THE EFFECTS OF JUVENILE HORMONE ON THE DEVELOPMENT, BEHAVIOUR AND ELECTROPHYSIOLOGY OF *LOCUSTA MIGRATORIA*

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ABSTRACT

Following recent research into the influence of juvenile hormone on migratory insect flight behaviour, a study of the effects of juvenile hormone on the larval marching behaviour of *Locusta migratoria migratorioides* (R & F) was undertaken. Firstly the walking behaviour of the 5th instar larvae was monitored on a free-running treadwheel that recorded all forward movement of the insect. A pattern of walking behaviour was evident throughout the 5th instar and also within a 3h period that agreed with published data from laboratory and field observations. However, the variation in behaviour recorded was high and a series of 4 treatwheels was developed to allow large numbers of animals to be tested. These simple wheels recorded only the total distance walked in each hour, and trials were limited to the third day of the instar. Treatments with synthetic JH I, II and III proved to be relatively inactive both morphogenetically and behaviourally (JH III only), while the JH - analogues, Law's mixture and ZR515, were found to have potent morphogenetic effects. Law's mixture was seen to reduce the level of walking observed in 5th instar larvae but showed some associated toxic effects; high doses of ZR515 increased the level of walking by over 50%. The antiallatotropic agent, precocene II, was employed as an 'anti - JH' within the 4th instar to produce precocious adultiform larvae. However, it was seen to induce potent JH - effects when applied in the 5th instar, and these animals also showed an increase in their walking behaviour. The adultiform larvae produced by treatment in the 4th instar showed walking behaviour that approached the low walking levels of normal adults. Their escape jump responses were dichotomous, and distinct, separate, signs of both larval and adult behaviours were observed in single individuals. These later findings were further explored at the neurophysiological level in an attempt to explain the suspected hyperactivity and sensitivity of these adultiform larvae. Spontaneous and stimulated neuronal activity levels were recorded in normal and treated animals and the tension developed in the extensor tibialis muscle during stimulation was also monitored. The results are discussed in relation to the current literature on the action of juvenile hormone in the locust.

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INTRODUCTION

Hormones have been known to be involved in vertebrate and invertebrate behaviour for many years. Truman and Riddiford (1974) have suggested in their review of the hormonal mechanisms underlying insect behaviour that because of the limitations on the size of their nervous system, the insects make greater use of hormones in the control of their behaviour than do any other invertebrates. A high level of interaction between the endocrine and nervous systems would allow more information to be contained in, and to be expressed by, the nervous system. Considerable attention has been paid to the role of hormones in insect reproductive behaviour, while a second major field of study has been the hormonal control of insect migratory behaviour (reviews: Truman and Riddiford, 1974; Goldsworthy, 1976; Rankin and Rankin, 1979).

Migratory behaviour is usually considered to be a population reaction to a variety of environmental events that results in the movement of some parts of the population, often over considerable distances. The migrant flying insects become highly sensitive to stimulii inducive to flight and less responsive or unresponsive to stimulii associated with reproduction and feeding (Kennedy, 1961). The physiological events involved in this change of sensitivity are dependant upon the insect's age and primarily on its developmental status. The quality of the external environment and diverse factors such as the population density and the changing seasons are also important. Thus, migration cannot be considered solely as the movement away from an unfavourable environment with the aim of reaching more favourable conditions. It is a more complex strategy that may only have advantages in the later stages of life or for the next generations (Johnson, 1969).

The migratory flight of many insects is influenced by the haemolymph titres of one or two hormones. In locusts three endocrine centres have been implicated both in the initiation of flight and in the adjustment of the fuel metabolism required by the rapid change from low energy resting behaviour to the heavy energy demands of flight (Goldsworthy, 1976). These centres are the corpora allata, the cerebral neurosecretory cells and the glandular lobes of the corpora cardiaca.

The corpora allata (CA) are paired endocrine glands that are linked by neuronal and neurosecretory cells to the CNS, via the corpora cardiaca.

They are the site of the synthesis and release of the juvenile hormones, of which three forms have been identified (review: Dahm *et al*, 1976). Which of these three forms are present in locusts is still uncertain, however: there is evidence that all three have some activity (Roussel, 1975, 1976a 1976b). While the work of Roussel (1976b), Lazarovici and Pener (1977) and Amerasinghe (1978) indicates that JH I is the more active form of juvenile hormone in all the criteria they examined, Caruelle *et al* (1979) and Huibregtse-Minderhoud (1979) found that the synthesis and release of JH III was greater than that of JH I or JH II. Peter *et al* (1979) reported that the protective functions of the JH - carrier protein appeared to be highly selective, and favoured JH III. In this introduction no attempt will be made to distinguish between these three forms of JH; their relative activities are discussed later (p. 48).

Juvenile hormone is released from the CA during the early instars and maintains the insect on its course of larval development (Wigglesworth, 1935, 1970). The CA cease to secrete JH in the penultimate instar (the 4th instar of gregarious *Locusta*) and the JH titre in the haemolymph during the last instar is at, or nearly at, zero. The absence of circulating JH initiates adult development and in the hemimetabolous insects the final moult to the adult stage allows the full development and expansion of the wings, the opening of the sexual organs, and various other morphological and physiological changes. JH is also active in the adults, however, and is involved in the maturation of the female reproductive organs and the oocytes. That JH is involved in the control of reproductive and migratory behaviours in various insects is now well established (reviews: Truman and Riddiford, 1974; Rankin and Rankin, 1979). Rankin and Riddiford (1978) have demonstrated that in *Oncopeltus* there is a low threshold above which JH initiates migratory flight, and a higher threshold above which the insect ceases migratory behaviour and enters the reproductive behavioural regime. In *Schistocerca* the CA are essential for the display of sexual behaviour, but in *Locusta* their control is not so complete (review: Truman and Riddiford, 1974).

The influence of JH on locust locomotor behaviour was first reported by Cassier (1963, 1964), who showed that CA implants increased the speed of movement and the phototropic responses of gregarious adult *Locusta*. Odhiambo (1965, 1966) reported that allatectomy of *Schistocerca* adults reduced spontaneous locomotor activity but Strong

(1968) reported that allatectomized *Locusta* did not show any marked reduction in locomotor activity. He argued (Strong, 1968) that the differences between these two observations were related to the relative influence of the CA on reproductive behaviour, and that reproductive behaviour constituted a major part of the normal locomotor activity of adult *Schistocerca*. However, Wajc and Pener (1971) reported less intense flight in allatectomized *Locusta* using a roundabout that excluded the possible influence of sexual behaviour on locomotion. Lee (Goldsworthy, 1976) has repeated these experiments and has determined that the flight of *Locusta* is affected by the CA: young allatectomized males flew less than control animals but when older these allatectomized animals flew better than the controls. This was because the flight behaviour declined less rapidly with age in the allatectomized adults than in the controls. Goldsworthy (1976) also reported that periodic topical applications of a synthetic JH reversed the initial effects of allatectomy on flight, but were less effective in the older animals.

It was therefore apparent that in *Locusta* the JH titre was positively correlated with adult flight behaviour, at least in the early adult life. The extent of the control was uncertain, however, and there is no published information on the influence of juvenile hormone on larval locomotor behaviour. It was therefore decided to study the marching behaviour of 5th instar *Locusta migratoria* larvae, and the possible control of this behaviour by JH.

The locust is easily cultured and for this work an ample supply was kindly provided by the Centre for Overseas Pest Research, London. It is also a robust insect and is well suited to experimental treatment. Further, being a major pest, its biology is well documented and it has now become accepted as one of the standard laboratory insects. The endocrinology, development, behaviour and neurophysiology have all been studied in some detail and there was therefore a good foundation on which to base this study. The 5th instar was chosen mainly because of the supposed absence of JH during this instar which would, if true, allow greater scope to manipulate JH levels by exogenous JH treatments. There is now some evidence that there is some JH circulating during the 5th instar, however (Baehr *et al*, 1979; Huibregtse-Minderhoud, 1979), and further evidence (p. 58 ; Johnson, 1973; Caruelle *et al*, 1979) that the CA may synthesize JH during this instar. As will be discussed later (p. 81), there were some disadvantages in using the 5th instar. The major disadvantage is the inherent difficulty in distinguishing between the effects

of JH on the CNS that result in temporary, plastic, changes in behaviour, and those effects on the behaviour that are the result of growth and differentiation of the CNS. There is a marked difference in the development of the locust during the early instars (1-4) from that in the final larval instar, when considerable muscular and behavioural developments precede the moult to the adult stage. At the start of the project it was felt that this difficulty could be overcome by achieving dose-responsive changes in behaviour, since one would not expect the developmental processes to be as easily or as rapidly modified as the behaviour. That these dose-responsive changes in behaviour have only partially been achieved (p. 79) emphasises that the hormonal control over this behaviour is not complete. It appears that to adequately study the problem of the hormonal control of larval behaviour one would have to compare the effects of hormones on the behaviour of both early and late instars, thereby distinguishing between the possible direct and indirect effects of such hormone treatments.

The larval stages of *Locusta* display a noticeable change in their locomotor behaviour during the course of each instar. In general they march little at either end of the instar but gradually reach a peak at some point in the mid-instar. There were various reports that the prothoracic glands were involved in the control of this behaviour (Carlisle and Ellis, 1963; Haskell and Moorhouse, 1963; Haskell, 1965), presumably by the release of the moulting hormone, ecdysone. It was thought that this was also related to the differences in marching behaviour observed between solitary and gregarious larvae. Solitary larvae are known to have larger, more persistent, prothoracic glands and display less active marching behaviour than gregarious larvae (Ellis, 1951; Ellis and Carlisle, 1961). Haskell and Moorhouse (1963) reported that homogenates of the prothoracic glands also reduced the CNS motor output to the metathoracic leg in *Schistocerca* adults, and this could be mimicked by the ecdysone-rich extracts of *Bombyx mori*. However, the solitary larvae also have higher JH titres than the gregarious larvae (Truman and Riddiford, 1974) and the studies of adult flight behaviour indicated that JH might enhance, rather than reduce, locomotor activity. Whether this would apply in the larval stages was unknown, however. As concluded by Truman and Riddiford (1974), "an attempt to explain the complex changes which occur during phase determination simply in terms of the concentration changes of these two hormones (ecdysone and

JH) is still premature and, indeed, may be too simplistic an approach."

It was therefore decided to first accurately quantify the marching behaviour of gregarious, 5th instar larvae. While Edney (1937), Ellis (1951, 1953, 1963) and Ellis and Ashall (1957) had reported changes in the locomotor activity during the 5th instar, there was some disagreement as to the exact timing of the changes in relation to the duration of the instar. Moorhouse *et al* (1978) had recently reported the design of an experimental treadwheel for use with locusts that allowed accurate analysis of their marching behaviour, and their system was adopted for this study. Thus, in the first section of the work, the walking behaviour of 5th instar larvae was determined over a period of 10,000s (nearly 3h) on each day of the instar. The recorded walking behaviour was examined both in terms of the changes observed during the instar and in terms of the changes observed within each session. The results are discussed and the rationale for the remainder of the study was developed from these early findings. It was determined that the most active walking behaviour occurred on the third day of the instar and that this behaviour was adequately described by a measure of the distance walked per unit time.

Therefore, in the second session the responses of the Day 3, 5th instar larvae to various JH treatments were determined, using a simple treadwheel recording only the number of wheel revolutions per hour. However, since hormone treatments at the start of the 5th instar do not have visible morphological effects until the following moult, it was only possible to quantify these effects after the behavioural trials had been made. Firstly, therefore, the developmental effects of the compounds to be used were studied, to allow an estimation of the changes in the haemolymph JH titres caused by the treatments. This data is presented and discussed in Section 2. The behavioural changes observed in such treated animals, on Day 3 of the instar, are discussed (Section 3) in relation to the morphological effects observed. In this way it was hoped to be able to estimate how the JH levels in the haemolymph were influencing the walking behaviour, at a time when no external evidence of the effectiveness of the JH - treatments was available.

The treatments used to enhance the JH levels were compared with treatments of an antiallatotropic agent, precocene II (Bowers *et al*, 1976). This compound destroys the CA in the 4th instar, inducing the formation of an aberrant developmental stage, that of the precocious

5th instar adultiform (Pener *et al*, 1978). The endocrinology of this compound was studied and the behaviour of the adultiform larvae was examined in relation to the observed developmental changes (Sections 2 and 3).

Finally, the activity levels of some of the motor neurones involved in the control of the metathoracic leg were recorded extra-cellularly in an attempt to determine the origin of some of the unusual behavioural traits observed in the adultiform larvae (Section 4). The spontaneous activity levels of neurones in two peripheral nerves were determined for groups of normal larvae, adultiform larvae and normal adults, and compared statistically. Extra-cellular recordings of the motor neurone involved in the rapid extension of the hind leg (FETi, Hoyle, 1978a) in response to brief air stimulation of the anal cercii were also made in each group. Lastly, the tension developed in the muscle responsible for kicking and jumping movements (the extensor tibialis muscle) was determined after electrical stimulation of the motor nerve carrying the FETi axon. These results are discussed in relation to the known neuroanatomy and physiology of the locust, and with regard to the observed behaviour of the animals.

The work reported in the thesis is summarized to indicate how the developmental processes involved in transition from the larval to the adult stage relate to the changing behaviour of the locust, and how the titre of JH in the haemolymph influences this behaviour.

SECTION 1 MATERIALS and METHODS

Animals

Mid - 4th instar *Locusta migratoria migratorioides* (R & F) were obtained from crowded cultures maintained by the Centre for Overseas Pest Research, London. Each batch of 50 - 150 larvae were kept in perspex cages (20cm in diameter and 35cm high) provided with one or two twigs and covered by a perforated zinc lid. The cages were kept at 28°C (± 1°C) with uncontrolled humidity. The room was illuminated by 60W tungsten filament bulbs shared between pairs of cages, providing light and radiant heat for 12h daily, with lights - on at 0700h local time. The locusts were fed on glass-house grown wheat seedlings provided at approximately the same time each morning. Although these seedlings dried considerably before the introduction of fresh food, locusts were observed to eat throughout the 24h, and effectively had food supplies throughout the day.

Each morning the stock cages were examined and all newly emerged 5th instar larvae were removed and kept at a fixed density of 5 animals per cage.

Pretreatment.

All food was removed from a cage three hours prior to a trial. At the end of this period a single, morphologically perfect individual was selected and fixed by the thoracic sternites to a chisel-like metal spike. It was unnecessary to anaethetize the locust, a rapid bond being achieved using a resin : wax mixture (60% pure beeswax, 40% XAM synthetic resin supplied by George Gurr; Steel, 1971). After fixing the bond strength was tested by allowing the locust a firm purchase against a solid surface. The spike was then secured above the treadwheel, the whole process taking from 30 - 60s.

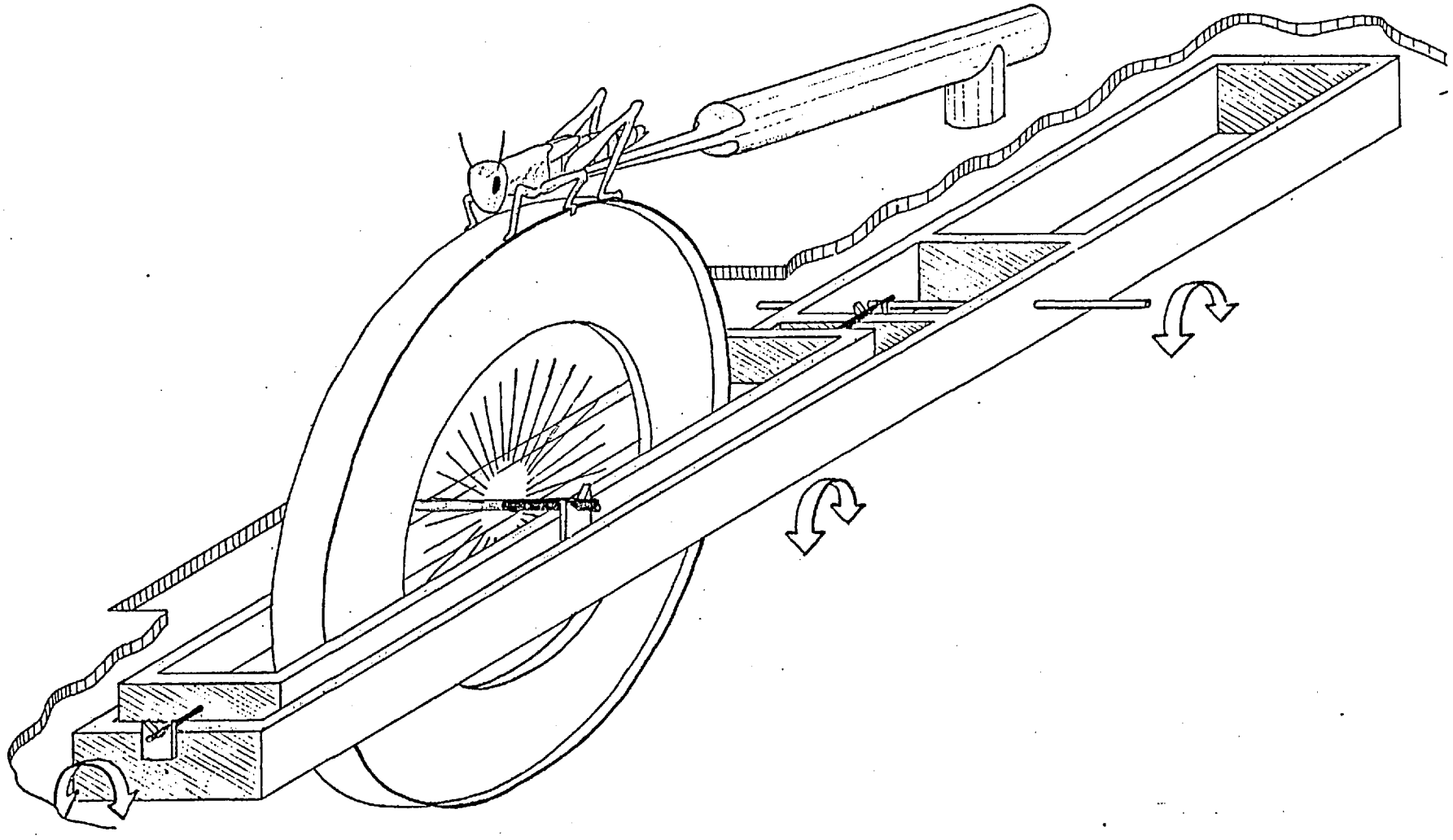
Recording of the rotational movement of the wheel began within 1 minute of positioning the locust and continued for 10^4s (nearly 3h). These recordings ran either from 1200h to 1500h or from 1500h to 1800h. At the end of each session the locust was removed and returned to its cage; records were kept of the instar durations of the locusts in each cage.

The treadwheel and arena.

The treadwheel was designed and built by Moorhouse *et al* (1978)

Figure 1

The treadwheel used in the determination of larval walking behaviour. The wheel is supported on a counterbalanced arm allowing vertical and lateral movement of the wheel (indicated by arrows). Rotation of the wheel is monitored by detection of the radial stripes breaking an infra-red light beam (not shown). The locust is held by a fixed metal arm bonded to the ventral thoracic sternites.



and was housed in an arena based on their design.

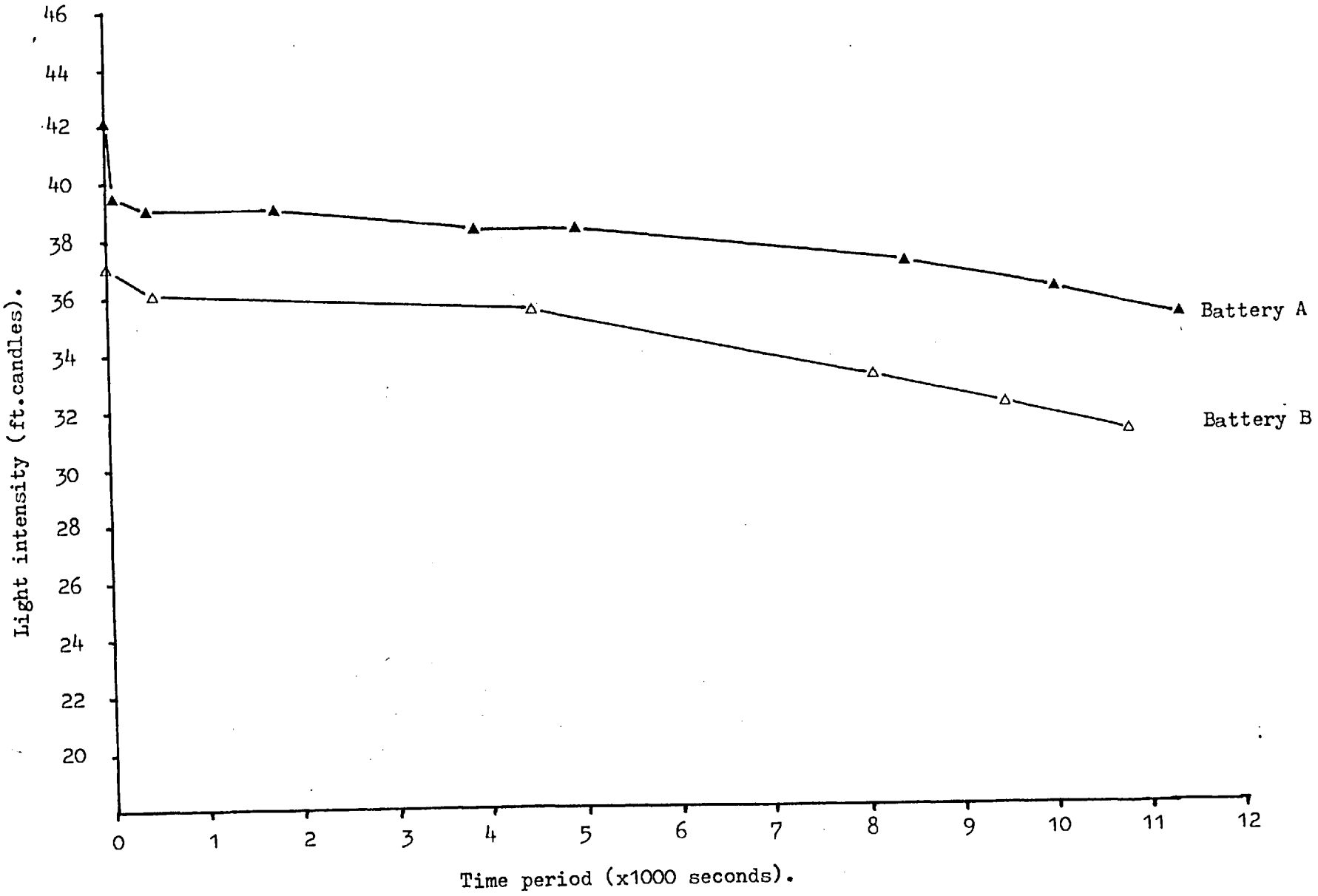
The wheel was constructed of high-density foam surrounding a central transparent disc marked with 500 equally spaced radii. This structure was surrounded by a cardboard rim 16mm wide with a renewable surface of masking tape. The final circumference of the wheel was 500mm (Figure 1). The wheel was mounted in a gimballed, counterbalanced frame providing almost frictionless rotation of the wheel. Limited vertical and lateral movement was also possible, with an upthrust at the wheel's centre equal to the average weight of a 5th instar locust. The locust was held above the wheel on a metal spike with its head vertically above the wheel's axis, so that it was walking slightly uphill; Moorhouse *et al* (1978) had found that this position favoured sustained walking. Although jumping off the wheel surface was possible and occasionally occurred during or immediately after positioning the locust on the wheel, it was rarely seen spontaneously, but jumping may account for infrequent bursts of exceptionally high 'walking' speeds being recorded. The wheel always returned to the locust following a jump, and there was no restriction to the vertical movements associated with normal walking and resting behaviours.

The wheel protruded 4cm through the floor of a matt black arena, 1m in diameter and surrounded by a matt black wall 15cm high. This was further surrounded by a white muslin curtain, 1m high and closed at the top by a white board. A black stripe 60cm high and 5cm wide was positioned vertically on the inside of this curtain directly in front of the locust. The arena was illuminated by a single quartz-halogen lamp suspended 90cm above the wheel and powered by a 12V car battery. This was the sole light source in the room and it proved difficult to see out through the muslin curtain. Some arm-waving tests indicated that the locust was unaware of movements more than 30cm outside the arena walls. The light intensity at the wheel was approximately 38ft candles, dropping by less than 10% during each trial. There was a sharp drop in intensity over the first few minutes after which the light level was relatively constant (Figure 2). The batteries were fully recharged before each trial.

The experimental room, like the culture room, was kept at 28°C ($\pm$ 0.5°C) and although the humidity was uncontrolled and ranged from 40 - 55% RH, it changed by less than 2% during a trial. The least controllable environmental factor was that of noise, and as locusts are known to be sensitive to sound, this may have had some influence on their

Figure 2

The intensity of illumination at the treadwheel surface provided by a quartz-halogen lamp powered by either of two 12V batteries. Abscissa : time, in 1000s periods; ordinate : light intensity (ft. candles).


46
44
42
40
38
36
34
32
30
28
26
24
22
20
Light intensity (ft.candles).
0
1
2
3
4
5
6
7
8
9
10
11
12
Time period (x1000 seconds).
Battery A
Battery B

behaviour. This effect was confirmed during initial experiments in which it was occasionally necessary to enter the room. The recording apparatus was therefore moved outside the room for the remainder of the experiments. The locusts were then undisturbed for the duration of each trial, although some external noise was still present.

Data collection and analysis

i) Data collection. An infra-red light emitting diode (LED) and photodiode were aligned on either side of the treadwheel frame to monitor the passage of the radial stripes as the wheel rotated. The signal from the photodiode was conditioned to give a short, discrete pulse for every millimeter of movement at the wheel's perimeter. No information on the direction of movement, or on lateral or vertical movements of the wheel were recorded. The signal was stored on magnetic tape using a Ferrograph Series 7 recorder, and later transferred to a master tape using a Racal Store 4 recorder. The signal was modified during this transfer from a complex, biphasic form to a single monophasic pulse. There was some error in the earlier transcriptions thought to result from using insufficiently clean tape heads and this produced an estimated 0.05 - 0.2% of false positives on the master copy; there were no false negatives. In later tapes, constituting the vast majority of all data collected, this error was absent or less than 0.01%

ii) Interpretation of the magnetic tape records. During the initial development of walking behaviour, and during poor walking periods, the locusts do not switch immediately from non-walking behaviour to walking behaviour; some non-walking behaviours such as biting at the wheel's edges can persist into the start of a walking bout. The change from walking to non-walking behaviour is more sudden and complete, however (Moorhouse *et al*, 1978). Other non-walking behaviours such as peering, grooming and even ventilatory movements can also cause the wheel to rotate, breaking the light beam and resulting in a pulse being recorded. Because of this, and the complication of some behaviours persisting into the start of a walking bout, the definition of walking was set so that at least 4 pulses must be recorded within 500ms before a walking bout is said to have begun. Walking then continues until the end of the first 500ms period that contains no pulses. This effectively set the minimum speed of walking behaviour at 2mm/s,

with a starting speed of 4mm/s. These values were chosen after a series of 18 recordings from a variety of good and bad walkers were analysed. Using pulse frequency and inter-pulse interval distributions it was hoped to determine a minimum speed of walking. The distributions were expected to be bimodal, with one peak corresponding to the frequency of pulses recorded during walking behaviour, and a second peak corresponding to the much lower frequency of the non-walking bouts. No clear minimum points could be determined, however (Figures 3-5), and the values used to define walking were those chosen by Moorhouse *et al* (1978) on the basis of similar tests. The consequences of this definition are discussed later (p. 18).

iii) The description of recorded walking behaviour. Each trial of 10^4 seconds (165 minutes) was divided into 10 periods of 1000s. Four parameters were used to describe the behaviour as recorded on magnetic tape, and these were determined both for the total 10^4s and for each 10^3s period. These will be referred to as follows:-

a) the distance walked per 10^3s (DW) and
b) the total distance walked in 10^4s (TDW), defined as the number of pulses recorded in all walking bouts;
c) the time spent in walking per 10^3S (TW) and
d) the total time spent in walking in 10^4s (TTW), defined as the sum of all walking bout durations;
e) the number of walking bouts per 10^3s (NB) and
f) the total number of walking bouts (TNB);
g) the speed of walking in each 10^3s (SW), calculated as DW/TW, and
h) the total speed of walking over 10^4s (TSW), calculated as TDW/TTW.

Because the last two parameters were calculated from the distance and time measurements, the total speed (TSW) is not equivalent to the sum of the subperiod speeds (SW). All other parameters are summatable, however.

Figure 3

The speeds of walking determined from the distance walked in 30s by three locusts, chosen as representative good (+), intermediate (•) and bad (o) walkers. Abscissa : time, in 1000s periods (upper axis) and in minutes (lower axis); ordinate : speed of walking (m/min).

