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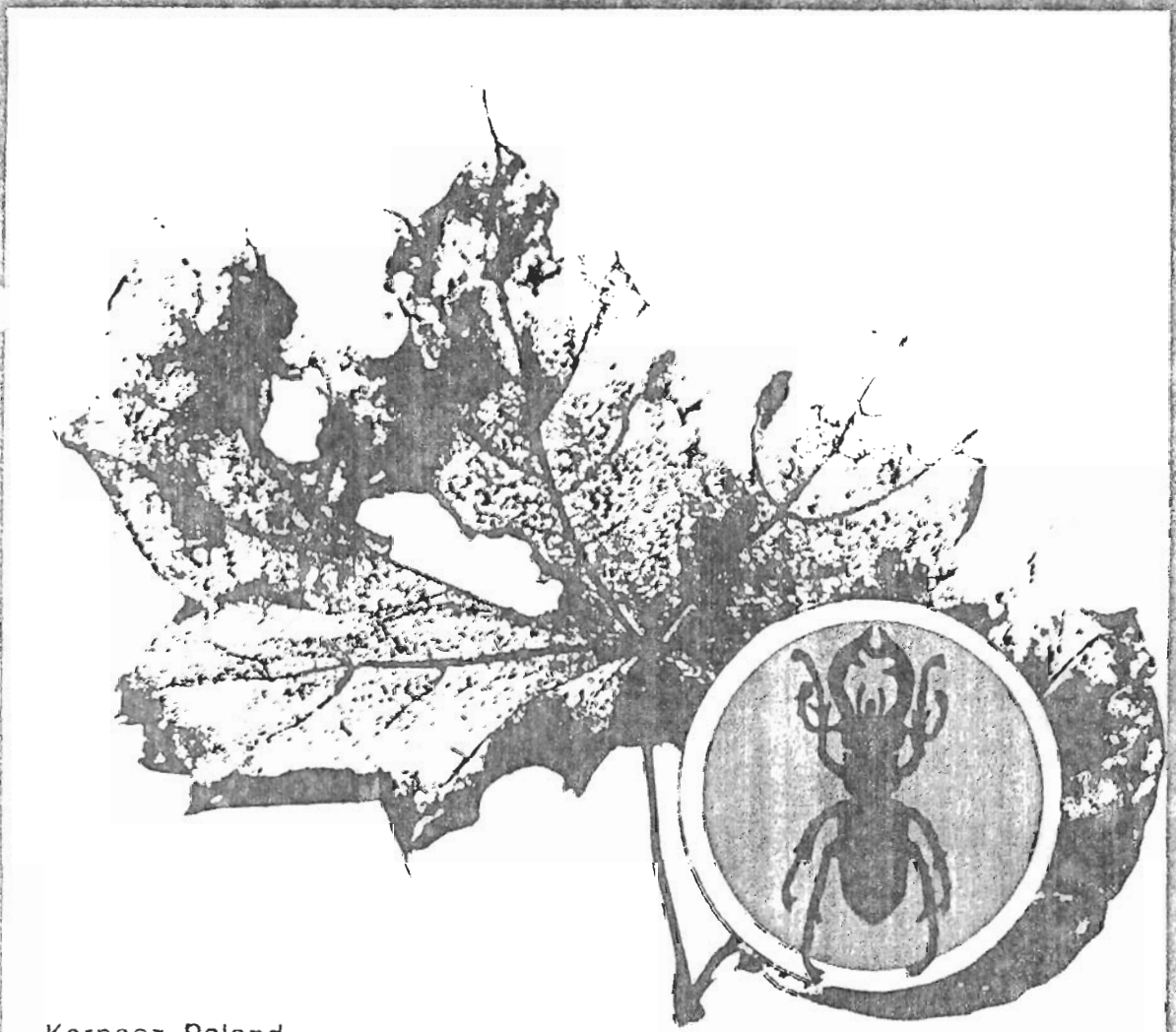

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REGULATION OF JUVENILE HORMONE ESTERASE IN THE CABBAGE LOOPER, TRICHOPLUSIA NI

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Abstract. The catabolism of juvenile hormone (JH) in Trichoplusia ni (Lepidoptera, Noctuidae) is catalyzed largely by a hemolymph and fat body juvenile hormone esterase (JHE). A large prewandering and a prepupation peak of hemolymph and fat body JHE activity is observed. JHE activity is largely due to a single enzyme identical in the hemolymph and fat body throughout the last larval instar. The prewandering JHE peak appears to be under neurosecretory control and is responsive to environmental factors while the second or prepupation JHE peak occurs in response to JH itself. There is additional evidence for inhibitory factors from the brain turning off JHE production. Evidence supports the hypothesis that JHE is produced by the larval fat body in response to the above factors. A survey of species from several families with regard to JHE titer and the isoelectric points of the enzymes involved indicates that the regulatory mechanisms elucidated for JHE in T. ni may be applicable to some but not all lepidopterous species.

