J. Exp. Biol. (1970), 53, 9–24
With 12 text-figures
Printed in Great Britain

ELECTROPHYSIOLOGICAL AND ENDOCRINOLOGICAL CORRELATES DURING THE METAMORPHIC DEGENERATION OF A MUSCLE FIBRE IN *GALLERIA MELLONELLA* (L.) (LEPIDOPTERA)

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(*Received* 20 *January* 1970)

INTRODUCTION

During the metamorphosis of certain Lepidoptera, including the greater wax moth, *Galleria mellonella* (L.), muscles can be made to degenerate precociously by denervating them (Finlayson, 1956, 1960; Randall, 1969). This has prompted the speculation that the peripheral innervation may regulate the normal pattern of muscle histolysis which occurs at metamorphosis; that if the neural factor is withdrawn the muscle degenerates, if it is sustained the muscle persists.

The relationship is of interest and may be analogous to that which prevails in vertebrates, where the 'trophic' influence of a nerve on its fully differentiated muscle has been abundantly documented (see Guth, 1968, for a review). The degree of resemblance is uncertain, however, because few systematic analyses of interactions between insect nerves and muscles during metamorphosis have been reported.

In a series of papers Lockshin & Williams (1964, 1965*a–d*) considered the mechanisms which regulate the cytolysis of the abdominal intersegmental muscles of the silk moth. They submit that these muscles are 'prepared' for degeneration during the first few days of adult development. At that time the synthesis of lysosome-associated cytolytic enzymes is brought about by exposure to ecdysone in the absence of juvenile hormone. Not until 3 weeks later, after the completion of adult development, is cytolysis triggered. According to these authors the signal for dissolution does not seem to be hormonal; instead, it is the cessation of nerve impulses from the ganglia which results in complete muscle degeneration within 48 hr. after adult emergence. Their studies suggested that if the nerves to these muscles were stimulated continuously, either electrically or by injecting parasympathicomimetic drugs, the histolysis of the muscles could be prevented or postponed.

Recently, Randall & Pipa (1969) investigated the cytomorphology of the principal planta retractor muscle of the proleg of *G. mellonella* during its metamorphosis. In the third abdominal segment it disappears between 19 and 45 hr. after pupal ecdysis (Randall, 1968). Cytological changes are evident in this muscle and its neural junctions soon after pupal emergence, and before it loses its contractility. A preliminary electrophysiological examination made in conjunction with the ultrastructural study sug-

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gested that discharge activity in the nerve to this muscle does not diminish until degenerative changes are conspicuous. Though the electrophysiological data were tentative and the degeneration of the principal planta retractor muscle was not quantified, the data seemed incompatible with the 'nerve silence' hypothesis advanced by Lockshin & Williams. This motivated us to examine closely certain of the electrophysiological and endocrinological relationships prevailing during its cytolysis.

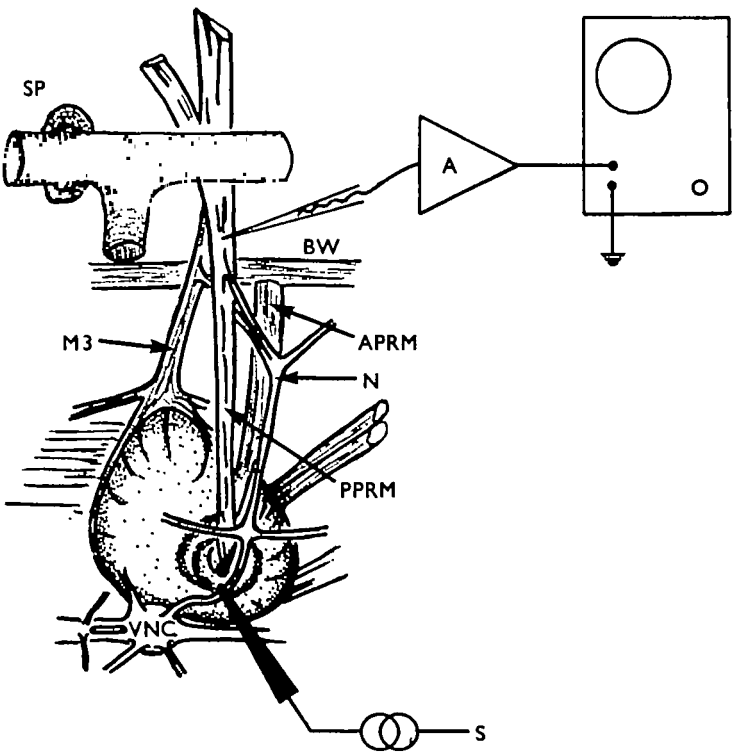

Fig. 1. A diagram (redrawn after Randall, 1968) showing muscles of the right proleg of the third abdominal segment of a *G. mellonella* larva, their innervation and the placement positions of the electrodes used in the present investigation. The principal planta retractor muscle (PPRM) is shown with the accessory planta retractor muscles (APRM) and muscle '3' (M_3). All of these muscles attach to the body wall (BW). PPRM is supplied by fine tracheae (not shown) which arise from the major tracheal trunk (T), posterior to the third abdominal spiracle (SP). Emanating from the posterolateral margin of the third abdominal ganglion (VNC) is the ventral nerve, a branch of which (N) contains seven axons. Only one of these axons innervates PPRM. Indirect electrical stimulation of PPRM was accomplished by placing a suction electrode (S) on the root of the ventral nerve. Intracellular glass microelectrode recordings of the PPRM's membrane resting and excitatory postsynaptic potentials were made using a Bioelectric Instruments neutralized input capacitance amplifier (A). The intracellularly recorded potentials were displayed on a Tektronix RM561 oscilloscope (O) and were photographed.

MATERIALS AND METHODS

Randall (1968), and Randall & Pipa (1969) have described in detail the anatomy and histology of the two principal planta retractor muscles of the first pair of prolegs of *G. mellonella*. Randall (1968) adopted Snodgrass' (1931) terminology, and designated this muscle 'PRVs' (= principal retractor of the vesica). In this report we shall use

the more direct abbreviation PPRM. A diagram showing the PPRM's innervation and position with respect to the planta and other proleg muscles is presented in Fig. 1. The muscle possesses markedly advantageous features: (1) it consists of a *single* fibre, and (2) this is supplied by a *single* motor neurone. Variability due to sampling different fibres of the same muscle at any one developmental stage is therefore eliminated.

We examined *in vivo* preparations of stage I larvae (as defined by Pipa, 1963; 218 preparations) and developing pupae of known age postecdysis (477 preparations). The insects were reared as described previously (Pipa, 1963). During stage I and throughout metamorphosis experimental and control insects were maintained at 33° ± 1° C. However, when electrophysiological measurements were being made using intracellular glass microelectrodes the preparations were examined at room temperature (22–25° C).