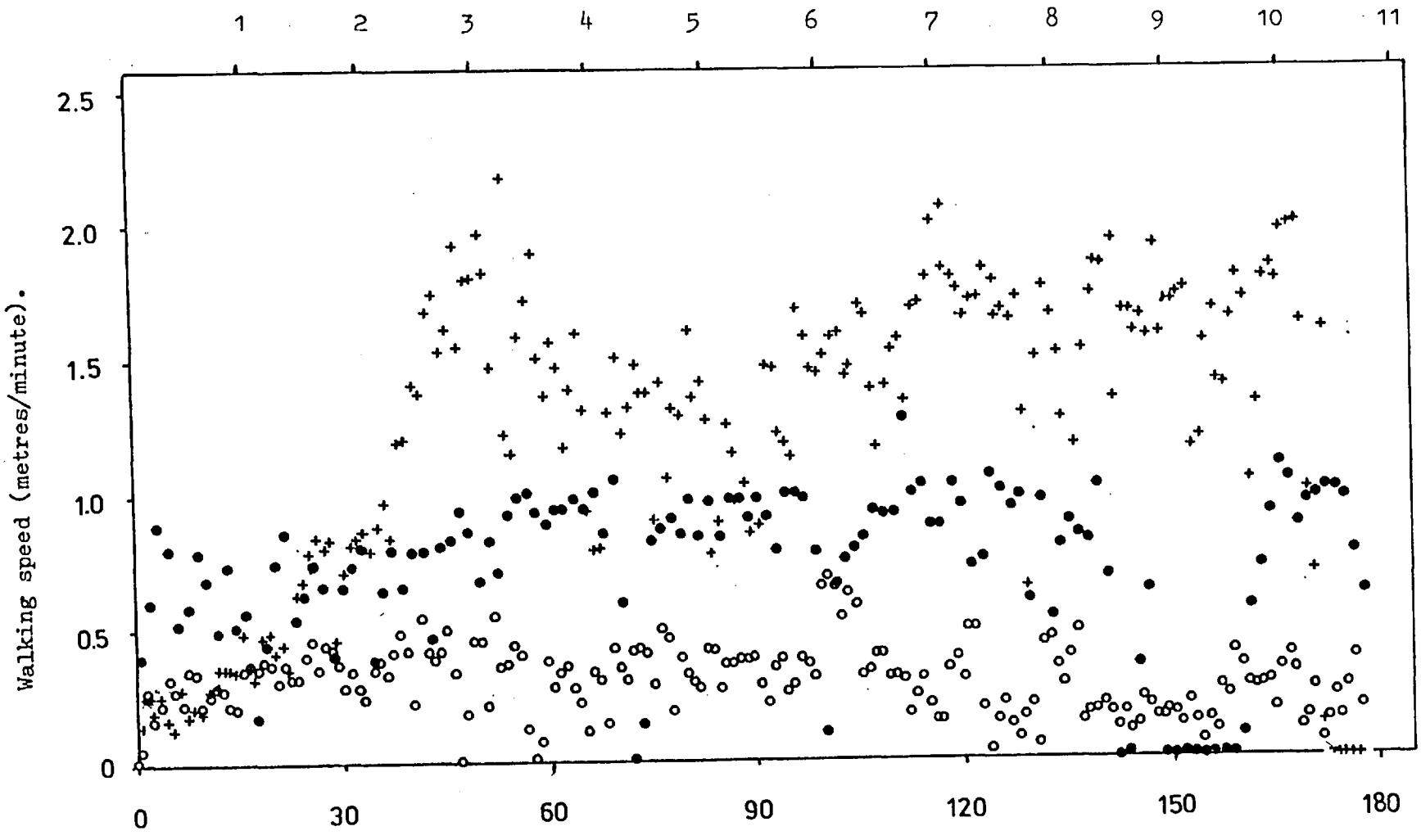

Time: Upper, seconds (x1000)
Lower, minutes.
1
2
3
4
5
6
7
8
9
10
11
Walking speed (metres/minute).
2.5
2.0
1.5
1.0
0.5
0
0
30
60
90
120
150
180

Figure 4

Frequency distributions of the walking speeds (mm/s) exhibited by the three locusts used in Fig. 3. The speed of walking was determined as the number of pulses per second over the duration of the 10^4s session and therefore included all pulses recorded on the magnetic tape, whether originating from walking or non-walking behaviours. Abscissa : walking speeds (mm/s); ordinate : frequency of occurrence of walking speeds, in arbitrary units.

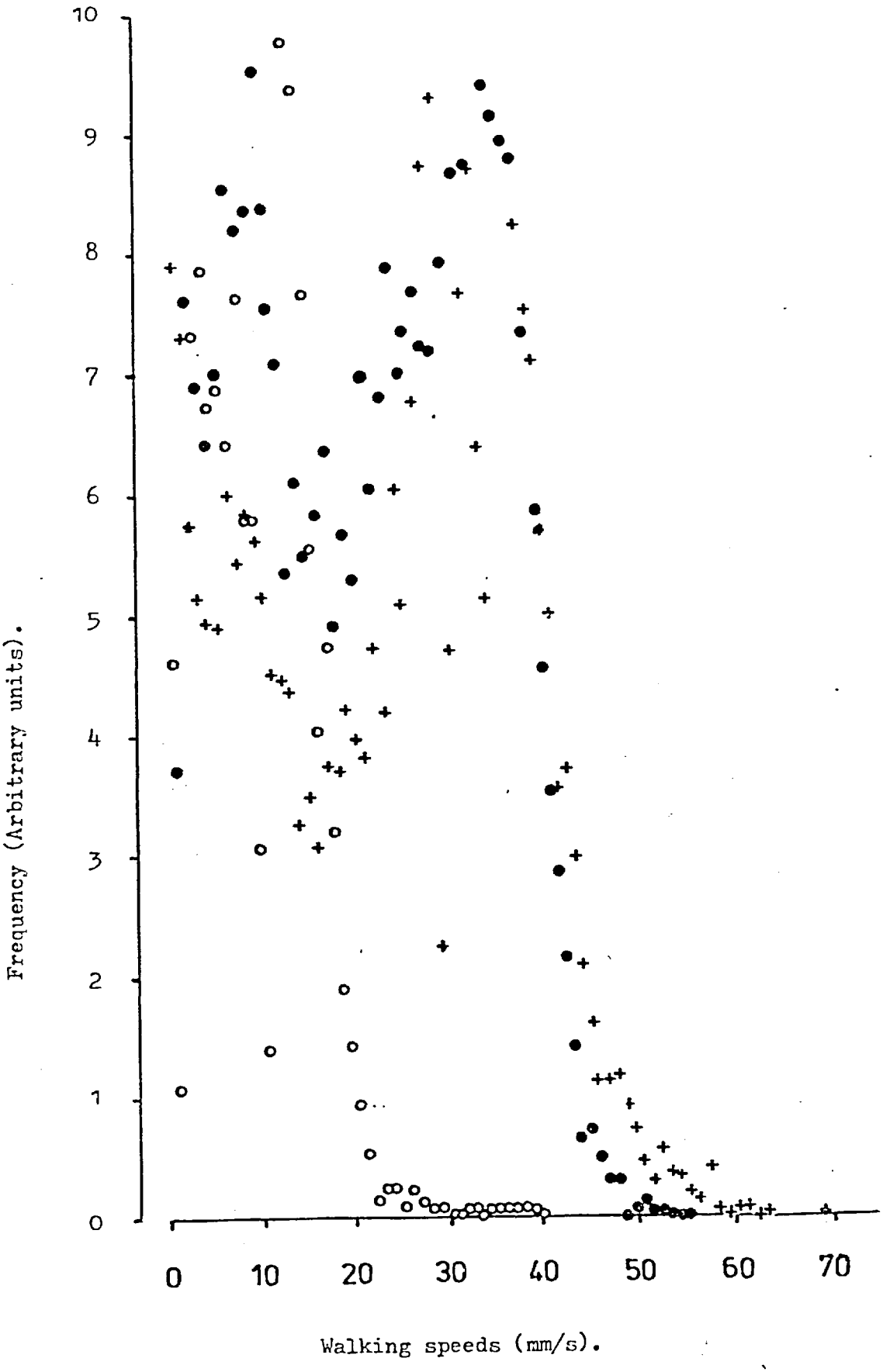
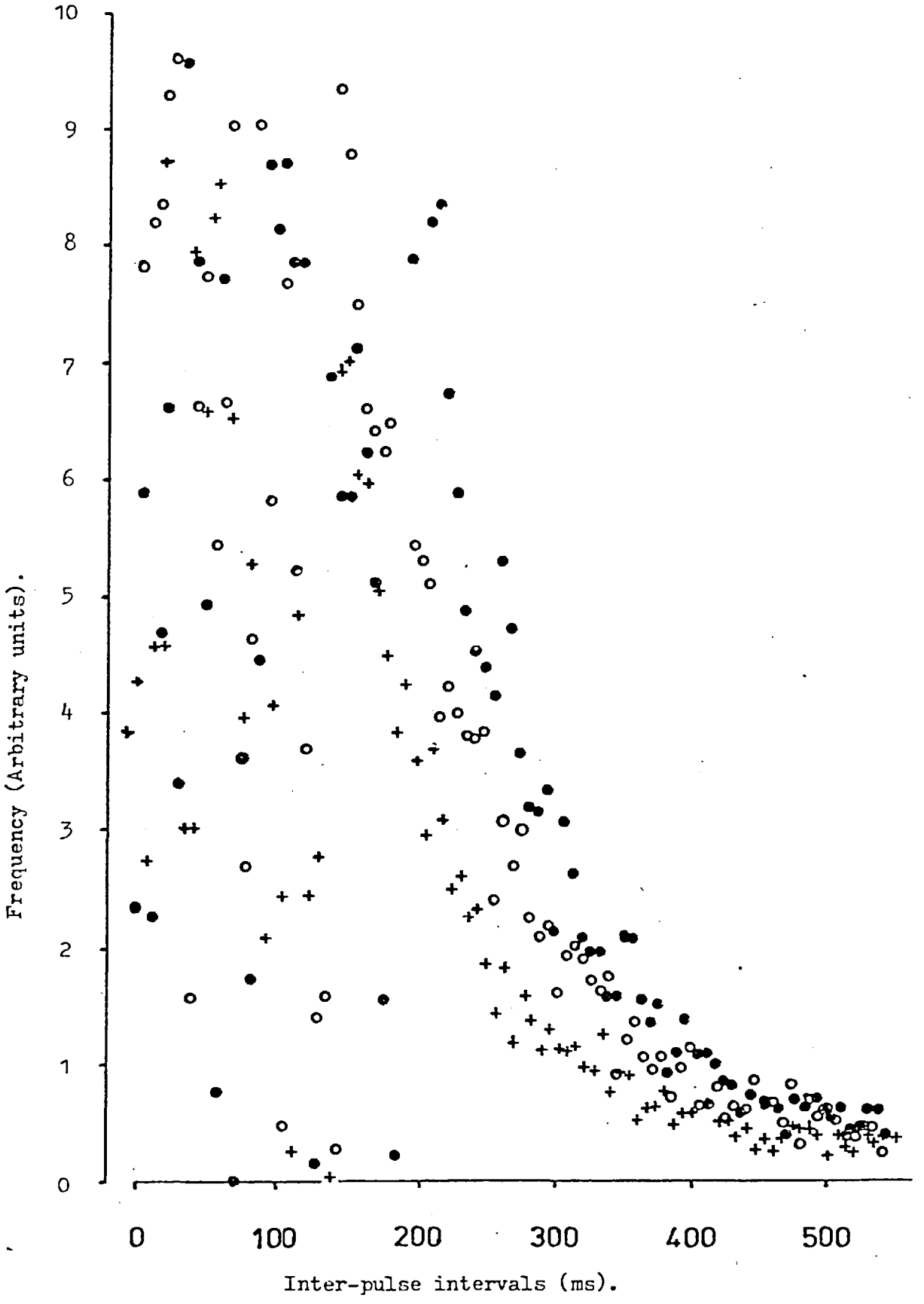

Frequency (Arbitrary units).
0
1
2
3
4
5
6
7
8
9
10
0
10
20
30
40
50
60
70
Walking speeds (mm/s).

Figure 5

Frequency distributions of the inter-pulse intervals determined in the three records used in Figs. 3 and 4. Abscissa : inter-pulse intervals (ms); ordinate : frequency of occurrence of inter-pulse intervals, in arbitrary units.


10
9
8
7
6
5
4
3
2
1
0
Frequency (Arbitrary units).
0
100
200
300
400
500
Inter-pulse intervals (ms).

SECTION 1 RESULTS

Under the conditions reported here the average duration of the 5th instar was 10 days; exceptionally it ranged from 8 to 13 days. Recordings were taken from 102 fifth instar larvae classified by age as shown below. Each group contained equal numbers of both sexes except for Day 2, which contained 6 females and 4 males.

Group	Age (h), 5th instar	Sample size
Day 0a	0 - 12	6
0b	12 - 24	6
1	24 - 48	10
2	48 - 72	10
3	72 - 96	10
4	96 - 120	10
5	120 - 144	10
6	144 - 168	10
7	168 - 192	10
8	192 - 216	10
9	216 - 240	10

Descriptions of the changes observed in the parameters used to quantify the walking behaviour are included in the following sections.

Sex differences in the marching behaviour.

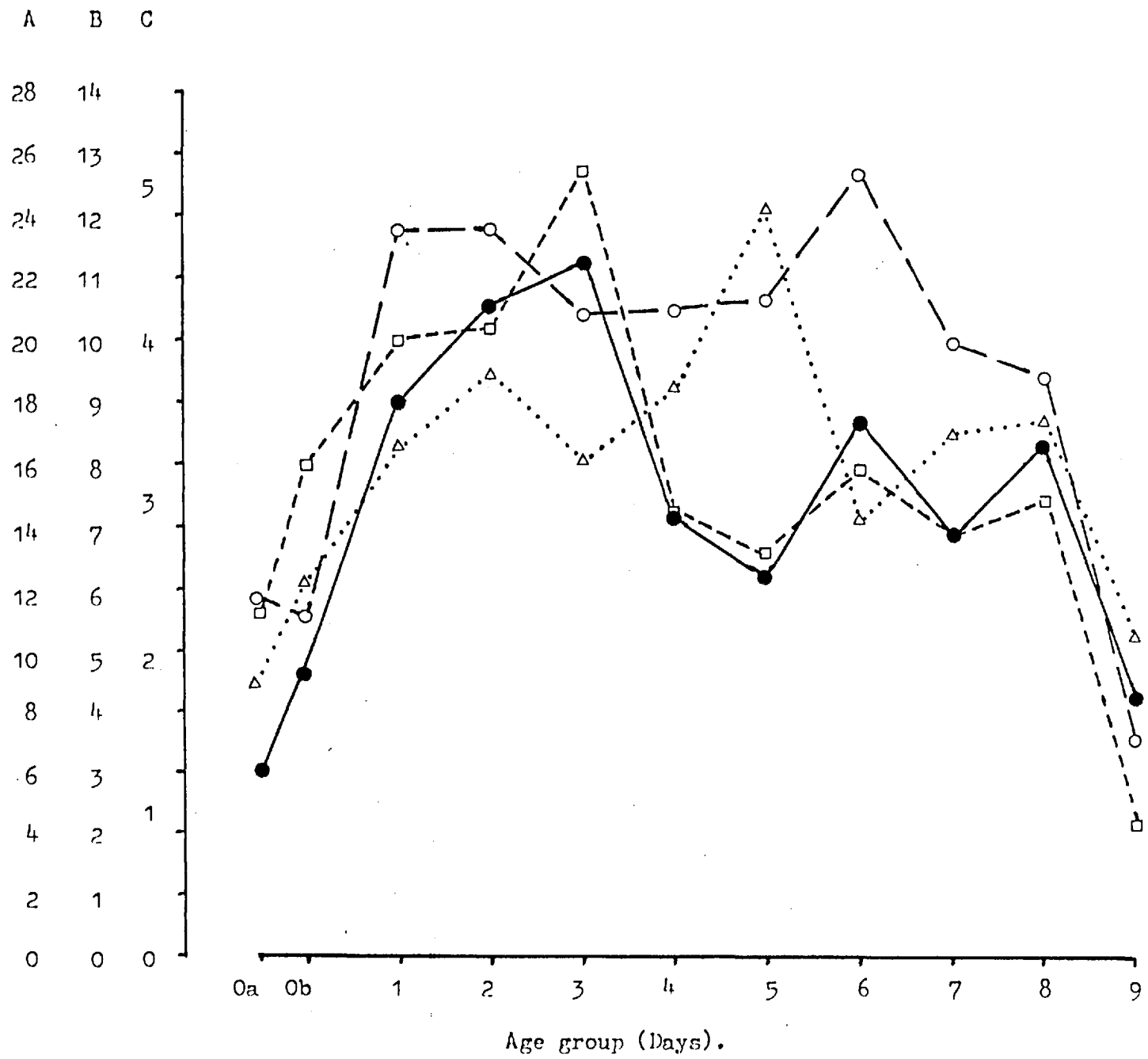
There was no evidence for a sex-difference in TDW in any age group ($n = 3$-6, $p \geq 0.111$, Mann-Whitney U-tests) or within the entire 102 locusts ($n = 50 : 52$, $p = 0.1446$, Mann-Whitney U-test). Although no statistical tests were undertaken using the other parameters of walking behaviour (TTW, TSW and TNB), there was no evidence to suggest that any sex-differences existed. The remaining sections therefore include data from both sexes without distinction.

Changes in the marching behaviour within the 5th instar.

i) Total distance walked. There was a marked change in the total distance walked within the 11 age groups tested over the course of the instar (Figure 6). Over the first 48h (Days 0a, 0b and 1) there was a rapid increase in TDW followed by a more gradual increase until a maximum on Day 3. TDW then fell over Days 4 and 5, but rose slightly during Days 6 - 8, possibly forming part of a

Figure 6

The average total distance walked (TDW), total time spent in walking (TTW), total speed of walking (TSW) and total number of walking bouts (TNB) determined over each 10^4s session in the 11 age groups. Days 0a and 0b contained 6 animals; Days 1-9 contained 10. Abscissa : age group, where Day 0a and 0b = 0-12h and 12-24h after ecdysis, Day 1 = 24-48h after ecdysis, etc; ordinates : TDW (●———●, Scale B, in 10m units); TTW (o— —o, Scale C, in 1000s units); TSW (▫------▫, Scale A, mm/s); TNB (▵·····▵, Scale B, in units of 100).


A
B
C
28
26
24
22
20
18
16
14
12
10
8
6
4
2
0
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
5
4
3
2
1
0
0a
0b
1
2
3
4
5
6
7
8
9
Age group (Days).

plateau in walking behaviour that may extend from Day 4 to Day 8. There was then a sharp fall to the lowest level of TDW on Day 9.

ii) Total time in walking. TTW rose rapidly during Days 0a, 0b and 1, reaching levels of 4200 - 5000s spent in walking during each session, equivalent to 42 - 50% of the 10^4s of the session (Figure 6). This level was maintained with some fluctuations from Day 1 to Day 6 and was followed by a slight fall on Days 7 and 8, and a sharp fall on Day 9 to the lowest level of TTW measured.

iii) Total speed of walking. TSW, determined as TDW/TTW, varied in a fashion similar to that of TDW, reflecting the relatively constant level of TTW during the middle of the instar (Figure 6).

iv) Total number of walking bouts. The total number of walking bouts recorded in each session varied considerably from the other parameters, although TNB showed an increase and decay in the same general fashion as the other parameters (Figure 6). Between particular age groups the relationship was less obvious however. For example, TNB fell from Day 2 to Day 3 while TDW and TSW both increased. The maximum TNB occurred on Day 5 when both TDW and TSW were at particularly low levels. It may be relevant that there was in general an inverse relationship between TNB and the other 3 parameters over Days 3 - 8. TNB fell sharply on Day 9, however, in common with the other parameters.

Changes in the marching behaviour within each 10^4s session.

i) Distance walked. While TDW followed a U-shaped curve with a rapid build up and fall at the start and end of the 5th instar, DW also changed within the 10^4 seconds of each session (Figures 7 - 17).

On Days 0a and 0b (Figures 7 and 8) DW increased over the first few 1000s periods, reaching peak levels after approximately 5000s (83 minutes). DW then fell until after 10000s walking had almost ceased. The next 3 age groups (Days 1 - 3, Figures 9 - 11) showed a similar, graded increase in walking with a peak in DW after 3000 - 5000s. However, DW did not fall to such low levels after the peak as seen in the first two age groups : the final values of DW on Days 1 - 3 were 33 - 66% of the peak values. The next 3 age groups (Days 4 - 6, Figures 12 - 14) showed little evidence of

Figures 7-17

The average distance walked (DW), time spent in walking (TW), speed of walking (SW) and number of walking bouts (NB) determined in each 1000s period for 10^4s, in 11 age groups spanning the 5th instar. Days 0a and 0b (Figs. 7 and 8) contained 6 animals; Days 1-9 (Figs. 9-17) contained 10 animals. Abscissae : time, in 1000s periods; ordinates : DW (●——●, Scale C, metres); TW (o— —o, Scale B, in 1000s units); SW (▫-----▫, Scale A, mm/s); NB (▵·····▵, Scale C, in units of ten).

Fig. 7.

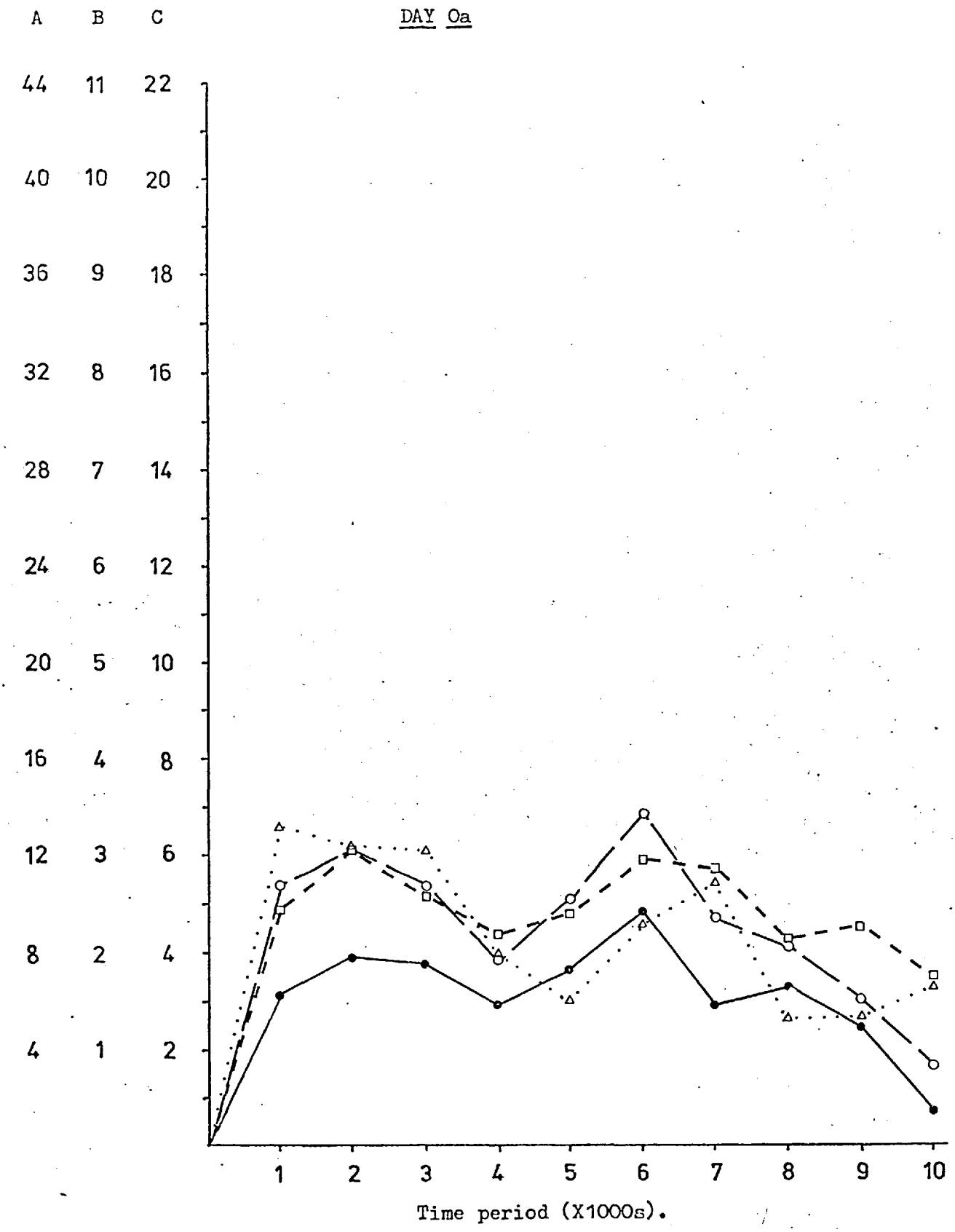

A B C
DAY Oa
44 11 22
40 10 20
36 9 18
32 8 16
28 7 14
24 6 12
20 5 10
16 4 8
12 3 6
8 2 4
4 1 2
1 2 3 4 5 6 7 8 9 10
Time period (X1000s).

Fig. 8.

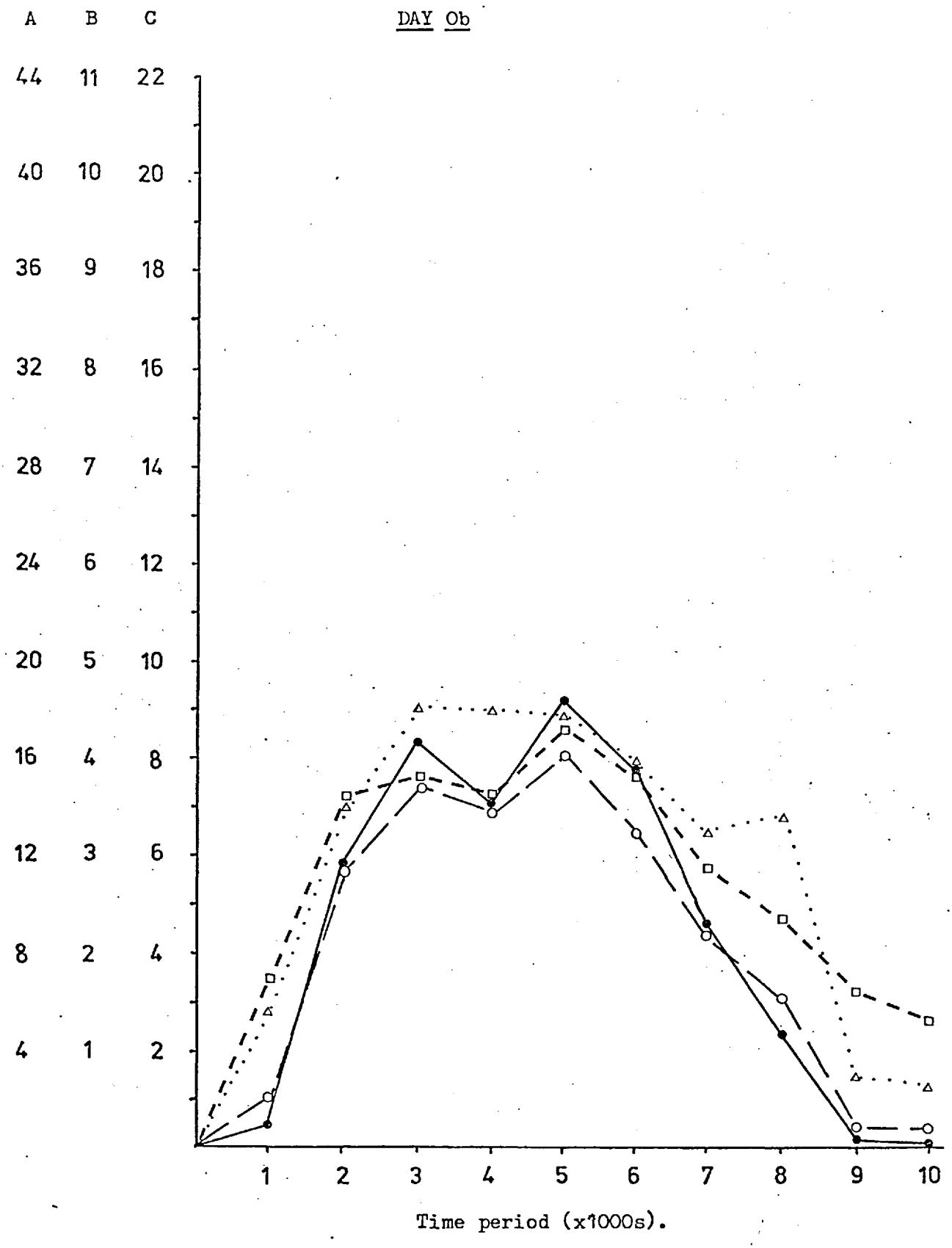

A B C
DAY Ob
44 11 22
40 10 20
36 9 18
32 8 16
28 7 14
24 6 12
20 5 10
16 4 8
12 3 6
8 2 4
4 1 2
1 2 3 4 5 6 7 8 9 10
Time period (x1000s).

Fig. 9.

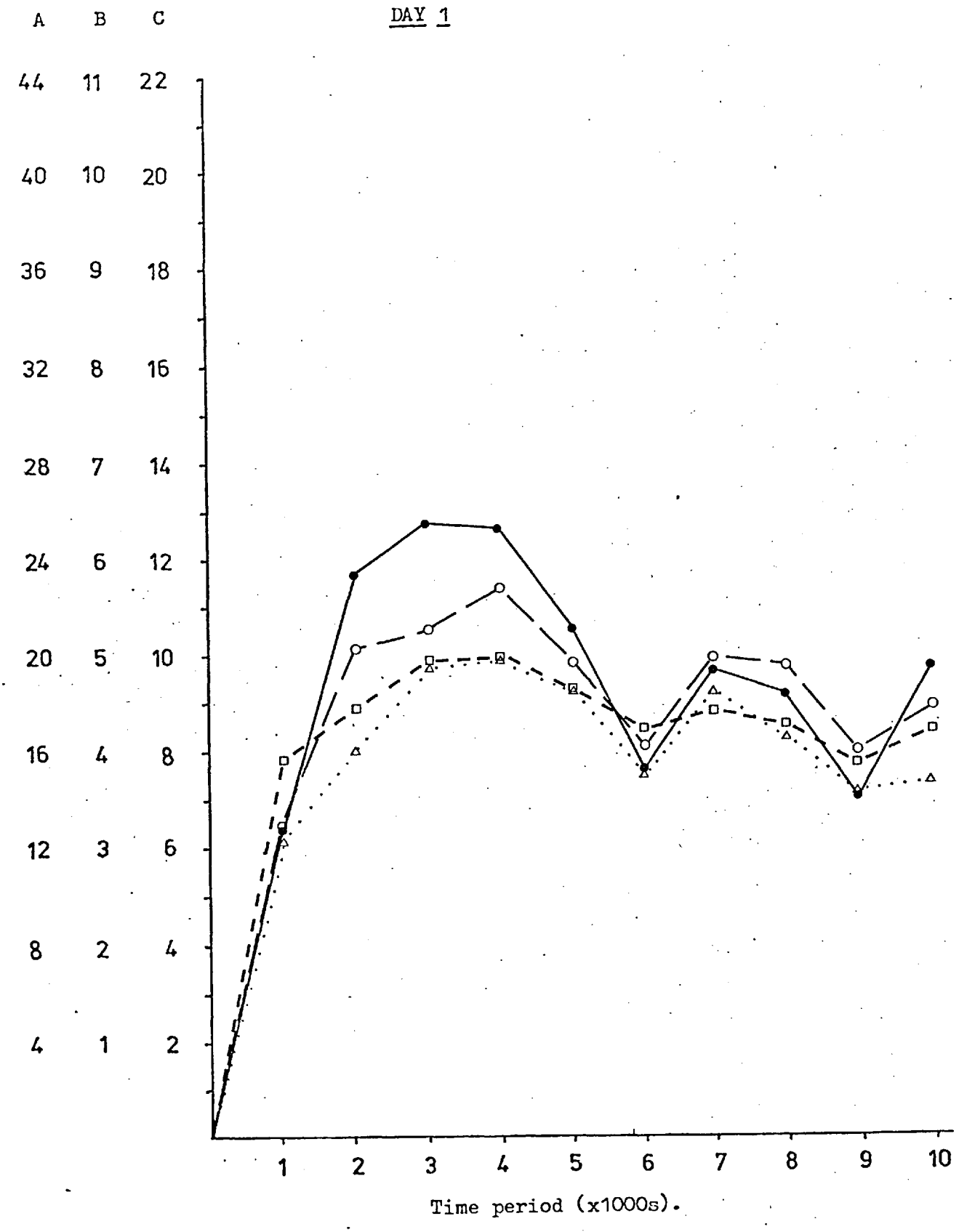

DAY 1
A
B
C
44
11
22
40
10
20
36
9
18
32
8
16
28
7
14
24
6
12
20
5
10
16
4
8
12
3
6
8
2
4
4
1
2
1
2
3
4
5
6
7
8
9
10
Time period (x1000s).