As evidenced by this text, the juvenile hormones (JHs) are important chemical mediators in the insects and they have probably been best studied in the Lepidoptera. Although definitive proof is still lacking, degradative metabolism as well as JH biosynthesis, release, transport and binding appear to be involved in JH action. Thus, our laboratory has investigated the enzymes involved in JH degradation and their regulation. The story is far from complete, but a clear picture has emerged on one major enzyme being involved in the primary step of JH degradation and on the apparent regulation of this enzyme by both epithelial and neuroendocrine mechanisms at various times during development. In this chapter, several years' work on the regulation of JH metabolism in the cabbage looper, Trichoplusia ni

(Hübner) will be summarized. Most of our discussion will be limited to T. ni since the applicability of our studies to other insect species is not yet known. We will, however, present some data which indicate that the situation observed in T. ni may be similar to that in several other Lepidoptera.

ROLE OF CATABOLISM IN JH REGULATION

Pathways of JH catabolism

The JHs have been shown to be catabolized by two major pathways in insects (Slade and Zibitt, 1972). Ester hydrolysis of the rather stable, conjugated methyl ester to JH acid is usually catalyzed by carboxylesterases while hydration of the 10,11-epoxide to a 1,2 glycol (JH diol) is usually catalyzed by membrane-bound epoxide hydrolases. The primary metabolites may be further catabolized to JH diol acid and/or conjugated (Fig. 1). Evidence to date indicates that all of the metabolites of JH are biologically inactive (for review, see Hammock and Quistad, 1976, 1980; deKort, 1981). The relative importance of these and other pathways of metabolism vary with the insect species and stage examined, but subsequent research has largely substantiated the hypothesis of Slade and Zibitt (1972) that ester hydrolysis is the major route of JH metabolism in the Lepidoptera (Weirich et al., 1973; Sanburg et al., 1974; Slade et al., 1976; Weirich and Wren, 1976; Vince and Gilbert, 1977; Hwang-Hsu et al., 1979; Mitsui et al., 1979; Reddy et al., 1979; Sparks et al., 1979a).

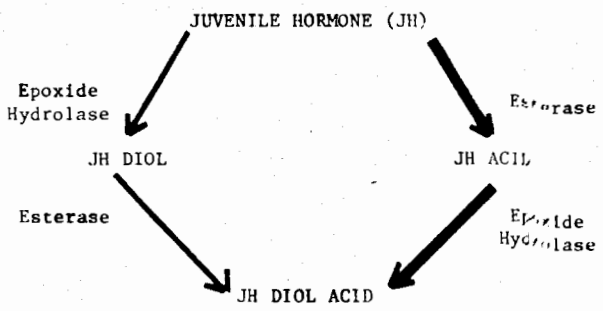

Fig. 1. Major pathways of juvenile hormone catabolism.

Compartmentalization of JH catabolism in *T. ni*

At this point, the discussion will be largely restricted to JH catabolism related to the larval-pupal transformation in T. ni. Unless otherwise noted, the data will refer to gate 1 animals which spent four days in the last (5th) larval instar. Trichoplusia ni has proven to be an excellent experimental animal for these studies because it is simple and inexpensive to rear and because synchronous subpopulations can be easily obtained.

Numerous physiological and morphological markers exist, facilitating very precise timing of developmental events. As a member of the family Noctuidae, it represents a group of the world's most damaging insects.

The metabolism of JH both in vivo and in vitro implicates ester hydrolysis as the major route of JH degradation. As indicated in Fig. 2, the hemolymph (HL) and fat body (FB) are very high in JH esterase (JHE) activity when expressed on a larval equivalent basis. Although JH catabolism in specific tissues may be developmentally significant in reducing the JH concentration in that tissue, the mass of the HL and FB coupled with the high specific activity of JHE in these tissues indicate that these two are the primary tissues involved in JH turnover in the last larval instar. Thus, our initial studies have been concentrated on these two compartments.