Each insect was pinned to a wax block, a dorsal longitudinal incision was made, and the digestive tract was removed. The PPRM of one of the first pair of prolegs was exposed for electrophysiological examination by pushing aside the major tracheal trunk (T, Fig. 1), being careful not to break the branches which join it to the muscle.

We used Baumann's (1968) '2–1' *Galleria* saline throughout. The saline contains the following ions in mM/l.: K^+ = 5, Na^+ = 128, Mg^{2+} = 2, and Cl^- = 141. It was buffered with 0·610 g/l. of trishydroxymethylamino methane, and was adjusted to pH 7·4 with 0·1 N-HCl.

Intracellular recordings of the PPRM

Membrane resting potentials and excitatory postsynaptic potentials (e.p.s.p.s) were obtained by using intracellularly placed 3 M-KCl glass microelectrodes (tip impedance = 15–20 Meg. Ω). The instrumentation utilized for recording is described in Fig. 1.

The intracellularly recorded e.p.s.p.s were evoked by stimulating the PPRM in one of two ways: (1) either by electrically exciting its nerve supply (Fig. 1) with a glass suction electrode (Florey & Kriebel, 1966), or (2) by stroking mechanoreceptors located on the last abdominal segment. The second method also initiates a complex reflex excitation of the ventral muscle groups associated with the third abdominal segment.

Electromyographic recordings from intact pupae

We explored the question of 'nerve silence' during pupal metamorphosis by making gross electromyographic recordings of ventral muscle activity in the third abdominal segment. The instrumentation system used is described in Fig. 2.

Programmed electrical stimulation of the PPRM during metamorphosis

We stimulated pupae electrically to determine whether the metamorphic degeneration of PPRM could be stopped or delayed. The instrumentation system devised to apply programmed stimuli of constant current to the nerve of the PPRM is described in Fig. 3. The administered stimuli ranged from 2–3 V., with an average current density of 10 μamp. These stimulus parameters were sufficient to cause the PPRM to contract. This was evidenced by the 'dimpling' of the proleg scars of pupae less than 2 hr. after ecdysis. The stimulus period and pulse frequency used to excite the pupal

PPRMs were determined by analysing electromyographic recordings from pupae of known age (Fig. 8). These data are noted in the legend for Fig. 3.

Pupae were selected for electrical stimulation within 30 min. after they had emerged. Each test insect was matched with a control which was also fitted with electrodes, but it was not electrically stimulated. After the 12-hr. stimulation period we took electro-

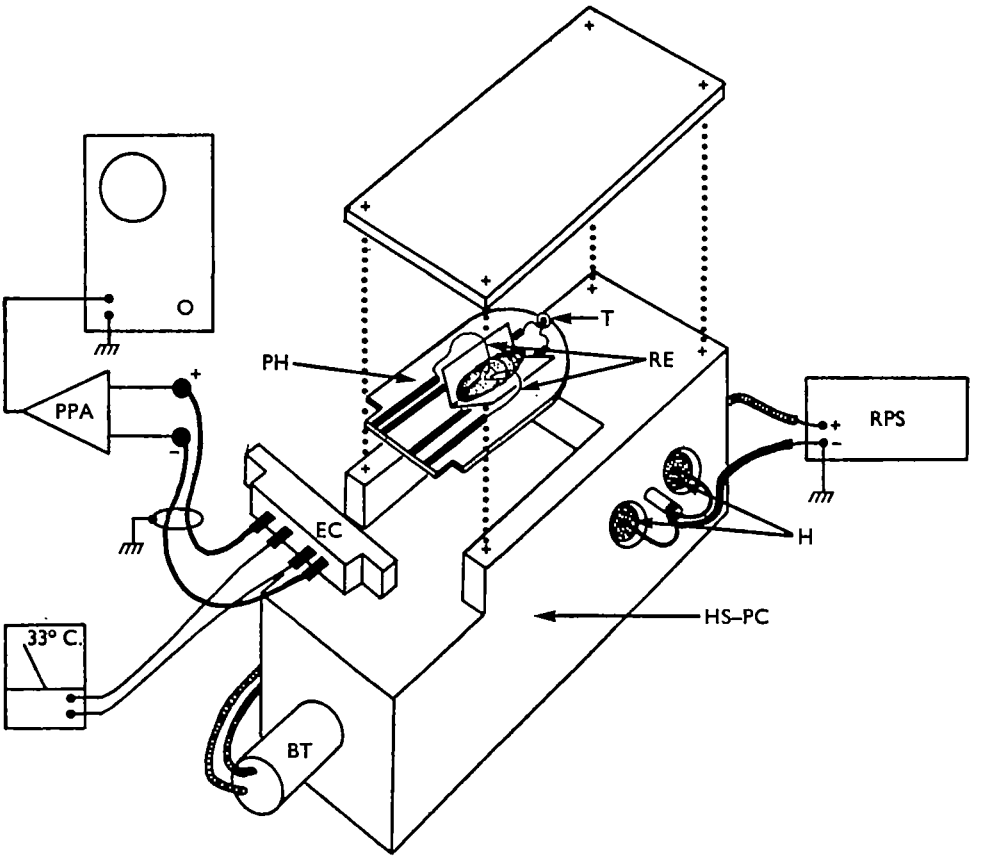

Fig. 2. Instrumentation for recording gross electromyographic discharge activity from muscles in the third abdominal segment of intact *G. mellonella* pupae during metamorphosis. Myographic recording electrodes (RE), prepared from 40 μ insulated copper wire, were inserted dorsally into the third abdominal segment within 30 min. after pupal ecdysis. Each pupa was loosely restrained in a V-shaped paper cradle attached to the pupa holder (PH). The pupa holder was a printed circuit board designed to accommodate a thermistor (T) and the recording electrodes. The thermistor recorded the air temperature within the cavity formed by the heat sink-pupal chamber (HS-PC) and the aluminium cover plate. The pupa holder made electrical contact with external instrumentation by an edge connector (EC). Electromyographic discharges amplified by a parametric pre-amplifier (PPA), were displayed on a Tektronix RM-561 oscilloscope (O), and were photographed by a 35 mm. continuous-moving film camera. Ambient temperature readings were monitored by a bridge network utilizing a galvanometer read-out (33° C.). Temperature stabilization of the pupa to $33° \pm 0{\cdot}5°$ C. was accomplished by a bi-metallic thermostat (BT) inserted longitudinally into the massive aluminium body of the heat sink-pupal chamber. The heat sink-pupal chamber was heated by two series-connected 4 Ω 10 W. resistors (H) which were 'press-fitted' into the aluminium block. A 4–10 V. precision-regulated d.c. power supply (RPS) was used to heat the two resistors. The regulated power supply not only contributed to the temperature stabilization, but made electromyographic recordings possible without 60 Hz interference. The pupal chamber was supported on a vibration-damped platform to eliminate peripheral excitation by mechanical disturbances.

physiological and birefringence measurements of the PPRMs from surviving specimens. The pupae were considered to be dead and were discarded if they failed to meet all of the following criteria: (1) had a heartbeat, (2) demonstrated spontaneously evoked e.p.s.p.s when intracellular glass microelectrode recordings were made from the ventral internal longitudinal and median muscles (see Randall, 1968, for nomenclature), and (3) demonstrated reflex-evoked e.p.s.p.s from these muscles when the terminal abdominal segment was stroked.