Fig. 10.

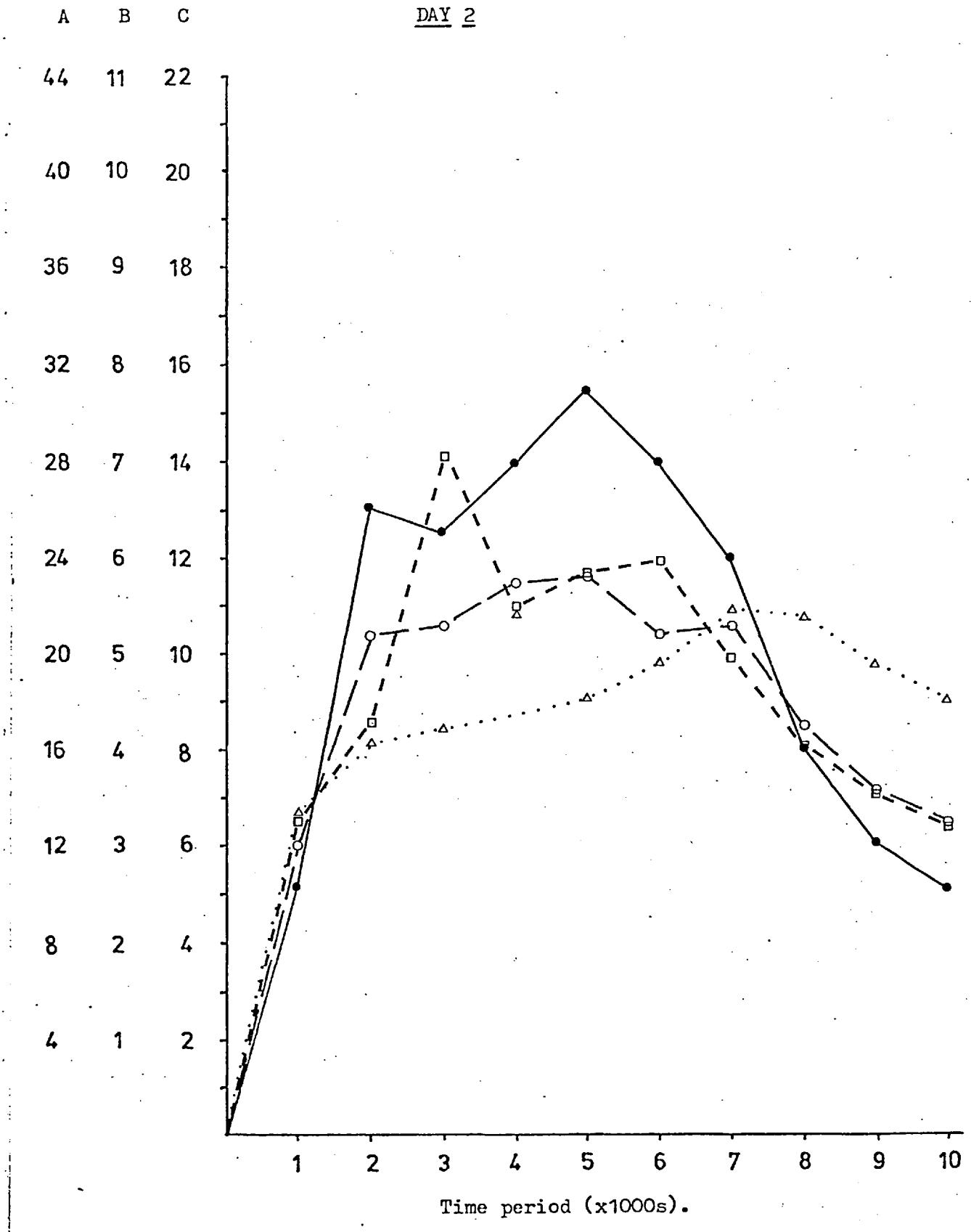

A B C
DAY 2
44 11 22
40 10 20
36 9 18
32 8 16
28 7 14
24 6 12
20 5 10
16 4 8
12 3 6
8 2 4
4 1 2
1 2 3 4 5 6 7 8 9 10
Time period (x1000s).

Fig. 11.

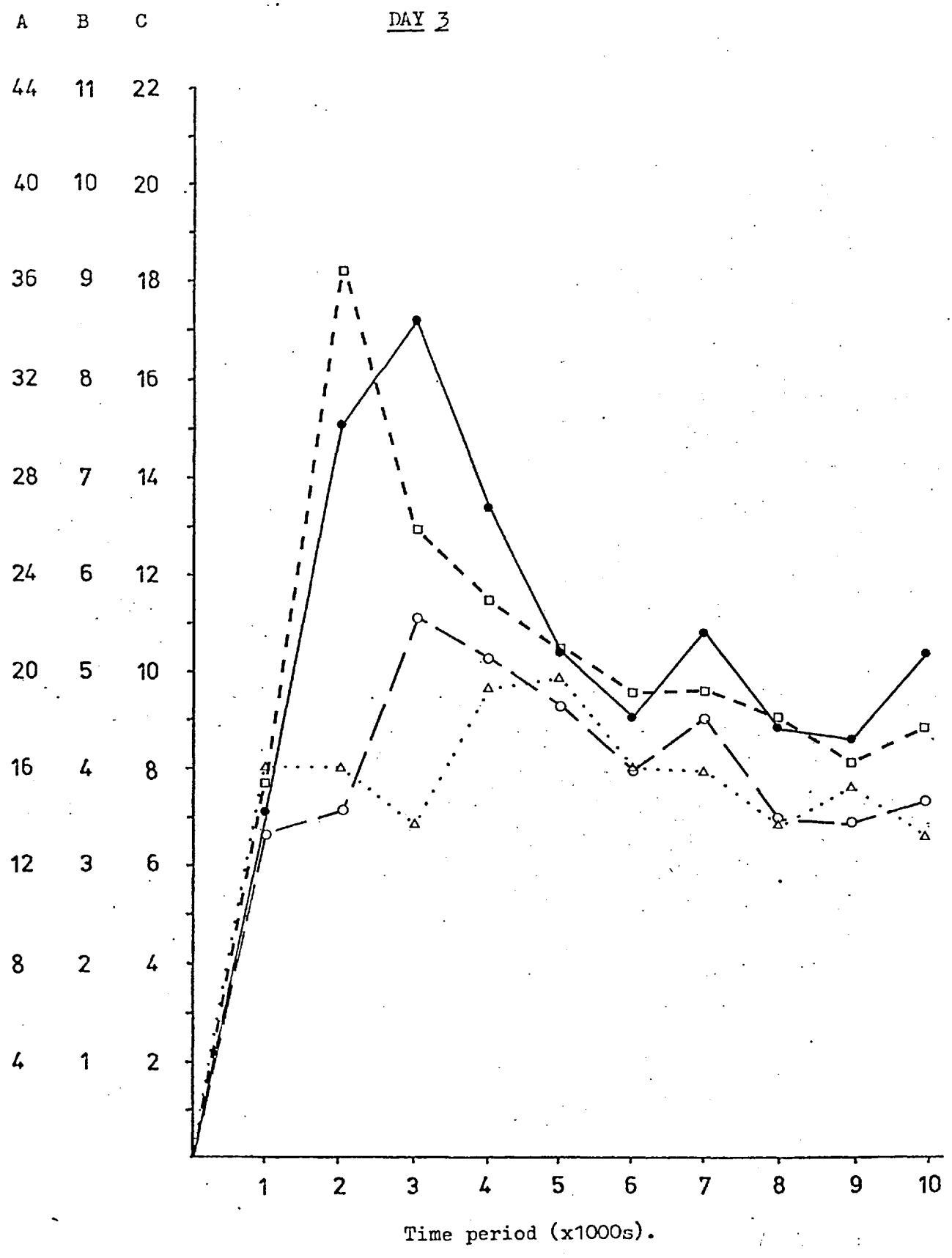

A B C
DAY 3
44 11 22
40 10 20
36 9 18
32 8 16
28 7 14
24 6 12
20 5 10
16 4 8
12 3 6
8 2 4
4 1 2
1 2 3 4 5 6 7 8 9 10
Time period (x1000s).

Fig. 12.

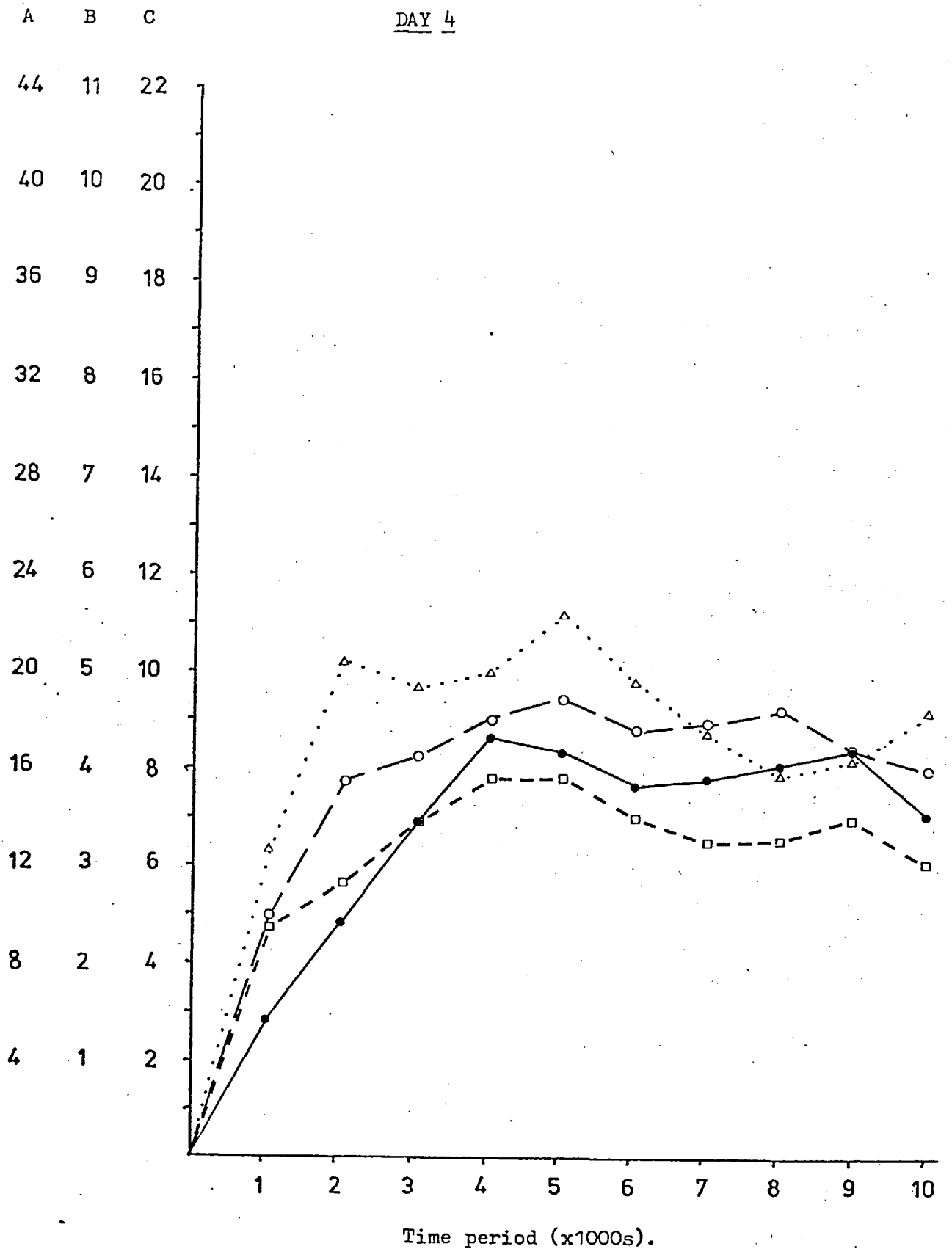

A
B
C
DAY 4
44
11
22
40
10
20
36
9
18
32
8
16
28
7
14
24
6
12
20
5
10
16
4
8
12
3
6
8
2
4
4
1
2
1
2
3
4
5
6
7
8
9
10
Time period (x1000s).

Fig. 13.

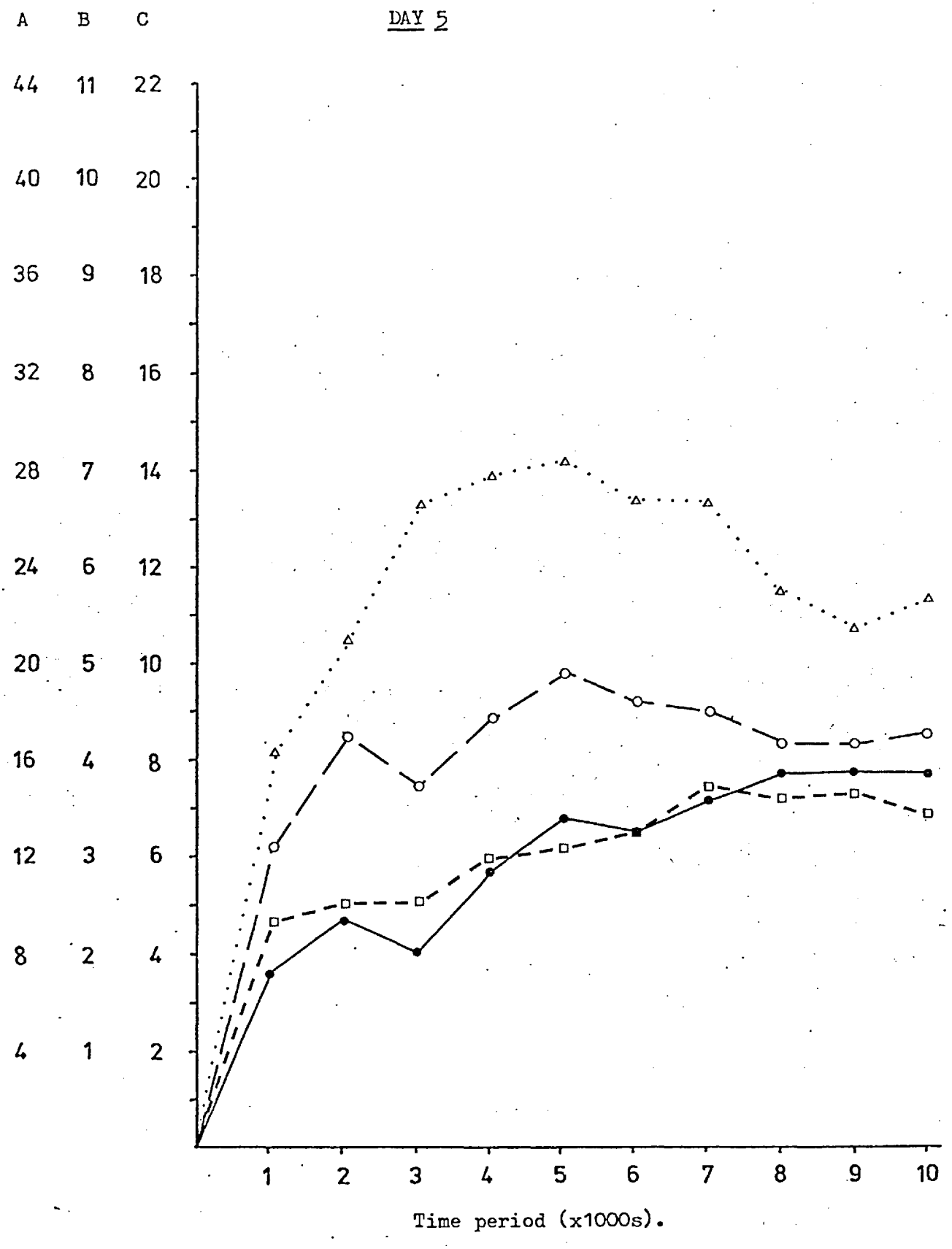

A
B
C
DAY 5
44
11
22
40
10
20
36
9
18
32
8
16
28
7
14
24
6
12
20
5
10
16
4
8
12
3
6
8
2
4
4
1
2
1
2
3
4
5
6
7
8
9
10
Time period (x1000s).

Fig. 14.

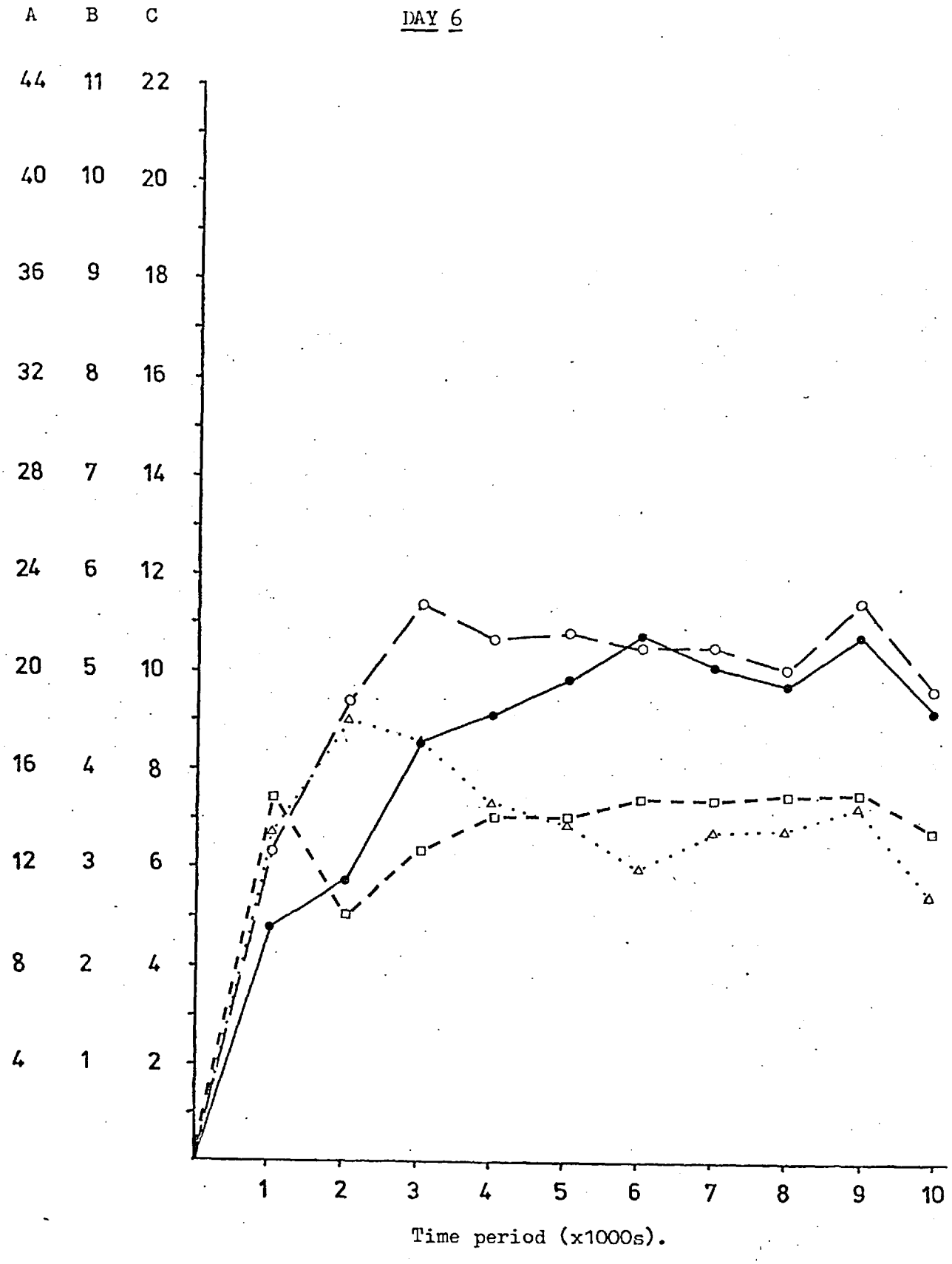

A B C
DAY 6
44 11 22
40 10 20
36 9 18
32 8 16
28 7 14
24 6 12
20 5 10
16 4 8
12 3 6
8 2 4
4 1 2
1 2 3 4 5 6 7 8 9 10
Time period (x1000s).

Fig. 15.

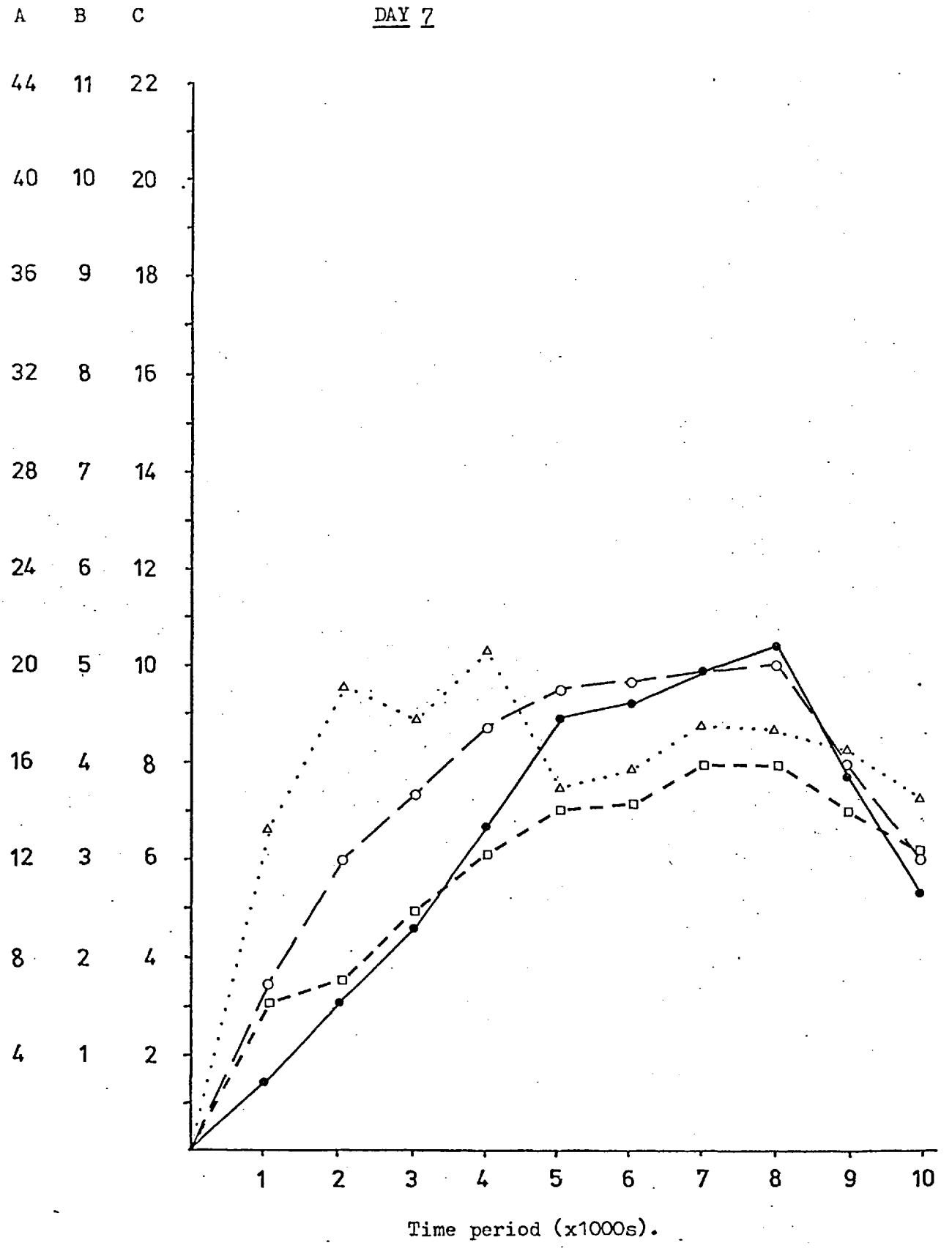

A B C
DAY 7
44 11 22
40 10 20
36 9 18
32 8 16
28 7 14
24 6 12
20 5 10
16 4 8
12 3 6
8 2 4
4 1 2
1 2 3 4 5 6 7 8 9 10
Time period (x1000s).

Fig. 16.

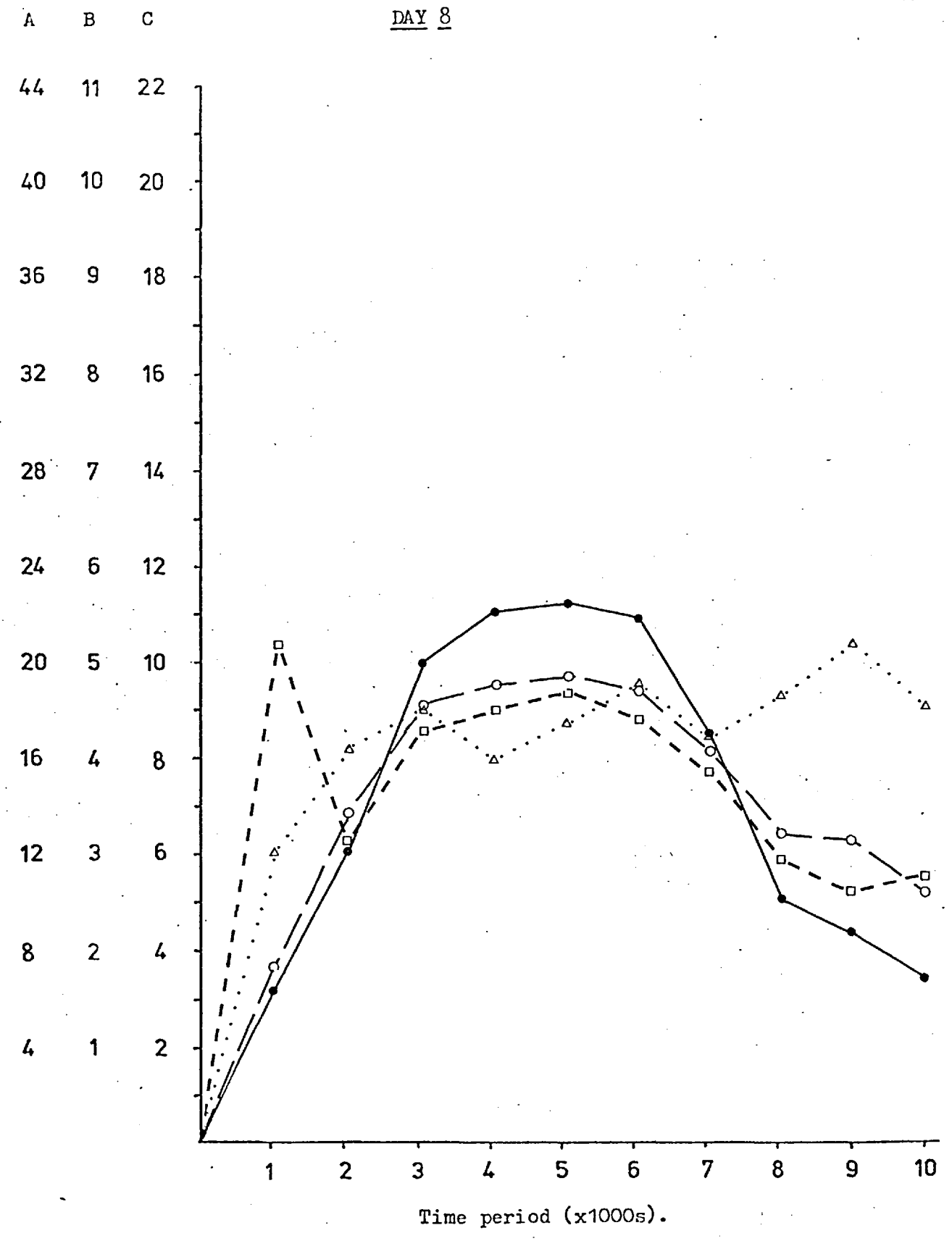
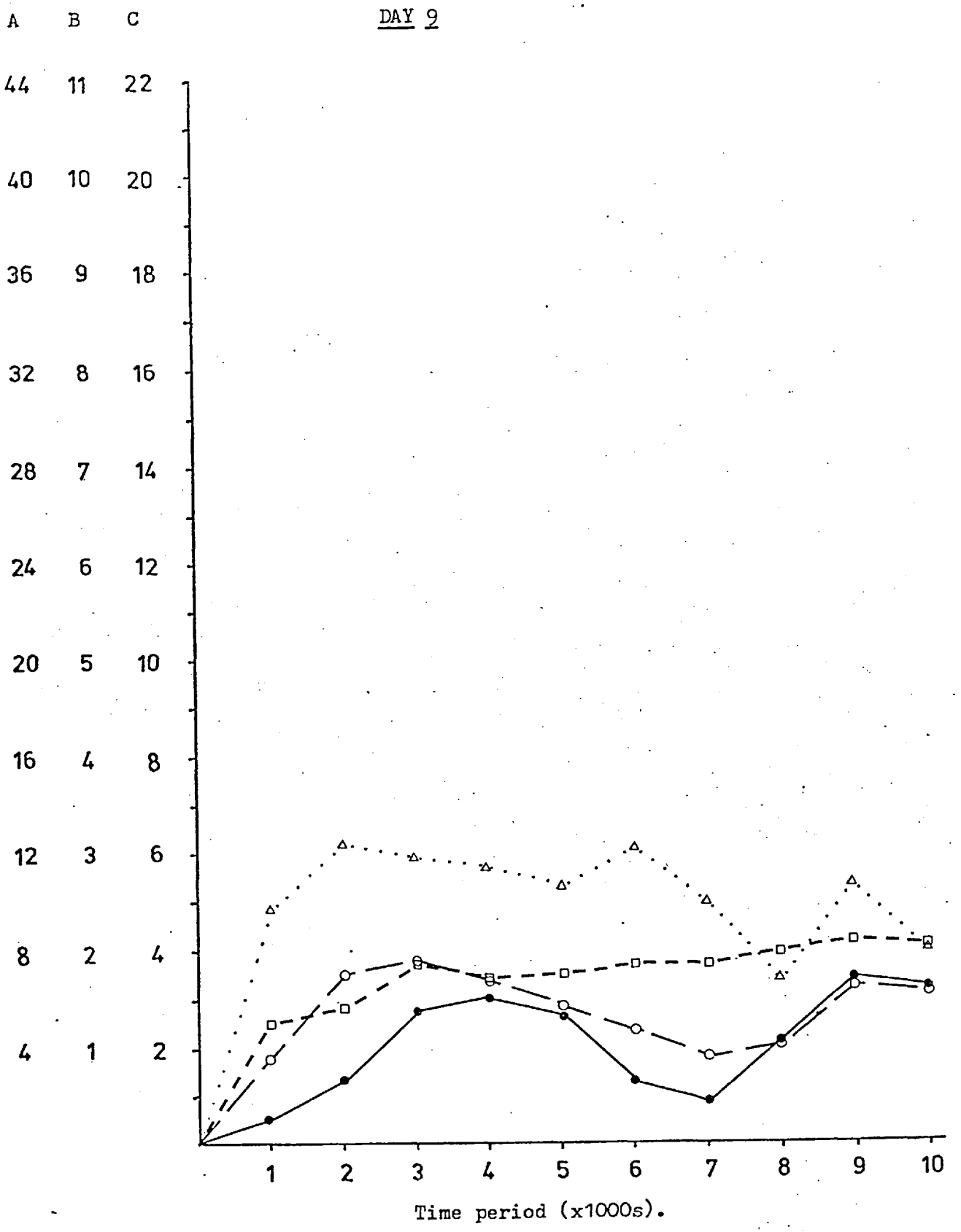

DAY 8
A
B
C
44
11
22
40
10
20
36
9
18
32
8
16
28
7
14
24
6
12
20
5
10
16
4
8
12
3
6
8
2
4
4
1
2
1
2
3
4
5
6
7
8
9
10
Time period (x1000s).