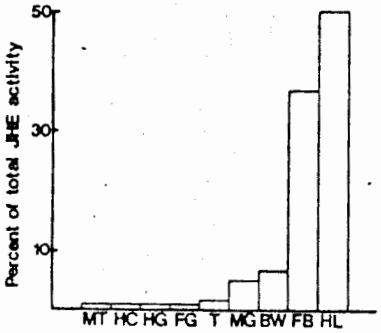

Fig. 2. Relative JHE activities in the tissues of last larval instar T. ni. Activities are expressed on a larval-equivalent basis. (MT = Malpigian tubules, HC = head capsule, HG = hindgut, FG = foregut, T = testes, MG = midgut, BW = body wall, FB = fat body, HL = hemolymph).

Titer of JH hydrolyzing enzymes

When the HL in T. ni was assayed for JHE activity during the last larval instar (L5) and pupal stage, two major peaks of activity were found: one on early day 2 (L5D2) and one on early day 4 (L5D4) of the last larval instar (Fig. 3). One minor peak of activity was also detected. Recent studies using morphological markers of development have demonstrated a very brief, but active burst of JHE at the L4 to L5 molt. Hemolymph JHE activity was low on early L5D1, L5D3 and during the pupal stage.

Fat body and midgut (MG) JHE activity appeared almost entirely in the 100,000X g supernatant, while epoxide hydrolase activity was entirely membrane-bound and largely in the 100,000X g pellet of both tissues. As previously reported with other Lepidoptera, epoxide hydrolase activity was highest during the wandering (L5D3) stage. JHE activity in the MG also peaked on L5D3 and was low on L5D1 and L5D4. Just as in the hemolymph,

the FB JHE activity peaked on early L5D2 and L5D4 (Fig. 3), providing circumstantial evidence for the FB as the site of production of HL JHE. The FB JHE declined more slowly than that in the HL on L5D3 and maintained a high but decreasing titer during the pupal stage. These data indicate that production and release of JHE may not be tightly coupled (Sparks et al., 1979a; Wing et al., 1980; Jones et al., unpublished).

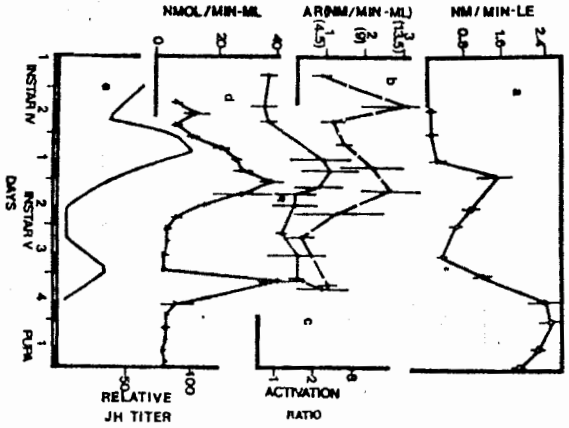

Fig. 3. a) Fat body JHE activity per larval equivalent (LE), b) Ratio of JHE activity in neck-ligated L5D1 last instar larvae implanted with various age donor brains to activity of sham larvae, c) Ratio of the activity in various age neck-ligated larvae injected with 4 brain-equivalent homogenates from L5D1 donors to activity of HOH injected larvae, d) Hemolymph JHE activity (Wing et al., 1980), e) Hypothesized hemolymph juvenile hormone titer as compiled from Fain and Riddiford (1975), Yagi (1976) and Sparks and Hammock (1979b).

Biological significance of JH catabolism

Weirich et al. (1973) speculated that the high JHE activity in last larval instar Manduca sexta correlated with the suspected decline in JH titer. As JH titers and JHE activity have been studied in other insects, this correlation seems to hold. A further correlation exists between the prepupal burst of JHE activity (L5D4 in T. ni) and the disappearance of a post-wandering increase in JH titer. However, Nijhout (1975) was correct in pointing out that a rough correlation of JHE activity with a decline in JH titer did not demonstrate a cause-effect relationship. In M. sexta, JHE activity in the hemolymph peaked well after the JH titer had significantly declined. Also, the demonstration that an esterase shows specificity for the JHs as substrates does not prove that its biological function is the catabolism of those JHs.

