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HORMONAL CONTROL OF MUSCLE ATROPHY AND DEGENERATION IN THE MOTH *ANTHERAEA POLYPHEMUS*

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SUMMARY

1. The intersegmental muscles of the giant silkmoth *Antheraea polyphemus* (Cramer) can undergo two forms of degenerative changes: a wasting atrophy that lasts about 6 days or rapid dissolution that is completed within 30 h.

2. Muscle atrophy is induced by a dramatic decline in the endogenous titres of the steroid moulting hormone 20-hydroxyecdysone.

3. 20-Hydroxyecdysone appears to act as a trophic factor for the muscles as infusion or injection of this steroid blocks further atrophy of the muscle.

4. The normal decline of 20-hydroxyecdysone also allows the muscles to become competent to respond to the peptide eclosion hormone.

5. Eclosion hormone is then released and acts directly on these muscles to induce rapid cell death which is morphologically and physiologically distinct from steroid-regulated atrophy.

INTRODUCTION

Programmed cell death is a fundamental component of development in most multicellular organisms (Saunders, 1966; Glucksman, 1951; Saunders & Fallon, 1967; Moon, 1981). It can allow tissues to migrate (Glucksman, 1951; Silver & Hughes, 1973), select structures to differentiate (Fallon & Cameron, 1977) and inappropriate neuronal connections to be removed (Chu-Wang & Oppenheim, 1975; Landmesser & Pilar, 1976; Truman & Schwartz, 1982). Cell death can also be the final result of a pathological condition (Webb, 1974; Harris, 1979). While the terminal events in cell death have been described in detail, there are few studies which have elucidated the factors responsible for their initiation.

We have examined the factors which regulate the programmed cell death of the intersegmental muscles (ISM) of the giant silkmoth *Antheraea polyphemus*. The ISM are a group of large embryonically derived muscles which span each of the abdominal segments of the larva. While the majority of the larval musculature degenerates during metamorphosis, the ISM are preserved in several of the abdominal segments

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(Finlayson, 1956). These muscles are then used by the pharate adult to extricate its from the pupal skin and cocoon at the time of adult eclosion (emergence). Following this behaviour, the ISM undergo rapid cell death and are completely degenerate in about 30 h (Finlayson, 1956). Some of the morphological and biochemical events which occur during cell death have been studied (for review, see Lockshin, 1981). However, the signals which initiate these events were not resolved.

In examining this system, we have found that two hormones, the steroid moulting hormone 20-hydroxyecdysone (20-HE) and the peptide eclosion hormone (EH), regulate muscle death. During the last days of adult development the endogenous titres of 20-HE decline. The withdrawal of this steroid removes a trophic support for the muscles, which then begin to atrophy. Following this decline in 20-HE, EH is released and acts directly on the ISM to induce the characteristic rapid cell death. A preliminary description of this work has been previously published (Schwartz & Truman, 1982).

MATERIALS AND METHODS

Animals

Diapausing pupae of the giant silkmoth *Antheraea polyphemus* were purchased from suppliers and stored at 4 °C. Individuals were transferred to an incubator at 25 °C with a photoperiod of 17L:7D to break diapause and initiate adult development. Animals were staged according to specific developmental markers as described by Walters (1970). Only males were used in this study.

Measurement of muscle mass

At the time of assay, the abdomens of test animals were opened mid-dorsally and pinned under saline (Weevers, 1966). After removal of the viscera, the lateral ISM from one side of segments 4–6 (see Fig. 1) were cut free and placed on pre-weighed foil. These samples were then dried for 24 h at 60 °C, and the foil was reweighed on a Mettler Ind. M5 balance. Muscle dry weight was measured to the nearest 5 μg.

20-Hydroxyecdysone

20-HE was purchased from Rohto Pharmaceutical Co., Osaka, Japan. A weighed amount of 20-HE was dissolved in saline (Ephrussi & Beadle, 1936) and the concentration checked spectrophotometrically at 240 nm (E_{240} = 12 670) (Meltzer, 1971). All injections were in volumes of 10–20 μl and were delivered through a Hamilton syringe. In some experiments, 20-HE was infused rather than injected. A length of PE-10 (Clay Adams) tubing was inserted through a small puncture wound in the dorsal thorax and waxed in place. Solutions were delivered at a constant rate through Hamilton syringes driven by an infusion pump (Sage Instruments).

Ecdysteroid radioimmunoassay

The intersegmental membrane between two abdominal segments was cut to allow blood collection. These samples were quickly frozen and stored at −20 °C until needed. At the time of assay, the haemolymph was extracted with four volumes of ice-cold 100 % ethanol and centrifuged to remove precipitated protein. The supernata

s then dried under filtered nitrogen and the ecdysteroid content determined by the radioimmunoassay procedure of Chang & O'Connor (1979) using ^{3}H-ecdysone as a radioligand. All points were assayed in triplicate. The antibody had a binding ratio of ecdysone: 20-hydroxyecdysone of about 4·6: 1.

Ligations

In some experiments, animals were ligated between the thorax and abdomen with a haemostat. The head and thorax were discarded, leaving the isolated abdomen secured by the haemostat. These isolated abdomens survived well for several weeks after ligation, and were held at 25 °C.

Surgical procedures

Prior to surgery, animals were anaesthetized with CO_2 (Williams, 1946). To remove the abdominal ganglia, a small square of ventral cuticle was removed, and the ganglia pulled out through the incision. The piece of cuticle was then replaced along with a crystal of phenylthiourea to prevent blood blackening (Williams, 1959). The cuticle was waxed in place with tacky-wax (Central Scientific Co., Inc., Chicago).

Eclosion hormone preparation

Two preparations of eclosion hormone were used for this study. The first was the supernatant of an aqueous extract of pharate adult *Manduca sexta* corpora cardiaca which had been heated at 80 °C for 5 min and then centrifuged to remove precipitated protein. For many experiments a more purified preparation of EH was used. The corpora cardiaca supernatant above was chromatographed on a Sephadex G-50 column and the active fractions then further purified by narrow gradient isoelectric focusing. Hormone was eluted from gel slices with 0·1 N acetic acid with 0·1 % bacitracin added. One unit of EH activity is defined as the amount of hormonal activity found in one pair of pharate adult *M. sexta* corpora cardiaca (Reynolds & Truman, 1980).

Histology

Intersegmental muscles were fixed *in situ* on ice for 1 h by flooding a dissected preparation with a mixture of 2 % formaldehyde and 0·5 % glutaraldehyde (Ladd) in 65 mM Millonig's phosphate buffer (pH 7·3) (Edwards, 1969). The ISM were then removed, cut into small pieces and post-fixed for 1 h in osmium tetroxide, also in Millonig's buffer. The tissue was then dehydrated through an acetone series, embedded in Epon 812, sectioned with glass knives on a Sorvall MT-1 microtome and stained (Richardson, Jarrett & Finke, 1960) for light microscopy.

RESULTS

Morphology and kinetics of ISM degeneration

The intersegmental muscles of *Antheraea polyphemus* individually span the third or fourth to sixth abdominal segments in developing adults. As seen in Fig. 1B, just ior to adult eclosion the ISM accounted for the bulk of the abdominal musculature.