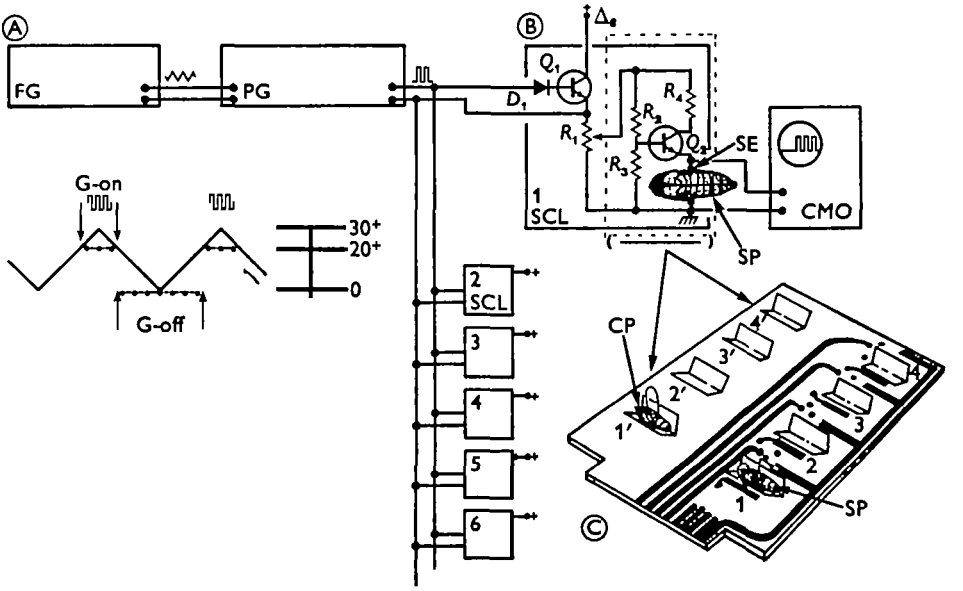

Fig. 3. The instrumentation system used in chronic, current-limited stimulation studies of *G. mellonella* pupae. Key to Fig. *A*, A portion of a linear triangle wave form derived from an Exact 240 function generator provides a d.c. gating potential to a Tektronix 160 series pulse-forming network. The on-gate period (20 sec.) is determined by a portion of the positive DC slope of the triangle wave threshold presented to the 160 pulse-forming network. The off-gate period or gate interval (2 min. 30 sec.) is a simple function of the triangle wave form's repetition rate or frequency. The gated, 160 pulse-forming network produced a train of pulses 1 msec. in duration with a repetition rate of 10 pulses/sec. The output pulses from the 160 pulse-forming network were used to control the current-limiting network seen in *B*. The current limiter consists of two stages, a switching emitter-follower and a current limiter. The emitter-follower stage, composed of Q_1 (Fairchild 2N3643), performs a dual function. First, in conjunction with the input diode (D_1) (Fairchild 1N3600) it serves to isolate the individual current limiters, i.e. limiters 1 to N, from each other. Secondly, it provides a method for regulating coarse stimulus amplitude. The coarse stimulus amplitude is determined initially by the absolute supply voltage applied to the collector of Q_1. Fine control of the stimulus amplitude is determined by R_1 (1 K) and the associated constant current-limiting network consisting of Q_2 (Fairchild 2N3643) and its component parts (R_2 = 3·6 K, R_3 = 2·2 K and R_4 = 10–33 K). The stimulus pulse appears between the emitter of Q_2 and ground. The stimulus was applied to the pupae via insulated 48 gauge copper wire electrodes inserted through the tergum of the third abdominal segment, as seen in *C*. The stimulus current was adjustable, and was initially monitored for each insect by a 50 Meg. Ω input impedance oscilloscope (CMO). By adjustment of the source potential applied to the collector of Q_1 and the potential drop across R_1 the stimulus current could be selectively limited for any pupa over a range of 0 to 50 μamp (stimulus currents of 5 to 10 μamp were generally employed). The pupa-stimulating circuit board (*C*) contained the current limiter (Q_2) and its associated components. The board with pupae was placed in a constant temperature oven maintained at 33° C. Note the V-shaped insect holders for each experimental pupa (SP) and their respective controls (CP). The remainder of the current-limiting module was mounted externally.

Endocrinological influences on PPRM degeneration

Within 1 hr. after they had emerged we transected pupae to obtain posterior fragments lacking all acknowledged endocrine glands. The details of the operation have been described elsewhere (Pipa, 1969). These fragments were removed from the incubator at hourly intervals; the PPRM's membrane resting potential and e.p.s.p.s were recorded, and the muscle's birefringence was measured.

In order to determine if the effects of the operation were due to an insufficient titre of hormone, we injected, shortly after surgery, into each fragment 0·6 μg. of synthetic 20-hydroxyecdysone (ecdysterone, Mann Research Laboratories, New York) dissolved in 2 μl. of Baumann's saline. Each test fragment was matched with a control that received 2 μl. of saline without the hormone. All injections were made with a 10 μl. capacity syringe (701) fitted with the Chaney Adaption (Hamilton Co., Inc., Whittier, California). At periodic intervals the experimental and control fragments were removed from the incubator, and electrophysiological measurements were made.

Birefringence measurements

We isolated the PPRM by carefully removing adjacent muscles and fat body. Next, the pupal exoskeleton was held closed along the mid-dorsal incision so as not to stretch the muscle, and the insect was fixed in Lillie's acetic alcohol formalin (Lillie, 1954). After *c.* 12 hr. we removed the PPRM from the pupa, washed it in distilled water and slide-mounted it in Farrant's glycerol gum arabic (Lillie, 1954).

We quantified the intensity of birefringence while observing the PPRM at a magnification of 200 diameters, with polarizer and analyser at right angles, and while using orthoscopic illumination (λ = 548 mμ). After orienting each branch of the Y-shaped muscle fibre in the position of maximum birefringence, the angular value for extinction (compensator azimuth) was determined using a λ/10 Brace-Köhler compensator. Because of wide variations between readings obtained at different regions of the same fibre, measurements were taken at the three extremities and the midsection, and these were averaged.