The above questions are partially answered by the use of selective inhibitors of JHE and other esterases which have served to indicate a causal relationship between an increase in the JHE activity and a suspected decrease in JH titer in T. ni. The results of a large screen of potential inhibitors of JHE are shown in Table 1. EPPAT is an irreversible inhibitor of both JH and α-naphthyl acetate (α-NA) hydrolyzing enzymes in T. ni HL and FB. By applying EPPAT topically to L5D1 and L5D2 larvae, the JHE activity in vivo could be dramatically reduced. Since EPPAT is a very poor inhibitor of acetylcholine esterase, its acute toxicity to T. ni is minimal. Following inhibition of the JHE, larvae spent much longer periods in the last larval instar just as if they were treated with JH or juvenoids. Developmental abberations resembling juvenoid treatment were also observed when the L5D4 peak was inhibited. EPPAT also significantly increased the half-life of radiolabeled JH in T. ni larvae. DFP and paraoxon provided partial controls for this experiment.

Table 1. Differential inhibition of hemolymph esterasea metabolizing juvenile hormone III and α-naphthyl acetate (JHE and α-NAE).

Inhibitor	I_{50} (M)		I_{50}JHE/I_{50}α-NAE
	JHE	α-NAE	
DFP	$>10^{-3}$	4×10^{-8}	>25,000
Paraoxon	2×10^{-6}	1×10^{-7}	20
EPPAT	1×10^{-6}	1×10^{-7}	10
TFT	1×10^{-7}	$\sim1\times10^{-4}$	∿0.001

DFP: O,O-diisopropyl phosphorofluoridate; paraoxon: O,O-diethyl, O-4-nitrophenylphosphate; EPPAT: O-ethyl, S-phenyl phosphoramidothiolate; and TFT: 1,1,1-trifluoromethyltetradecan-2-one. Data from Sparks and Hammock, 1980b.

This evidence clearly supports a biological role for JHE in T. ni, but additional research is needed. EPPAT inhibits numerous carboxylesterases in T. ni, while DFP is acutely toxic. Thus, more selective inhibitors of JHE and less toxic inhibitors of other carboxyesterases are needed for more definitive experiments. The trifluoromethylketone (TFT) is a potent selective inhibitor of JHE, but its reversible nature limits its usefulness in in vivo experiments. Thus, the tools to clearly distinguish among the various factors involved in catabolic regulation of the JH titer, in vivo, are still lacking (Hammock et al., 1977b; Sparks et al., 1979b; Sparks and Hammock 1979a, 1980b; Wing et al., 1980; Sparks, unpublished).

Role of carrier protein in JH catabolism

A single, low molecular weight carrier protein appears to exist in the HL of T. ni as has been reported in M. sexta (Goodman and Gilbert, 1974; Kramer et al., 1974). The binding activity in T. ni larvae focused at a similar pH when HL from each day of the last larval instar and several other developmental stages were analyzed by isoelectric focusing. When total HL JH binding activity was monitored during the last larval instar of T. ni, only minor changes were observed with peak activity occurring between mid-L5D2 and mid-L5D4 similar to the HL protein content. Numerous roles have been suggested for the JH carrier, as reviewed by Kramer and Law (1980), which hypothesized that it serves to insure an equal distribution of JH throughout the insect and a rapid response to changes in the rate of JH production or catabolism. Thus, the carrier protein probably serves, in part, to make the influence of JHEs on JH titer more dramatic in T. ni, stabilizing JH when JHEs are absent and increasing JH turnover when they are present (Sparks and Hammock 1979b; Wing et al., 1980; Sparks, unpublished).

SITE OF JHE PRODUCTION

Biochemical evidence for the presence of a single hemolymph JHE

The concept of JH being catabolized by both general and specific JHEs was developed for M. sexta by Sanburg et al. (1975). This concept appears particularly applicable to Galleria mellonella where several esterases are capable of rapidly hydrolyzing JH, but in T. ni a single enzyme referred to as JHE appears to be largely responsible for JH hydrolysis. The carboxylesterases detectable using a stain of α-NA contribute less than 1% of the total JH hydrolyzing activity in L5D2 hemolymph.

Numerous attempts to distinguish more than one JHE in T. ni hemolymph have failed including partial purification of the enzyme from L5D2 hemolymph. The L5D2 and L5D4 peaks of JHE activity as well as JHE artificially induced by treatment of the appropriate stage with head factor or juvenoids (as discussed below) also could not be distinguished based on numerous techniques including gel filtration, anion exchange chromatography, electrophoresis, inhibition and precipitation. Analytical isoelectric focusing proved to be an especially powerful tool for these studies. Under conditions where proteins with pI's differing by 0.06 pH units could be clearly distinguished, all hemolymph JHE's from T. ni were identical (Sparks and Hammock, 1979a; Wing et al., 1980).