By 36 h after eclosion (Fig. 1A), the ISM were completely gone, revealing in their absence, the small external muscles. These latter muscles were formed during adult development, and survived for the life of the adult.

Degeneration of the ISM was quantified by measuring the dry weight of the muscles. Following eclosion there was a precipitous linear loss of cell mass (Fig. 3). The muscles lost contractility by about 18 h, and by 25 h they were reduced to flimsy bags of membranes.

The programmed death of the intersegmental muscles could be prevented by isolating the abdomen from the head and thorax of animals prior to, but not after, eclosion (Lockshin, 1969). Although the ISM from abdomens which were isolated prior to eclosion did not show a rapid degeneration, they nevertheless underwent a progressive atrophy during the subsequent 6 days (Fig. 4). By late on the 5th day, these atrophying muscles eventually lost contractility.

Accompanying the different kinetics of loss of cell mass in degenerating and atrophying muscle, was a marked difference in cellular ultrastructure. Before eclosion, the ISM displayed the features characteristic of arthropod skeletal muscle, including numerous peripheral nuclei, long bands of mitochondria and a repeating 2·5 μm sarcomere structure (Fig. 2A). This normal appearance was lost, however, when the animals completed eclosion and rapid ISM degeneration began. Twenty-four hours after eclosion (Fig. 2B), no trace of the contractile apparatus was apparent. Instead, the fibres were composed of whorls of internalized membranes, distorted mitochondria and an aggregation of muscle nuclei. This distortion and destruction of the fibres was not due solely to the substantial loss of cellular mass. The mass of atrophying ISM from abdomens isolated prior to eclosion and held for 3 days was the same as that of the degenerating muscle described above, yet the microscopic appearance of the former was quite different (Fig. 2C). Although these isolated muscles showed a greatly decreased fibre diameter, a reduction in the number of mitochondria and centrally located nuclei, they nevertheless had normal repeating sarcomeres. This last feature correlates well with the observation that these muscles were still contractile (Fig. 4).

The above data suggest that the ISM of *A. polyphemus* can undergo two types of degenerative changes. The first is a rapid programmed cell death in which cellular mass and organization are lost during the 28 h period following normal eclosion. The second occurs in pre-ecdysial isolated abdomens and is characterized by a slow atrophy of the fibres without dramatic derangement of cellular ultrastructure or the rapid loss of contractility.

Regulation of rapid ISM degeneration

In the silkmoths, adult eclosion is triggered by a peptide hormone, the eclosion hormone (EH), which is released from centres in the head (Truman, 1980). When

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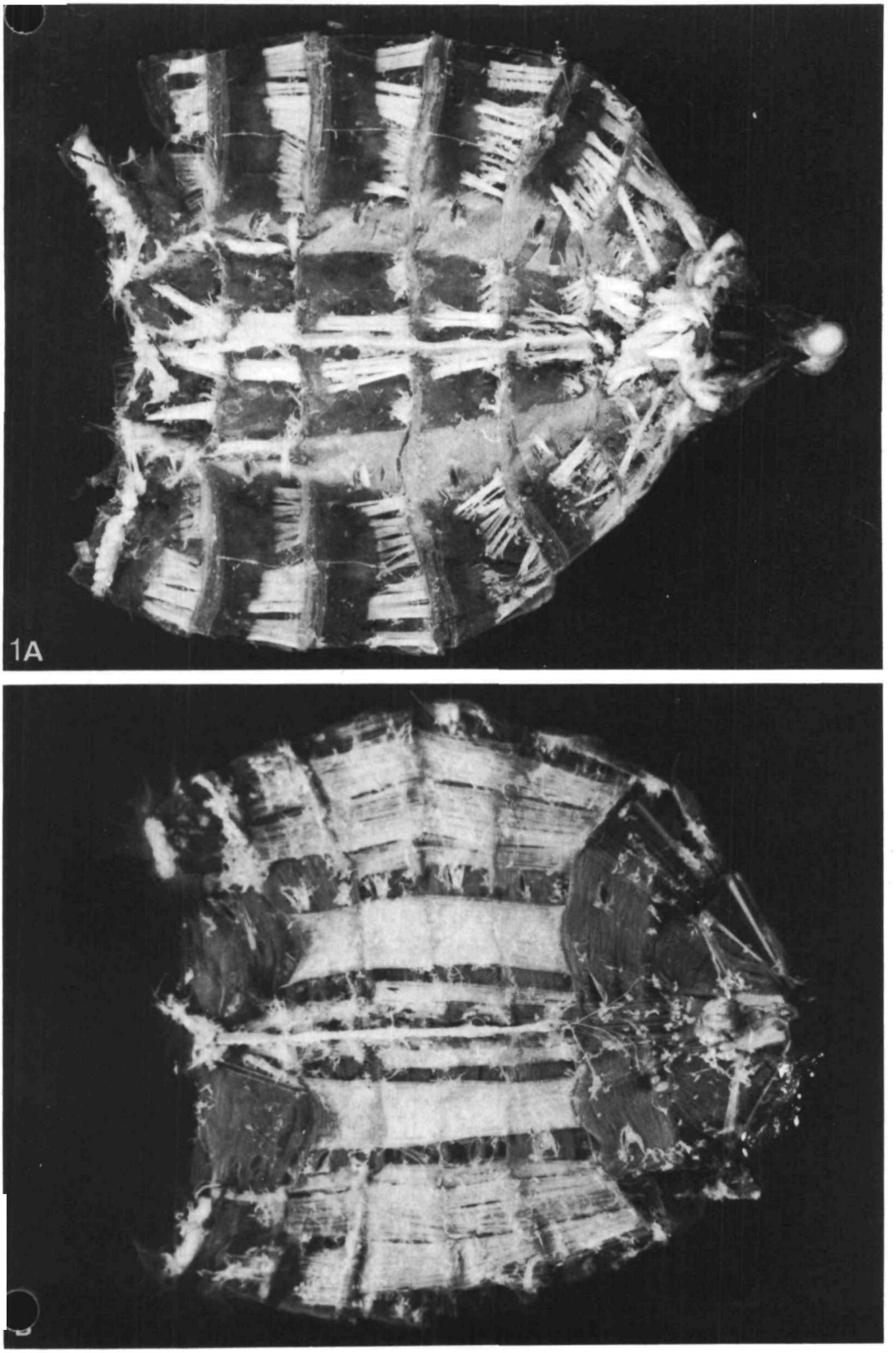
Fig. 1. Dissection showing the abdominal musculature of *Antheraea polyphemus*. Abdomens were slit mid-dorsally and eviscerated to expose the skeletal musculature. (A) Abdomen 36 h after eclosion. The ISM have completely degenerated, leaving only the small external muscles lining the abdomen. (B) A comparable preparation on the last day of adult development. At this stage the ISM are preserved and contractile. Magnification, approximately 4·5 times.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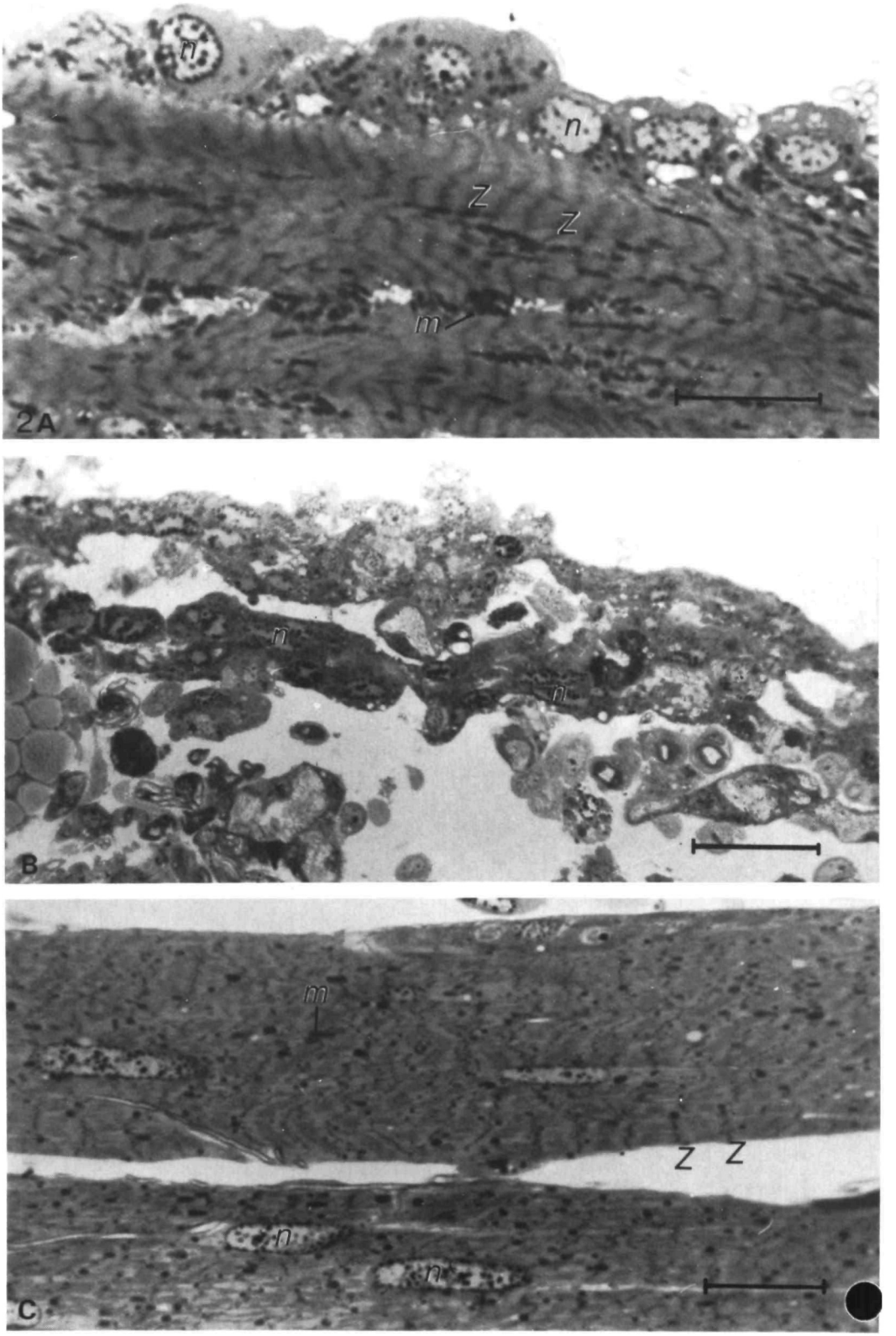
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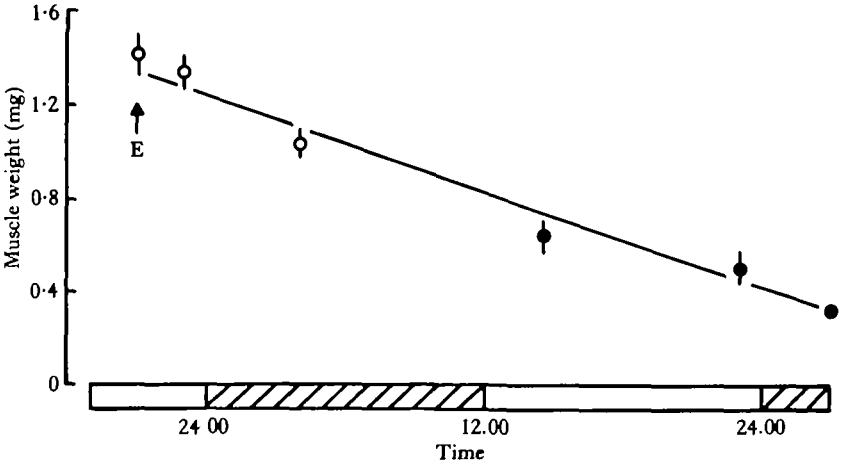