RESULTS

Electrophysiology and birefringence of the PPRM of intact larvae and pupae

When the ventral nerve which supplies the PPRM is stimulated via a suction electrode (Fig. 1), only one type of e.p.s.p. can be recorded intracellularly. We applied finely graded variations in the amplitude, duration and frequency of stimuli to the ventral nerve, but failed to demonstrate the presence of more than one axon to this muscle fibre. This supports the conclusion reached from earlier histological studies of the PPRM (Randall & Pipa, 1969).

Intracellularly recorded PPRM membrane resting potentials of stage I larvae differ considerably from one preparation to the next. In the insect's haemolymph these range from a high value of 60 mV. to a low value of 40 mV. Similarly, the e.p.s.p.s of stage I larvae vary from 40 mV. (Fig. 4) to 20 mV.

As metamorphosis progresses the PPRM membrane resting potential (Fig. 5) and e.p.s.p.s elicited by mechanical stimulation (Figs. 4, 5) decline in amplitude. At 11–12 hr. after pupal ecdysis the membrane resting potentials were generally below

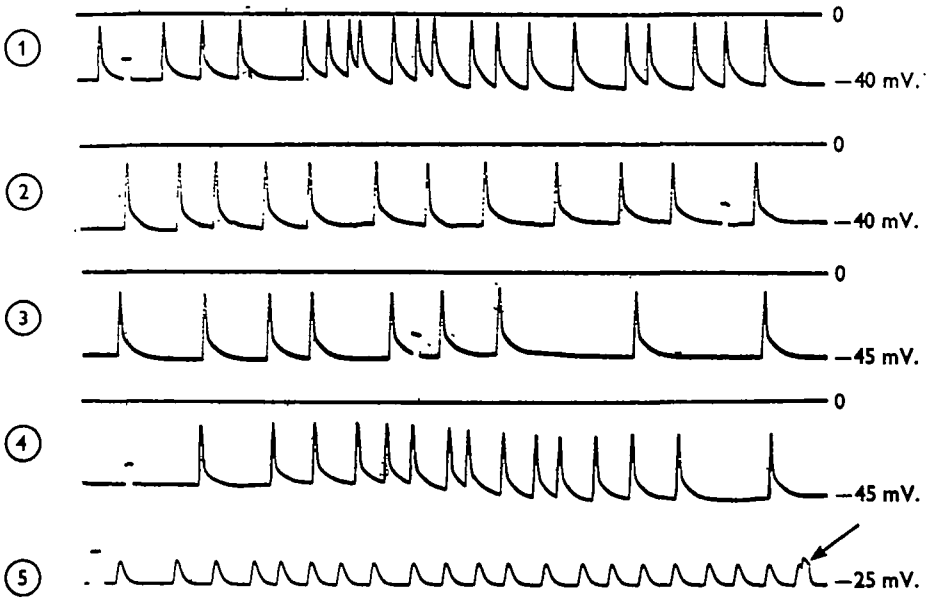

Fig. 4. Intracellular glass microelectrode recordings of reflex-evoked excitatory postsynaptic potentials from the first proleg PPRM of *G. mellonella*. Traces 1, 2 and 3 are from three different stage I larvae; traces 4 and 5 are typical of records obtained from mechanically stimulated pupae at 3 and 7 hr. after ecdysis. The calibration pulse contained in each trace record is the same for all traces, i.e. 10 mV., 5 msec. The arrow denotes an artifact produced when the calibration pulse summated with an e.p.s.p.

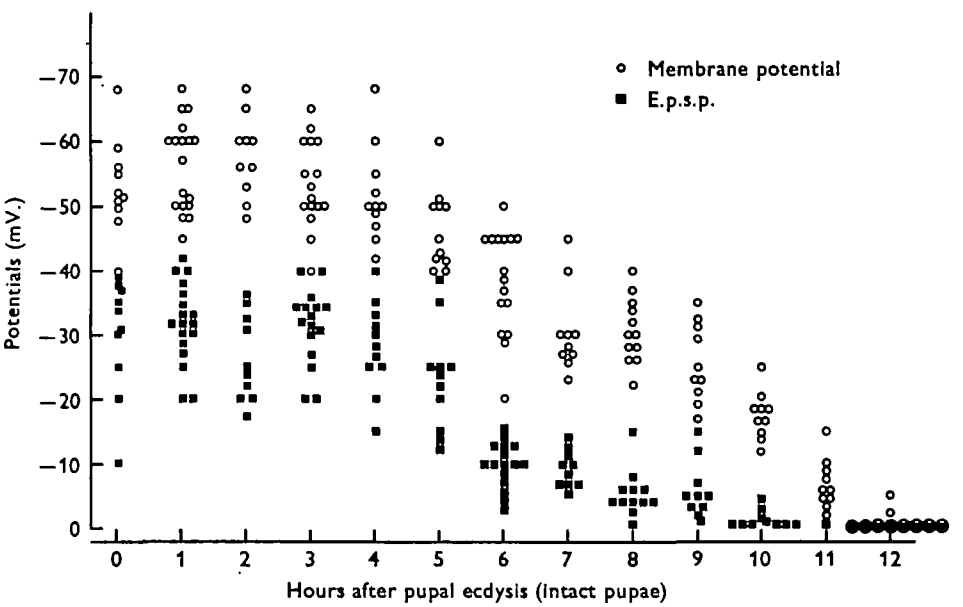

Fig. 5. The decremental changes in membrane resting potential and excitatory postsynaptic potential (e.p.s.p.) recorded from the first proleg PPRM of *G. mellonella* 0–12 hr. after pupal ecdysis. The e.p.s.p.s (see Fig. 1) were evoked in the preparations by peripheral tactile stimulation. Note that at 11–12 hr. after pupal ecdysis e.p.s.p.s could no longer be elicited.

18 mV., and e.p.s.p.s could not be recorded from the muscle. A marked attenuation of e.p.s.p. amplitude occurs between 4 and 6 hr. after pupal ecdysis, and this diminished amplitude is evident in spontaneously contracting PPRMs, as well as in the PPRMs which are induced to contract by stimulating mechanoreceptors of the pupae. Because e.p.s.p.s can be recorded constantly during the first 10 hr. after pupal ecdysis, the existence of functional neuromuscular junctions at that time is confirmed. Since e.p.s.p.s can be elicited by touching mechanoreceptors on the last abdominal segment of the pupa, appropriate central connections must also be operative during that interval.