Fat body as the site of JHE production

Using saturnid pupae, Whitmore et al. (1972, 1974) provided evidence that the FB was the site of production of some hemolymph esterases. Nowock and Gilbert (1976) and Reddy et al. (1979) also provided evidence that the FB produced JHE in M. sexta and G. mellonella, respectively. The FB is certainly the expected source of JHE since its role in protein synthesis in insects is well established. Although unequivocal evidence is lacking, the FB appears to be the major site of HL JHE production in T. ni.

The high level of FB JHE (Fig. 2) and the close correlation between HL and FB JHE titers during the last larval instar (Fig. 3) provide circumstantial evidence for the above hypothesis. As discussed below, when HL JHE is artificially induced in either the larval or pupal stage, an increase in FB JHE is also observed. When the same biochemical techniques that were used to compare the HL esterases were applied to a comparison of HL and tissue esterases, HL and FB JHE activity appeared to be due to the same single enzyme. Even when techniques were used to retard protein degradation, the low JHE activity in the midgut was attributable to a similar, but clearly different, enzyme. Short term organ culture of T. ni FB yields JHE release rates which correlate with HL levels as was earlier shown in M. sexta by Nowock and Gilbert (1976). Hopefully, such work will soon yield unequivocal evidence regarding the location of JHE production as well as its regulation (Wing et al., unpublished).

REGULATION OF JH ESTERASE

Although due to the same enzyme, the two peaks of JHE in the last larval instar of T. ni appear to be under at least two different modes of endocrine regulation. Surgical and other experiments have determined that both nervous tissue and the corpora allata (CA) are involved. The regulation of the L5D2 and L5D4 JHE peaks (Fig. 3) will be discussed separately below.

Stimulatory control of the L5D2 JHE peak

Sparks and Hammock (1979b) obtained evidence from ligation experiments that a stimulatory factor from the head, which was not JH, was largely responsible for the L5D2 JHE peak (Fig. 3). When various head and body tissues were bioassayed by injection of homogenates (inducing factor content) or implantation (inducing factor output) into neck-ligated L5D1 larvae, both the brain (BR) and subesophageal ganglion (SEG), but not the prothoracic or abdominal ganglia, caused significant increases in the HL JHE activity (Jones et al., 1980). The ability to induce JHE

was in the order: BR or SEG (implanted separately) <BR + SEG (implanted together but disconnected) <BR + SEG (implanted as the intact complex (Fig. 4a). When the content and output of the inducing factor was determined during development, high activity was found in both tissues during the L4 to L5 intermolt period and on early L5D2. The first peak of inducing factor corresponded with the small peak of JHE activity in the late L4 and the second occurred at the same time as the L5D2 JHE peak (Fig. 3).

Using late L5D1 brain homogenates as the source of inducing factor, the responsiveness of T. ni abdomens to the factor was bioassayed during development. The homogenate recipients were sensitive to the factor in the early last instar, but recipients were much less sensitive during the L4-L5 intermolt. This observation explains how a high inducing factor titer stimulates a large in vivo HL JHE peak in L5D2, but only a small brief burst of JHE at the L4-L5 molt (Fig. 3).

In addition to direct regulation by the BR-SEG complex, the L5D2 peak appears to be indirectly influenced by JH. Topical application of JH or juvenoids to L5D2 larvae significantly increased the HL JHE titer. This effect was prevented by prior head ligation, indicating the influence of JH on this JHE peak was mediated through the head (Sparks and Hammock, 1979b; Jones et al., 1980; Jones et al., unpublished).

Inhibitory control of the L5D2 JHE peak

The continued presence of the inducing factor from the BR-SEG complex was found necessary for JHE production and release; however, a brain centered JHE inhibin seems to be involved in the decline of the L5D2 peak. Injection of L5D3 brain homogenates into ligated L5D1 larvae resulted in a significant decline in JHE titer. The apparent production of a JHE inhibin may explain why neck ligation of mid-L5 T. ni resulted in a higher JHE titer than that occurring in normal larvae. Additional work is clearly needed on the interaction of the JHE inducing factor, JHE inhibin, and other hormones during the last larval instar (Jones et al., 1980).