Fig. 3. Loss of ISM mass in normally eclosing adults. The open symbols represent contractile muscles, the closed symbols non-contractile ones. Values are mean (±S.E.) dry weight of the muscles; 5–6 animals per point; E, eclosion.

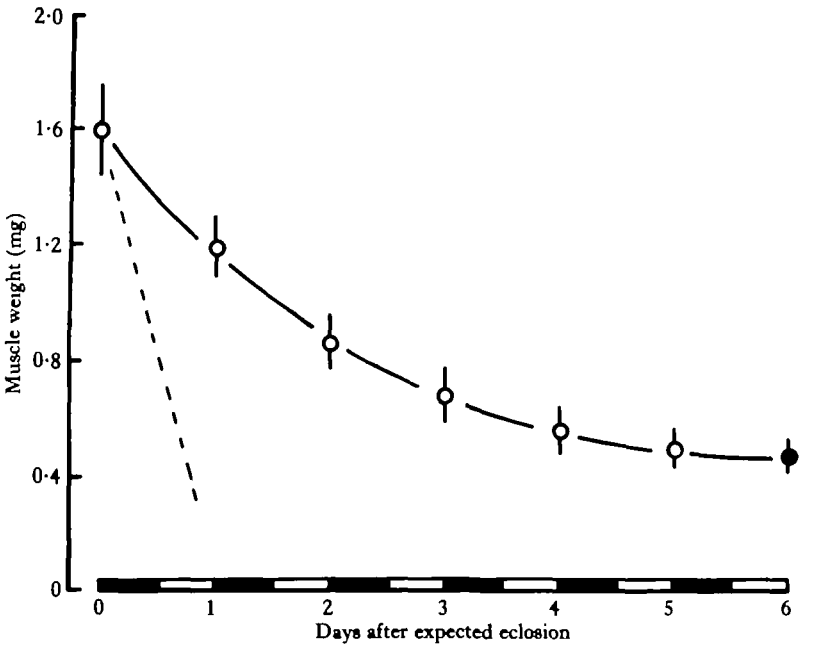

Fig. 4. Loss of ISM mass in abdomens isolated early on day 17 prior to eclosion. Open symbols represent contractile muscles; closed symbol, non-contractile ones. Values are mean dry weight (±S.E.) for 5–6 animals per point. Dashed line represents data from Fig. 3.