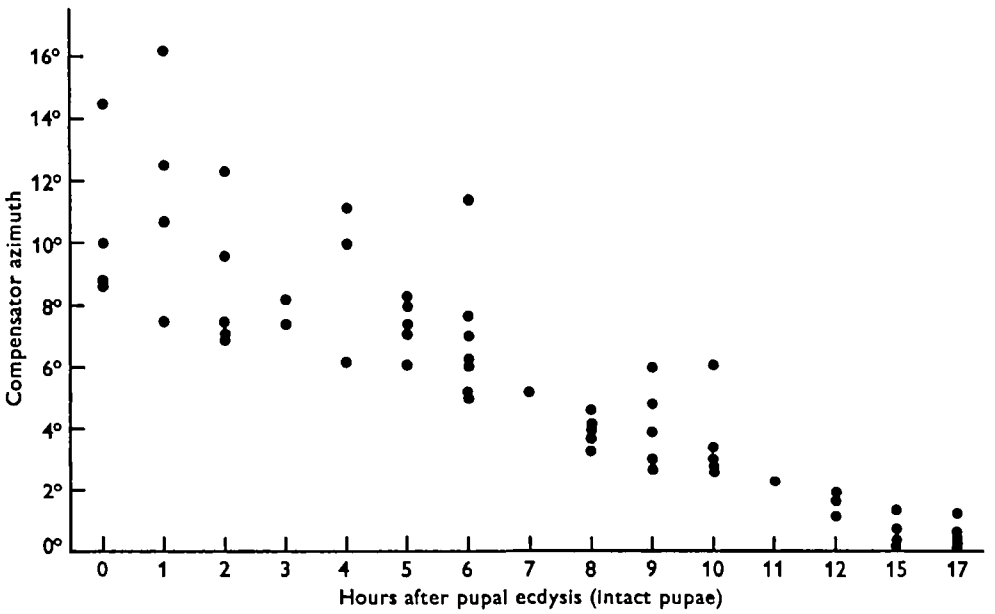

Fig. 6. Decrease of the intensity of birefringence of the first proleg PPRM during metamorphosis of *G. mellonella* pupae. Each point represents the mean of measurements taken at each of four locations on one muscle fibre.

Concurrent with the decline in e.p.s.p. amplitude the intensity of birefringence of the PPRM gradually diminishes, and it becomes minimal between 10 and 15 hr. after ecdysis (Fig. 6). By then, cytomorphological signs of PPRM degeneration are pronounced (Randall & Pipa, 1969) and the muscle volume has decreased dramatically.

During the first 12 hr. after ecdysis a close correlation exists between two measurements of PPRM integrity: the intensity of its birefringence, and the amplitude of its membrane resting potential (Fig. 7). The PPRMs with birefringence values of 2·5°–3·5° invariably have membrane resting potentials at or below 18 mV. Also, by comparing Fig. 5 with Fig. 6 it is evident that e.p.s.p.s usually could not be recorded from PPRMs which had birefringence values as low as this.

As noted previously (Randall & Pipa, 1969), the PPRM demonstrates histological signs of degeneration at its cuticular attachments before these signs have become pronounced at the midsection of the muscle, suggesting that the incidence of degeneration may not be uniform along the length of the fibre. Because of this likelihood we sought differences in membrane resting potentials along the axis of PPRM during

metamorphosis. After numerous attempts we concluded that if such variations existed they were below the resolving capabilities of our instrumentation. Unfortunately, in pupae older than 6 hr. postecdysis, when such study might have been more meaningful, the width of the PPRM was so reduced near its attachments that it was not technically feasible to make such measurements.

Usherwood (1961, 1963) has shown that changes in the form and frequency of spontaneous miniature end plate potentials will occur after the nerve supply to the extensor tibiae muscle of the locust, *Schistocerca gregaria*, has been severed. This suggested that these characteristics might be useful in defining the metamorphic

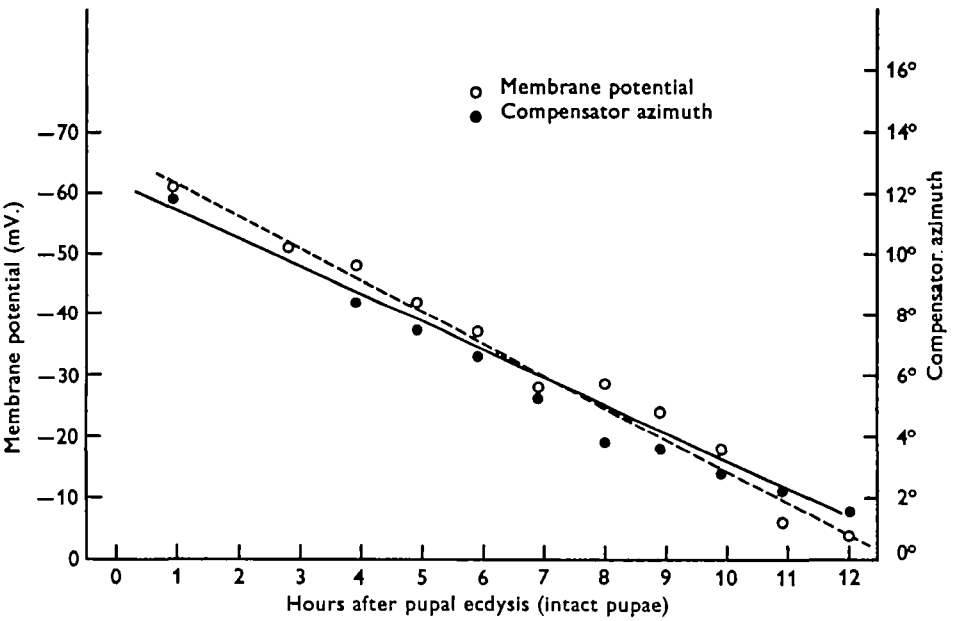

Fig. 7. The correlation of the membrane resting potential with the birefringence intensity during the metamorphic degeneration of the first proleg PPRM in intact *G. mellonella* pupae. Each point represents the mean standard deviation of values given in Figs. 5 and 6.

degeneration of the PPRM. Although we tried to record miniature end plate potentials from larvae and pupae, we were unsuccessful. As noted in the studies of Belton (1969) and Randall & Pipa (1969), the neuromuscular junctions of *Galleria* are completely surrounded by highly convoluted plasma membranes. These may have prevented us from attaining adequate juxtaposition of our recording micreoelectrodes.

Electromyographic discharge activity in the third abdominal segment during pupal metamorphosis

During the first 18 hr. after pupal ecdysis continuous electromyographic recordings were made from the third abdominal segment which contains the PPRMs. The data (Fig. 8) were characteristic for six preparations. In two additional trials continuous recordings were made starting within 30 min. after pupal ecdysis and ending at adult emergence. The periodic discharge patterns evident beyond 18 hr. postecdysis clearly resemble those seen from 2 to 18 hr. postecdysis.

