silk gland, body wall, gut, fat body, gonad and imaginal wing discs from day-4 last instar are recorded in the table. For purposes of comparison, JH esterase activity of hemolymph is also included. The data presented here show that JHE activity of silk gland and body wall, whether presented as nmoles JHA per g protein or g wet weight of tissue, is lower than that of hemolymph and other tissues studied. The data also show that the JHE activity of gonad, gut, fat body, and wing discs is higher than that of hemolymph if presented as nmoles/g protein.

Changes in imaginal wing disc JHE activity during the course of the last larval instar are presented in the accompanying figure, and the data show that on day 1 JHE activity is very low, and is less than 10% of the day-2 level. The enzyme activity in wing discs peaked on day 2 and declined to a very low, nearly undetectable, level by day 5.

The data presented in this report show that JH esterase activity is present in many insect larval tissues in addition to hemolymph and fat body. It is not surprising that JHE activity in fat body is the highest of all tissues studied because fat body has been shown to be the source of hemolymph esterase in *Cecropia* as well as in the wax moths[10,15]. However, it is interesting to note that JHE activity in gonads, midgut and imaginal wing discs of day-4 last instar *Galleria* larva is also relatively high. All these tissues are sensitive to JH and their differentiation is dependent on the removal of JH. The relatively low JHE activity in silk gland and particularly in the tissues of the body wall is very surprising. But the apparent low JHE activity in these tissues may be a function of the high proportion of secretory proteins in these tissues.

The fact that JHE activity in wing discs peaks on day 2, 2 days before the peak enzyme activity in hemolymph, may have a bearing on the association between the enzyme activity and reprogramming and differentiation of this tissue. Studies on *Plodia* show that even a small amount of exogenous JH affects differentiation of wing discs both in vitro and in vivo[16].

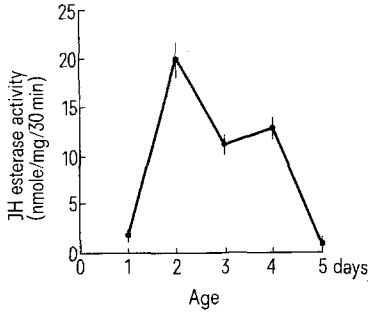
In *Plodia,* and in *Galleria,* imaginal wing discs become less sensitive to JH very early during the last larval instar, long before the chitogenous epithelium becomes insensitive to JH[16,17].

The observation that the wing disc JHE activity reaches its peak earlier than the hemolymph esterase activity may have a bearing on the early reprogramming of wing disc cells. By day 5 when the discs are no longer sensitive to JH they possess no JHE activity. The apparent close correlation between changes in sensitivity to JH, reprogramming of the genome, and JHE titers of larval wing discs is consistent with the current concepts that pupal differentiation occurs only after the removal of endogenous JH from their target tissues. Furthermore, the early appearance of JH esterase that is correlated with the early reprogramming of the disc cells even before the 1 peak of ecdysone suggests absence of JH per se may be a primary factor in reprogramming of the genome[6] and that JHE represents a specific developmentally significant enzyme.

JH esterase activity in different tissues of day-4 last instar *Galleria* larva

Tissue	JH esterase activity nmoles/g protein/min	nmoles/g tissue/min*
Body wall	3.7± 0	0.679±0.0
Silk gland	10.0± 2	0.634±0.2
Midgut**	243.3±33	0.776±0.03
Fat body	898.0± 0	35.63 ±0.0
Hemolymph	90.0±10	62.63 ±1.0
Gonad	112.0± 9	52.5 ±4.4
Wing discs	446.7±33	–

JH esterase activity was measured according to the method described by Weirich et al.[3] except for data on fat body and gonad for which the procedure described by Vince and Gilbert[13] was used. * Because of losses during homogenization and because different amounts of fluid may remain adherent to the tissues the calculations based on fresh weight of tissue may be less reliable than those based on the amount of protein in the extract. Wing discs were too small to weigh. ** JH esterase activity of midgut from day-3 penultimate instar larvae is very low and could not be measured.



Age-related changes in the imaginal wing disc JHE activity during the last instar in *Galleria* larvae.

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