Fig. 2. Morphology of intact, degenerating and atrophying *Antheraea polyphemus* ISM. (A) Light micrograph of the ISM at the time of adult eclosion. The muscles are contractile and display features typical of arthropod skeletal muscle. (B) Light micrograph of the ISM 24 h after eclosion. The muscles at this stage are completely degenerate. (C) Light micrograph of the ISM from pre-ecdysial isolated abdomens, examined 3 days after normal eclosion. The scale represents 25 μm; *n*, nucleus; *Z*, Z line; *m*, mitochondria.

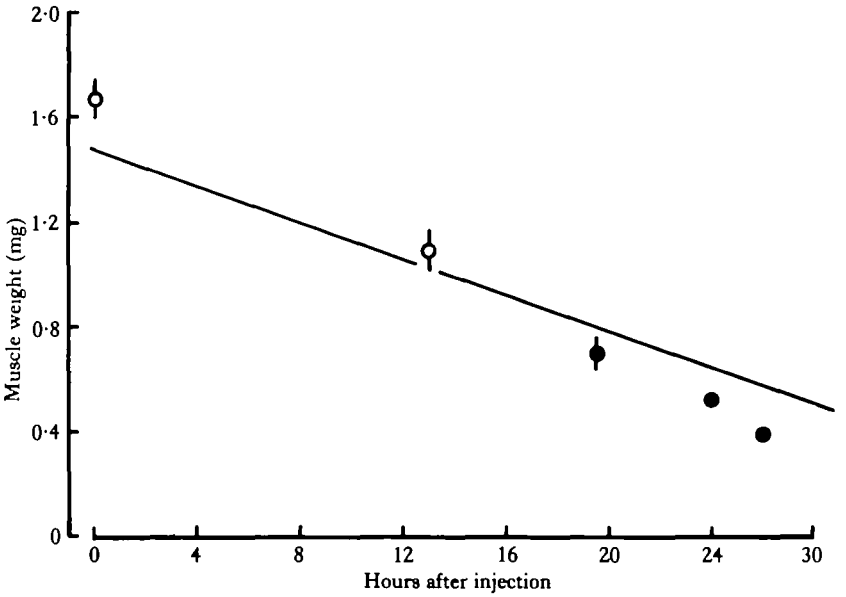

Fig. 5. The ability of EH (1 unit) to cause degeneration of the ISM in isolated abdomens. Abdomens were injected immediately after isolation on day 17. Open symbols represent contractile ISM, closed symbols reflect the non-contractile muscles. The line is the least squares regression for muscle loss in normally eclosing adults from Fig. 3. Values are means ± S.E. for 4–6 animals per point.

abdomens are isolated prior to EH release, abdominal structures are denied exposure to this peptide. As described above, in such isolated abdomens the ISM did not undergo rapid degeneration, but instead atrophied. Injection of these preparations with EH resulted in rapid ISM degeneration which was qualitatively and quantitatively identical to that seen in normally eclosing adults (Fig. 5). The ability of EH to induce ISM breakdown appeared to be all-or-none and was initiated with a threshold of approximately 0·1 units (Fig. 6). Since the amount of EH released at eclosion in the moths is at least 0·5 units (Truman *et al.* 1981), circulating levels of EH are supramaximal for the induction of ISM degeneration.

ISM degeneration appeared to be specifically induced by EH or extracts which contained this peptide. As seen in Table 1, isolated abdomens treated with saline, ISM homogenates, proctolin or perivisceral organ extracts (a rich source of bursicon, Taghert & Truman, 1982) failed to show rapid muscle degeneration.

Table 1. *Effect of test substances on ISM degeneration in* Antheraea polyphemus

Material	Dose per abdomen	Muscle weight* (mg)	% Contractile
Saline	10 µl	1·20 ± 0·10 (4)	100
Perivisceral organ	1 VNC equivalent	1·11 ± 0·08 (5)	100
ISM homogenate	1/6 segment	1·22 ± 0·05 (3)	100
Proctolin	10^{-7} mol	1·30 ± 0·35 (5)	100
Corpora cardiaca extract	1 unit	0·46 ± 0·04 (7)	0
Purified EH	1 unit	0·55 ± 0·10 (4)	0

* Isolated abdomens were held at 25 °C for 24 h after injection.
Values are the mean ± S.E. (N) dry weight of the lateral ISM.

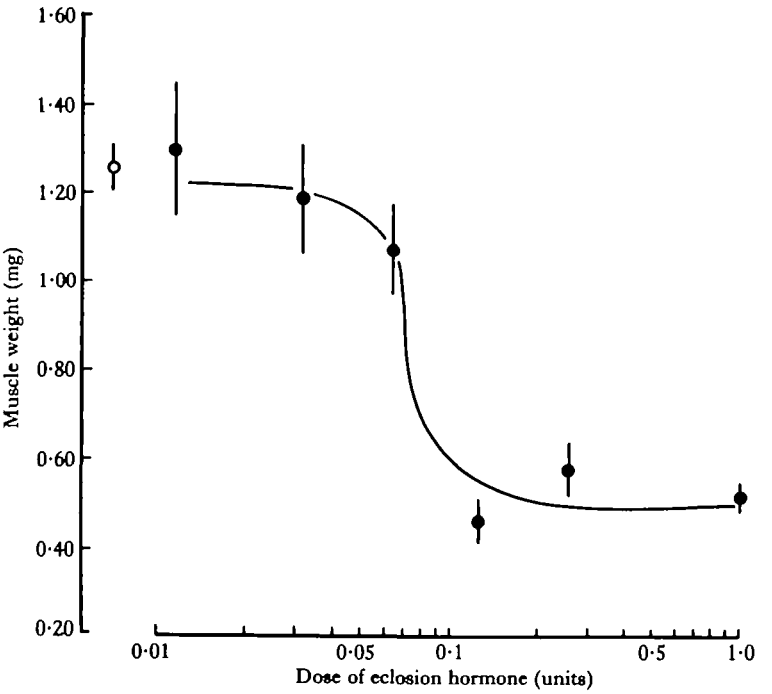

Fig. 6. A dose-response curve for the effectiveness of EH in inducing ISM degeneration in isolated abdomens. Muscle weight was determined 24 h after injection. Abdomens were isolated at the same time as in Fig. 5. Open symbol represents uninjected controls. Values are expressed as mean dry weight (±S.E.) for 4–6 animals per group.

Site of EH action

The ability of EH to induce ISM degeneration could be due to a direct action of the peptide on the muscles themselves or indirectly through the hormone's action on the CNS. Earlier reports had suggested that cessation of motoneurone activity was responsible for triggering muscle death (Lockshin & Williams, 1965*a*,*b*). Since EH has been demonstrated to have direct effects on the CNS (Truman, 1978), such a mechanism appeared plausible. This hypothesis was initially tested by injecting isolated abdomens with 5 μg (approximate haemolymph concentration of 16 μM) of

Table 2. *Role of the CNS in eclosion hormone induced ISM degeneration in* Antheraea polyphemus

	Controls		EH treated*	
Treatment†	Muscle weight (*N*)	% Contractile	Muscle weight (*N*)	% Contractile
None	1·21 ± 0·10 (4)	100	0·53 ± 0·03 (4)	0
TTX	1·27 ± 0·22 (4)	100	0·61 ± 0·01 (5)	0
−CNS 1 day	1·05 ± 0·12 (4)	100	0·56 ± 0·08 (6)	0
−CNS 21 days	0·98 ± 0·07 (5)	100	0·47 ± 0·17 (4)	0

* Preparations treated with 1 unit of eclosion hormone.