Although electromyographic activity diminishes markedly during the first 2 hr. postecdysis (Fig. 8), there is continuous nerve discharge activity in this segment throughout metamorphosis. This is evidenced by the myograms of the ventral longitudinal muscles and their associates. While it could be argued that the electromyograms provide an uncertain indication of the central nervous system's control (Runion & Usherwood, 1966), the electromyographic discharges did correlate with visually monitored PPRM contractions during the first 9 hr. after pupal ecdysis, i.e. when degeneration of the PPRMs is clearly in progress. During this time the PPRMs will invariably contract following contraction of the ventral intersegmental muscles and the ventral internal longitudinals.

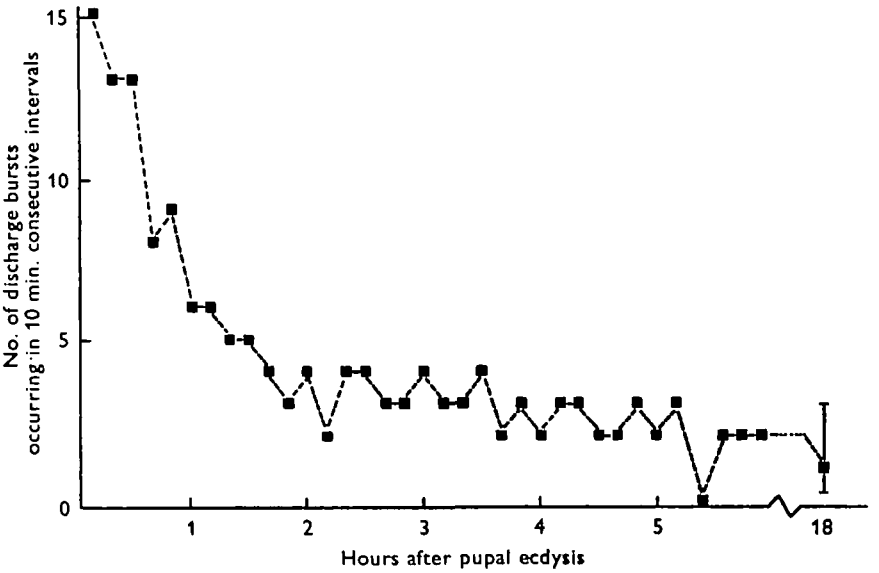

Fig. 8. The number of electromyographic bursts recorded from the musculature of the third abdominal (first proleg-bearing) segment of *G. mellonella* during the initial 18 hr. after pupal ecdysis. Recording electrodes were inserted dorsally into the segment. The number of discharge bursts during each consecutive 10 min. sampling period was counted throughout the first 18 hr. A myographic burst consisted of 4–8 individual muscle-unit discharges. See Fig. 2 for a description of the instrumentation used in obtaining these data.

Electrical stimulation during the degeneration of the PPRM

The experiments were conducted as described in Fig. 3. The programmed, individual stimulus, current-limiting networks were effective in preventing accidental electrocution of pupae during the 12-hr. experimental period. Only seven of the 65 chronically stimulated pupae studied were suspected of having been electrocuted, and these were discarded.

We were unable to demonstrate a sparing effect of electrical stimulation on muscle degeneration in this experiment. The resting potentials of the PPRMs from chronically stimulated pupae averaged 2·4 mV. (N = 58; S.D. = ±0·45 mV.); those from the controls, 2·6 mV. (N = 65; S.D. = ±0·38 mV.). The birefringence measurements (compensator azimuth values) from chronically stimulated PPRMs averaged 3·5° (N = 19; S.D. = ±2·5°), compared with 3·4° for the controls (N = 20; S.D. = ±2·5°).

Potentiation of the metamorphic degeneration of the PPRM by ecdysterone

When pupae are deprived of all acknowledged endocrines by transection the degradation of the PPRM's membrane resting potential and of the e.p.s.p.s is delayed. In the intact pupae the membrane resting potential can seldom be recorded beyond 11 hr. after ecdysis (Fig. 5), but in the posterior fragments it can be measured consistently at 21 and 22 hr. (Fig. 9). Although e.p.s.p.s from the PPRMs of intact pupae likewise

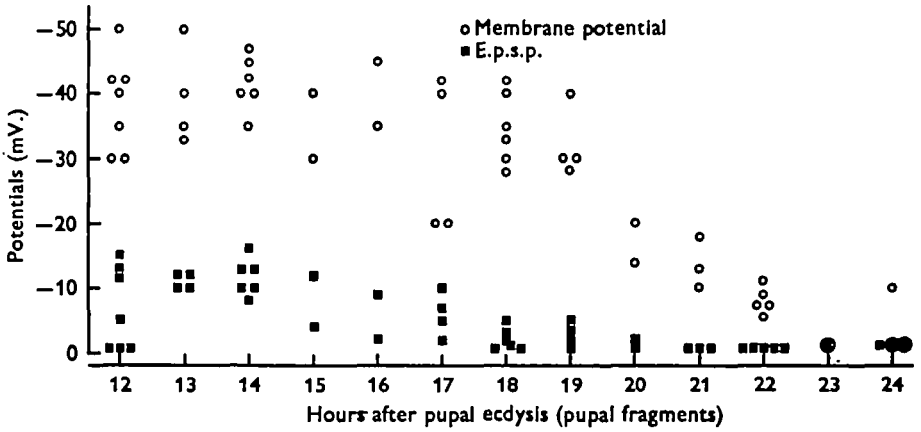

Fig. 9. Diminution of membrane resting potentials and e.p.s.p.s of the first proleg PPRM in *G. mellonella* pupae which had been deprived of all acknowledged endocrines within 1 hr. after ecdysis. The pupae were transected behind the prothorax and the wound was closed with a plastic slip held in place with wax. The posterior fragments were kept at 33° ± 1° C. until removed for examination. The e.p.s.p.s were evoked by peripheral tactile stimulation. Note the delay in the falling-off of these potentials compared to those from whole pupae (Fig. 5).

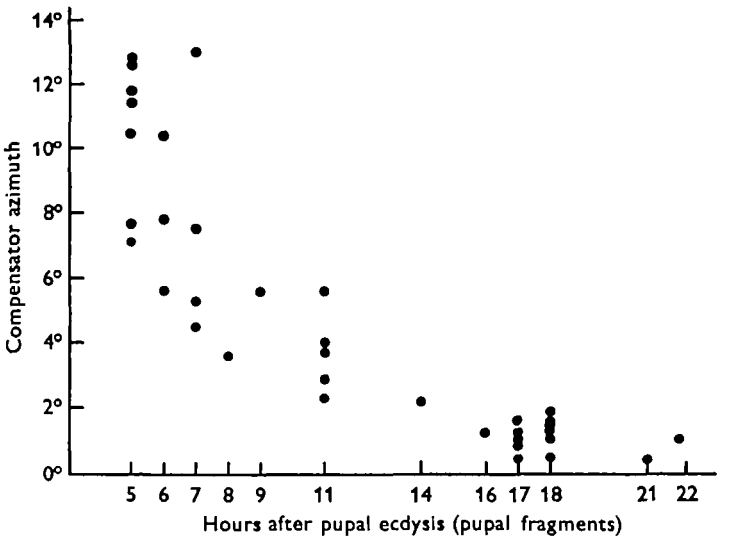

Fig. 10. The decrease in the birefringence of the first proleg PPRM of *G. mellonella* pupae which had been deprived of all acknowledged endocrines as noted for Fig. 9. Compare with Fig. 6.