Fig. 17.


A B C
DAY 9
44 11 22
40 10 20
36 9 18
32 8 16
28 7 14
24 6 12
20 5 10
16 4 8
12 3 6
8 2 4
4 1 2
1 2 3 4 5 6 7 8 9 10
Time period (x1000s).

the peak in DW being reached within the recorded period of 10^4s. DW rose sharply within the first half of the session but then remained at that level, or even increased slightly over the second half of the session, with the final values of DW generally as high as those recorded 5000s earlier. Days 7 and 8 (Figures 15 and 16) again showed strong peaks in DW after 8000 and 5000s respectively, with final DW values 50% and 30% of the peak values recorded. On the final day of the instar, Day 9 (Figure 17), DW rose gradually to a peak after 4000s, then fell and rose again until after 9000 - 10000s DW was at its highest value. The bimodal form of this curve was not supported statistically (p. 15).

ii) Time in walking. Within each 10^4s session TW varied in a similar fashion to DW. As indicated in Table 1, TW and DW are significantly correlated in all age groups with associated statistical probabilities of $p \leq 0.001$ in all but two of the groups. However TW showed less variation than DW amongst the 11 age groups. The peak values of TW increased from Day 0a to Day 3 but these increases were smaller than those for DW (Figures 7 - 11); when DW fell to relatively low levels over Days 4 - 6 (Figures 12 - 14) TW remained close to the levels seen on Days 2 and 3. Nonetheless, the overall shape of the curve of TW changed in a similar manner to that of DW on all days.

iii) Speed of walking. The curves for SW varied in a similar fashion to those of DW and TW. Table 1 shows the high correlation between SW and DW; SW and TW were less highly correlated. Although Days 0a, 0b and 1 (Figures 7 - 9) showed a gradual rise and fall in SW, there was a sharp peak in SW after 3000s on Days 2 and 3 (Figures 10 and 11), reaching a particularly high value of 38mm/s on Day 3. Days 6 and 8 (Figures 14 and 16) also showed a small early peak in SW followed by the main peak predicted by DW levels. These extra peaks are responsible for the low correlation coefficients between SW and DW and between SW and TW on these Days. There was no bimodal form to SW on Day 9 (Figure 17).

iv) Number of walking bouts. The changes in NB during each 10^4s session were not closely correlated with those in the other three parameters (Table 1), except on Days 0b and 1 (Figures 8 and 9). However, NB tended to follow the same general pattern of changes,

Table 1 The correlation coefficients and their associated statistical probability levels calculated between all possible pairs of the parameters determined for each of the ten 1000 s periods of the total 10^4s session, within 11 age groups spanning the 5th instar. Each correlation coefficient was calculated from average values, determined every 1000 s, each being the average from 6 or 10 animals. The probability levels are given as $p_r \leq 0.1$ (+); $p_r \leq 0.05$ (++) and $p_r \leq 0.01$ (+++).

Age group	DW : TW		DW : NB		DW : SW		TW : NB		TW : SW		NB : SW	
	r	p_r	r	p_r	r	p_r	r	p_r	r	p_r	r	p_r
Day 0a	.951	+++	.379		.790	+++	.600	+	.878	+++	.666	++
0b	.994	+++	.933	+++	.990	+++	.962	+++	.992	+++	.942	+++
1	.926	+++	.840	+++	.925	+++	.910	+++	.875	+++	.921	+++
2	.978	+++	.265		.836	+++	.406		.831	+++	.186	
3	.677	++	.005		.808	+++	.334		.242		.127	
4	.931	+++	.481		.892	+++	.642	++	.840	+++	.668	++
5	.643	++	.283		.957	+++	.761	+++	.554	+	.298	
6	.805	+++	-.403		.525	+	.180		.021		-.701	++
7	.951	+++	.137		.972	+++	.366		.889	+++	.080	
8	.970	+++	.135		.526	+	.343		.311		-.634	++
9	.695	+++	-.097		.664	++	.475		.176		-.350	

with some notable exceptions. The obvious peaks in DW, TW and SW seen on Days 2 and 3 (Figures 10 and 11) were not pronounced in NB; from Day 1 onwards the value of NB rose in the 1st half of the session but maintained the peak level during the second half of the session. In this respect it appears that NB reached on Day 1 the pattern shown by the other parameters on Days 4 - 6. The level of NB remained relatively high on Day 9, indicating that the low levels of marching recorded on this day were associated with frequent walking bouts of short duration.

The correlation between behavioural measures.

Analysis of the 11 age groups showed a strong correlation between some of the measurements used to describe the marching behaviour of the locusts (Table 2). The TDW values determined for the locusts in each age group were significantly correlated with the TTW and TSW values in all eleven age groups ($r \geq 0.76$, $p_r < 0.001$); the remaining possible correlation coefficients were not significant in all age groups. The TDW and TNB values were only significantly correlated in one age group (Day 0a), while the TNB and TSW values showed no statistically significant correlation coefficients in any age group. However, as indicated in Table 2, some of these remaining correlation coefficients had values of p_r of between 0.05 and 0.1. Furthermore, the 10 average values for each parameter determined over the 1000s periods were positively correlated, as indicated in Table 1 and Figures 7 - 17. In all cases the parameter DW (an average of 6 or 10 individuals' DW values) was strongly correlated with TW and SW. The correlation between NB and TW was high but NB was only poorly correlated with DW and SW.

The high statistical significance of these correlation coefficients indicates that the parameters TDW and DW are valid descriptions of the walking behaviour of the locusts and can, in isolation, be used in further analysis of the observed changes in behaviour. The following statistical analyses therefore include only data on the distance walked by the locusts.

Statistical analyses of the changes in walking behaviour

i) Analysis of the changes in TDW. The TDW values determined for the 6 or 10 locusts in each age group showed significant differences between the 11 age groups ($p < 0.001$, Kruskal - Wallis test for non-related samples). Comparison of all the possible pairs of age groups using the Mann-Whitney U-test confirmed the observations

Table 2 The correlation coefficients and their associated statistical probability levels calculated between all possible pairs of parameters, determined over 10^4 s, within 11 age groups spanning the 5th instar. Each age group consisted of 10 animals except for Days 0a and 0b that consisted of only 6 animals. The probability levels are given as $p_r \leq 0.1$ (+), $p_r \leq 0.05$ (++) and $p_r \leq 0.01$ (+++).

Age Group	TDW : TTW		TDW : TNB		TDW : TSW		TTW : TNB		TTW : TSW		TNB : TSW	
	r	p_r	r	p_r	r	p_r	r	p_r	r	p_r	r	p_r
Day 0a	.994	+++	.76	++	.844	+++	.80	++	.835	+++	.670	+
0b	.950	+++	.14		.953	+++	.42		.966	+++	.340	
1	.778	+++	0.0		.923	+++	-0.14		.493	+	-0.04	
2	.887	+++	-0.10		.922	+++	.06		.692	++	-0.12	
3	.887	+++	.31		.727	+++	.35		.417		.122	
4	.916	+++	.52	+	.968	+++	.64	++	.805	+++	.395	
5	.810	+++	.30		.926	+++	.69	++	.577	++	.018	
6	.930	+++	.17		.828	+++	.36		.598	++	-0.26	
7	.935	+++	.52	+	.984	+++	.69	++	.906	+++	.550	+
8	.955	+++	-0.63	+	.913	+++	.28		.805	+++	-0.30	
9	.966	+++	.11		.970	+++	.31		.957	+++	.108	

Table 3 The probability values associated with the differences between the total distance walked (TDW) values for all possible pairs of age groups, as calculated by the Mann-Whitney U-test. Each age group contained 10 animals except for Days 0a and 0b which contained only 6 animals. Increasingly significant probability values are indicated by the symbols —, ═ and ≡.

Age Group	0a	0b	1	2	3	4	5	6	7	8
0b	0.24									
1	0.02	0.05								
2	0.05	0.10	>0.1							
3	0.02	0.10	>0.1	>0.1						
4	0.10	>0.1	>0.1	>0.1	>0.1					
5	0.10	>0.1	>0.1	>0.1	0.10	>0.1				
6	>0.1	>0.1	>0.1	>0.1	>0.1	>0.1	>0.1			
7	>0.1	>0.1	>0.1	>0.1	>0.1	>0.1	>0.1	>0.1		
8	>0.1	>0.1	>0.1	>0.1	>0.1	>0.1	>0.1	>0.1	>0.1	
9	>0.1	0.10	0.002	0.002	0.002	0.002	0.002	0.02	0.05	0.02

made earlier (Table 3):

a) The TDW's on Day 0a were significantly lower than those of Days 1, 2 and 3 ($p < 0.05$);

b) The TDW's on Day 0b were significantly lower than those of Day 1 ($p < 0.05$);

c) The TDW's on Day 9 were significantly lower than those of Days 1 - 8 inclusive ($p < 0.05$);

d) The remaining possible pairs of age groups showed no significant differences in TDW at the $p = 0.05$ level.

To summarize, there is a significant rise in the total distance walked by 5th instar larvae over the first 2 days of the instar. This rise is followed by a central peak in the walking behaviour that extends from Day 2 to Day 8. There is then a significant fall in walking on the final day of the instar (Day 9), when the lowest levels of TDW were recorded.

ii) Analysis of the changes in DW. The changes in DW over the 10 component 1000s periods were analysed in two steps. Firstly, a Friedman 2-way analysis of variance was performed on all age groups, comparing the DW values recorded for the 6 or 10 locusts in that group over each of the 10 periods. Those age groups that showed significant differences between the 10 periods ($p \leq 0.05$; Table 4) were further analysed using the Mann-Whitney U-test between all possible pairs of 1000s periods (Table 5).

Days 0a, 1, 2, 7 and 8 showed significant differences in the first test. The variation in walking shown by the individual locusts was so great that no significant differences could be demonstrated in the other groups. It is perhaps relevant that Days 3 and 6, on either side of the central group of 'non-significant' Days, showed statistical differences at the $p = 0.1$ level. It appears that there was a graded change in the value of p before and after the central peak of walking on Days 4 and 5.

In those groups that showed significant differences at the $p = 0.05$ level, the second test confirmed a trend that was apparent in all age groups. In the first 1000s period DW was significantly lower than in the best walking periods (3000 - 7000s). This varied in extent from Day 7, where differences were seen between the 1st and the 4th - 8th periods at the $p = 0.05$ level, to Day 1 where only

Table 4 The probability values associated with the differences between the 10 distance walked (DW) values determined within the 10^4s session, in 11 age groups spanning the 5th instar, as calculated by the Friedman 2-way analysis of variance. Each DW value was the average from 6 or 10 animals.

Age Group	Chi-squared value	Probability level (p)	Statistical significance ($p \leq 0.05$)
Day 0a	12.1	>0.10	
0b	29.891	<0.001	+
1	17.798	<0.05	+
2	53.367	< 0.001	+
3	15.534	< 0.10	
4	11.689	> 0.10	
5	8.684	>0.10	
6	15.556	< 0.10	
7	27.322	<0.005	+
8	21.512	<0.025	+
9	0.262	>0.995	

Table 5 The probability values associated with the differences in DW values for all possible pairs of 1000 s periods, as calculated by the Mann-Whitney U-test, for those 5 age groups that show statistically significant differences in Table 4. Only probability levels of $p \leq 0.10$ are shown as $p \leq 0.1$ (+), $p \leq 0.05$ (++), and $p \leq 0.01$ (+++).

Day 0b

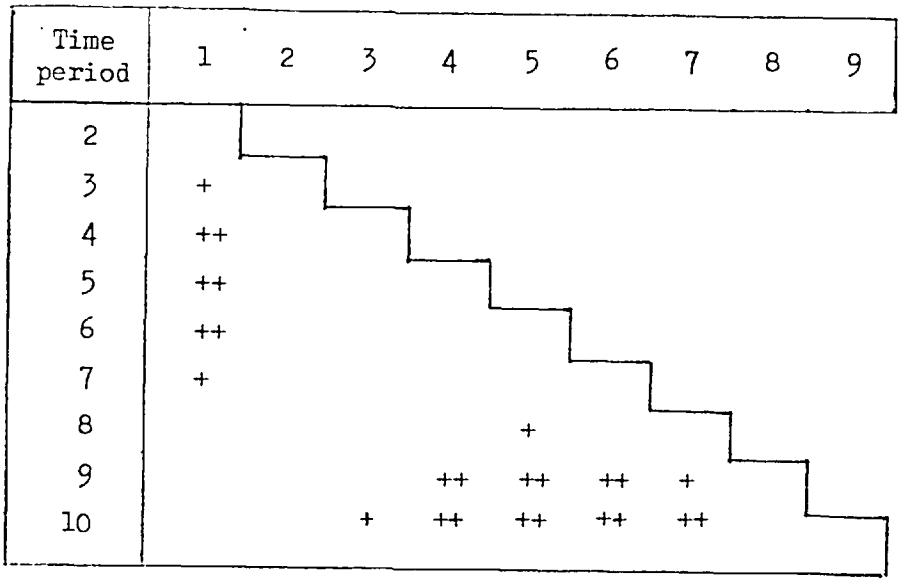
Time period	1	2	3	4	5	6	7	8	9
2									
3	++								
4	++								
5	++								
6	++								
7									
8									
9		++	++	++	++	++	++	+	
10	+	++	+++	++	+++	++	++	++	

Day 1

Time period	1	2	3	4	5	6	7	8	9
2									
3									
4	+								
5									
6									
7									
8									
9									
10									

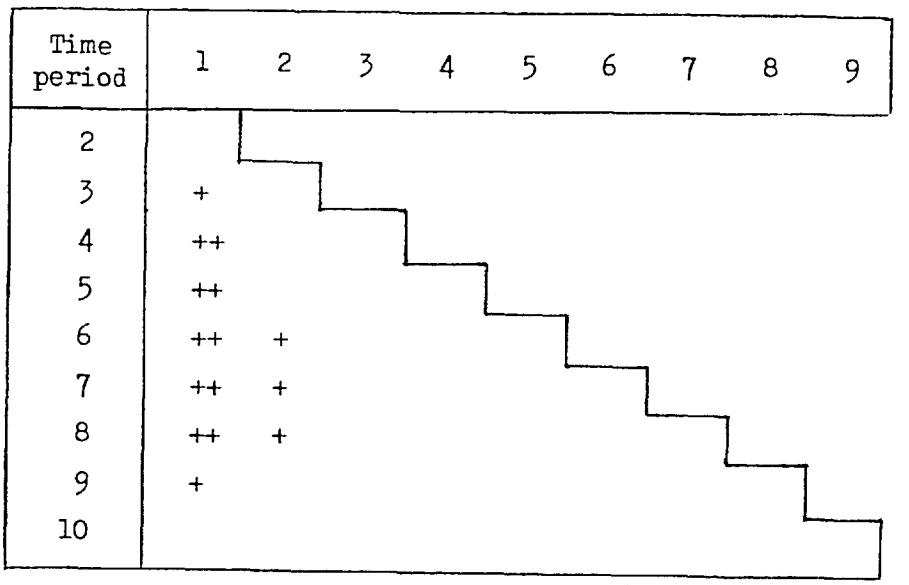
Table 5 (continued)

Day 2



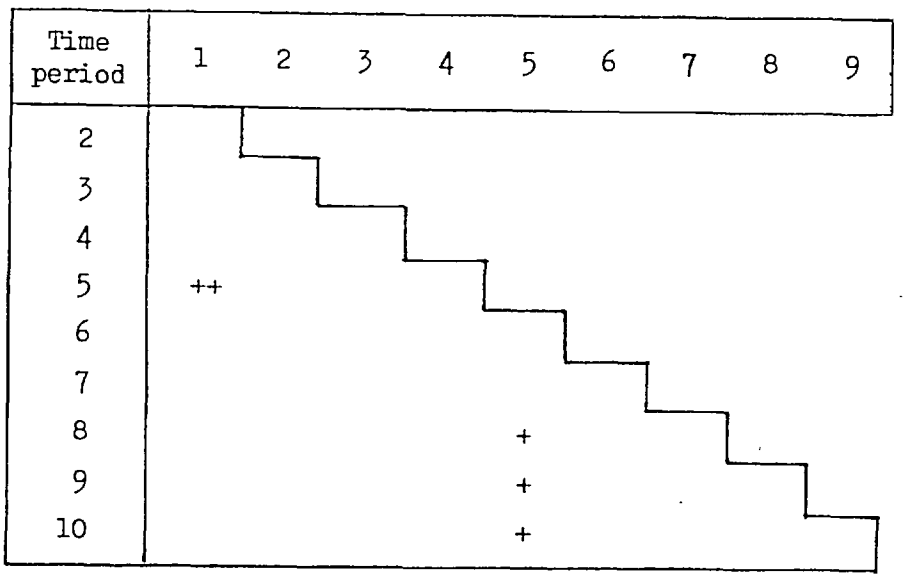
Time period	1	2	3	4	5	6	7	8	9
2									
3	+								
4	++								
5	++								
6	++								
7	+								
8					+				
9				++	++	++	+		
10			+	++	++	++	++		

Day 7



Time period	1	2	3	4	5	6	7	8	9
2									
3	+								
4	++								
5	++								
6	++	+							
7	++	+							
8	++	+							
9	+								
10									

Day 8



Time period	1	2	3	4	5	6	7	8	9
2									
3									
4									
5	++								
6									
7									
8					+				
9					+				
10					+				

one difference could be seen at the $p = 0.1$ level. Following this build up in DW at the start of the session, the last 2 periods of the session were significantly lower than the peak periods in some groups.

To summarize, the DW in each 1000s period increased significantly over the first few periods until a peak was reached. In early and late age groups (Days 0a, 0b, 1, 2, 8 and 9) the distance walked then fell off, reaching particularly low levels by the end of the session on Days 0a and 0b. There was an indication that the peak in DW occurred progressively later in the session within Days 3 - 7, but returned to its mid - session position on Days 8 and 9.

An examination of two possible sources of variation.

Two attempts were made to determine the sources of the large variation in the distances walked by individuals within each age group.

i) Starvation. It was thought that increasing the period of starvation before a trial from 3 to 24h might act selectively on the poorest walkers. Locomotor activity is known to be related to hunger in locusts (Edney, 1937; Ellis, 1951) and it seemed that increasing the period of starvation might selectively enhance the walking behaviour of the least active animals, thus reducing the total variation within a group. The average time spent in walking on Day 3, after 3h starvation was 42%. Good walkers within this and other age groups often spent up to 80% of each 1000s period in walking and it seemed unlikely that much improvement on these levels could be achieved. By contrast the poor walkers of Day 3 spent less than 40% of each 1000s period in walking, and in some cases as little as 5%.

However, a group of 10 Day 3 locusts were tested after a total of 24h starvation with no appreciable reduction in the standard deviation about the mean. Both the distance walked and the time spent in walking increased considerably but the standard deviations also increased (Table 6). All four behavioural parameters were compared statistically but there were no significant differences between them ($p \geq 0.10$, Mann-Whitney U-tests). The frequency distributions of the 100 TW values determined in each group were similar, although the 24h - starved group showed a slightly greater frequency of high TW values.

Table 6 The TDW, TTW, TNB and TSW values determined in two groups of Day 3 animals starved for either 3h or 24h prior to being tested on the treadwheel. Each group contained 10 animals and the values are given as the mean, standard deviation about the mean and the ratio of mean and standard deviation.

Parameter	Statistic	Period of starvation	
		3h	24h
TDW (m)	Mean	112.0105	129.5912
	S.D.	69.323	78.129
	$\bar{x}$/S.D.	1.616	1.659
TTW (s)	Mean	4155.00	4955.24
	S.D.	1639.54	2321.00
	$\bar{x}$/S.D.	2.534	2.135
TNB	Mean	798.3	723.9
	S.D.	264.1	265.8
	$\bar{x}$/S.D.	3.023	2.723
TSW (mm/s)	Mean	25.417	24.779
	S.D.	10.405	7.867
	$\bar{x}$/S.D.	2.443	3.150

ii) Developmental status. A second possible source of variation was related to the method used in selection of each age group. Each group was selected from animals having moulted within a 24h period, and although the majority moulted during the 12h of darkness, a 24h difference in ages after ecdysis was theoretically possible. The range of physiological ages (i.e. the extent of development) would increase in each group as the instar progressed, as individuals developed at their own rates. By the mid - to late - instar (Days 5 - 7) groups might contain animals that were either still at the middle of their 5th instar development or were nearing the end. However, by Day 8, and particularly by Day 9, the selection of animals for the behaviour trials would be severely limited as all the rapidly developing larvae would have initiated ecdysis to adulthood, and would not therefore be selected. Frequency distribution histograms (not included) of the TDW's within each age group supported this : those from Days 5 - 8 showed a larger spread of TDW values than those of the earlier age groups. The spread of TDW values on Day 9 was minimal.

All 102 animals were therefore classified as good, average or bad walkers : good walkers had TDW's of at least twice the mean TDW for that age group; bad walkers had TDW's that were less than half the mean TDW; the remainder were average walkers. The 11 age groups were then combined into two groups : Days 0 - 3 were included in 'the first half of the instar', up to the peak in walking activity; Days 3 - 9 were included in 'the second half'. The average instar duration for each cage of 5 locusts was taken to apply to all individuals within that cage, since no individual instar durations were recorded. It was then possible to relate the numbers of good, bad or average walkers to the occurrance of short, average or long instar durations. Finally, it was assumed that physiologically advanced individuals, with short instar durations, would be good walkers in the first half of the instar and bad walkers in the second, as they would have reached their peak in walking activity sooner than average. The reverse conditions were assumed for physiologically retarded individuals with long instar periods.

However, disregarding all average walkers (n = 57), there were no apparent differences in the numbers of good or bad walkers present in the advanced or retarded groups. 16 cases supported the supposition that developmental status influenced walking, while 19 refuted it; 10 cases were found to belong to the 'average' instar duration group.

SECTION 1 DISCUSSION

The criterion used to define walking behaviour had a number of important implications. As described, a minimum speed of walking of 2mm/s, with a starting speed of 4mm/s was selected. The duration of each walking bout was also measured to the nearest half second. In rapid marching, walking is interrupted at frequent intervals by bouts of non-walking behaviour which became shorter in duration as the intensity of walking increases (Moorhouse *et al* 1978). The start and end of each walking bout is sudden during periods of strong walking, and the loss of information due to the definition of walking behaviour was minimal. However, in less active walking behaviour, as seen in the early and late instar (and in some individuals at all stages of the instar), the loss was more severe. Movement at speeds of less than 4mm/s was not recorded as walking and the inherent 500ms phase delay in analysis could even allow the recording of a walking bout of 500ms duration with a distance walked of zero. This was possible if a triggering series of 4 or more pulses was immediately followed by a non-walking period. The walking bout discriminator would be activated at the end of the first 500ms period containing the 4 or more pulses, but the pulse-counter would not include these triggering pulses in the measure of the distance walked.

In total the number of walking pulses ignored was at least four times the number of walking bouts; this does not include the additional loss of information arising from walking activity of less than 2mm/s, bouts of walking that do not reach 4mm/s and all non-walking behaviours. As a percentage of the total number of pulses recorded before analysis, the number of pulses ignored during analysis varied from 5 to 54% (mean = 17.12%, n = 13), and was negatively correlated with the total number of pulses recorded ($r = -0.86$, $p > 0.001$). The definition of walking therefore selectively discriminated against the lowest walking activity and in the worst cases at least half of the information recorded was lost in analysis. While reduction of the threshold levels used to trigger and maintain the walking discriminator would tend to reduce this loss, it would also erroneously include a greater percentage of non-walking behaviours into walking bouts. Comparison of the magnetic tape records with visual observations of the locust appears to be necessary for the accurate distinction between walking and non-walking behaviours in this system.

The correlation coefficients determined between the parameters used to describe the recorded data indicated that the single measure of the distance walked (DW or TDW) most accurately described the walking behaviour at all stages of the instar. At the peak in walking on Day 3 the correlations between individuals' TDW and TTW and TSW values were highly significant; the average DW values for the 10 locusts in that group were also highly significantly correlated with the TW and SW values. These high correlation coefficients were also seen at all other stages of the instar. It was therefore decided that studies into the physiological mechanisms of the walking behaviour (see Section 2) could be made using wheels recording a measure of the distance walked in 2 or 3 hours without distinction between walking and non-walking behaviours, and without recording the time spent in walking or the number of walking bouts.

The change in marching activity within the 5th instar of *Locusta* has been described from field observations and from detailed laboratory actograph experiments. While some early reports indicated that marching behaviour in the gregarious phase was in response to adverse conditions, field observations (Uvarov, 1928) and laboratory experiments (Edney, 1937; Ellis, 1951) indicated that the behaviour was an activity spontaneously engaged in by the majority of larvae unless feeding or resting. Various environmental cues such as humidity, temperature (Edney, 1937; Ellis, 1951), hunger and crowding (Ellis, 1951, 1953, 1954) affect marching behaviour, but the animals have been observed to march even under optimal conditions and have been reported to march through dense vegetation without stopping to feed (Uvarov, 1928). The work reported here confirms both that *Locusta* larvae march under constant laboratory conditions, and that marching occurs in well fed animals but is enhanced by hunger. Optomotor stimulation is also regarded as a major factor in the promotion of marching in unrestrained animals (Kennedy, 1951; Ellis, 1953, 1954; Ellis and Hoyle, 1954) but these experiments show that intense and sustained walking behaviour can occur in the complete absence of these stimulii.

Edney (1937) reported a gradual increase in locomotion activity in solitary larvae in the first 3 days of the 4th and 5th instars, with a peak half way through the instar. The activity then diminished until it was almost zero 24 - 36h before the moult. However, animals showed intense locomotor activity on the last day of the instar if no suitable

perch for moulting was present. Ellis (1951) reported a similar peak in the marching behaviour of crowded mid - 4th instar larvae, but no marching or feeding occurred for one day before the moult. Hoyle (1956) showed a related reduction in jump lengths over the last 3 days of the instar.

The experimental design employed here was considerably more critical than that used by other workers since the locusts tested were isolated, in stable conditions, and the walking behaviour could be very accurately recorded. However, interpretation of the data was complicated by the extreme variation in the behaviour exhibited, also reported by Edney (1937). At any stage the differences between the individuals were often as great or greater than the differences seen at different stages in the instar. The variation could not be reduced by 24h starvation, and analysis involving an estimation of the developmental status of each animal was not successful.

One of the sources of variation in any culture of locusts is the variation in the phase-status of the individuals in the culture. Even though the parental stocks are crowded and the larvae are maintained in crowded conditions, there is usually some variation in the extent of the larval gregariousness. This is true even in animals known to have emerged from a single egg pod (Blackith and Albrecht, 1961; for a review of the consequences of, and inheritance of, phase characteristics, see Uvarov, 1966). While the range of phase - status is much smaller than the differences between fully gregarious and solitary animals, this may be a significant source of variation in the walking behaviour that is known to be phase - related (Ellis, 1951, 1953, 1959, 1964). This variation in phase - status may also account for some of the large variations observed in all aspects of this study, including morphological responses to JH - treatments (p. 53) and physiological responses of individual neurones (p. 97).

The large standard deviations in the behaviour of each group necessitates the use of large sample sizes for statistical analysis of the changes observed; in this work the maximum group size of 10 was limited by the time involved in each trial. The changes reported in the marching behaviour both over the instar and over each session were therefore statistically weak: those groups showing significant differences in their behaviour were scattered among many 'non-significant' groups. Thus it is only possible to describe trends in the 5th instar behaviour supported where possible by statistically significant results.

This lack of reliable results was not unexpected as the experimental design minimized the disturbance to the locusts while allowing as unrestricted behaviour as possible. The walking was therefore relatively unstimulated, although it was strengthened by the 'railroading' effects of the wheel and the visual field (Moorhouse *et al*, 1978).

Under these conditions gregarious 5th instar larvae showed a build up in marching behaviour that reached a peak on Day 3 of the 10 day instar. The marching activity fell off gradually, with some evidence of a plateau in the distance walked and in the time spent in walking, until the penultimate day of the instar. Only on the final day, Day 9, was walking behaviour greatly reduced. At the peak of activity on Days 2 and 3 maximal distances were achieved in relatively few, long walking bouts of high average speeds. The time spent in walking was a less important factor in determining the best walking periods.

There was some evidence of a change in the pattern of the walking behaviour exhibited over the (approximately) 3h sessions that was related to the age of the locusts. Early 5th instar larvae rapidly built up to intense activity levels within the first 3000s (50 minutes), but these levels were not sustained: by the end of the session the walking activity had almost ceased. In contrast, mid-instar larvae could sustain high walking levels for long periods, and on Days 5 and 6 walking was still increasing by the end of the session. The last two age groups showed a fall in walking after about 2h, in the same fashion as the early instar larvae. Many developmental factors may be involved in these changes in sustained activity: feeding is known to reach a maximum in the mid - to late - instar (Ellis, 1951; Davey, 1954); the cuticle undergoes a number of changes before and after ecdysis (Duarte, 1939; Malek, 1958a, 1958b), and is at its maximum strength in the mid-instar; muscular development and responsiveness also change at each end of the instar (Hoyle, 1956). Finally, the availability of fuel supplies for marching may be analogous to the situation in adults, where adipokinetic hormone release after some 30 minutes of flight is necessary to switch the animal from the initial carbohydrate metabolism to lipid metabolism (review: Goldsworthy, 1976). Such a related control system could account both for the changing pattern of marching over the 10^4s session and might also influence the changes during the instar itself.