† All preparations were abdomens isolated from pharate adults. Muscles were tested and weighed 24 h after treatment and are expressed as mean dry weight ± S.E. (*N*).

the neurotoxic drug tetrodotoxin (TTX) (Moore, Blaustein, Anderson & Narahas[redacted] 1967). Within 10 min these abdomens underwent permanent flaccid paralysis. When examined 24 h later, these preparations had fully contractile ISM (Table 2). When comparably treated abdomens were injected with EH following paralysis, the ISM underwent rapid degeneration. These data suggest that neither the patterning nor the complete cessation of motoneurone electrical activity induces degeneration.

Since the motoneurones could also provide the muscles with some sort of chemical trophic support, we removed the entire CNS from isolated abdomens on the last day of adult development. While slight ISM atrophy was seen 24 h later, the muscles were fully contractile in these denervated abdomens. These muscles responded to EH by showing the characteristic loss of mass following EH injection (Table 2).

To control for the possible action of EH on the distal stumps of the motoneurones, the abdomens of diapausing pupae were denervated and the animals allowed to undergo the normal 3-week period of adult development. As originally reported by Finlayson (1960), such chronically denervated ISMs persisted through adult development and were contractile at the expected time of eclosion. On the last day of adult development, just prior to EH release, the abdomens were isolated from these animals and held for 24 h either with or without EH injection. The ISM from uninjected abdomens showed some atrophy relative to normal animals, but they were contractile and spanned the segment (Table 2). By contrast, the EH-injected abdomens showed extensive muscle degeneration. These data suggest that the ability of EH to induce

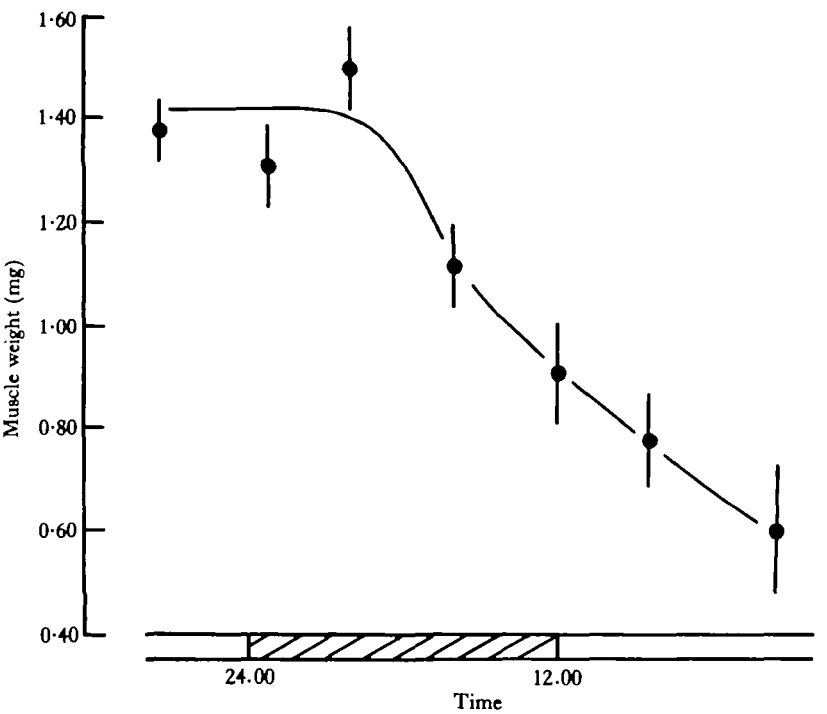

Fig. 7. Onset of ISM sensitivity to EH during the last 36 h of adult development. Abdomens were ligated at the time shown and injected with 1 unit of EH. The muscles were removed 24 h after the eclosion of control animals, and are expressed as mean dry weight (±S.E.) for 6–9 animals per point.

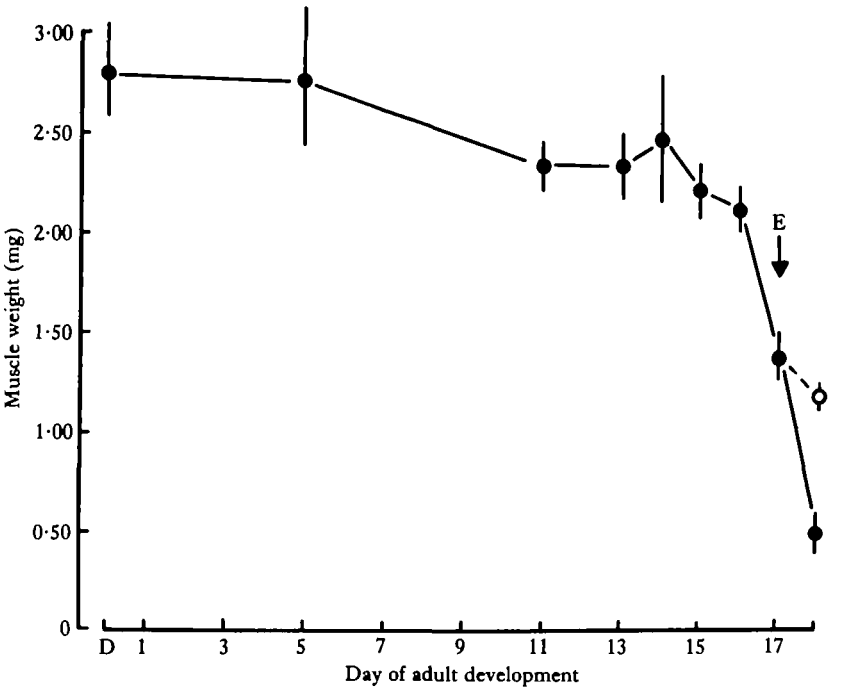

Fig. 8. The change in ISM dry weight of *Antheraea polyphemus* during the course of adult development. Filled symbols, intact animals; open symbol, abdomens isolated before ecdysis on day 17. E, time of adult eclosion; D, diapausing animals. Values are means ± S.E. for 5 animals per point.

ISM degeneration is not mediated through the nervous system but is a direct action on the muscles.

Onset of ISM responsiveness to EH

The ability of the ISM to respond to EH arises on the last day of adult development. *A. polyphemus* were taken on the day before eclosion (day 16), their abdomens ligated with a haemostat, and then injected with a supramaximal dose of EH. Twenty-four hours later, the ISM from these animals had a dry mass of 1·68 ± 0·14 mg ($N = 4$) compared to a mass of 1·54 ± 0·07 mg ($N = 5$) for uninjected control abdomens. To determine more precisely the time at which the ISM acquired EH sensitivity, animals were selected during the last 36 h of adult development, and at various times, ligated and injected with 1·0 unit of EH. The muscles were then examined the day following the emergence of control animals. As seen in Fig. 7, animals only responded to the injected hormone early on the last day of development. There was a time-dependent acquisition of hormone responsiveness, with maximal sensitivity occurring a few hours prior to the normal emergence of the animals. These data suggest that some event occurs between days 16 and 17 of adult development to allow the ISM to respond to EH.