Control of the L5D4 JHE peak

Topical application of JH and juvenoids to late last instar larvae resulted in a significant increase in HL and FB JHE in both normal and ligated late L5 larvae. Before a critical period on L5D3, juvenoids have no direct effect on JHE titers in isolated abdomens, but after this time a dose-dependent increase follows juvenoid treatment. The effect was clearly dependent on juvenoid structure with ethyl in contrast to methyl branches yielding the highest activity. The effect could be

inhibited by coapplication of the JH-antagonist ETB indicating interaction at the receptor level (Sparks et al., 1979b). In vivo studies using radiolabeled JH further demonstrated that the JH doses needed to induce normal JHE levels led to HL JH titers approaching those reported in normal Lepidoptera.

These data suggested that the L5D4 peak was, at least in part, directly induced by JH from the CA. To test this hypothesis, L5D3 (7 hr ALO) larvae were allatectomized which reduced the JHE titer during the second peak to less than 10% of normal, while removal and immediate reimplantation of the CA (or juvenoid application) resulted in a normal L5D4 JHE peak. Allatectomy and ligation at later times had a decreasing effect on the L5D4 peak indicating that the endocrine event (presumably JH release) occurred roughly 12.hr before the JHE peak (Fig. 4b).

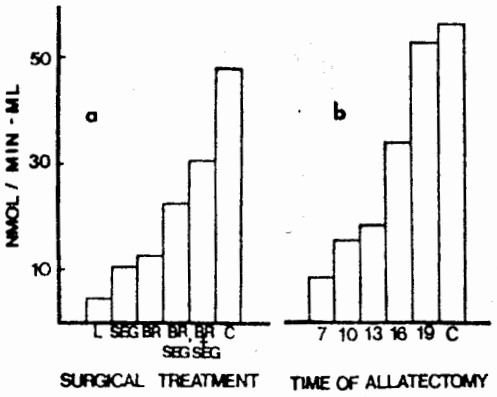

Fig. 4. a) Hemolymph JHE activity in neck-ligated L5D1 larvae receiving various surgical treatments (L = ligated only; SEG; BR; BR, SEG; BR + SEG = implantation of L5D1 subesophageal ganglion, brain, brain and subesophageal ganglion disconnected and intact brain and subesophageal ganglion complex, respectively; c = unligated control. b) Effect of time of allatectomy (hrs after lights on ALO) of L5D3 larvae on JHE activity. Larvae were bled on L5D4 5 hr ALO and the hemolymph assayed for JHE activity. c) Sham-operated control.

No other tissue besides the CA has yet been found to stimulate the L5D4 JHE peak. Neither the brain nor the SEG from L5D4 larvae, whether injected as homogenates or implanted intact into ligated L5D1 larvae, resulted in a significant increase in JHE. Although late last instar larvae are responsive to the inducing factor (Fig. 3), the factor was not found to be present in normal larvae at this time, implicating JH as the major factor involved in inducing the L5D4 JHE peak. Since HL JHE can be rapidly and quantitatively measured, its induction in isolated abdomens by implanted CA may be a good assay for JH output from CA (Sparks and Hammock, 1979b; Jones, unpublished).

As mentioned earlier, a synchronous increase in FB and HL JHE levels are observed following juvenoid treatment. Based on blood volume

experiments, the total JHE in the hemolymph following such treatment cannot be explained by JHE release from the FB alone. Production of JHE, whether by de novo synthesis or modification of an inactive pro-enzyme, must occur in addition to JHE release. Using lactate dehydrogenase as a marker for FB cytosol, this JHE release has been demonstrated to be a specific phenomenon and not simply due to FB histolysis for either the L5D2 or L5D4 peak (Wing et al., 1980).

There is some evidence that a JHE inhibin is involved in the decline in the L5D4 JHE peak as was found for the L5D2 peak. Removal of the brain during the early part of the L5D4 JHE decline and implantation into neck-ligated larvae of a similar stage resulted in a significant increase in the rate of JHE decline (Jones et al., 1980).

Influence of dietary factors on JHE

In larvae that are either deprived of food or else placed on an inadequate diet, the HL JHE declines even more rapidly than in ligated larvae. This observation supports the contention that JHE activity is under negative as well as positive regulation during the early last instar. Placing starved larvae on an adequate diet led to a subsequent increase in HL JHE. No single factor appeared totally responsible for maintaining a high JHE titer, but protein is clearly the most important clue in T. ni. The concept that the lack of nutritional intermediates necessary for JHE production leads to the rapid decline in JHE activity is overly simplistic. The decline in HL JHE clearly occurred too rapidly to be a starvation effect and HL and FB protein content remained high. The effect of starvation on HL JHE has also been duplicated in M. sexta (Riddiford and Hammock, unpublished) where a concomitant increase in JH titer has been demonstrated (Cymborowski et al., 1979). This indirect influence of diet on JHE appears to be one way in which the insect coordinates its development with its environment. It also raises the possibility that there may be a tight link between JH production by the CA and JHE production by the FB.