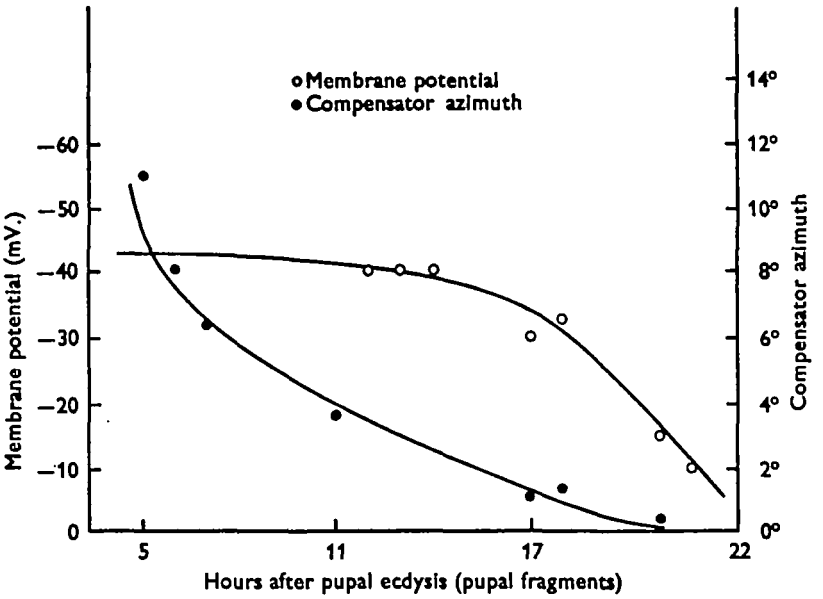

Fig. 11. The relationship of the membrane resting potential to birefringence intensity during metamorphic degeneration of the first proleg PPRM in transected *G. mellonella* pupae. Each point represents the mean standard deviation of values given in Figs. 9 and 10. Compare with Fig. 7.

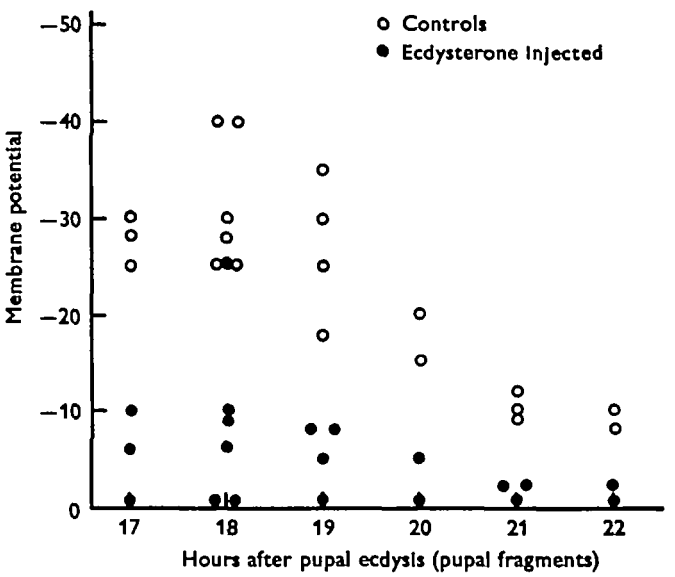

Fig. 12. The potentiating effect of 20-hydroxyecdysone on the attenuation of the membrane resting potential of the first proleg PPRM in *G. mellonella* pupae which had been transected within 1 hr. after ecdysis. The hormone dissolved in physiological saline was injected into each pupal fragment shortly after surgery. Controls received the same volume of saline as that injected into the experimentals, but without the hormone.

cannot be detected beyond 11 hr. postecdysis (Fig. 5), they can be recorded at 19 to 20 hr. in the posterior fragments (Fig. 9). In agreement with observations made on intact pupae, when the membrane resting potential of the PPRMs from the posterior fragments drops below 18 mV., e.p.s.p.s cannot be recorded, nor can these muscles be seen to contract following contraction of the ventral internal longitudinal muscles.

The intensity of birefringence of the PPRM from pupal fragments becomes minimal between 11 and 17 hr. after ecdysis (Fig. 10). These data are similar to those obtained from intact pupae (Fig. 6), showing that the removal of the brain, corpora allata, corpora cardiaca and prothoracic glands has little effect on the rate at which the micellar orientation of the contractile proteins is disrupted.

The close correspondence between the PPRM membrane resting potential and birefringence evident in intact pupae (Fig. 7) is not apparent in pupal fragments (Fig. 11). This simply reflects the observations noted above; the operation severely retards the deterioration of the permeability properties of the muscle membrane, but not the loss of muscle birefringence.

In order to determine whether the retarded decline of the PPRM membrane resting potential in transected pupae is caused by an inadequate hormone titre, 20-hydroxyecdysone was injected. The mortality in this experiment was about 13%. The data (Fig. 12) clearly indicate that the hormone can potentiate the loss of the muscle's membrane resting potential. The rate of attenuation of e.p.s.p.s, too, is influenced by administering this substance. In the controls e.p.s.p.s are observable up to 20 hr. after ecdysis, but in the fragments which receive 20-hydroxyecdysone they cannot be detected at 17 hr., when the membrane resting potential is below 18 mV.

DISCUSSION

Certain lepidopteran muscles will not differentiate during metamorphosis unless their innervation is left intact (Kopeč, 1923; Williams & Schneiderman, 1952; Nüesch, 1952, 1954). Also, fully differentiated muscles which normally survive the histolytic phase of pupal development can be caused to regress during that time by depriving them of their innervation in the larva (Finlayson, 1956, 1960; Randall, 1969). Although these observations imply a metabolic dependency of a muscle on its nerve supply, the nature of the influence is obscure, and it may not be of one kind.

For the intersegmental muscles which persist in the silkworm pupa but degenerate in the adult it has been proposed that the final signal for the onset of histolysis consists of a curtailment of efferent nerve impulses (Lockshin & Williams, 1965*b*). It was because we were uncertain whether this hypothesis could be invoked to explain the initiation of muscle degeneration which occurs in a lepidopteran pupa that we decided to examine the PPRMs of the first pair of prolegs of *G. mellonella*. These two muscles were promising subjects for the study; each of them is a single fibre innervated by one axon, and the cytomorphological features of their cytolysis had been investigated (Randall & Pipa, 1969).