Regulation of the muscle atrophy by the ecdysteroids

Thus far, examination of the fate of the ISM has focused principally on the last day of adult development (day 17). As seen in Fig. 8, the ISM already showed a marked reduction in mass before this time. The mass of the ISM remained relatively constant

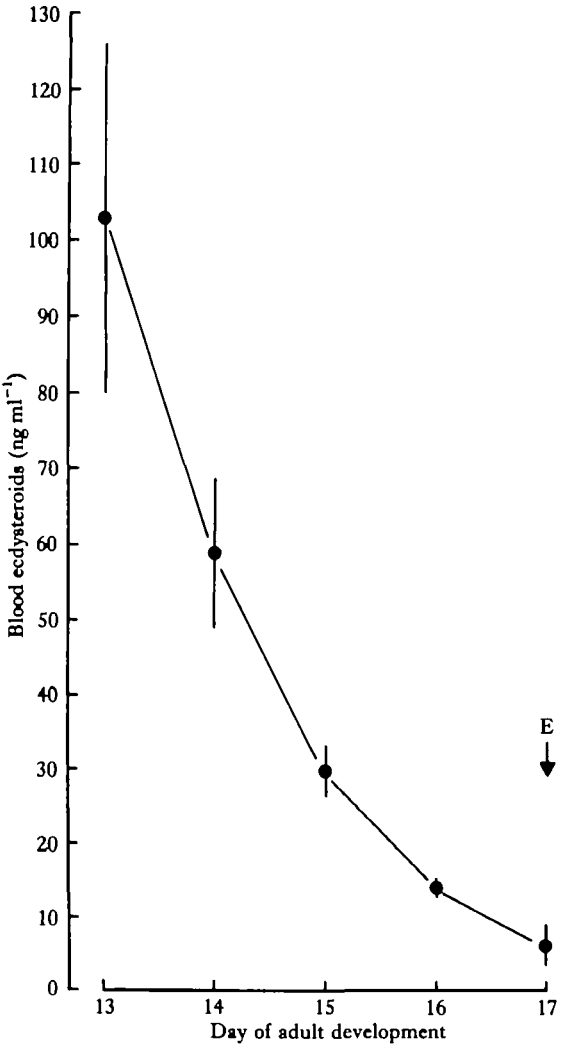

Fig. 9. Titre of ecysteroids in the haemolymph of *Antheraea polyphemus* during the last 5 days of adult development. Ecdysteroids were measured by radioimmunoassay and are expressed in ecdysone equivalents ml^{-1} haemolymph. Values are means ± S.E. for 5 animals per point; E, time of eclosion.

throughout adult development until day 16, when it began to decline. In intact insects, the appearance of EH on the next day resulted in the rapid degeneration of the muscles, but if the abdomens were isolated prior to peptide release, the muscles simply continued on the course of weight loss depicted in Fig. 4.

One indication of the possible hormonal regulation of ISM atrophy came from experiments on *Tenebrio molitor* (Sláma, 1980) and *Manduca sexta* (Schwartz & Truman, 1983; Truman, Rountree, Reiss & Schwartz, 1983). In these insects, the decline in circulating ecdysteroids at the end of adult development regulates the terminal events of metamorphosis, including the onset of behavioural responsiveness to EH. We therefore examined the potential role of this hormone in the changes that occur in the ISM late in development.

Fig. 9 shows that in *A. polyphemus*, the haemolymph titre of ecdysteroids undergoes a marked decline during the last 5 days of adult development. On day 13, the haemolymph titre was 103 ± 23 ng ecdysone equivalents ml^{-1}, whereas by adult eclosion 4 days later, it had fallen to a value of $6{\cdot}6 \pm 2{\cdot}7$ ng ml^{-1}. Thus the onset of ISM atrophy is associated with a normal decline in the ecdysteroid titre.

To examine the potential relationship between a declining haemolymph titre of the ecdysteroids and the state of the ISM, animals were selected at various times during the last 1·5 days of adult development, injected with 20-HE (25 μg) and then examined 24 h after eclosion. Insects injected with 20-HE on day 16 of development showed normal eclosion behaviour the next day but failed to show ISM degeneration. Injection of the same dose of 20-HE at progressively later times in development became less effective during the night of day 17, so that by the following photophase, steroid treatment no longer preserved the muscles (Fig. 10). Coincidently, the time at which the animals became steroid-independent corresponds to the time that they acquire EH sensitivity.

The ability of 20-HE to preserve the ISM was dose-dependent. Animals were injected with various doses of 20-HE at lights-off on day 16, the last time at which maximal muscle retention could be caused by this steroid. Doses less than 10 μg had no effect on the normal course of muscle degeneration (Fig. 11). Above this threshold dose, however, 20-HE caused complete preservation of the muscles at the time of examination.

When given as single doses, the levels of 20-HE required to preserve the ISM were relatively large. We attempted a more physiological way of administering the steroid to the insect by means of continuous infusion. Day 15 animals were cannulated and

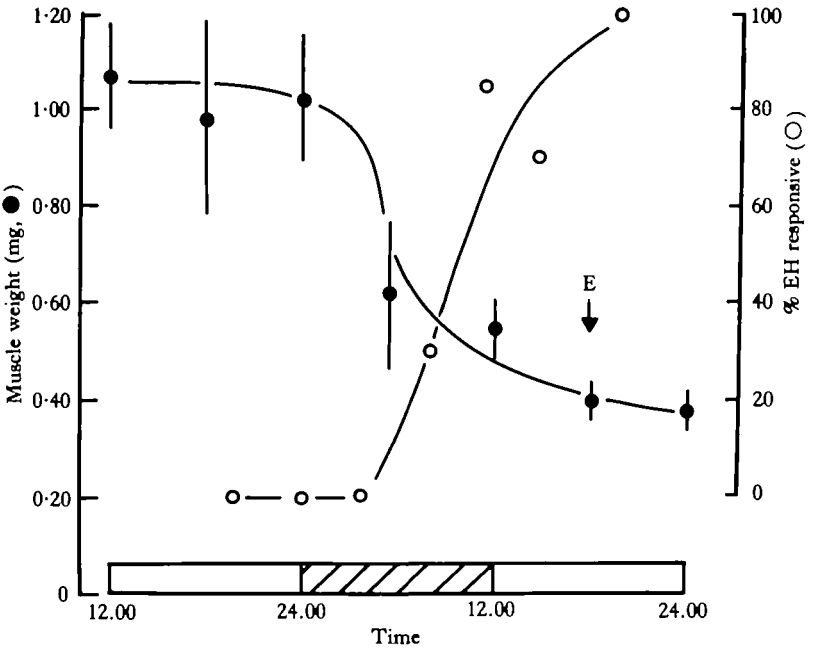

Fig. 10. Sensitivity of the ISM to preservation by ecdysteroid injections during the last 36 h of adult development. Intact animals were injected with 25 μg of 20-HE at the times indicated and held for 24 h after normal eclosion (E). EH sensitivity was determined by injecting control-isolated abdomens with 1 unit of EH at the time indicated. Values are means ± S.E. for 5–6 animals per point.