The most active walking behaviour was observed on Day 3 and the change in activity over the session agreed with the changes reported

by Moorhouse *et al*, (1978) under similar conditions. They reported an increase in activity over the first hour that was sustained during the second hour and gradually decreased in the third. This pattern was most evident here on Day 2, while Day 3 showed an earlier, sharper peak in the distance walked in each 1000s. A similar pattern in locomotor activity has been reported in the field (Kennedy, 1939; Ellis and Ashall, 1957) and in the laboratory (Ellis, 1951). The major difference in behaviour was that normal marching is generally accompanied by numerous long, low, leaps; leaping rarely occurred on the treadwheel except during the first few minutes of the session. This reduced behaviour may be due to the lack of optomotor stimulii and the lack of tactile stimulii normally provided by other locusts and by the moving environment. Otherwise, it is apparent that the behaviour recorded on this treadwheel was close to the normal behaviour exhibited in the field and in unrestricted laboratory experiments. The measure of the distance walked by the locusts per unit time closely described the most significant changes observed in the walking behaviour.

SECTION 2. MATERIALS and METHODS

Animals

Mid-third or mid-fourth instar *Locusta migratoria migratorioides* were obtained from crowded cultures maintained by the Centre for Overseas Pest Research, London. These stocks were kept as previously described (p. 7). Insects were removed at known intervals either at 0-24h, 0-4h or 0-2h post-ecdysis. Following precocene treatment the 4th instar larvae were kept at densities of 20-30 per cage; all others were kept at densities of 5-10 per cage.

Each cage was supplied daily with abundent quantities of fresh food and for the majority of the year received only glass-house grown wheat seedlings. However, when the numbers of locusts were particularly high, the wheat supply was supplemented by locally grown bamboo leaves during the winter, on grass during the summer. Both these supplementary food stuffs tended to dessicate more rapidly than the wheat, but locusts were seen to be feeding throughout the day, indicating that the food was acceptable even after 24h. Locusts used for behavioural tests were fed exclusively on wheat seedlings until after the tests (92-120h after ecdysis).

As before (p. 7), the locusts were kept under a 12:12 LD light regime with lights on at 7.00am. However, the lighting power supply was changed in June 1979 (approximately half way through this study) from the normal 240 V AC supply to a 240V DC supply. There is evidence to suggest that locusts can perceive the 100Hz 'mains-ripple' emitted by tungsten-filament bulbs running on normal AC supplies (Miall, 1978). While the effects of fluctuating light levels on locust behaviour are unknown, AC lighting can cause a disruption of normal flight and feeding behaviours in certain insect species (Renner, 1957; Van Praagh, 1975). The room used for behavioural tests remained AC - illuminated throughout the study.

Animals were treated with various doses of hormones (p. 24) and their behaviour tested either on Day 3 of that instar or on Day 3 of the following instar. After emergence to the adult stage the locusts were either immediately sacrificed and morphometically examined, or they were maintained for 6 or more days before being sacrificed. Precocene-treated locusts were killed 6-10 days after emergence to allow for possible growth of the CA before dissection. JH-treated animals were killed shortly after emergence unless their morphology was such that a supernumerary moult could be expected. In this case they were maintained for 9-15 days; even if no external signs

of an extra moult could be seen, the double cuticular layers could occasionally be seen during dissection and this was recorded as an attempt at a supernumerary moult.

Records were kept of all mortalities and dead animals were removed daily. Instar lengths were recorded and individuals were identified using a series of dots of non-toxic enamel paint on the hind femur.

Hormones

The sources and chemical structures of the hormones and hormone-analogues used are given below.

i) Juvenile hormones JH I, JH II and JH III were obtained from Calbiochem as A grade juvenile hormones in solution in hexane:

a) JH I: 3, 11-dimethyl -10- epoxy -7- ethyl-trans, trans -2, 6-tridecadienoic acid, methyl ester; MW: 294.4

b) JH II: 10-epoxy- 3, 7, 11 - trimethyl-trans, trans - 2,6-tridecadienoic, methyl ester; MW: 280.4

c) JH III:10-epoxy - 3, 7, 11-trimethyl - 2,6-trans, trans-dodecadienoic acid, methyl ester; MW: 266.4

ii) JH - analogues

a) Law's mixture : 10-epoxy-7- ethyl -3, 11-dimethyl-trans, trans -2, 6-tridecadienic acid. Obtained from Calbiochem as B grade synthetic juvenile hormone. Its activity was stated to be 'about 1% that of pure natural juvenile hormone and at least 100 times more active than dodecyl methyl ether.'

b) ZR515: Isopropyl-11-methoxy- 3, 7, 11-trimethyl -2, 4 - dodecadianoate; 89% pure. Generously supplied by the Zoecon Corporation.

iii) Precocene II: 6, 7-dimethoxy -2,2-dimethyl -2H-1-benzo-pyran; 99% pure. Obtained from the Aldrich Chemical Company.

Determination of possible carrier fluids for hormone injection.

Topical applications of labile compounds suffer a number of disadvantages. Hormonal compounds are often photolabile and are usually volatile, such that the exact quantities entering the tissues or remaining on the cuticle after topical applications are unknown. Carrier fluids such as acetone or hexane disrupt the cuticular wax layers and may deposit some of the compound within the cuticle, where it may be stored indefinitely, released slowly into the haemolymph or even lost during ecdysis. Part of a topical dose may also be lost by friction with the cage, its contents or

other animals. Additional effects, such as the chilling of the body during evaporation of the carrier fluid, cannot be disregarded. Finally, many carrier fluids and hormonal compounds have a distinct odour, which may affect the immediate behaviour of the animal, and the effects of these compounds entering via the respiratory system - either as a vapour or as a spreading film - are difficult to estimate.

Internal treatments also have disadvantages but the possibility of injecting a hormone directly into the haemolymph avoids many of the problems stated above. The main problems involved are that the animals suffers some distress and tissue damage, that the carrier fluids are often toxic, and that, without the protection of haemolytic carrier proteins, the hormones can become irreversibly bound onto fatty tissues near the point of injection. Since the volumes of hormones and analogues used are small, accurate doses require the mixing of the hormone in a larger volume of a non-toxic fluid that has no effects in common with the test compound. Physiological salines are the obvious choice, but many insect hormones have low solubilities in water. To overcome this oils and oil mixtures have been used, such as peanut oil, caster oil and olive oil. However, some oils can display hormonal activity (Johnson, 1972), and in addition, Gilbert and Schneiderman (1958) have reported that many diverse substances show JH activity in invertebrates. A number of solvents were tested and their suitability as carrier fluids are summarized below.

i) Acetone Injections of 1µℓ acetone into mid - 5th instar larvae produced temporary behavioural effects such as twitching and tetanus of the hind leg and loss of co-ordination. The animals recovered quickly and showed no long-term effects other than slight tissue damage at the site of the injection.
Injections of 10µℓ acetone in Day 0 5th instar larvae produced immediate severe effects; muscle-rigidity, loss of co-ordination and even complete immobility. The animal apparently recovered quickly, but after 5h were seen to be moving slowly, and one had shed a hind leg. Tissue discolouration was evident near the injection site within 24h, and half of the sample did not recover from the motionless stage.

ii) Hexane. Injection of 1µℓ or 10µℓ hexane gave comparable results to those stated for acetone. One locust died within 48h of treatment with 10µℓ hexane.

iii) Rissela 17 oil. Volumes of 10µℓ, 15µℓ and 20µℓ were injected into early 5th instar larvae but no effects were evident other than the usual excitability after handling. After 24 h slight tissue

discolouration was occasionally seen near the injection site. Mortalities after the following moult were slightly higher than normal, but less than 5%.

iv) Dimethyl farmamide. An injection of 20µℓ of dimethyl farmamide into mid - 5th instar larvae produced immediate severe effects, with muscle rigidity, regurgitation of the gut contents and loss of co-ordination. The locusts appeared distressed and were observed to pull the hind leg on the side of the injection around towards the head, using the other legs on that side. Some animals were seen to bite at the leg and after 24h a number had shed the hind leg ipsilateral to the injection. After 5 minutes all animals were motionless and remained so until death some 2 - 5 days later.

Although Rissela oil appeared to be the most suitable candidate, precocene II was found to be insoluble in this oil, and the JH - analogues only slightly soluble. A number of acetone : oil or hexane : oil mixtures were therefore tested.

v) Acetone : Rissela oil. Numerous 1 : 10 v/v acetone : oil mixtures were tested. Up to 20µℓ of the mixture was apparently behaviourally inactive, although some temporary effects similar to those produced with 1µℓ acetone were observed. No mortalities occurred and tissue damage at the site of the injection was seen in a minority of the sample.

One group receiving 5µℓ of a 1 : 5 acetone : oil mixture in the mid - 5th instar showed no immediate or delayed effects, although another group of 24 animals treated 0 - 24h into the 4th instar showed high mortalities at the 5th instar - adult moult, after a successful 4th - 5th instar moult (p. 40).

vi) Hexane : oil. Mixtures of 1 : 10 and 1 : 5 hexane : oil showed no differences in effect to the acetone : oil mixtures, when applied to mid - 5th instar larvae. There were no delayed mortalities.

vii) Acetone : insect saline. A number of mid - 5th instar larvae received injections of 2.5µℓ of a 1 : 5 v/v mixture of acetone and insect saline (Usherwood and Machili, 1968). There were no obvious immediate or delayed effects, and little evidence of tissue discolouration at the site of the injection.

Mode of application.

The various dosages used are outlined in Tables 8, 9, 10, 12 and 15 showing both the numbers of locusts receiving treatment and the time and

mode of application employed.

i) Topical applications. All hormones were applied by micro-syringe such that the liquid film spread dorsally over the abdomen and beneath the wing buds. The hormones were dissolved in either acetone (for all JH - analogues and for precocene) or in hexane (for the A grade juvenile hormones), and were applied in 5µℓ volumes. Where necessary when using the Law's mixture, the hormone was applied directly without acetone, in one or two 5µℓ treatments separated by at least 30 minutes. Care was needed at all high doses to allow the film of hormone to dry before release of the locust.

ii) Internal applications. A limited number of larvae were treated with JH III or precocene II by injection in an acetone : oil mixture. The hormones were first dissolved in acetone and then mixed in a 1 : 10 v/v ratio with Rissela oil to achieve the required dosage in 5µℓ of the mixture. The carrier was injected into the lateral abdomen by insertion between two sclerites, and care was taken to ensure that no leakage occurred after withdrawal of the needle.

A number of mid-5th instar larvae were injected with 43 or 214µg of ZR515 carried in 0.5µℓ acetone and mixed in insect saline to a total volume of 2.5µℓ. The technique used was the same as described above.

Morphometrics

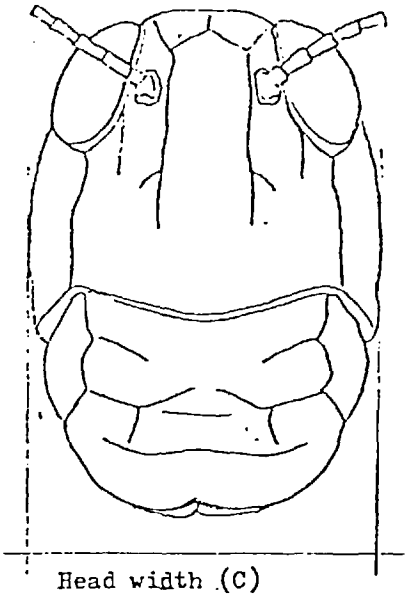
A number of measurements were made on the larvae and adults to quantify the morphological abnormalities produced. These measurements were chosen to characterize the obvious changes in head, thorax and wing morphology, and were based on those made by Dirsh (1953).

All measurements were made on dead locusts, although the animals were not of a standard age when sacrificed. The measurements were made by visual comparison with 1-mm squared graph paper and were taken to the nearest 0.5mm. Dirsh (1953) recommends the use of calipers for measurement but the morphological changes observed were large enough to allow the less accurate method used here. The measurements used are indicated in Fig 18. Elytron length was defined as the distance from the proximal notch to the most distal part of the wing; no attempt was made to straighten the twisted or crumpled wings of JH-treated adults. Head length was defined as the distance from the vertex to the lowest point of the labrum. The other measurements are self-explanatory.

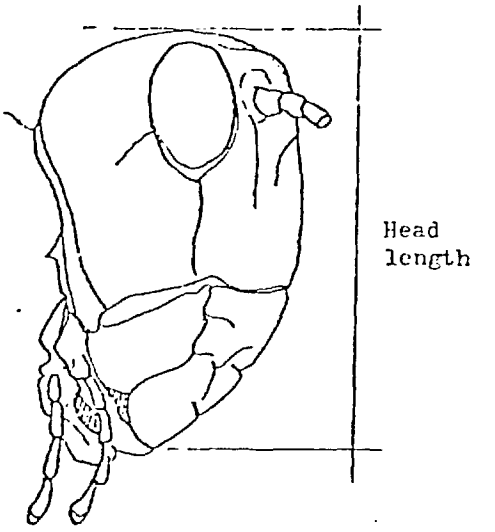
The degree of abdominal distension was variable due to both the gut

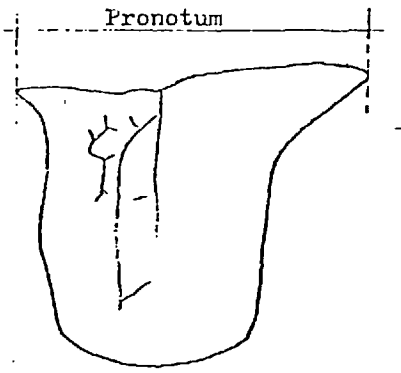
Figure 18

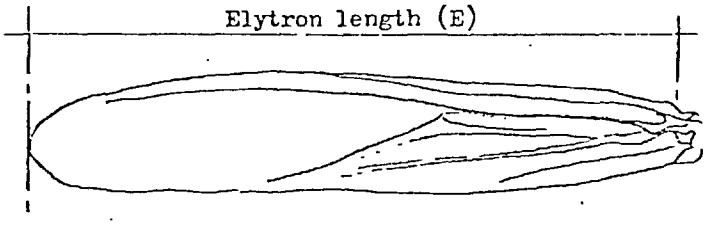
Measurements taken in morphometric studies of precocious adultiform larvae, juvenile adultoids and normal locusts. All measurements were taken to the nearest 0.5mm, by eye.



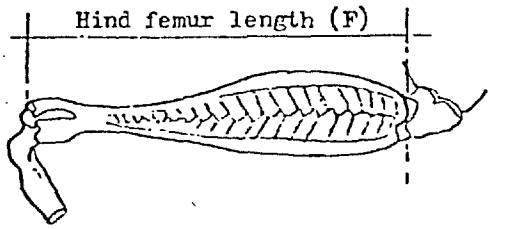
Head width (C)

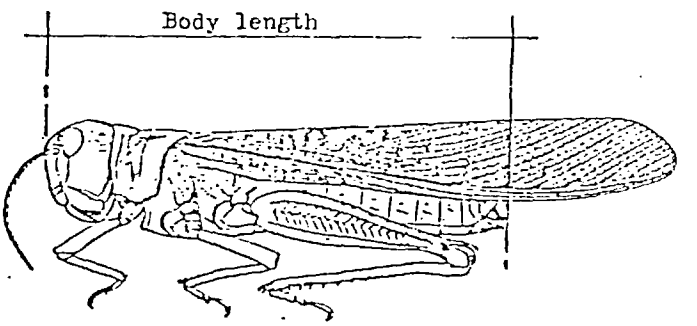






Above and right: Albrecht (1953)
Below: Uvarov (1966)





contents and the posture adopted during death. Although most measurements are independent of age (assuming that the cuticle is inelastic), this measurement is probably age dependent and was the most variable.

Dirsh (1953) recommends the use of the ratio of maximum head width (C) to hind femur length (F) as the most indicative, single characteristic of phase-status. Both this ratio and the ratios F/C and E/F (elytron length/ femur length) are also commonly used (Uvarov, 1966); all three ratios are given in Tables 11, 14 and 17.

Scoring of the morphogenetic effects produced.

Because treatment with precocene and JH have opposing morphological effects, namely the production of premature adultiforms and larval adultoids, two separate scoring systems were adopted. These are considered separately here, as one is a measure of the adult characters displayed by larvae while the other is a measure of the larval characters displayed by adults.

i) Score of precocene - induced adultiforms. The morphometric studies on the precocious adultiforms considered three classes of morphology : normal or outwardly normal 5th instar larvae; semi-developed adultiform larvae with small, rigid wings held vertically above the abdomen and partial adult colouration; and fully developed adultiform larvae with well formed wings that were partially or fully folded down on the abdomen, and with full adult colouration. Behavioural studies on these adultiforms included a distinction between two forms of the full developed adultiforms; for this reason all semi-developed adultiforms will be referred to as Type 1, and fully developed adultiforms as Type 2/3.

ii) Score of JH - induced adultoids. Two scoring systems have been used here to measure the extent of juvenilization displayed after JH or JH - analogue treatments. The first was one used by Tang (1976) and considered wing deformation, limb deformation and larval colouration. The system was used in the early parts of this study, before it became apparent that the scores provided were not sufficiently definative and certain characters or combinations of characters were being ignored. A more detailed system was therefore developed that was based on an additive score of wing deformation, pronotum deformation and larval colouration. Each was scored as being absent, slight medium or pronounced, and scores were adopted that represented the combinations of characters displayed. Thus certain possible scores were uncommon, and others never used. The two score systems are compared in Table 28.

All juvenile adultoids produced by treatment with Law's mixture were scored with both systems. The dose - response curves for each are similar in shape (Fig. 19) and indicate that the different weighting of characters implied in each scoring system have only a minor influence on the shape of the dose - response curves. For morphometric classification, however, the relationship between scores and C/F ratios are discussed in detail later (p. 53).

Estimation of corpora allata volumes.

The corpora allata of *Locusta migratoria* grow throughout the first few weeks of adult life (Johnson, 1972); measurement of the volume of these glands should therefore be standardized by age. The morphologically normal adults produced within the control groups and the lowest precocene dosage groups were killed and stored in aqueous Bouin's solution 8 - 11 days after emergence. The adultiforms produced were killed and fixed 17 - 23 days after emergence; however since the CA were severely degenerated after precocene treatment, this delay served to increase the reliability of CA identification during dissection. The juvenile adultoids produced by precocene treatment (p. 35) and some ZR515 - induced adultoids, as well as all control animals were killed and dissected 6 - 10 days after emergence.

Freshly killed animals were dissected under insect saline while those stored in Bouin's solution were washed and dissected under industrial methylated spirits. Dissections were carried out under a stereo - dissecting microscope with good lighting. Under these conditions the CA could be identified in 100% of the freshly killed control groups, and in 89% of the preserved control groups. Either the left or right gland was measured since there is no evidence of hetrogenous development of the CA in *Locusta* (Weaver, 1979). The volume of the gland was estimated by visual measurement of the length and width of the gland, by comparison with a wire of 0.55mm diameter; the measurements were taken to the nearest 0.1mm. Analysis of 16 visual estimates of the size of the glands that were subsequently isolated and measured under a compound microscope revealed a constant error in size judgement over the range of gland sizes encountered. The gland volumes were calculated after correction for this error assuming that the glands were ovoid in shape.

i) Volume of an ovoid = $4/3 . a . b^2$, where a and b are the longest and shortest axes of the ovoid;

ii) Mean error in estimation of dimension a = 1.396 ± 0.313;
Mean error in estimation of dimension b = 1.437 ± 0.614;

iii) Therefore the estimated volume of the gland = $12.075.x.y^2$

where x and y are the estimated longest and shortest axes of the gland.

SECTION 2. RESULTS

A. Morphogenetic effects of JH and JH - analogues

Juvenile hormones (JH I, II and III)

High dosages (i.e. 50 - 200µg) of JH I, II and III, applied topically or internally in the early 5th instar had very slight morphogenetic effects. Higher doses may have been more effective but it was not considered worthwhile to determine a complete dose - response curve for each compound. Firstly, there was no evidence of any dose - response relationships from the two dosages tested for JH II and III, and the level of morphogenetic activity of JH I was extremely low. Secondly, it would have been prohibitively expensive to use the compounds at such high dosages, even if effective, when compared to the other JH - analogues. The data is given in Table 8 and no further analysis was made on the effects of JH I and II. JH III was tested more extensively and sufficient numbers of animals were treated to ensure that the low morphogenetic activity was not due to errors made in application or to errors due to low sample sizes.

Two samples of JH III (Calbiochem Lot No. 800554) were tested and stored separately. Neither were effective in *Locusta*, but one sample was tested against the bean aphid, *Megoura viciae*, and found to display full JH activity in this species (J. Hardie, unpublished observations). There was no evidence of a significant difference in the duration of the 5th instar of groups treated with JH I, JH II, JH III or acetone alone. The group treated with 100µg JH III showed no significant morphological differences to the control group. Treatments topically with the hormone (JH III) dissolved in either 5µl acetone or hexane, or injection of the hormone carried in either 1µl hexane or 5µl 1 : 10 acetone : oil were equally ineffective.

There was no evidence of sex differences in morphological deformation, in the numbers of adultoids produced, or in the duration of the 5th instar in all treatment and control groups.

Law's mixture

i) Dose-responses. Increasing dosages of Law's mixture, applied topically 0 - 24h into the 5th instar, progressively induced greater numbers of adultoids, and increasingly affected adultoids. The highest dose applied, of 10,690µg, produced exclusively supernumerary larvae; these were killed before they could display any attempt at an additional moult, but from later evidence, using ZR515, it is likely that many of these supernumeraries would have attempted a further moult if allowed the time. Two dose - response curves were determined (Fig. 19) using both Tang's (1976) scoring system and the system developed here; the curves show

Table 7 Mode and time of application of control treatments, applied in either the 4th or 5th instar. The morphological score of juvenilization and the instar durations (as means for 5th instar treatments; medians for 4th instar treatments) are also given.

4th Instar treatments

Mode of Application	Treatment	Time of Application (hours)	Sample Size	Morphological Score	4th and 5th instar durations (median, days)	
Topical	5µl acetone	0-24	105	-	6	9
Injected	5µl 1:10 v/v acetone:oil	0-24	25	-	9	10

5th Instar treatments

Mode of Application	Treatment	Time of Application (hours)	Sample Size	Morphological Score ± 1 S.D.	4th and 5th instar durations (mean ± 1 S.D.)
Topical	5µl acetone	4-8	14	0.385 ± 0.650	8.000 ± 0.0
"	"	0-24	47	0.194 ± 0.577	10.080 ± 1.105
"	"	72-96	26	0.130 ± 0.346	8.583 ± 0.789
Injected	5µl 1:5 v/v hexane : oil	0-24	5	0.175 ± 0.500	-
Injected	5µl 1:10 v/v acetone:oil	0-24	5	0.250 ± 0.500	8.500 ± 0.577
Injected	5µl 1:10 v/v acetone/saline	72-96	32	0.219 ± 0.491	9.100 ± 0.620

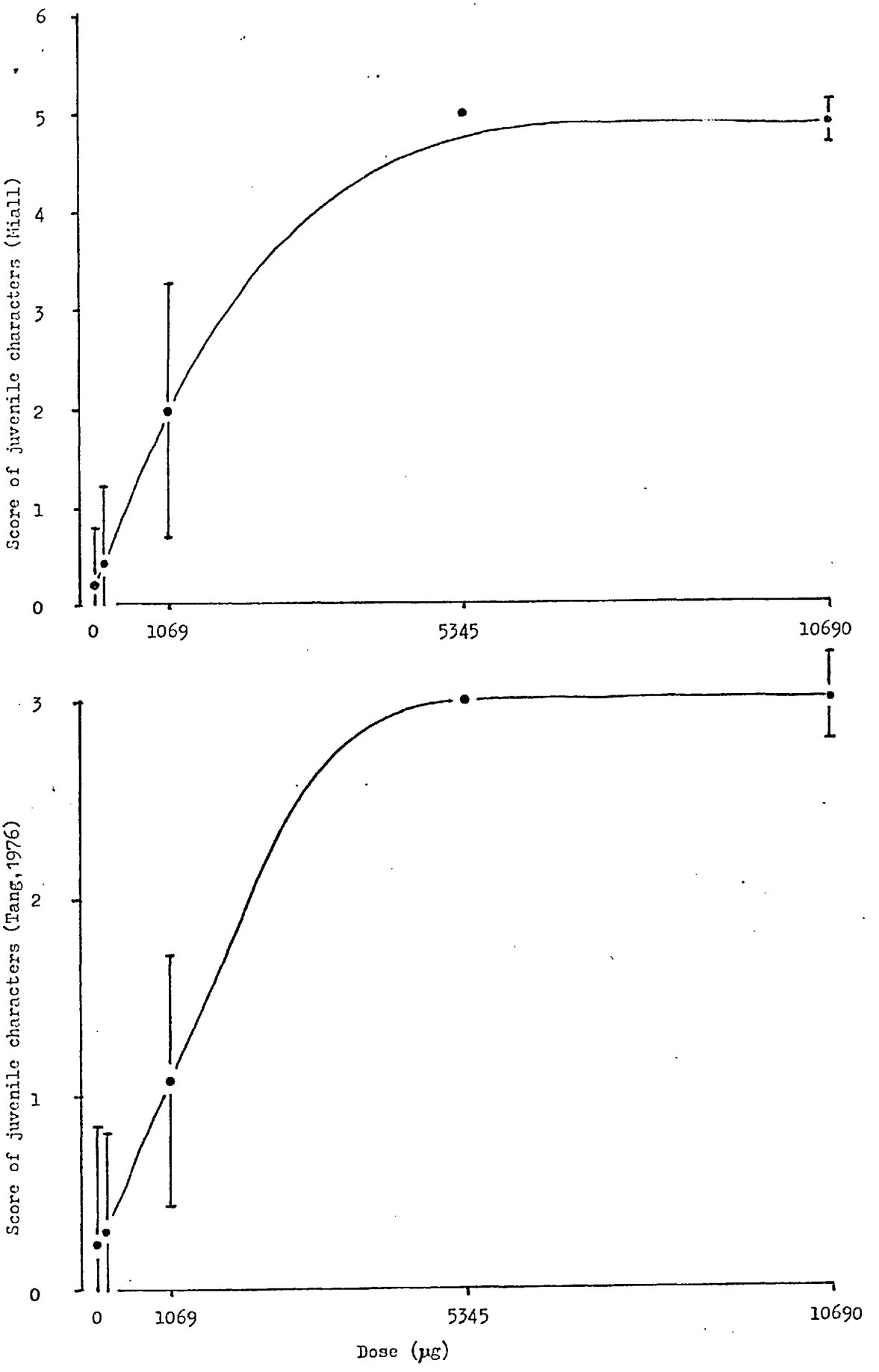
Table 8 The mode, timing and dose of juvenile hormone applied in the 5th instar. The 5th instar duration and the degree of juvenilization (morphological score) are also given.

Hormone	Mode of Application	Time of Application (hours, 5th)	Dose (µg)	Sample Size	Morphological Score ± 1 S.D.	5th instar duration (days ± 1 S.D.
JH I	Topical	12-16	50	5	0.20 ± 0.447	8.40 ± 0.548
JH I	"	"	100	5	0.40 ± 0.548	8.20 ± 0.447
JH II	"	"	50	5	0.80 ± 0.447	7.80 ± 0.447
JH II	"	"	100	4	0.25 ± 0.500	8.00 ± 0.0
JH III	"	0-24	100	66	0.606 ± 1.357	11.36 ± 1.604
JH III	"	"	200	5	0.0 ± 0.0	9.50 ± 0.577
JH III	Injected (*)	"	100	5	0.0 ± 0.0	10.80 ± 0.447
JH III	Injected (+)	"	100	10	0.0 ± 0.0	10.10 ± 1.524

(*) Injected hormone carried in 1µl hexane
(+) Injected hormone carried in 5µl 1:10 v/v acetone : oil

Figure 19

Dose response curves of the juvenilization induced by Law's mixture, determined using the scoring system of Tang (1976) and a system developed during this study. Ordinate : score of the juvenile characters retained by the adultoids; abscissa : dose of Law's mixture (µg) applied 0-24h into the 5th instar. Bars : $\pm$ 1 standard deviation of the mean.


Score of juvenile characters (Kiall)
0
1
2
3
4
5
6
0
1069
5345
10690
Score of juvenile characters (Tang, 1976)
0
1
2
3
0
1069
5345
10690
Dose (μg)

good similarities, in shape and it seems that the individual biases of each scoring system are of little importance if sufficiently affected adultoids are being characterized.

No sex differences in either the percentage of adultoids produced or the severity of their morphogenetic responses were evident.

ii) Morphometrics The adultoids and supernumerary larvae produced by treatment with Law's mixture were morphologically similar to those produced by the other JH - analogues used. Two differences were noticed in retrospect, but no data was collected to confirm them : Law's mixture adultoids were observed to display stronger larval colouration and were often more heavily melanized than other adultoids; there was also a higher percentage of severely affected adultoids that could not escape from the old cuticle during the 5th - adult moult. Although both these phenomena were observed after treatment with ZR515 and with precocene II (p. 35), they were never as frequent as seen following Law's mixture treatment.

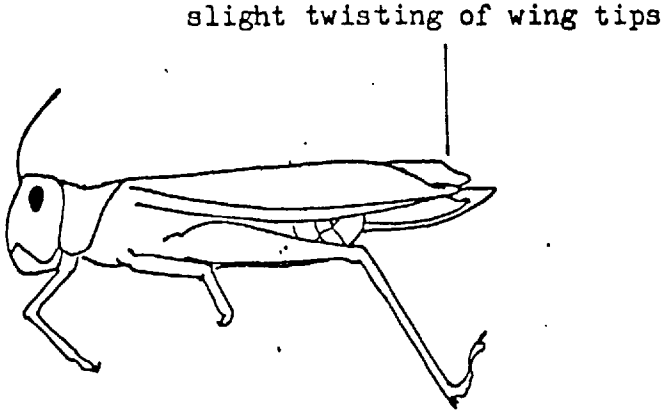
The adultoids produced displayed a wide range of morphogenetic characteristics which will be summarized below, and are also presented in Figs. 20 and 21. The most common indication of juvenilization was the abnormal twisting of the wing tips of the adults. This ranged from a slight deformation of the fore-wings to marked curling of both fore - and hind - wings. Deformation of the pronotal shield was first evident as a slight arching and in more severe cases as deep creases, often with the posterior edge curled beneath the main portion of the shield. In addition to various combinations of these characters, pronounced juvenilization was apparent from the retention of larval colouration into the adultoid stage. Although colour-blindness caused difficulty for the author in seeing the green pigmentation characteristic of juvenile adultoids (Tang, 1976), the more severely affected animals showed strong retention of the larval colouration, and this was used in scoring the juvenilization of adultoids. Larval colouration was never observed without the deformation of the wings and pronotum, and was considered as an indication of strong juvenilization.