JH CATABOLISM IN OTHER LEPIDOPTERA

Insects are notoriously diverse in many respects, and the generalization of our data on JHE regulation in T. ni to other insects, especially those outside of the order Lepidoptera should be very cautious. For reviews, the reader is referred to Kramer and Law (1980), Sparks and Hammock (1980b), Hammock and Quistad (1980), and numerous chapters in this text, especially the chapter by Kumaran.

Hemolymph JHE titers in other Lepidoptera

Although the model of JHE regulation in T. ni presented here may not be universal even for the Lepidoptera, it is evident that several other species at least have similar JHE profiles during development. For instance, eight species representing six families exhibited 2 major JHE peaks during the last larval instar (T. ni and Spodoptera exigua, Noctuidae; Ephestia elutella, Pyralidae; Hemileuca nevadensis, Saturniidae; Pectinophora gossypiella, Gelechiidae; Orgyia vetusta, Liparidae; M. sexta, Sphingidae; Estigmene acraea, Arctiidae). As in T. ni (Fig. 3) the first (prewandering) peak reached a maximum just prior to the cessation of feeding and the second (prepupation) peak occurred just before the pupal molt. In S. exigua and T. ni the prepupal JHE peak was equal to or slightly higher than the prewandering peak. In P. gossypiella and E. elutella the prepupal JHE was substantially higher than prewandering JHE, while in H. nevadensis, O. vetusta, and M. sexta the prepupation peak of JHE was relatively small. In two other species monitored in this study (G. mellonella, Pyralidae; Junonia coenia, Nymphalidae), a rise in JHE was observed which correlated with the cessation of feeding, but the JHE titer then declined to low levels as pupation approached.

Biochemical similarities of lepidopteran HL JHEs

When HL samples corresponding to the prewandering JHE peak in last instar larvae of several species were examined by analytical isoelectric focusing, JHE activity focused in an acidic pH range. The hemolymph of many of the species examined displayed a single peak of JHE activity (T. ni, S. exigua, Heliothis virescens, Noctuidae; E. elutella, Pyralidae; P. gossypiella, Gelechiidae). One species tested had two peaks of activity in the pH 4.0-6.5 range (J. coenia, Nymphalidae) and another had three peaks (O. vetusta, Liparidae). In most cases, a single peak of JH binding activity was also found.

HL from T. ni, M. sexta, S. exigua, H. virescens, G. mellonella, J. coenia and O. vetusta were treated with $1X10^{-4}$ M DFP, EPPAT and TFT both before and after isoelectric focusing (as in Table 1). It was found that all of the HL samples examined before electrofocusing except J. coenia were negligibly inhibited by DFP, but they were totally inhibited by EPPAT and TFT. J. coenia HL, which had very low levels of JHE activity relative to the other samples, was largely, but not totally inhibited by all three compounds. All active fractions after isoelectrofocusing were completely inhibited by TFT and EPPAT, but two active fractions from O. vetusta were also partially inhibited by DFP.

Similarities in JHE regulation among the Lepidoptera

JHE regulation in G. mellonella has also been well studied and a comparison of this chapter with that by Kumaran (this volume) will illustrate both similarities and differences between G. mellonella and T. ni. Cauterization of the brain region of M. sexta containing the group 2 median neurosecretory cells resulted in only a slightly lower than normal prewandering peak (Bhaskaran and Jones, unpublished), indicating that other brain or nervous tissue is involved at that time. Cauterization of the group 4 cells did not prevent a high JH-induced supernumerary molt, arguing against a hypothetical JHE inhibin produced here since such an inhibition was not released by this cauterization. As in T. ni, ligation or starvation rapidly reduces the prewandering JHE, while the JHE titer is somewhat reduced but still very active in allatectomized or black mutant larvae (Bhaskaran and Jones, unpublished; Riddiford and Hammock, unpublished). M. sexta does not respond to juvenoid application with increased JHE until just before pupation (just prior to darkening of the metathoracic bars). After this time, late instar larvae and pupae whether black mutant, allatectomized or normal, respond to juvenoid treatment with a dose-dependent increase in hemolymph JHE. The prepupation JHE peak is greatly reduced or absent in allatectomized M. sexta (Riddiford and Hammock, unpublished). Thus, JHE regulation in M. sexta appears similar in many regards to that summarized below for T. ni.