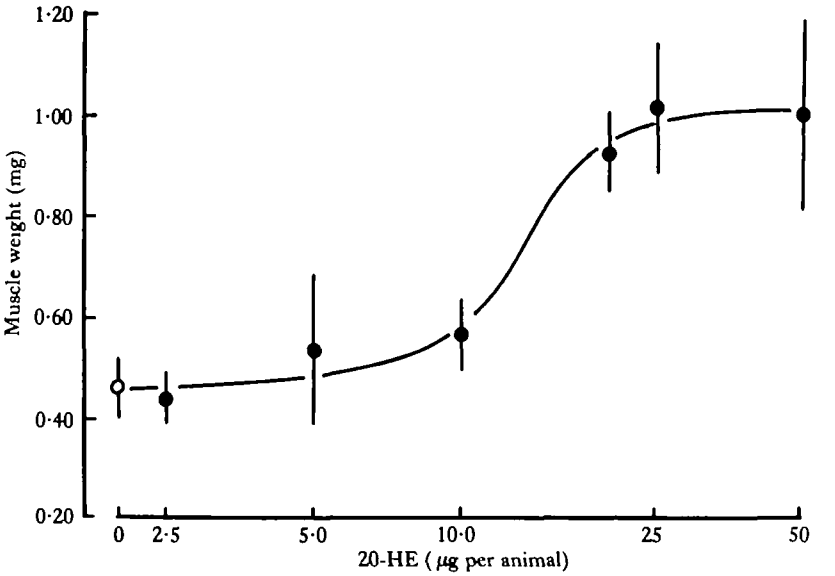

Fig. 11. Dose-response curve for the effectiveness of 20-HE in preserving the ISM. Animals were injected at lights-off on the day preceding eclosion (day 16) with various doses of 20-HE and then held until 24 h after normal eclosion. The open symbol is the muscle mass of uninjected controls; the solid symbols are 20-HE treated animals. Values are means ± S.E. for 5–6 animals per point.

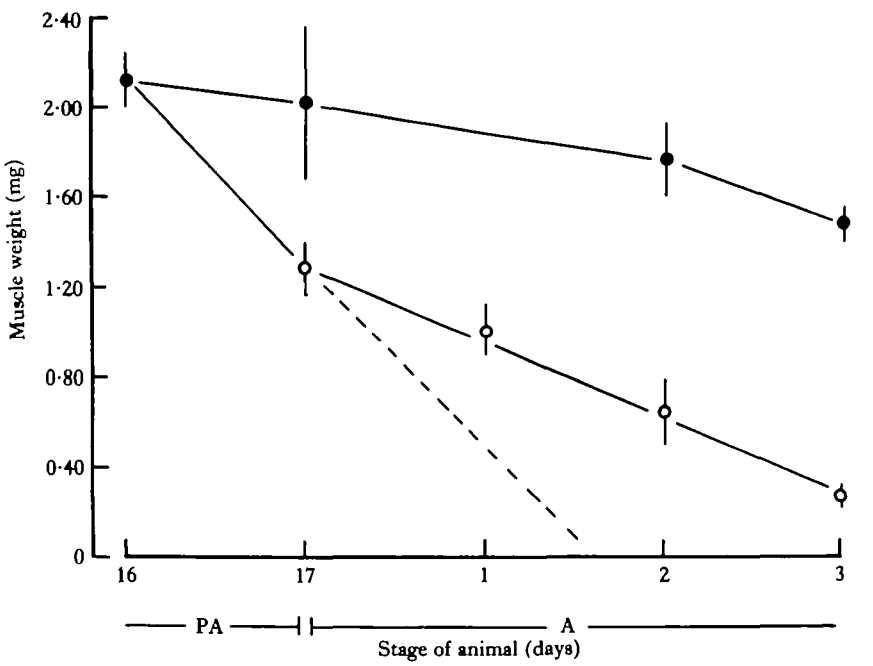

Fig. 12. The rates of ISM atrophy in 20-HE treated animals. The dashed line represents the normal loss of mass from intact normally eclosing adults. Open circles, animals injected with 25 μg of 20-HE on day 16; closed circles, animals infused with 30 ng g^{-1} h^{-1} of 20-HE starting 2 days before eclosion and continuing for up to 5 days. Pharate adult stage denoted by PA; adult stage denoted by A. Mean ± S.E. of 5–7 animals per group.

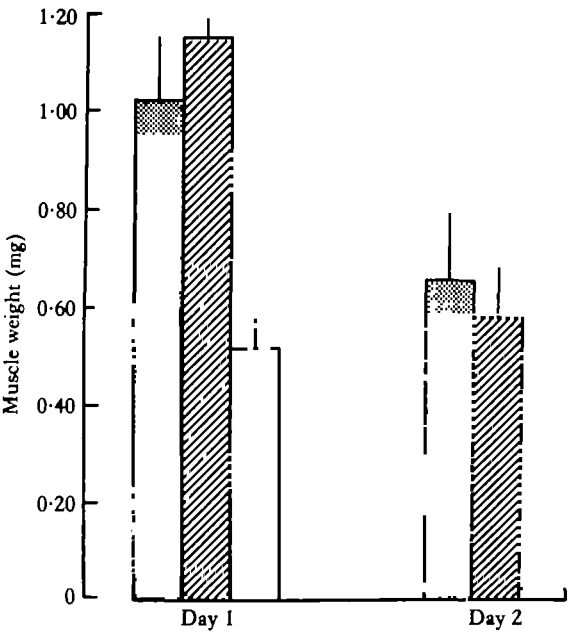

Fig. 13. Treatment with 20-HE renders the ISM insensitive to EH. Histograms show the dry weight of the ISM of isolated abdomens after various endocrine manipulations. Open bars, intact normally eclosing animals; hatched bars, animals injected with 25 μg 20-HE on day 16: striped bars, animals pretreated with 20-HE and injected with 1 unit of EH on the day preceding assay. The x-axis reflects the day of adult life when the muscles were examined. Mean ± S.E. of 4–5 animals per group.

infused with approximately 30 ng g^{-1} h^{-1}. These animals were viable throughout the infusion period, but treatment blocked many of the terminal events of adult development, including resorption of the moulting fluid and eclosion. When these infused animals were examined three days after the scheduled time of eclosion, they had fully contractile ISM which showed no signs of either atrophy or degeneration (Fig. 12).

It should be noted that single injections of 20-HE on day 16 did not preserve the ISM indefinitely. As seen in Fig. 12, there was some loss of mass at ecdysis and this decline continued throughout adult life, albeit at a much slower rate than that seen in untreated moths showing rapid ISM degeneration. The moths treated at this time showed a normal eclosion so they presumably released EH. However, their muscles appeared incapable of responding to the peptide. This phenomenon was further examined by injecting intact animals on day 16 and then challenging them with 1 unit of EH after their normal eclosion. As seen in Fig. 13, pre-treatment with 20-HE prevented the ISM from showing an enhanced rate of degeneration when exposed to supramaximal doses of the peptide.