The most severely affected adultoids displayed all these characters maximally : they possessed severely crumpled wings, generally much shorter than those of the adult and often very darkly melanized, as are the larval wing-buds. The wings rarely opposed correctly, and in

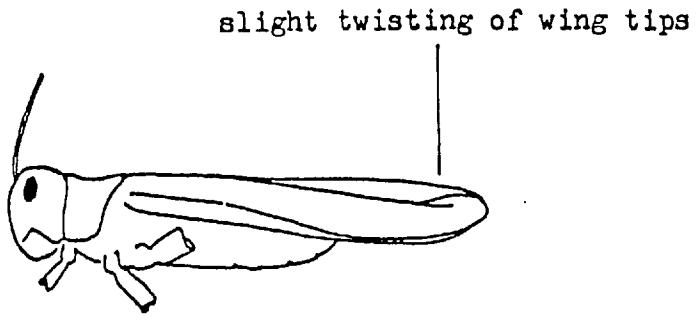
Figures 20-21

Annotated diagrams indicating the progressive characters evident in Law's mixture induced adultoids; both lateral and dorsal views are given for each example.

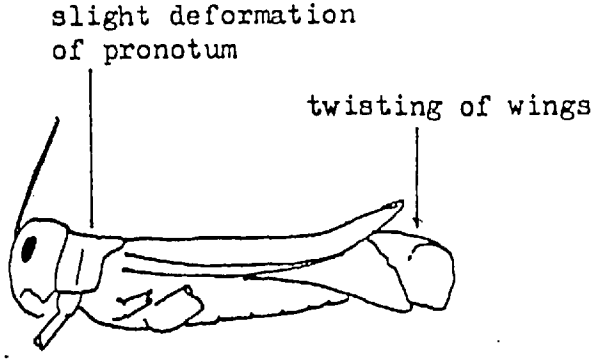
(a)



(b)



(c)



(d)

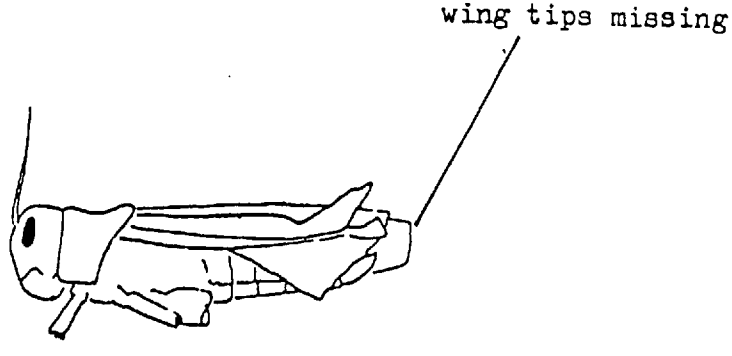
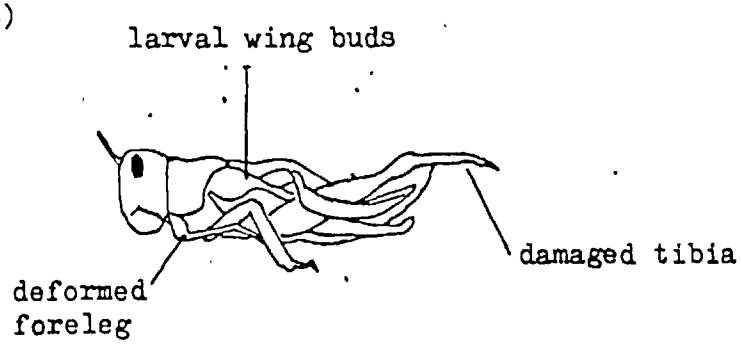
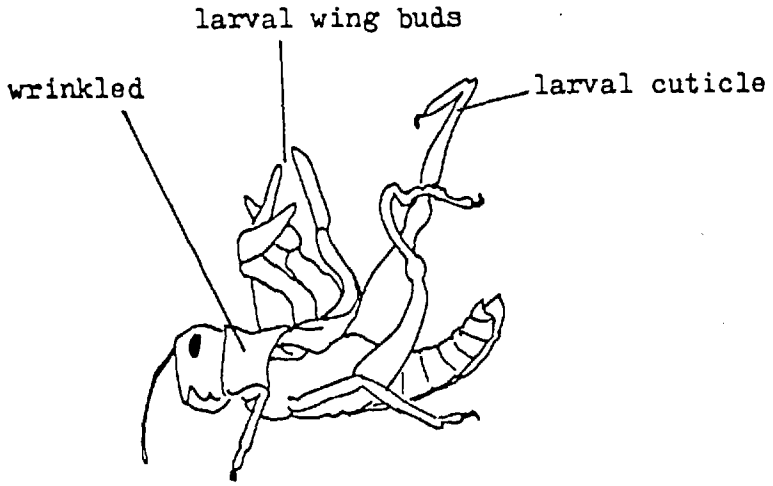


Fig. 20a-d.

(e)


larval wing buds
damaged tibia
deformed
foreleg

(f)


larval wing buds
wrinkled
larval cuticle

(g)

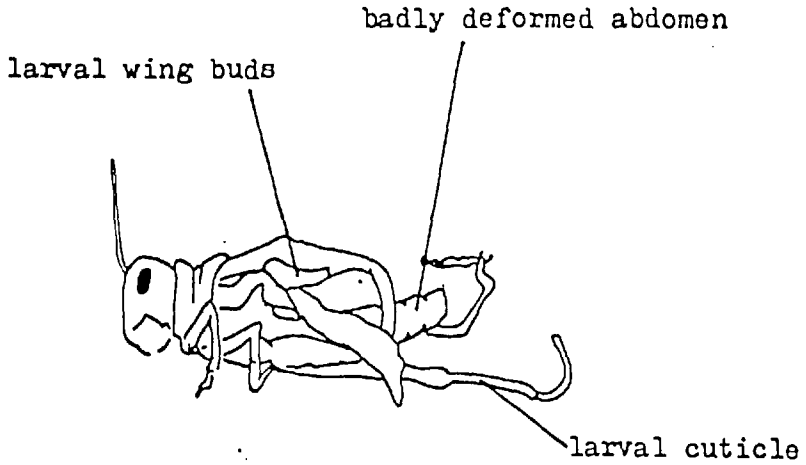
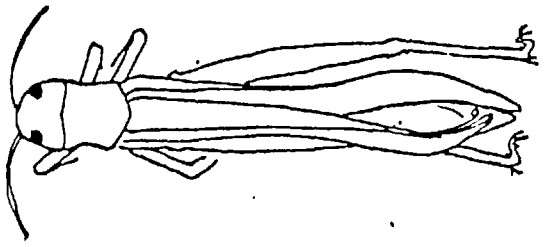
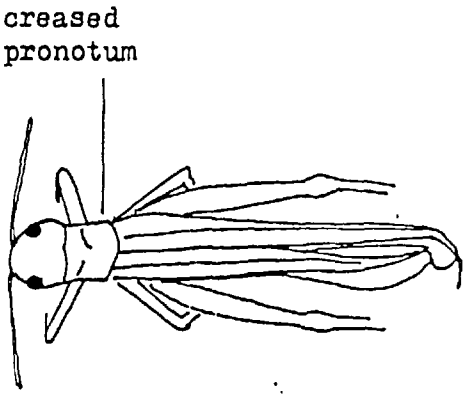

badly deformed abdomen
larval wing buds
larval cuticle

Fig. 20e-f.

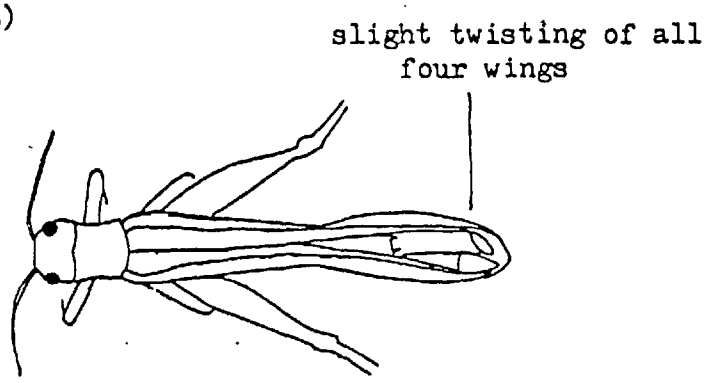
(a)



(c)



(b)



(d)

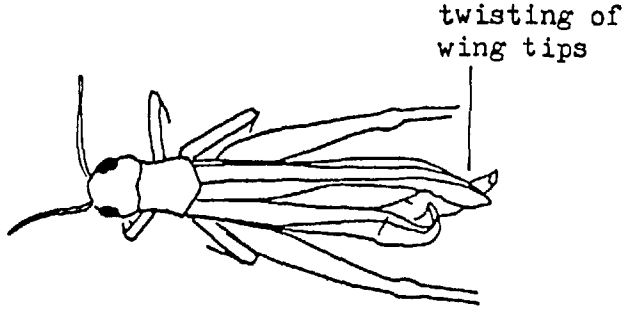
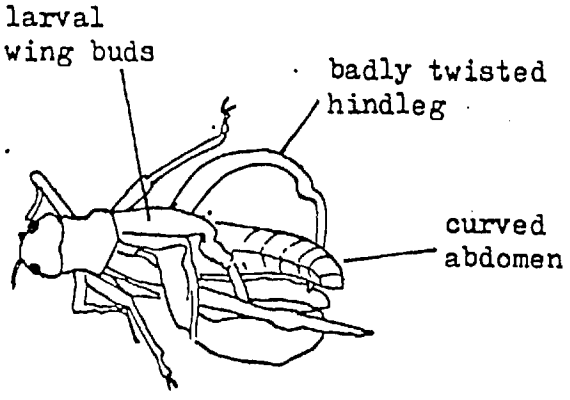
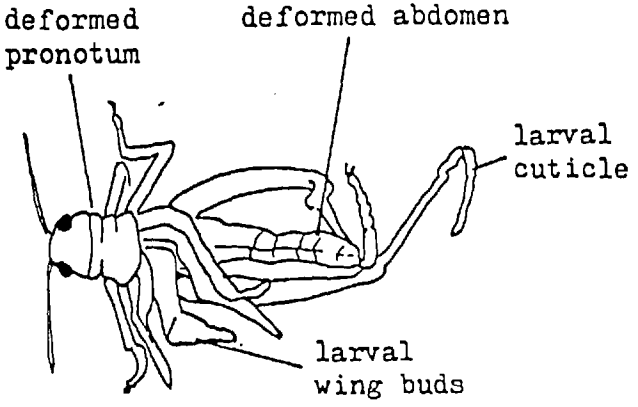


Fig. 21a-d.

(e)


larval
wing buds
badly twisted
hindleg
curved
abdomen

(g)


deformed
pronotum
deformed abdomen
larval
cuticle
larval
wing buds

(f)

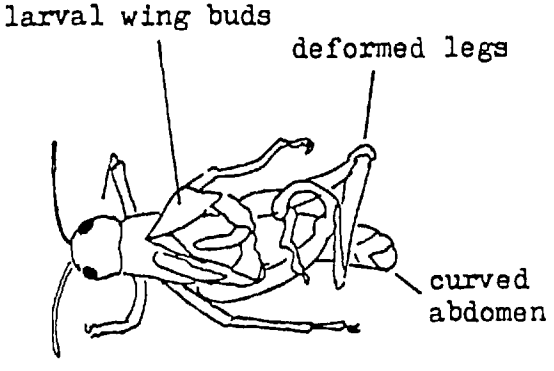

larval wing buds
deformed legs
curved
abdomen

Fig. 21e-f.

many cases were held in a vertical plane, as are the over-developed wing buds of precocious adultiforms. The pronotum was creased and often curved beneath the posterior edge; the pronotum cuticle was occasionally roughened, in one or two cases appearing almost granular. The head capsule was larger than that of a normal adult, but the body length was somewhat smaller. As a result the proportion of head to body appeared more typical of the larval form rather than of the adult. The colouration was typical of the larvae, with a combination of orange and black areas on the thorax and head. The melanization was occasionally strongly pronounced and the entire head and thorax heavily pigmented in some cases.

No morphometric measurements were made on these adultoids, but they appeared to be typical of adultoids and supernumerary larvae produced by Tang (1976) using Law's mixture, various Zoecon JH - analogues and also CA implantations. They were also similar to adultoids produced by ZR515, described in detail later.

iii) Instar duration. The duration of the 5th instar was reduced significantly following treatment with Law's mixture ($p < 0.001$, 1 - way analysis of variance) with a negative correlation between instar length and dose ($r = -0.246$, $p < 0.05$, $n = 135$). The lowest dose of 107µg resulted in little advance of the moult but higher doses, associated with almost exclusive production of supernumerary larvae, significantly advanced the moult (Table 9a). Within each dosage group the instar durations were correlated with the extent of juvenilization (Table 9b).

It appears that supernumerary larvae produced by low doses were associated with a delayed moult to the adultoid stage, whereas supernumerary larvae produced at high doses were associated with an advanced moult. The intermediate dosage group showed no significant correlation between instar duration and morphogenetic deformation.

ZR515

i) Dose - responses. Increasing dosages of the Zoecon Corp. JH - analogue ZR515 demonstrated a clear dose - response relationship in the induction of juvenile characters at the larval - adult moult (Table 10). A dose - response curve for topical applications was determined using the system described (p. 28), and is given in Fig. 22. Both the number of adultoids and the severity of their morphogenetic deformation increased with increasing doses, and topical applications of 425µg, applied 0 - 24h into the 5th instar produced almost exclusively supernumerary larvae, many of which attempted an additional moult

Table 9(a) The mode, timing and dose of Law's mixture applied in the 5th instar. The 5th instar duration and the degree of juvenilization (given as two morphological scores) are also given.

Mode of Application	Time of Application (hours, 5th)	Dose (µg)	Sample Size	Morphological Score ± 1 S.D. (Miall's system)	Morphological Score ± 1 S.D. (Tang's system)	5th instar duration (days ± 1 S.D.)
Topical	0-24h	107	41	0.378 ± 0.797	0.300 ± 0.516	10.658 ± 2.163
"	"	1069	43	1.977 ± 1.300	1.071 ± 0.640	9.452 ± 1.261
"	"	5345	5	5.000 ± 0.0	3.000 ± 0.0	
"	"	10690	39	4.910 ± 0.226	3.000 ± 0.226	9.436 ± 2.257
Injected(*)	"	1069	10	5.000 ± 0.0	3.000 ± 0.0	

* Injected compound carried in 5µl 1:10 v/v acetone:oil

Table 9(b) The correlation coefficients determined between the 5th instar duration and the morphological score of juvenilization after treatment in the early 5th instar with three doses of Law's mixture.

Dose (µg)	Sample Size	Correlation Coeff. (r)	Probability Associated (p)
107	29	+ 0.811	0.001
1069	31	- 0.164	0.05
10690	32	- 0.599	0.001

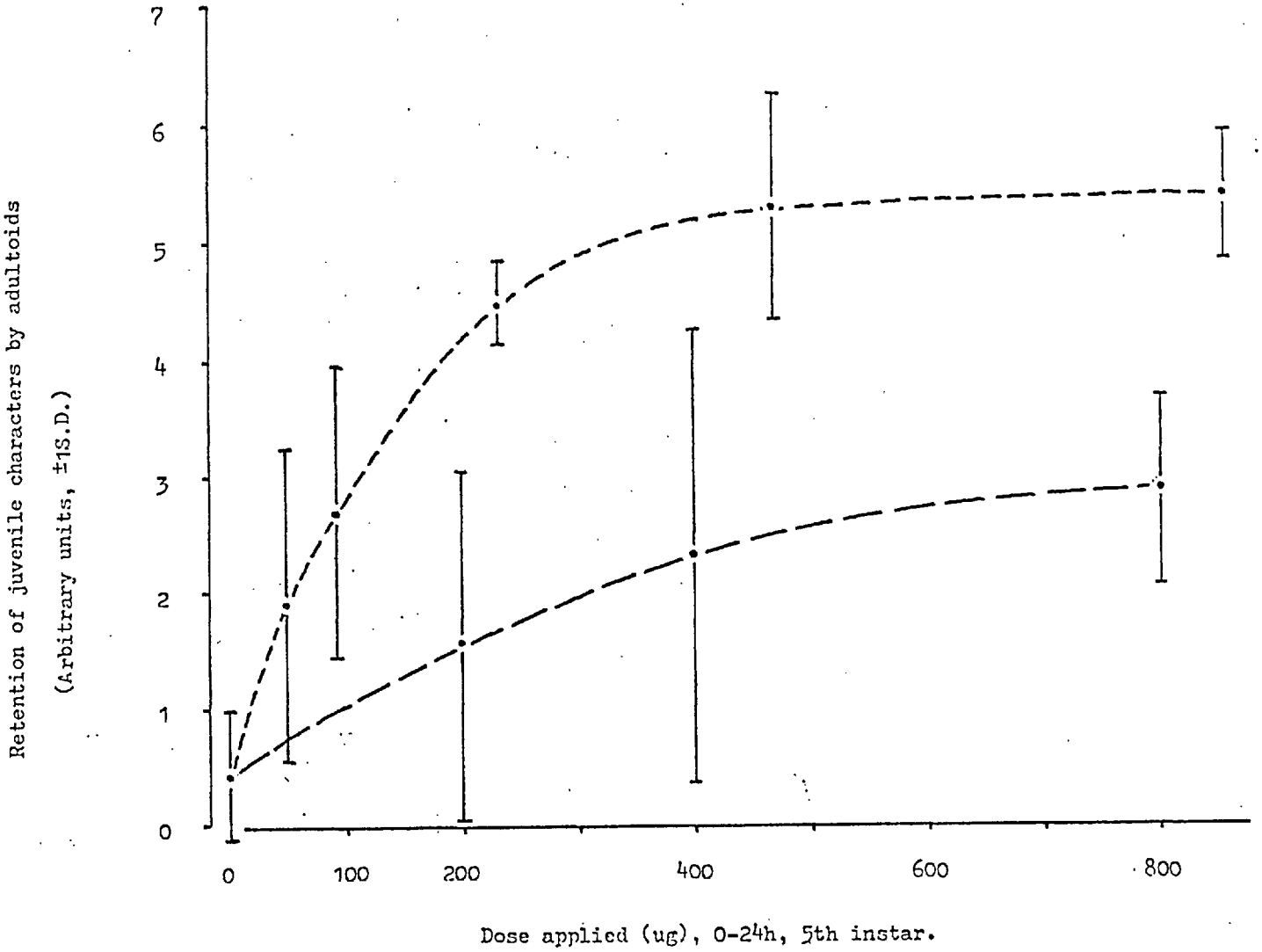
Table 10 The mode, timing and dose of the juvenile hormone ZR515 applied in the 5th instar. The 5th instar duration and the degree of juvenilization (morphological score) are also given.

Mode of Application	Time of Application (hours, 5th)	Dose (µg)	Sample Size	Morphological Score ± 1 S.D.	5th instar duration (days ± 1 S.D.)
Topical	0-24	43	30	2.483 ± 1.214	8.545 ± 0.617
"	"	85.5	18	2.667 ± 1.237	8.667 ± 1.085
"	"	213	5	4.500 ± 0.354	7.000 ± 0.707
"	"	427	25	5.130 ± 0.527	8.958 ± 1.805
"	"	855	5	5.400 ± 0.548	7.400 ± 1.140
"	0- 4	85.5	11	3.045 ± 1.274	7.273 ± 0.786
"	4- 8	"	11	2.454 ± 1.011	8.364 ± 0.504
"	8-12	"	11	2.864 ± 1.362	7.545 ± 0.522
"	12-16	"	5	2.800 ± 1.255	7.000 ± 0.0
"	16-20	"	5	2.900 ± 1.557	7.400 ± 0.548
"	20-24	"	15	4.100 ± 0.822	7.600 ± 0.548
"	24-28	"	5	4.300 ± 0.758	7.400 ± 0.548
"	20-24	43	15	2.200 ± 0.960	8.867 ± 0.640
"	48-72	"	16	3.000 ± 0.796	8.687 ± 0.602
"	72-96	427	30	See text	9.023 ± 0.706
Injected(*)	72-96	43	29	0.276 ± 0.528	
"	72-96	213	33	0.838 ± 1.211	8.391 ± 0.783

(*) Injected analogue carried in 5µl 1:9 v/v acetone:insect saline.

Figure 22

Dose-response curves determined after treatment 0-24h into the 5th instar with ZR515 (●- - - -●) and precocene II (●——●). Bars : ± 1 standard deviation of the mean.


Retention of juvenile characters by adultoids
(Arbitrary units, ±1S.D.)
0
1
2
3
4
5
6
7
0
100
200
400
600
800
Dose applied (ug), 0-24h, 5th instar.

some days after the moult to the supernumerary stage. A further 30 5th instar larvae were treated topically with 425µg, 72 - 96h after ecdysis, about 6 months after those experiments quoted here. Many of these animals had difficulty in emerging from the old cuticle at the moult following treatment (5th instar - adult); while some of these may be due to their treatment during behavioural tests, which included glueing a clip over the pronotum,eight animals were not used in behavioural experiments and 5 of these did not complete ecdysis.

A number of 5th instar larvae were treated 72 - 96h after ecdysis with either 43 or 213µg ZR515, injected in a 1 : 9 acetone : insect saline solution. Controls received the acetone:saline mixture alone. The control group showed no morphogenetic effects (score = 0.21875 $\pm$ 0.4908, n = 32); the 43µg group were similar to the control group (score = 0.2758 $\pm$ 0.5276, n = 29); and the high dosage group showed only slight morphogenetic deformation (score = 0.83824 $\pm$ 1.2106, n = 33).

ii) Time - responses. A number of 5th instar larvae were treated with a standard dose of 85.5µg ZR515, topically applied at known periods after emergence. The morphogenetic response was scored after the following moult, but no clear time - response relationship was evident after treatment over the first 28 hours of the instar (Fig. 23). Treatment with a standard dose of 43µg, applied 0 - 24h or 48 - 72h into the 5th instar also showed no decline in the activity of ZR515 over the first 3 days of the instar (scores : 2.483 $\pm$ 1.214 and 3.00 $\pm$ 0.796, n = 30 and 16 respectively).

iii) Morphometrics. The morphological effects of this JH - analogue were similar to those produced by Law's mixture treatments or by precocene II when applied to act as a JH - mimic. All the animals treated 0 - 24h into the 5th instar were killed and morphometrically measured after the larval-adult moult. This data was analysed for several morphologically defined groups, producing a description of the changes in each parameter over the scale 0 - 6 (Table 11), and confirmed the observations of the morphogenetic effects of Law's mixture on 5th instar locusts.

The body length tended to fall with increasing score, while the head capsule width and length increased. This was particularly evident in the females, and gave the adultoids noticably larval proportions. Elytron length fell with increasing score in both sexes, shortening by over 40% from the score 0 group to the score 6 group. Pronotum length tended to fall at intermediate scores, and was lowest at scores of 3.5 in both sexes. All groups with scores of 3.5 and above tended to

Figure 23

Time response histogram determined after timed applications of 83µg ZR515 applied within the first 28h of the 5th instar. Bars : 1 standard deviation of the mean.

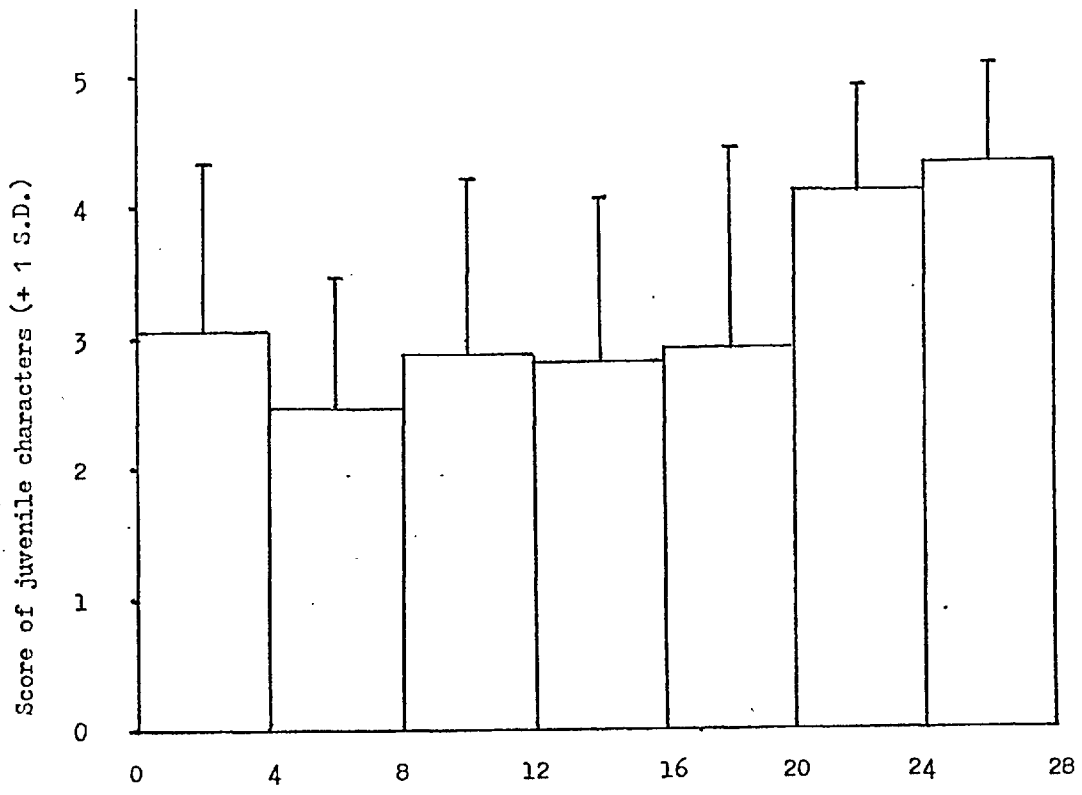

Score of juvenile characters (+ 1 S.D.)
0
1
2
3
4
5
0
4
8
12
16
20
24
28
Time of application of ZR515 in hours after the moult to the fifth instar.

Table 11

The morphological measures of body characters determined in groups of juvenile adultoids classified by their morphological scores (LEVEL = score x 10). The adultoids were induced by ZR515 treatments using doses of 43-855µg, applied within the first 96h of the 5th instar. Values for males and females are given separately; males on the left, females on the right. The sample sizes (N), means and standard deviations are given for each group. All measurements are in millimetres x 10 (i.e. 482.5 = 48.25 mm).