Our data convince us that the metamorphic degeneration of the PPRM is not signalled by cessation of nerve impulses from the ganglion. Nerve discharge activity to the PPRM can be elicited until 11 hr. after ecdysis by brushing mechanoreceptors located on the last segment of the pupal abdomen. During this interval degenerative

changes in the muscle become pronounced: the intensity of birefringence is reduced and the membrane resting potential and e.p.s.p.s are attenuated. Clearly, the PPRM can be excited via a reflex pathway during the time the muscle is regressing, and there is no reason to suspect that this will not occur naturally. Furthermore, we found that the PPRM contracts whenever the pupa contracts certain ventral intersegmental muscles of the third abdominal segment. Because electromyographic records obtained during the first 18 hr. after pupal ecdysis suggest considerable ventral intersegmental muscle activity, presumably the PPRM is also being excited regularly while it is degenerating.

The small size of this preparation prevented us from employing direct neurogram recording techniques (Runion & Usherwood, 1966) to test the functional state of the nerve supplying the PPRM. Randall & Pipa (1969) reported 20 to 29 axons within the ventral nerve (Fig. 1) and this number increases markedly by 24 hr. after ecdysis. Therefore, even if neurograms could have been recorded, they would have been difficult to interpret.

If metamorphic degeneration of the PPRM were caused by a cessation of efferent nerve impulses one would anticipate that the process could be retarded or stopped by electrically stimulating the nerve which supplies this muscle. Indeed, Lockshin & Williams (1965*b*) report achieving this, and consider the data to be their most convincing evidence. In our study, however, no such 'sparing effect' could be detected. We began stimulating PPRM before its e.p.s.p.s had become attenuated, and we were certain that the electrical stimuli we applied would cause the muscle to contract; yet, at the end of the test period, the membrane resting potentials and birefringence of the muscle were virtually identical in the stimulated and control pupae.

In their experiments using chronic electrical stimulation Lockshin and Williams (1965*b*) used stimulus amplitudes of 6–10 V. and reported that 50% of the stimulated surviving preparations had muscles which were 'fully preserved'. Though they noted that about half of their animals had died during the experiment, they did not indicate what criteria they used to assure that the muscles from the survivors were not moribund. We have found that stimulus amplitudes higher than 7 V. will cause necrosis in tissues adjacent to the stimulating electrode. In about half of our pupae stimulated at such high voltages the PPRMs showed no e.p.s.p.s or membrane resting potentials at the end of the test period, though these muscles were still present. We suspect, on the basis of our results, that the 'sparing effect' noted in the experiments of Lockshin & Williams (1965*b*) may have been due to the electrocution of their muscle preparations.

Irrespective of this difficulty, the likelihood remains that our interpretations and those of Lockshin & Williams (1965*b*) reflect the existence of different 'signalling' mechanisms for muscle degeneration in the two species. It is conceivable, for example, that motor end plates along the PPRM lose their ability to transmit impulses at different times, and that this regulates what seems to be a non-uniform degeneration along the length of the muscle (Randall & Pipa, 1969). Had we been able to record miniature end plate potentials from PPRM this hypothesis might have been supported. In the absence of such data, however, it must remain conjectural. Of course, the possibility also exists that the 'trophic' influence of the axon supplying PPRM (Randall, 1969) is neurohumoral, not bioelectrical.

The decline in the intensity of the PPRM birefringence is not retarded significantly by transecting *G. mellonella* pupae just behind the prothorax soon after they have

emerged. Evidently, the histolysis of this muscle will continue although all acknowledged sources of hormones have been removed. This is similar to the results reported by Finlayson (1956), who found that certain abdominal intersegmental muscles which normally degenerate in the newly emerged adult cecropia silkmoth will also regress after the abdomen has been isolated from the rest of its body. Lockshin (1969) finds that if the abdomen of *Antheraea polyphemus* is isolated after adult emergence the intersegmental muscles will degenerate, but he notes that when the abdomen of the pharate adult is isolated with a minimum of trauma these muscles are frequently retained beyond the time of expected histolysis.

For *G. mellonella* pupae there is experimental evidence that transection lowers the relative titre of ecdysone (Pipa, 1969). Nevertheless, the assumption that PPRM histolysis will continue in the absence of this hormone is unwarranted. The amount of ecdysone which persists within these posterior fragments is unknown.

In contrast to the negligible effect transection has on the decline of the PPRM birefringence, the operation significantly delays the attenuation of this muscle's membrane resting potential and of the e.p.s.p.s. Because the retardation is alleviated by injecting 20-hydroxyecdysone it would seem that the metamorphic degeneration of these properties is normally enhanced by this steroid hormone, or by one of its analogues.

SUMMARY

1. Excitatory postsynaptic potentials (e.p.s.p.s), membrane resting potentials, and intensity of birefringence were measured from the two principal planta retractor muscles (PPRMs) of the first pair of prolegs during metamorphosis of *Galleria mellonella*. This was done to determine whether cessation of efferent nerve impulses to these single fibres signals their degeneration.

2. E.p.s.p.s were elicited reflexively by brushing the insect's last abdominal segment. Though they become attenuated, e.p.s.p.s can be recorded until 11–12 hr. after pupal ecdysis. By this time the membrane resting potential has dropped from *c.* 55 mV. to less than 18 mV., and the intensity of birefringence has become minimal.

3. Continuous electromyographic records taken during the initial 18 hr. after pupal ecdysis suggest that the PPRMs are being excited regularly as they degenerate.

4. No 'sparing effect' on the degeneration of the PPRMs could be detected when programmed, current-limited stimuli were administered to pupae via chronically implanted electrodes.

5. Removing all acknowledged endocrines by transecting newly emerged pupae has negligible effect on the decrease of the PPRM birefringence, but greatly retards the degradation of the membrane resting potential and e.p.s.p.s. This retardation can be alleviated by injecting the steriod hormone 20-hydroxyecdysone.

We thank Dr Roderick Craig for providing us with additional laboratory space, and for his helpful counsel. We also gratefully acknowledge the assistance of Dr David Bentley, Zoology Department, University of California, Berkeley, who kindly reviewed our data and offered suggestions, and of Mr Clyde Brown, Senior Laboratory Technician, Department of Soils and Plant Nutrition, University of California, Berkeley, who permitted us to use his machine shop facilities. The study was supported

by U.S. Public Health Service Research Grant Ro1 NB–03845 from the National Institute of Neurological Diseases and Stroke awarded to R. L. Pipa.

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