DISCUSSION

Rapid post-ecdysial ISM degeneration

The rapid degeneration of the ISM in *A. polyphemus* has been extensively examined both morphologically and electrophysiologically by Lockshin and co-workers (for

review see Lockshin, 1981). Within the first 2 h following eclosion, there is a doubli[illegible] of the number of lysosomes within the cytoplasm of the ISM (Lockshin & Beaulaton, 1974). Lysosome production continues to increase markedly throughout the course of degeneration and ultimately these structures represent the major organelle within the dying fibres. During these early hours of cell death there is a random loss of myofibrillar protein from the sarcomere (Beaulaton & Lockshin, 1977). This general reduction in fibre size continues, and by 20 h following eclosion the muscles are no longer contractile (Lockshin & Beaulaton, 1979; L. M. Schwartz, L. Bolles & W. Almers, in preparation). The inability of these degenerating fibres to contract correlates well with their completely distorted ultrastructure. The final events in this degenerative process are the release of the nuclei into the haemolymph and the disintegration of the remaining membranes (Lockshin & Beaulaton, 1974).

The factors involved in triggering the death of the ISM have been less well defined. Lockshin & Williams (1965*b*) suggested that the motoneurones which innervate the ISM become electrically quiescent following eclosion and that it is this change in firing pattern which is responsible for triggering muscle death. Their hypothesis was supported by the observations that electrical or pharmacological induction of CNS hyperactivity prevented normal ISM degeneration from occurring while paralysis with TTX resulted in muscle degeneration in intact animals (Lockshin, 1971).

The data presented in this paper suggest that the death of the ISM is a direct response to an endocrine signal. Lockshin (1969) first observed that abdomen isolation prior to eclosion in *A. polyphemus* prevented normal cell death, while ligations after emergence were ineffective. He concluded that this manipulation interrupted some signal from the anterior end of the insect needed for the normal breakdown of these muscles. This signal appears to be the peptide eclosion hormone. Firstly, abdominal isolation was only able to 'save' the ISM when performed prior to EH release. Secondly, injection of EH into isolated abdomens induced ISM degeneration which was indistinguishable from that seen in normally eclosing adults. Thirdly, this action appeared specific to EH, as other biologically active insect factors were without effect on the ISM. Lastly, EH was able to invoke ISM degeneration at a dose which was well below haemolymph titres just prior to eclosion.

EH appears to cause degeneration by a direct action on the ISM. The ISM in abdomens isolated before ecdysis and treated with TTX to remove motor stimulation of the muscles, did not degenerate. Moreover, the removal of the entire abdominal CNS either from insects just prior to eclosion or from diapausing pupae, did not result in ISM death. Importantly, the ISM of the insects in all of these treatment groups retained their capacity to degenerate when challenged with EH. Denervated muscles even showed the normal changes in cGMP metabolism which follow EH exposure (Schwartz, 1982; L. M. Schwartz & J. W. Truman, in preparation).

A direct action of EH on the ISM is not inconsistent with Lockshin's observations. Lockshin observed for example that TTX injection into intact animals during adult development could induce ISM degeneration, but only at the time of normal eclosion (Lockshin, 1971). Since the actions of TTX appear to be peripheral in insects (Gammon, 1978), these animals presumably released EH, which then acted on the ISM. The observation that CNS hyperactivity could preserve the ISM past their normal time of degeneration (Lockshin & Williams, 1965*a*) may reflect a fail-sa[illegible]

mechanism by which animals can prevent the inappropriate breakdown of their muscles if they have difficulty extricating themselves from the old pupal cuticle and cocoon at eclosion. Such CNS preservation of cells destined to die is seen in the hawkmoth *Manduca sexta*, where motoneurones can be retained past their normal time of death by preventing the newly ecdysed moth from completing the full sequence of behaviour seen at emergence (Truman, 1983).

Pre-ecdysial ISM atrophy

The mass of the ISM appears to be constant throughout most of adult development until day 16, when the muscles enter a phase of gradual atrophy (Lockshin & Beaulaton, 1974) (see Fig. 8). In *M. sexta*, this reduction in the mass of the ISM during the final days of adult development acts to diminish the net force generated by the ISM, although the force per fibre cross-sectional area remains constant (L. M. Schwartz, L. Bolles & W. Almers, in preparation). Perhaps this reduction may prevent the ISM from pulling away from their attachment points along the weakened pupal cuticle at a time when the latter is degraded in preparation for adult eclosion.

The cellular events which underlie this muscle atrophy are very different from those involved in the rapid degeneration. Preliminary electron micrographic examination of these muscles (L. M. Schwartz & W. Wolfgang, unpublished observations) did not reveal the presence of significant numbers of lysosomes which could account for this extensive loss of protein. One possible mechanism for this loss of cellular material may involve the regular 'house-keeping' maintenance of the muscle which becomes disrupted, and thus allows endogenous protein degradation to outstrip synthesis. Such a situation would invoke a general loss of cellular material, resulting in atrophy.

While the biochemical steps involved in muscle atrophy in this system are unknown, a number of lines of evidence suggest that the factor responsible for regulating this atrophy is the insect moulting hormone, 20-HE. The initiation of muscle atrophy in *A. polyphemus* occurs during the decline in the endogenous ecdysteroid titre. This normal phase of atrophy could be prevented by artificially elevating the haemolymph ecdysteroid titre with either injection or infusion of this steroid. Such steroid-treated animals showed a pronounced retention of their ISM well past the time when control animals of comparable age had lost theirs. These animals also failed to respond to their own or exogenous EH. The time at which the ISM became EH responsive coincided with that when they became insensitive to 20-HE. Thus, the decline in the steroid titre commits the muscles to an adult differentiative programme (i.e. degeneration) which is initiated by EH at eclosion. Interestingly, in *M. sexta*, the decline in the steroid titre also determines the timing of EH release (Truman *et al.* 1983). The decline in the circulating titre of the ecdysteroids therefore insures that the degeneration of the ISM occurs at a developmentally appropriate time. Another well documented example of steroid withdrawal controlling the onset of peptide responsiveness in muscle occurs in pre-parturition rats, where myometrium sensitivity to oxytocin is dependent on a decline in the endogenous circulating titres of the steroid progesterone (Soloff, Alexandrova & Fernstrom, 1979). In this system, the declining steroid appears to trigger the synthesis of oxytocin receptors in the target tissue, thus conferring on it the capacity to respond to the peptide (Alexandrova & Soloff, 1980).

Hormonal control of muscle degeneration has been observed in several vertebr[illegible] and invertebrate systems. Zachery & Hoffman (1980) have demonstrated *in vitro* ecdysteroid regulation of selective muscle degeneration in *Calliphora* larvae at the time of pupariation. In some insects, such as the blood sucking bug *Rhodnius*, the ISM undergo cyclic atrophy and regrowth with each nymphal moult, presumably in response to changes in the ecdysteroid titre (Wigglesworth, 1956). Such steroid-dependent cyclic changes in muscle mass can be induced in the levator ani muscle of rats. This muscle is maintained by circulating titres of testosterone (Čihák, Gutmann & Hanzlíková, 1970) and atrophies when the animal is castrated. If steroid is provided exogenously, the muscle regains its normal mass and function (Vyskočil & Gutmann, 1977).

It is quite possible that the events involved in the regulation of ISM degeneration may be analogous to events associated with both the maintenance and degeneration of vertebrate muscle. Given the large size of the ISM, their synchronous, rapid degeneration and their defined endocrine triggers, these muscles may constitute a fruitful model system for the study of mechanisms of muscular dysfunction.

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