(a) Body length (mm x10)

LEVEL	N	MEAN	ST. DEV.	LEVEL	N	MEAN	ST. DEV.
0	2	482.5	10.6	10	6	529.2	24.0
10	6	466.7	18.6	15	2	530.0	7.1
15	1	465.0	0.0	20	4	497.5	38.4
20	5	465.0	7.9	25	15	520.3	29.5
25	13	482.7	25.8	30	6	500.8	28.4
30	7	451.4	12.1	35	2	455.0	99.0
35	3	438.3	10.4	40	2	535.0	7.1
40	6	453.3	20.4	45	5	511.0	26.8
45	7	432.1	25.8	50	9	497.8	37.8
50	15	450.3	19.5				
60	1	490.0	0.0				

(b) Pronotum-length (mm x10)

LEVEL	N	MEAN	ST. DEV.	LEVEL	N	MEAN	ST. DEV.
10	3	81.67	5.77	10	4	92.50	2.89
15	1	90.00	0.00	15	2	95.00	0.00
20	6	88.33	4.08	20	4	90.00	4.08
25	12	86.25	5.28	25	12	95.83	5.57
30	7	83.57	6.27	30	6	92.50	6.12
35	2	75.00	0.00	35	2	82.50	3.54
40	4	77.50	8.66	40	2	90.00	0.00
45	7	80.00	4.08	45	5	93.00	5.70
50	15	81.67	9.76	50	9	95.00	11.99

(c) Elytron length (mm x10)

LEVEL	N	MEAN	ST. DEV.	LEVEL	N	MEAN	ST. DEV.
0	2	520.0	49.5	10	6	535.8	11.6
10	6	497.5	19.7	15	2	465.0	63.6
15	1	395.0	0.0	20	4	481.3	46.1
20	6	441.7	29.9	25	15	460.3	42.6
25	13	425.8	31.9	30	6	455.0	24.1
30	7	419.3	25.1	35	2	380.0	28.3
35	3	380.0	18.0	40	2	352.5	38.9
40	6	355.8	48.3	45	5	405.0	18.4
45	7	359.3	36.6	50	9	363.3	20.0
50	13	341.5	35.3	60	1	380.0	0.0
60	2	330.0	42.4				

(d) Hind femur length (mm x10)

LEVEL	N	MEAN	ST. DEV.	LEVEL	N	MEAN	ST. DEV.
0	2	232.50	3.54	10	6	247.50	5.24
10	6	234.17	8.61	15	2	245.00	0.00
15	1	230.00	0.00	20	4	242.50	8.66
20	6	236.67	4.08	25	15	249.67	7.43
25	13	235.38	6.28	30	6	246.67	5.16
30	7	236.43	9.45	35	2	242.50	3.54
35	3	226.67	7.64	40	2	245.00	7.07
40	6	231.67	6.83	45	5	248.00	5.70
45	7	227.86	11.13	50	9	243.33	14.58
50	14	233.57	5.35	60	1	265.00	0.00
60	3	238.33	10.41				

(e) Head capsule width, C (mm x10)

LEVEL	N	MEAN	ST. DEV.	LEVEL	N	MEAN	ST. DEV.
0	2	85.00	7.07	10	6	90.83	2.04
10	6	83.33	2.58	15	2	90.00	0.00
15	1	80.00	0.00	20	4	88.75	4.79
20	6	82.50	2.74	25	15	90.33	4.42
25	13	83.46	3.76	30	6	92.50	2.74
30	7	84.29	3.45	35	2	90.00	0.00
35	3	80.00	0.00	40	2	90.00	0.00
40	6	83.33	2.58	45	5	95.00	3.54
45	7	82.86	2.67	50	9	93.89	4.86
50	15	83.67	3.52	60	1	105.00	0.00
60	3	91.67	2.89				

(f) Head capsule length (mm x10)

LEVEL	N	MEAN	ST. DEV.	LEVEL	N	MEAN	ST. DEV.
0	2	117.50	3.54	10	6	125.00	3.16
10	6	115.83	3.76	15	2	127.50	3.54
15	1	115.00	0.00	20	4	125.00	0.00
20	6	116.67	4.08	25	15	126.33	5.50
25	13	116.15	4.16	30	6	127.50	2.74
30	7	113.57	4.76	35	2	127.50	3.54
35	3	111.67	2.89	40	2	125.00	7.07
40	6	116.67	4.08	45	5	128.00	4.47
45	7	112.86	6.36	50	9	127.22	5.07
50	15	117.00	4.55	60	1	140.00	0.00
60	3	126.67	10.41				

(g) C/F Ratio

LEVEL	N	MEAN	ST. DEV.
0	2	.3654	.0249
10	6	.3561	.0112
15	1	.3478	0.0000
20	6	.3486	.0103
25	13	.3546	.0140
30	7	.3565	.0055
35	3	.3532	.0118
40	6	.3600	.0165
45	7	.3642	.0160
50	14	.3565	.0161
60	3	.3848	.0059

LEVEL	N	MEAN	ST. DEV.
10	6	.3671	.0098
15	2	.3673	0.0000
20	4	.3660	.0138
25	15	.3618	.0142
30	6	.3752	.0157
35	2	.3712	.0054
40	5	.3675	.0106
45	5	.3831	.0113
50	9	.3862	.0133
60	1	.3962	0.0000

(h) F/C Ratio

LEVEL	N	MEAN	ST. DEV.
0	2	2.743	.187
10	6	2.811	.085
15	1	2.875	0.000
20	6	2.871	.086
25	13	2.824	.115
30	7	2.805	.043
35	3	2.833	.095
40	6	2.782	.125
45	7	2.751	.119
50	14	2.811	.131
60	3	2.599	.039

LEVEL	N	MEAN	ST. DEV.
10	6	2.7256	.0730
15	2	2.7222	0.0000
20	4	2.7355	.1055
25	15	2.7679	.1109
30	6	2.6691	.1118
35	2	2.6944	.0393
40	5	2.7222	.0786
45	5	2.6123	.0776
50	9	2.5921	.0922
60	1	2.5238	0.0000

(i) E/F Ratio

LEVEL	N	MEAN	ST. DEV.	LEVEL	N	MEAN	ST. DEV.
0	2	0.235	.179	10	66	2.166	.070
10	66	2.126	.098	15	20	1.898	.260
15	1	1.717	0.000	20	4	1.984	.162
20	6	1.868	.144	25	15	1.846	.188
25	19	1.809	.126	30	66	1.845	.106
30	7	1.774	.093	35	20	1.566	.094
35	8	1.678	.104	40	20	1.437	.117
40	6	1.538	.215	45	5	1.633	.074
45	7	1.579	.165	50	9	1.496	.083
50	19	1.463	.145	60	1	1.434	0.000
60	2	1.383	.258				

have severely creased pronotal shields but the supernumerary larvae (scores 5.0 and 6.0) tended to display an enlarged pronotum and so raised the mean pronotum length parameter. Femur length did not change significantly or systematically with score; it is known to change with the degree of phase - shift in *Locusta* (Dirsh, 1953) and the E/F, C/F and F/C ratios were developed as indicators of phase-status. These ratios did change with the degree of juvenilization, and the E/F and F/C ratios fell significantly with increasing score. As expected, the C/F ratio increased with increasing score.

iv) Instar duration. There was no clear evidence of a delay or advance in the moult following treatment with ZR515; although two dosage groups showed an advance in the moult, the remaining groups showed little change (Table 10). There was a significant negative correlation between the 5th instar duration and the resultant morphological score, however, ($r = -0.254$, $p < 0.05$, $n = 95$), but this was not evident within each dosage group. There was no sex difference in the instar duration within any dosage group.

v) Supernumerary moult. Some supernumeraries attempted an additional moult after a number of days. The duration of this extra 6th instar was 13.11 days ($\pm$ 2.37 days, $n = 9$) and it is likely that many other supernumeraries would have also attempted a further moult, had they not been killed for morphometric studies before Day 13. The moult was most often evident as a shedding of the abdominal cuticle, revealing normal adult pigmentation beneath. Less frequently, the thoracic cuticle was shed, but the legs and wings were rarely completely freed from the old cuticle. The head capsule cuticle occasionally split dorsally as in a normal moult, but was rarely shed. All animals had apparently normal adult cuticle developing beneath the larval cuticle; there was no evidence that a further supernumerary instar would occur. Most animals were severely crippled by the attempted moult and were killed or died within 2 days; no data is therefore given about their life - span.

Precocene II

It became evident during this project that, in terms of the normal developmental sequence in *Locusta*, precocene can have two opposing effects : when applied in the early instars (1 - 4), or in the adult, precocene acts to destroy the CA and thus remove the source of endogenous JH (Bowers, 1976; Pener *et al* 1978). To this extent precocene is effectively an anti - JH, and will be considered as such in later sections (p. 39). However, when applied to the fifth (final) instar, precocene can attack the CA but simultaneously acts as a JH - analogue. For this reason the morphological

effects of precocene II, applied in the early 5th instar, are given within the general heading of 'morphological effects of JH - analogues.'

i) Dose - responses. The juvenilization caused by topical applications of precocene II, administered 0 - 24h into the 5th instar, was scored using the same system as was used for the JH - analogue ZR515 (p. 28). The effect was clearly dose - responsive (Fig. 22), but although high dosages were used, resulting in the majority of animals displaying marked juvenile characters, the maximum response was apparently not induced after even 800µg doses. Maximal responses are indicated as for ZR515 treatment by a plateau in the dose - response curve.

Both the number of adultoids produced and their gross morphological deformities appeared to be indistinguishable from those induced by ZR515 - treatment. However, because of the probable non - linearity of the scoring system, it is difficult to compare the effectiveness of each compound. At the highest doses used ZR515 produced exclusively supernumerary larvae, many of which would be expected to attempt an additional moult. At similarly high dosages, precocene induced less severe morphological changes, and only a minority of full supernumerary larvae. Precocene II acted as a more potent JH - analogue than all of the JH's (JH I, II and III) and was considerably more potent than the Law's mixture analogue.

However as in all treatments of JH's, JH - analogues and precocene, the morphogenetic effects are very variable in *Locusta*, and within any dosage group there was a spread of responses from animals showing no visible morphological deformities to the production of full supernumerary larvae. For this reason the majority of adultoids and supernumerary larvae studied morphometrically were produced at low doses, using particularly large treatment groups (Table 12). There was no evidence to suggest that the adultoids produced at different dosages differed in any respect. There was no evidence of any sex - difference in response to precocene.

ii) Time - responses The proportion of full supernumerary larvae produced at even high doses was small compared with that produced by ZR515, and remained at about the same level at all doses. Thus, it was felt that there may have been a critical, brief, window within the first 24 hours of the 5th instar when treatment would be particularly effective. A wide age - distribution was suspected within each treatment group selected 0 - 24h after ecdysis. It was likely that only a small number of larvae within each group were being treated at this

Table 12 The timing and doses of precocene II topically applied in the 5th instar. The 5th instar duration and the degree of juvenilization (morphological score) are also given.

Time of Application (hours, 5th)	Dose (µg)	Sample Size	Morphological Score ± 1 S.D.	5th instar duration (days ± 1 S.D.)
0-24	200	45	1.560 ± 1.520	8.682 ± 0.883
"	400	17	2.324 ± 1.940	11.588 ± 2.320
"	800	9	2.889 ± 0.821	8.111 ± 0.928
12-24	100	10	2.900 ± 2.010	8.300 ± 0.823
20-24	"	19	1.611 ± 1.750	8.555 ± 0.784
0- 2	200	10	0.100 ± 0.316	7.700 ± 0.675
2- 4	"	9	0.278 ± 0.833	6.778 ± 0.667
4- 6	"	10	0.200 ± 0.422	7.800 ± 0.789
6- 8	"	12	0.542 ± 0.782	7.833 ± 0.718
8-12	"	12	2.583 ± 1.960	8.833 ± 2.082
14-16	"	14	3.214 ± 1.780	12.071 ± 1.072
18-22	"	13	4.000 ± 2.260	10.600 ± 1.183
20-24	"	18	1.824 ± 1.499	8.824 ± 0.113
24-28	"	10	1.600 ± 1.630	9.000 ± 2.211
28-32	"	11	1.182 ± 1.330	8.977 ± 0.459
32-36	"	9	0.556 ± 0.527	7.667 ± 0.500
36-40	"	12	2.000 ± 2.090	8.583 ± 0.669
40-44	"	10	1.000 ± 1.250	8.500 ± 1.269
24-48	"	13	1.231 ± 1.510	8.000 ± 0.555
48-72	"	13	0.846 ± 0.689	7.846 ± 0.375
72-96	"	21	0.548 ± 0.893	9.333 ± 0.483
0- 4	400	11	0.182 ± 0.405	9.909 ± 0.944
4- 8	"	19	0.132 ± 1.025	8.632 ± 0.684
14-18	"	10	2.750 ± 1.532	7.800 ± 1.033
22-26	"	4	1.250 ± 0.957	10.000 ± 0.816

critical time, and that the age - distribution might have been directly related to the wide distribution of morphological types produced.

A series of animals was therefore selected within 2 or 4 hours of the moult to the 5th instar and 12 groups were treated with a standard 200µg dose of precocene topically applied at known times after selection (Table 12). The resultant juvenilization was scored after the larval - adult moult and the results are given in Fig. 24. It can be seen that there was a period 8 - 24h after the moult when topical treatments were most effective; within the most severely affected group, treated at 18 - 22h, nearly half the animals (6 out of 15) attempted an additional moult some 10 - 15 days after the moult to the supernumerary form. After the first 24h of the 5th instar the morphogenetic responsiveness to precocene declined and treatment 72 - 96h into the instar was scarcely effective. It should be noted that even within the most closely age - selected groups collected within 2h of the moult there was still a considerable spread of morphological types produced. Thus the age of the locusts at the time of treatment was not the sole cause of the variation in the extent of juvenilization.

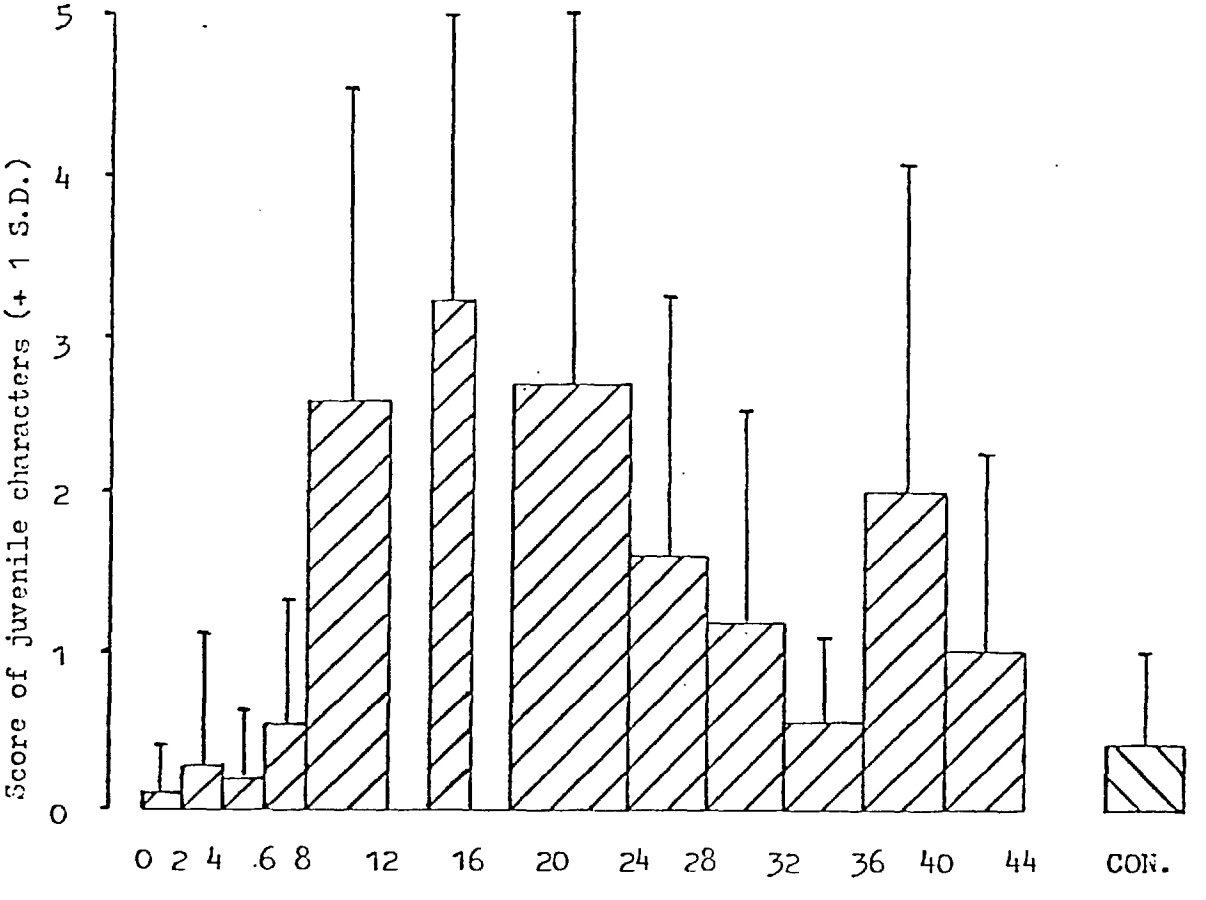
A number of larvae were treated at timed intervals with 400µg precocene and although this series was not complete (Table 12), the maximum score of juvenile characters occurred in a group treated 14 - 18h after the moult. This agrees with the peak observed after 200µg treatments, and also indicates that even 400µg cannot swamp the system to the extent where a sensitive period could not be detected.

iii) Morphometrics. As mentioned previously, the proportion of animals showing severe juvenilization was low at all doses and certain morphological types or scores were under - represented. While all animals treated with precocene in the early 5th instar (n >350) were morphometrically examined, there were no indications of any differences in the various morphological types produced that were related to the timing or the dosage used. The 200µg group (n = 247) was considered to include all the typical morphological responses observed, and was analysed in more detail.

Firstly the data was analysed by the time of application over the first 44h of the instar, and the C/F ratios determined for each sex are given in Fig. 25. This data shows the close relationship between scores and C/F ratios (compare Figs. 24 and 25), particularly for the females. However, the changes observed over the 44h period (Fig. 25) were indicative only of the changing proportion of supernumeraries within each group;

Figure 24

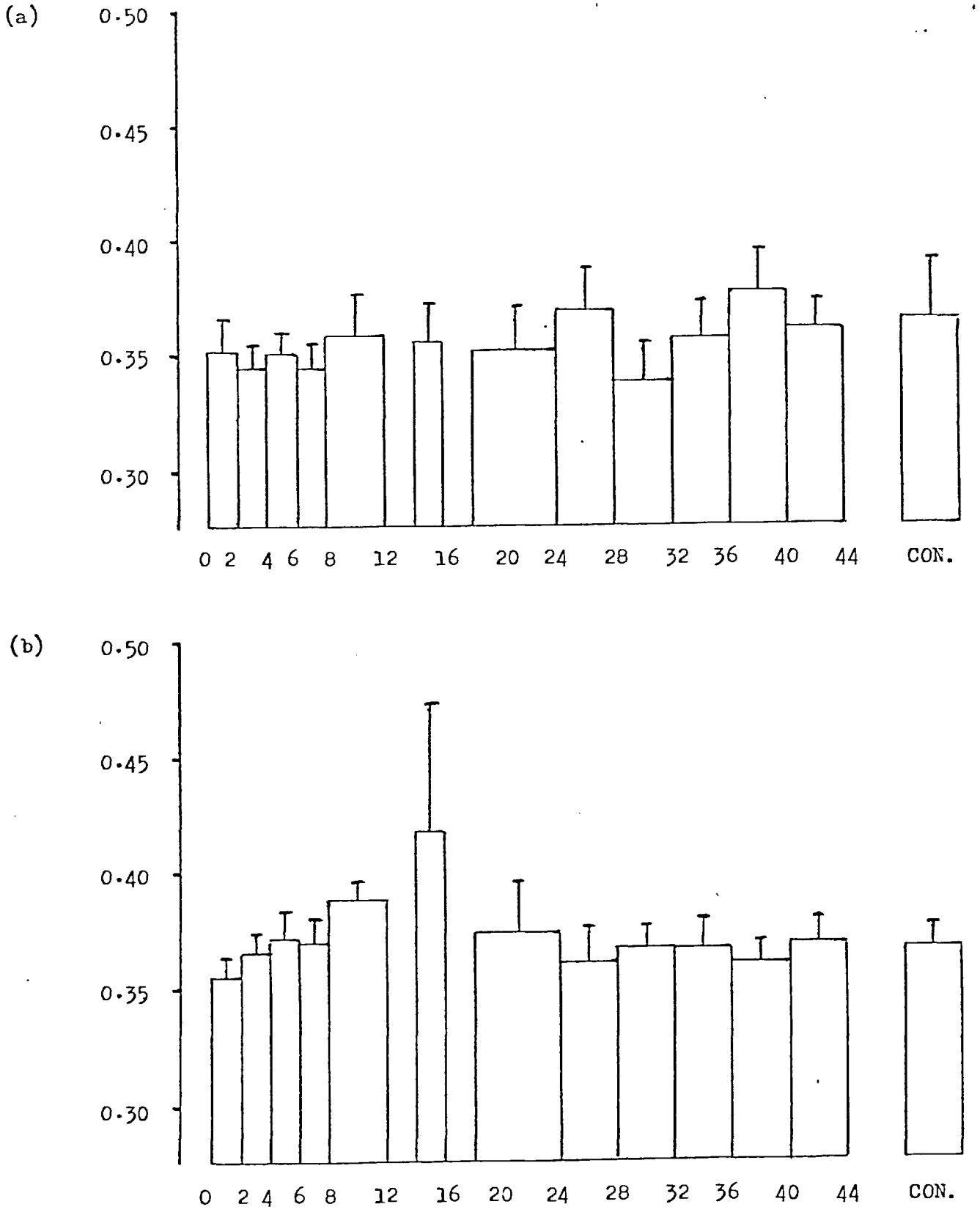
Time-response histogram determined after timed applications of 200µg precocene II applied within the first 44h of the 5th instar. Bars : 1 standard deviation of the mean.



Time of precocene treatment, 0-44h, 5th instar.

Figure 25

Ratios of head capsule width (C) to hind femur length (F) determined in (a) male and (b) female adultoids induced after timed applications of 200µg precocene II applied within the first 44h of the 5th instar. Bars : 1 standard deviation of the mean.



Time of precocene treatment, 0-44h, 5th instar.

when each morphological type (or score) was examined alone there were no significant changes in the C/F ratio over the 12 groups treated 0 - 44h after emergence (Table 13). It was concluded that the combination of these 12 groups did not bias the sample in any way, and the following section includes data from all 247 animals treated with 200µg precocene, 0 - 96h into the 5th instar (Table 14).

As described for the adultoids induced by ZR515 or Law's mixture, the increasing juvenilization of the adultoids was evident first as a deformation of the wing tips and pronotum and later as an increase in head capsule size and retention of larval colouration. The males showed a slight fall in body length with increasing score, but this was not evident in the females. Wing lengths dropped significantly in both sexes, but hind femur length showed little systematic change with increasing scores. Pronotum length was at a minimum in score groups of 3.5 - 4.5 and increased in the supernumeraries, although the pronotum was still severely creased in these high score groups. Head length showed slight increases in both sexes and head width increased with increasing score. The F/C and E/F ratios fell significantly with increasing score while the C/F ratio increased. The correlation coefficients calculated between the scores and the C/F ratios for both sexes were statistically significant (males: $r = 0.470$, $p<0.01$, $n = 124$; females: $r = 0.256$, $p <0.05$, $n = 123$).

iv) Instar duration. There was a significant change in the 5th instar duration after treatment with precocene in the early instar (Table 12). The situation was confused, however, since nearly half the treatment groups showed a significant delay correlated with increasing morphogenetic score, while the remainder showed a significant advance. Within those groups treated 0 - 24h into the instar and used to determine a dose - response curve, each dosage group and also the combined data from all doses showed a significant negative correlation between score and instar duration ($p \leq 0.05$ in all cases). In the 12 groups used for determination of the time response curve (receiving 200µg precocene, 0 - 44h into the instar) there was a significantly positive correlation coefficient between the mean scores and mean instar durations for each group ($r = 0.864$, $p <0.001$, $n = 12$), as indicated in Table 12. However, those groups treated at the critical period, 8 - 20h into the instar, showed particularly delayed moults, but within each group the highly affected animals almost invariably emerged from the 5th instar first. In other words, although the mean instar duration for the groups was

Table 13 An analysis of variance of the C/F ratios determined within each of the morphological score groups produced following precocene II treatment in relation to the time of treatment in the early 5th instar.

Males

Morphological Score group	Sample Size	F-ratio	Degrees of Freedom	Associated probability (p)
0	46	1.38	15 : 30	0.05
1	51	2.14	13 : 37	0.05 *
2	7	1.70	4 : 2	0.05
5	7	26.97	3 : 3	0.05 *

Females

0	42	1.25	11 : 30	0.05
1	37	1.21	12 : 24	0.05
2	12	0.88	3 : 8	0.05
3	3	624.60	1 : 1	0.05 *
4.5	8	16.13	3 : 4	0.01 **
5	7	9.28	4 : 2	0.05
6	6	0.34	2 : 3	0.05

Table 14

The morphological measures of body characters determined in groups of juvenile adultoids classified by their morphological scores (LEVEL = score x 10). The adultoids were induced by precocene II treatments using doses of 200µg, applied within the first 72h of the 5th instar. Values for males and females are given separately; males on the left, females on the right. The sample sizes (N), means and standard deviations are given for each group. All measurements are in millimetres x 10 (i.e. 483.6 = 48.36 mm).

(a) Body length (mm x10)

LEVEL	N	MEAN	ST. DEV.	LEVEL	N	MEAN	ST. DEV.
0	46	483.6	13.3	0	42	520.0	20.3
10	51	480.0	13.9	10	37	526.1	17.9
20	7	499.3	35.3	20	12	510.8	18.7
25	4	476.3	16.5	25	7	514.3	15.9
30	2	492.5	31.8	30	3	535.0	25.0
40	2	470.0	7.1	35	1	530.0	0.0
45	2	460.0	7.1	45	9	506.3	30.9
50	7	472.1	20.8	50	7	512.9	22.1
60	2	467.5	31.8	60	5	516.0	20.7

(b) Pronotum length (mm x10)

LEVEL	N	MEAN	ST. DEV.	LEVEL	N	MEAN	ST. DEV.
0	36	94.86	4.39	0	30	99.50	4.61
10	29	93.75	4.00	10	24	99.79	4.03
20	6	95.83	8.61	20	1	95.00	0.00
25	3	90.00	8.66	25	4	98.75	11.09
30	2	77.50	3.54	30	1	100.00	0.00
40	3	76.67	18.93	35	1	85.00	0.00
45	2	75.00	0.00	45	9	96.67	7.64
50	7	90.71	12.05	50	4	100.00	13.54
60	2	92.50	3.54	60	5	97.00	13.96

(c) Elytron length (mm x10)

LEVEL	N	MEAN	ST. DEV.	LEVEL	N	MEAN	ST. DEV.
0	46	504.6	16.4	0	42	543.3	17.1
10	51	496.6	19.1	10	37	538.2	15.6
20	7	462.9	37.1	20	12	504.2	58.3
25	4	435.0	23.1	25	7	510.7	28.5
30	2	487.5	24.7	30	2	457.5	10.6
40	2	442.5	60.1	35	1	340.0	0.0
45	2	367.5	24.7	45	8	418.8	62.7
50	7	373.6	44.1	50	7	385.0	51.4
60	2	347.5	38.9	60	6	350.0	60.9

(d) Hind femur length (mm x10)

LEVEL	N	MEAN	ST. DEV.	LEVEL	N	MEAN	ST. DEV.
0	46	235.11	7.11	0	42	244.3	9.2
10	51	232.65	8.80	10	37	243.5	8.3
20	7	229.29	14.56	20	12	247.5	17.1
25	4	233.75	10.31	25	7	247.1	9.9
30	2	242.50	3.54	30	2	237.5	10.6
40	2	235.00	0.00	35	1	260.0	0.0
45	2	232.50	3.54	45	8	239.8	5.4
50	7	233.57	8.52	50	7	240.7	8.4
60	2	235.00	0.00	60	6	253.3	24.6

(e) Head capsule width, C (mm x10)

LEVEL	N	MEAN	ST. DEV.	LEVEL	N	MEAN	ST. DEV.
0	46	88.83	3.27	0	42	89.05	2.97
10	51	88.06	3.49	10	37	88.92	3.15
20	7	88.86	5.67	20	12	91.25	5.28
25	4	86.25	4.79	25	7	96.43	11.44
30	2	87.50	3.54	30	3	91.67	5.77
40	3	81.67	14.43	35	1	95.00	0.00
45	2	85.00	0.00	45	8	91.25	2.31
50	7	89.29	3.45	50	7	93.57	2.44
60	2	90.00	0.00	60	6	97.50	2.74

(f) Head capsule length (mm x10)

LEVEL	N	MEAN	ST. DEV.	LEVEL	N	MEAN	ST. DEV.
0	29	117.41	3.92	0	26	126.54	6.45
10	45	117.11	5.17	10	34	125.44	4.83
20	7	116.43	4.76	20	12	131.67	7.18
25	4	117.50	2.89	25	5	130.00	9.35
30	2	117.50	3.54	30	3	133.33	2.89
40	3	111.67	18.93	35	1	125.00	0.00
45	2	115.00	0.00	45	8	131.25	5.82
50	6	121.67	4.08	50	6	133.33	4.08
60	2	122.50	3.54	60	6	129.17	3.76

(g) C/F Ratio

LEVEL	N	MEAN	ST. DEV.	LEVEL	N	MEAN	ST. DEV.
0	46	.3524	.0132	0	42	.3549	.0144
10	51	.3530	.0167	10	37	.3653	.0115
20	7	.3614	.0121	20	12	.3694	.0199
25	4	.3693	.0209	25	7	.3905	.0494
30	2	.3610	.0198	30	2	.3787	.0129
40	2	.3830	0.0000	35	1	.3654	0.0000
45	2	.3656	.0056	45	3	.3822	.0057
50	7	.3925	.0175	50	7	.3839	.0067
60	2	.3830	0.0000	60	5	.3877	.0355

(h) F/C Ratio

LEVEL	N	MEAN	ST. DEV.	LEVEL	N	MEAN	ST. DEV.
0	46	2.841	.105	0	42	2.745	.105
10	51	2.839	.134	10	37	2.740	.089
20	7	2.769	.093	20	12	2.714	.141
25	4	2.715	.160	25	7	2.589	.271
30	2	2.775	.153	30	2	2.642	.090
40	2	2.611	0.000	35	1	2.737	0.000
45	2	2.735	.042	45	3	2.617	.039
50	7	2.619	.123	50	7	2.572	.044
60	2	2.611	0.000	60	5	2.600	.276

(i) E/F Ratio

LEVEL	N	MEAN	ST. DEV.	LEVEL	N	MEAN	ST. DEV.
0	46	2.147	.077	0	42	2.236	.078
10	51	2.136	.089	10	37	2.192	.092
20	7	2.027	.211	20	12	2.035	.170
25	4	1.861	.072	25	7	2.071	.164
30	2	2.010	.073	30	2	1.929	.131
40	2	1.883	.256	35	1	1.309	0.000
45	2	1.582	.130	45	3	1.754	.259
50	7	1.602	.208	50	7	1.602	.227
60	2	1.479	.155	60	6	1.406	.354

positively correlated with score, within each group the relationship was reversed and agreed with the negative relationship observed at other doses (see above).

v) Supernumerary moult. Some of the locusts treated with precocene in the early 5th instar were sufficiently juvenilized to display full supernumerary characteristics and to attempt a further moult a number of days after the larval: adultoid moult. As was seen in ZR515 - induced supernumeraries, few were successful, and the moult was usually evident as a shedding of abdominal and occasionally thoracic cuticle. The head was rarely disturbed, and both the legs and wings were usually caught in the 'larval' cuticle ; the animals were severely crippled and were either killed or died within a few days of the moult. As in the ZR515 - treatments, all the supernumeraries that attempted an additional moult showed normal adult colouration beneath the supernumerary cuticle, and would not be expected to attempt any further moults. While many of the groups containing supernumerary larvae were not maintained long after the larval : adultoid moult, all those locusts used in the determination of the time - response curve to 200µg precocene were kept until any signs of further moults were evident.

Of the 21 full supernumerary larvae produced within this series (n = 247), 8 displayed attempted moults after a 6th instar of 12.375 days (± 1.8468). One of the eight was in the group treated 14 - 16h in the instar, and 6 of the remainder were produced after treatment at 18 - 22h. It is probable that some of the other supernumeraries may also have attempted further moults, had they not been killed for morphometric study.

B. Morphogenetic effects of anti - JH treatment

Both topical and internal treatments of precocene II were effective in acting morphogenetically as anti - juvenile hormones when applied in the early 4th instar. Cages of approximately 25 4th instar larvae were treated within the first 24h of the instar and were examined daily for the following 25 - 30 days (Table 15). Larvae moulting to the next instar were marked with a series of paint - spots, and were scored for the extent of the precocious development (see Methods, pp. 28). The life tables constructed for each dosage group are given in Fig. 26a and 26b.

Internal applications.

Twenty five larvae were injected with 100µg precocene II, carried in 5µg of a 1 : 10 acetone : oil mix. By Day 24, 31.6% of the group had died, with

Table 15 The mode, timing and dose of precocene II applied in the 4th instar. The median values of the 4th and 5th instars, where these were observed, are given.

Mode of Application	Time of Application (hours,4th)	Dose (µg)	Sample Size	4th instar duration (median,days)	5th instar duration (median,days)
Topical	0-24	50	25	6	10
"	"	100	53	7	10
"	"	200	122	7	10
"	"	400	51	8	11
Injected(*)	"	100	25	8	10

(*) Injected precocene carried in 5µl 1:10 v/v acetone:oil

Figure 26

Life tables indicating the moulting, morphological type and mortalities observed in locusts treated (a) topically and (b) internally with precocene II, applied at various doses, 0-24h into the 4th instar. Ordinates : the percentage incidence of each type observed each day after treatment; abscissae : time in days after treatment. IV, V and Ad. : areas representing normal 4th, 5th and adult stages; cross-hatched areas representing precocious adultiform larvae; black areas representing mortalities.