MODEL OF JHE REGULATION IN T. NI

The results summarized above can be used to formulate a partial model of JHE regulation in the last larval instar of T. ni (Fig. 5).

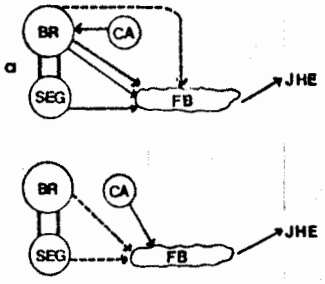

Fig. 5. Modes of T. ni JHE regulation of the first (a) and second (b) hemolymph JHE peaks during the last instar. (——— direct stimulatory factor, ////// indirect stimulatory factor, — — — direct inhibitory factor).

During the L4-L5 intermolt period, the brain and subesophageal ganglion release large amounts of an inducing factor which acts on a poorly responsive fat body (Fig. 3). The fat body produces only a small amount

of JHE seen as the L4-L5 intermolt peak. This JHE activity may be correlated with a drop in JH titer sometimes observed during the larval-larval molt and may serve to "prime" the fat body to become more responsive to the inducing factor following the molt. During late L5D1, the brain and subesophageal ganglion again release an inducing factor which stimulates a responsive fat body to release relatively large amounts of JHE into the hemolymph. Unmetabolized JH may feed back to the head region to cause additional release of inducing factor. After mid-L5D2 the apparent neuroendocrine stimulation of JHE is relaxed and a brain-centered JHE inhibin is released resulting in a declining JHE titer in both the hemolymph and fat body.

In the late last instar, a burst of JH from the corpora allata directly induces another peak of hemolymph JHE. As the corpora allata turn off JH production in preparation for pupation, a brain-centered JHE inhibin once again effects a rapid decline in hemolymph, but not fat body, JHE just prior to pupation. Thus, the same protein (JHE) is under at least two completely different mechanisms of endocrine regulation in the early and late last larval instar.

Such a seemingly complex system of regulation may have biological significance. The timing of the developmental events involved are clearly crucial for the larva's survival. In the early last instar, regulation by a neuroendocrine mechanism which will allow the insect to be responsive to its environment is important. The larva, at this stage, is not committed to a fixed schedule leading to pupation. The larva can extend the time in the feeding stage of the last instar and may possibly even undergo a supernumerary instar to allow additional feeding. Thus, if it appears unlikely that the larva can acquire sufficient nutrients for successful development, the appearance of JHE in the hemolymph may be delayed or reduced. This lack of catabolic enzymes helps to maintain a high JH titer which will delay PTTH release or lead to a supernumerary instar.

The high JHE titer may serve to clear the last traces of JH from the larva allowing PTTH release. Once PTTH release has occurred in the last larval instar, the developmental events leading directly to pupation are set in motion. JHE must be removed from the hemolymph if the prepupal burst of JH is to be effective. The role of this JH burst is still being debated (Kiguchi and Riddiford, 1978; Cymborowski and Stolarz, 1979), but JH must clearly be removed from the hemolymph before pupation. Thus, an efficient system for JH removal exists in T. ni with an increase in JHE titer following half of a day after prepupal induction of the fat body by JH. This system leads to a very precisely timed burst of JHE immediately before pupation. In M. sexta the change in the sensitivity

of the fat body to JH rather than the post-PTTH appearance of JH seems to time the second JHE peak, but the ultimate result is identical.

The mechanism by which the fat-body becomes responsive to first the inducing factor and secondly to JH is still unknown. If these mechanisms could be elucidated and artificially stimulated to occur, precocious development should follow. For instance, JH produced in early instars would then be expected to induce JHE leading to clearance of the JH required for normal development. Thus, the intricacies of the insect endocrine system continue to provide interesting possibilities for the disruption of the development of man's most able competitors.

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DISCUSSION

B. Cymborowski: Do you have any idea what this head factor might be?

B.D.H: We have not done too much work on it. Our data to date is consistent with it being a peptide neurohormone, for instance it is trypsin and pronase sensitive. I hope that Krishna or I can follow up on these observations in the future.