Fig.26a.

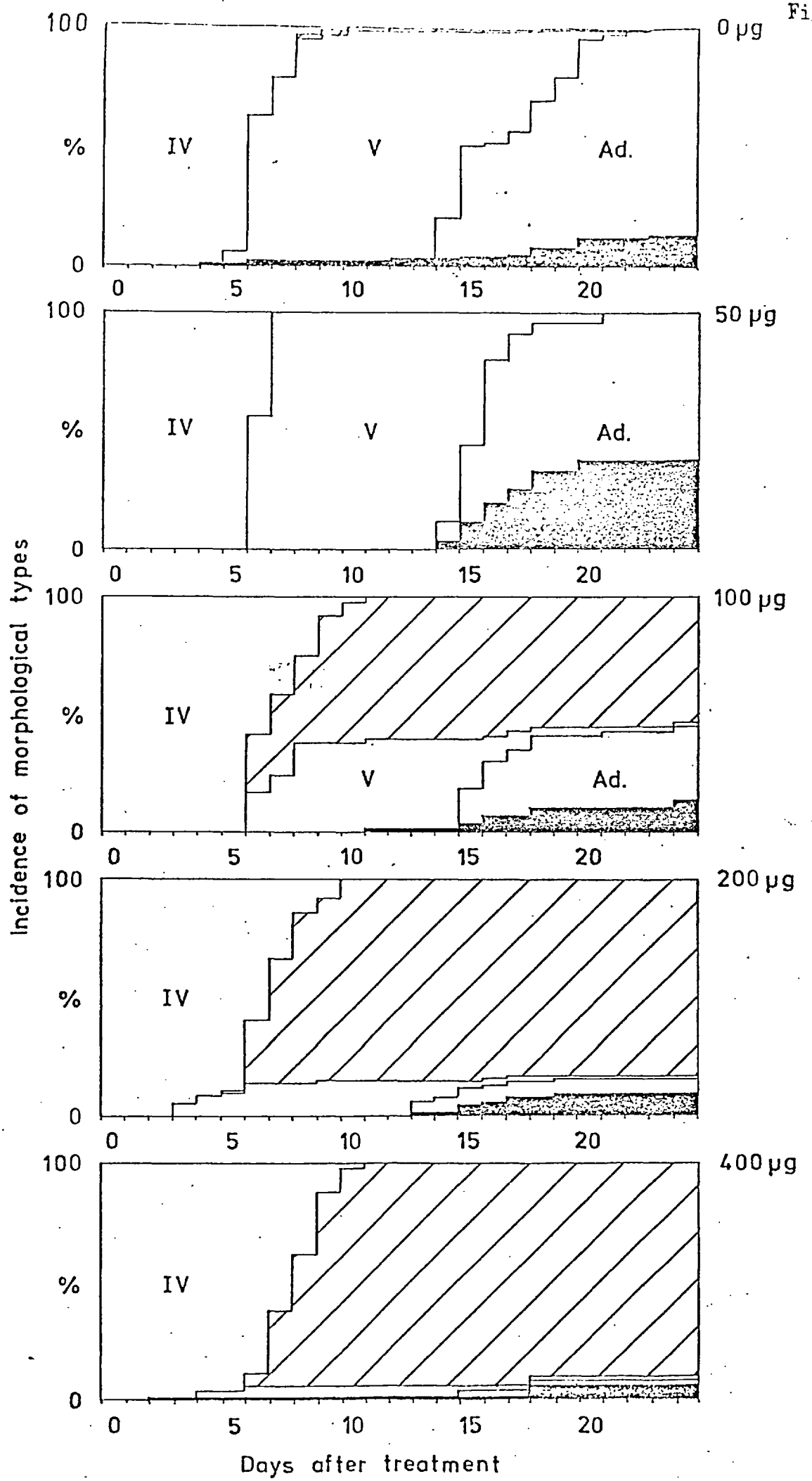
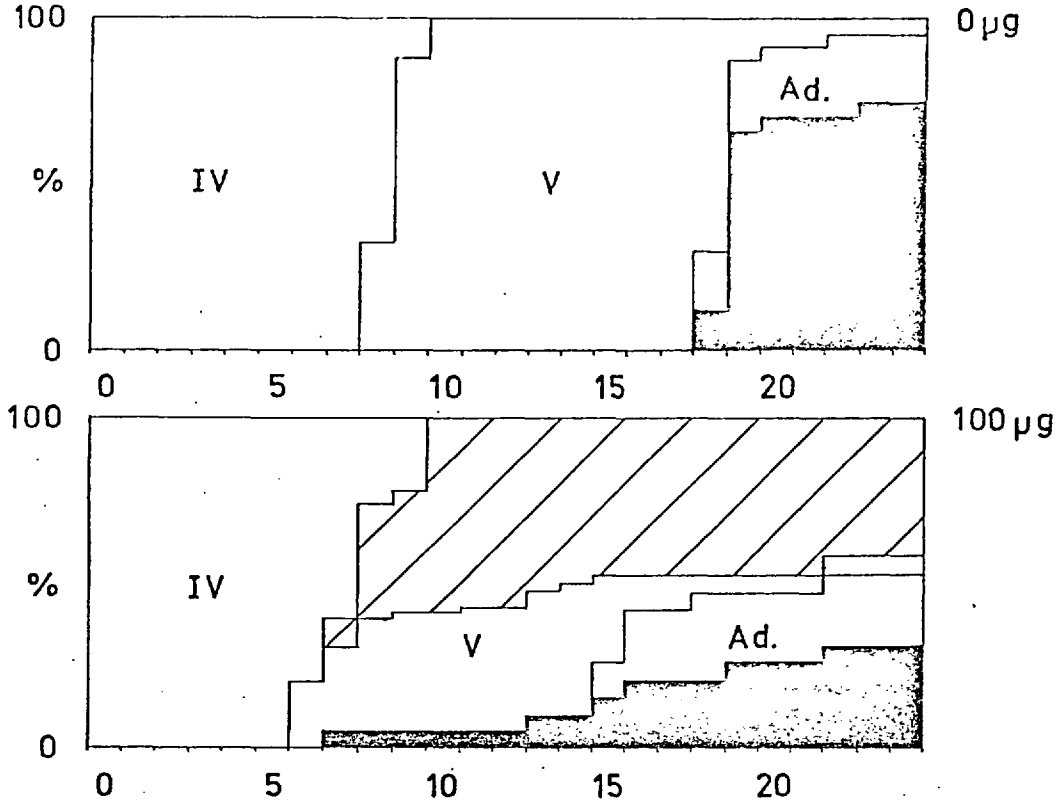

0 µg
50 µg
100 µg
200 µg
400 µg
100
%
0
IV
V
Ad.
0
5
10
15
20
Incidence of morphological types
Days after treatment

Fig. 26b.



no visible signs of disease. Although no evidence of an attempted ecdysis could be found, there was a slight increase in mortalities at the time of the expected moult (Fig. 26b). From the 8 apparently normal 5th instar larvae produced, 4 moulted to apparently normal adults, 3 died and one remained as a permanent 5th instar until Day 25, when the group was killed. 42% of the surviving locusts were permanent, precocious 5th instar adultiforms, displaying a range of morphological characteristics.

No attempt was made to determine a dose - response curve for this form of treatment, and no behavioural trials were undertaken, since a control group of 25 4th instar larvae showed high mortalities at the final moult, after an injection of 5µℓ of a 1 : 10 acetone : oil mix (Fig. 26b).

Topical applications

Topical applications of precocene II produced a dose - dependent proportion of precocious, 5th instar adultiforms. The resulting animals were of three major types : apparently normal 5th instar larvae (type 0); larvae with very few signs of precocious development (type 1); and adultiforms showing medium to severe precocious development (types 2 and 3). The majority of the type 0 animals continued the normal developmental sequence, moulting after a slight delay to apparently normal adults. The other groups (1 - 3) showed no further development : they were permanent 5th instar adultiforms.

i) Dose - responses Fig. 26a indicates that with increasing doses of precocene II, above 50 µg, an increasing proportion of 4th instar larvae moult to permanent, precocious adultiforms. Both 200µg and 400µg doses produced almost exclusively adultiform larvae; within each effective dosage group (100µg - 400µg) at least one type 0 larva did not display the expected moult to the adult stage. In the 200µg group, this represented 1 of 7 apparently normal larvae, in the 400µg, 1 of 2.

Type 1 adultiforms displayed enlarged wing - buds, and partial or complete adult colouration; this group corresponds to the types 1 and 2 reported by Pener *et al* (1978). The types 2 and 3 adultiforms had more fully developed wings that displayed veination and folding. The wings were manually mobile, but were only seen to be spontaneously moved by the type 3 adultiforms. Both groups had full adult colouration and correspond to types 3 and 4 of Pener. The relative abundances of each type produced are indicated in Table 16.

ii) Time - responses. All treatments of precocene were applied 0 - 24h into the 4th instar. Although selective timing of the treatment to within a few hours might reveal a maximally sensitive period, the very

Table 16 Percentage frequencies of the occurrence of morphological types of precocious adultiform larvae following treatment in the 4th instar with precocene II

Dose (µg)	Sample Size	% Occurrence of types : 0	1	2/3
0	41	100	0	0
50	12	100	0	0
100	22	31.82	13.64	54.54
200	69	10.15	13.04	69.56
400	42	2.38	4.79	92.86

high proportions of severely affected adultiforms produced by 200µg and 400µg doses argue against this. No attempt was therefore made to determine the time - dependent responses of 4th instar larvae to precocene treatment.

iii) Morphometrics. The morphogenetic effects of precocene II, applied in the 4th instar as an anti - JH are qualitatively similar to those reported by Pener *et al* (1978). A series of morphological measurements were taken of the adultiforms, after they were killed some 25 - 30 days after treatment (Table 17).

The adultiforms had a body length slightly greater than that of the normal 5th instar larvae, but considerably less than that of the normal adults. This measurement was particularly variable, however, as it was influenced by both the gut contents and the posture adopted during death. Elytron lengths were considerably longer in the adultiforms and the most severely affected adultiforms produced (types 2 and 3) frequently had tattered wing - tips such that the true length of the wings was greater than that measured. The hind leg dimensions did not change significantly compared with normal 5th instar dimensions. The head capsule tended to be smaller than normal in the adultiforms and as body length was slightly increased, the adultiforms tended to have adult, rather than larval proportions. Finally two ratios of measurements were calculated and showed a clear shift from normal larval values towards normal adult values.

iv) Instar durations. Precocene treatment at the start of the 4th instar caused a slight increase in the duration of the 4th instar, before the moult to either apparently normal 5th instar larvae (type 0) or to precocious adultiforms (types 1 - 3). The type 0 larvae displayed a slightly delayed moult to the adult stage but a small proportion (1 - 2%) showed no further moult, and remained as healthy, permanent larvae until they were killed 17 - 23 days after the 4th - 5th instar moult. The delay in both the 4th instar, and in the 5th instar of those type 0 larvae that moulted to the adult, was dose - dependant (Table 15, Fig. 26a and b). All adultiforms (types 1 - 3) were permanent, and showed no visible signs of further moults. There was a slight increase in mortalities amongst these adultiforms at the time of the expected 5th : adult moult, however, that may indicate the unsuccessful initiation of ecdysis.

Finally, the 4th instar duration was positively correlated with the degree of precocious development displayed in the 5th instar : the type

Table 17 The morphological measures of body characters determined in normal 5th instar larvae, precocious adultiform larvae and normal adults. The precocious, 5th instar adultiforms were induced by treatments of 50-400µg precocene II, topically applied within the first 24h of the 4th instar; they are grouped into Types 1 and 2/3 according to their wing development and cuticular pigmentation (see Text). All measurements are in millimetres $\pm$ 1 standard deviation.

Dimension	Sex	Normal fifth instar larvae ♂ n = 15 ♀ n = 11	Type 1 adultiforms ♀ n = 11	Type 2/3 adultiforms ♂ n = 40 ♀ n = 30	Normal adults ♂ n = 16 ♀ n = 10
Body length	♂	34.17 ± 1.98	38.29 ± 1.87	36.13 ± 1.16	45.66 ± 2.14
	♀	34.86 ± 2.20		38.08 ± 1.01	50.25 ± 1.14
Elytron (E)	♂	11.13 ± 0.55	13.21 ± 0.91	16.85 ± 2.17	48.47 ± 1.85
	♀	11.73 ± 0.68		16.72 ± 2.66	53.45 ± 0.96
Hind Femur (F)	♂	17.00 ± 0.46	17.43 ± 0.67	17.00 ± 0.47	22.72 ± 0.73
	♀	17.82 ± 0.46		17.55 ± 0.46	23.90 ± 0.57
Hind Tibia	♂	16.23 ± 0.59	16.29 ± 0.86	15.91 ± 0.48	21.12 ± 0.72
	♀	16.73 ± 0.34		16.43 ± 0.52	22.00 ± 0.62
Head length	♂	10.20 ± 0.32	9.79 ± 0.27	9.40 ± 0.34	11.63 ± 0.29
	♀	10.77 ± 0.26		9.93 ± 0.34	12.30 ± 0.26
Head width (C)	♂	7.07 ± 0.26	7.00 ± 0.29	6.50 ± 0.30	7.84 ± 0.30
	♀	7.41 ± 0.20		6.95 ± 0.27	8.50 ± 0.00
E/F Ratio	♂	0.655 ± 0.025	0.756 ± 0.058	0.991 ± 0.127	2.135 ± 0.088
	♀	0.658 ± 0.029		0.952 ± 0.151	2.238 ± 0.067
C/F Ratio	♂	0.416 ± 0.016	0.397 ± 0.015	0.383 ± 0.019	0.345 ± 0.011
	♀	0.416 ± 0.013		0.397 ± 0.013	0.356 ± 0.009

0 larvae emerged before the types 1 - 3 adultiforms in all treatment groups.

v) Adultiform flight - muscle anatomy. A limited number of normal 5th instar larvae, 5th instar adultiform larvae and normal adults were dissected to expose the thoracic musculature. All dissections were made on animals 2 - 3 days after the previous moult.

In all respects the muscles appeared to be consistent in their development with the state of precociousness indicated externally. Typical muscle development of the three types seen are given in Fig. 27, where the muscles are labelled according to the system used by Albrecht (1953). All 5th instar larvae and all type 0 and 1 adultiforms had thin transparent sheets of proto-muscle, that could not easily be distinguished into separate muscle blocks. All type 2 and 3 adultiforms showed apparently well developed flight muscles that were opaque and easily separated into muscle blocks. Although the thorax and muscles were smaller than in normal adults, the muscles were complete in number and position and type 3 animals had been seen to display vigorous wing flapping (p. 40).

C. Corpora allata degeneration following precocene treatment.

Although precocene II was morphologically active as an anti - JH during the 4th instar, and as a JH - analogue during the 5th instar, it was active in causing the degeneration of the corpora allata in both instances.

Treatment in the early 4th instar.

Topical applications of more than 50µg of precocene were effective in causing acute degeneration of the CA. Doses of 50µg were ineffective, and the CA volumes were not significantly different from those of the control groups. ($p = 0.4217$, $n = 19:15$, 2-sample t-test). The CA degeneration did not appear to be dose - dependent above the 50µg level, however, and there were no significant differences between the CA volumes of the 200µg and 400µg groups ($p = 0.9668$, $n = 10:7$, 2-sample t-test). Of 22 animals examined after treatment with 100µg the CA could only be seen in 7 specimens, all of type 0 (i.e. animals that had moulted to a normal 5th instar larva, and then to a normal adult). The CA were not visible in any of the 10 type 1 - 3 adultiforms examined (Table 18). Of 25 animals examined after treatment with 200µg, the CA were seen in only 3 type 0 adults and in one type 1 adultiform. Only one permanent 5th instar larva (p. 40) was available for dissection : the CA could not be found.

The group means for the CA volumes given in Table 18 show a sharp drop

Figure 27

Diagrams of (a) adult, (b) adultiform and (c) larval thoracic musculature; each diagram is a lateral view of the vertically bissected thorax, orientated with the anterior end to the right. Muscle blocks are numbered as in Albrecht (1953).

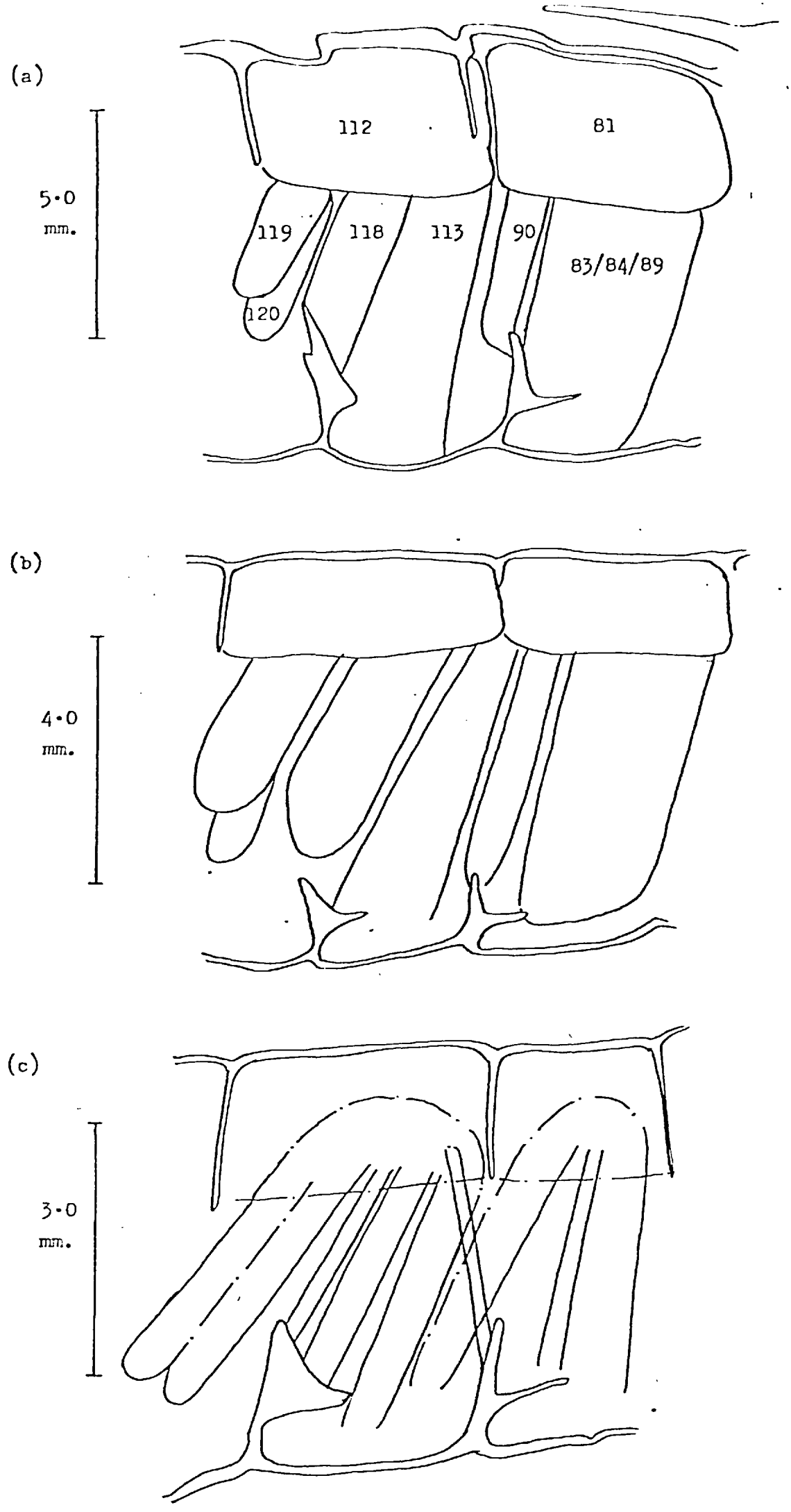

(a)
5·0
mm.
112
81
119
118
113
90
83/84/89
120
(b)
4·0
mm.
(c)
3·0
mm.

Table 18 The CA volumes determined, and the percentage of each dosage group in which the CA could be found during dissections, following precocene II treatment in the 4th instar. "CA volumes" are given including and excluding those animals in which the CA could not be found.

a) Percentages of each sample in which the CA could be observed, for each morphological group

Dose (µg)	Sample Size	Morphological type; no. and % with CA							
		0		1		2/3		Permanent 5th	
		n	%	n	%	n	%	n	%
0	19	19	89	-	-	-	-	-	-
50	15	15	93	-	-	-	-	-	-
100	22	10	70	4	0	8	0	-	-
200	25	7	43	5	20	13	0	1	0

b) Mean CA volumes for each dosage group, and mean volumes of those CA measured in each dosage group

Dose (µg)	Mean CA volumes		Mean visible CA volumes	
	n	(mm^3 ± 1 S.D.)	n	(mm^3 ± 1 S.D.)
0	19	0.479 ± 0.249	17	0.535 ± 0.195
50	15	0.420 ± 0.170	14	0.450 ± 0.128
100	22	0.062 ± 0.117	7	0.194 ± 0.133
200	25	0.045 ± 0.128	4	0.282 ± 0.206

at doses above 50µg. There was also a drop in the mean CA volumes within those animals in which the CA could be seen. In other words, although some animals had intact CA after precocene treatment, the glands were significantly smaller than those of the control group.

No sex differences could be seen in CA - degeneration by precocene treatment.

Treatment in the early 5th instar.

Topical applications of precocene II at specific time intervals within the first 3 days of the 5th instar demonstrated a clear anti - allatotropic action. In most respects the time - response curve (Fig 28) corresponded to that determined for the juvenilization of 5th instar larvae by precocene; very early treatment was effective in CA - degeneration, however, without causing significant juvenilization (compare Figs. 24 and 28).

i) Dose - responses. The mean CA volumes of groups treated 0 - 24h into the 5th instar are given in Table 19, and show a clear dose - related reduction in gland volumes. The proportion of each group in which the CA could be seen during dissection was not dose - related however. Analysis of the CA volumes of those animals in which the CA were seen showed a reduction in gland size at high doses.

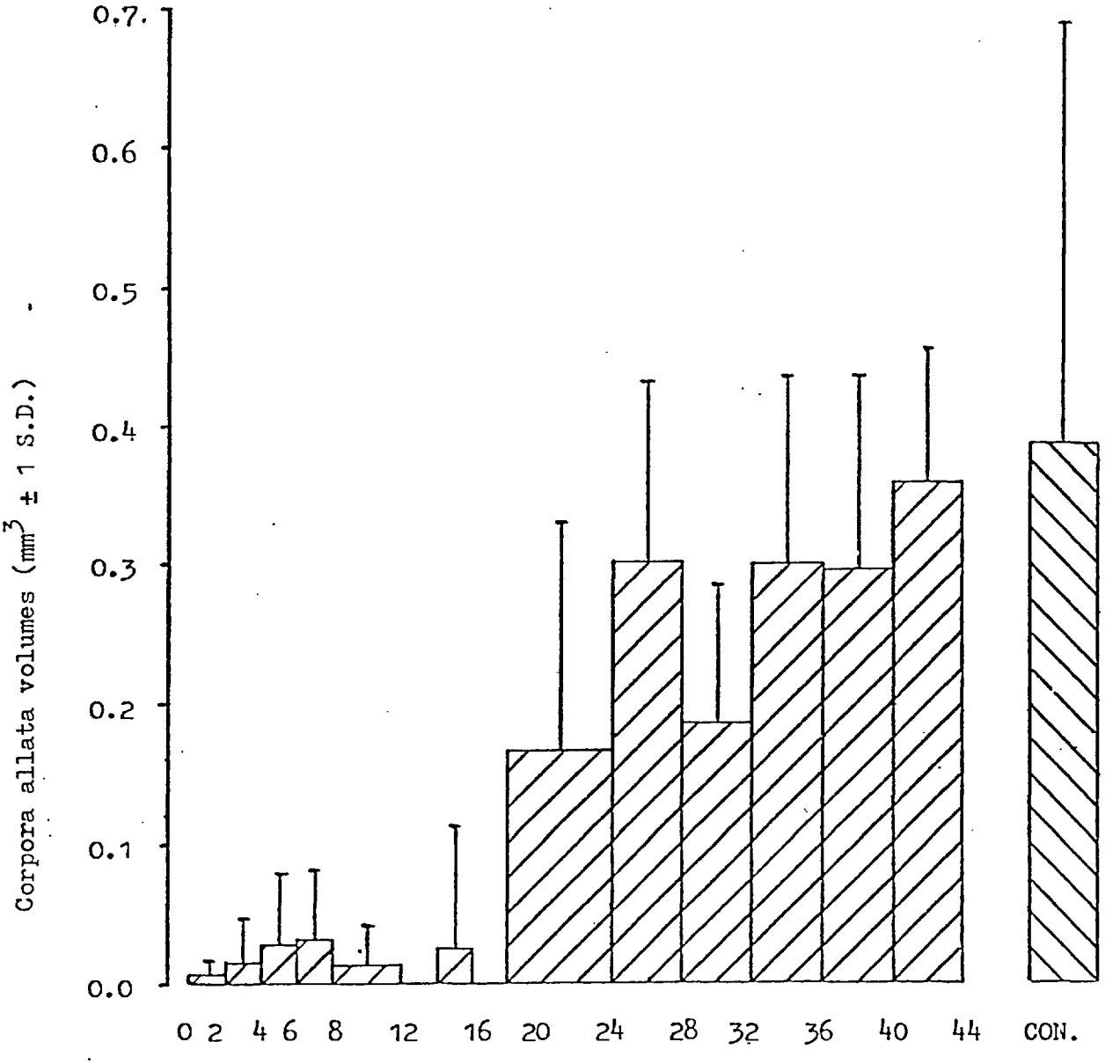
ii) Time - responses. The closely age - structured groups used to determine a time - response curve for juvenilization by precocene (p. 36) were dissected as 6 - 10 day old adults and a time - related degeneration of the CA could be seen (Fig. 28). Treatments within the first 18h of the instar resulted in almost complete degeneration of the CA. After this the sensitivity of the glands to precocene - attack diminished, until treatment 40 - 44h into the instar caused no significant reduction in CA volumes. By plotting the reciprocal values of the mean CA volumes given in Fig. 28, one can get an indication of the extent of the CA degeneration caused (Fig. 29). There was a possible double peak of CA sensitivity, with one peak occurring within the first 4h of the instar, and a second 8 - 12h into the instar.

A limited series of groups were treated with 400µg precocene, but the CA volumes determined showed little evidence of a peak in CA sensitivity within the first 26h of the instar. (Table 20).

There were no sex differences in CA degeneration in any of the groups treated within the 5th instar.

Figure 28

Time response histogram of CA volumes determined in adultoids after timed applications of 200µg precocene II applied within the first 44h of the 5th instar. CA volumes are given as the mean volumes of single glands. Bars : 1 standard deviation of the mean.



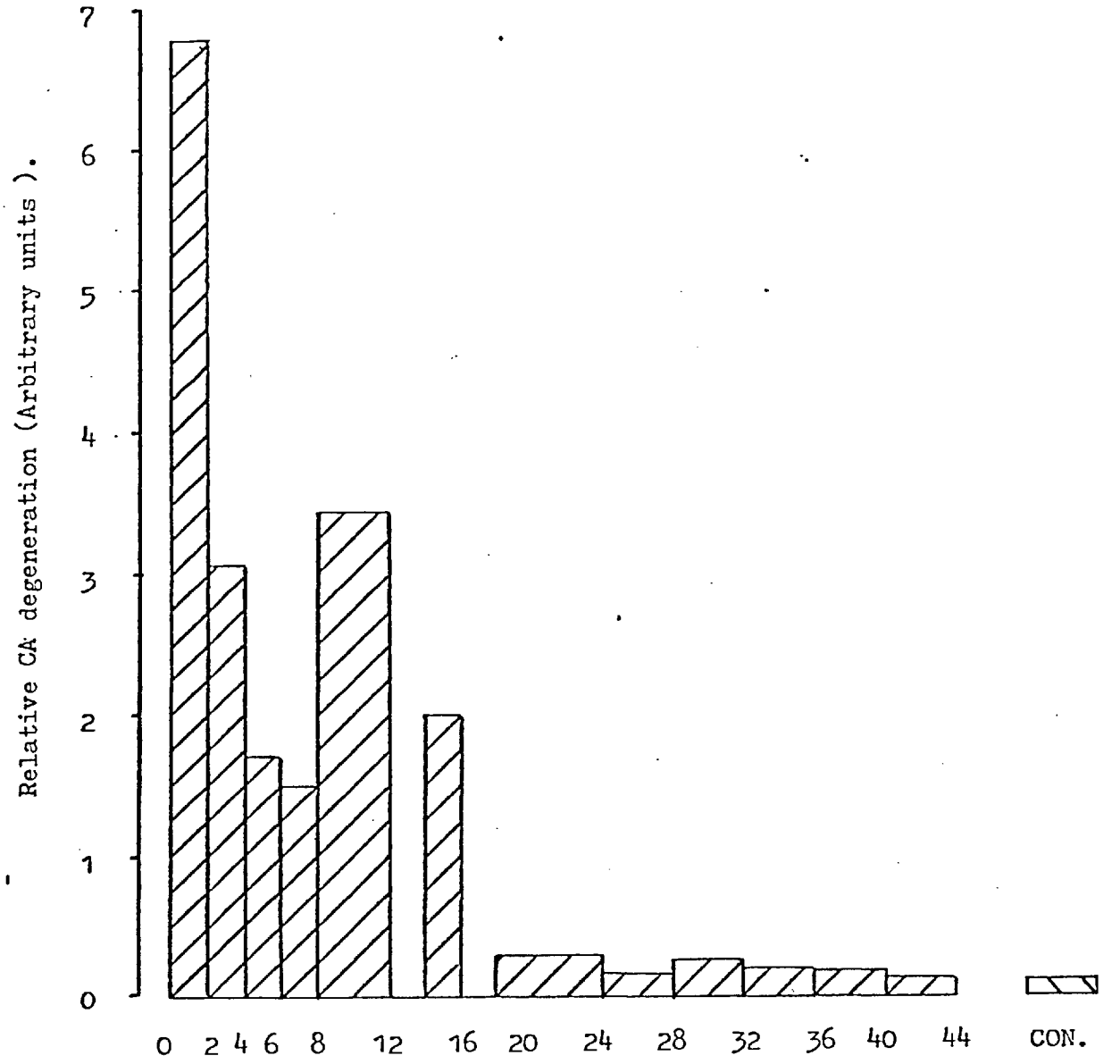
Time of precocene treatment, 0-44h, 5th instar.

Table 19 The CA volumes determined, and the numbers of each dosage group in which the CA could be found during dissections, following precocene II treatment in the 5th instar. "CA volumes" are given including and excluding those animals in which the CA could not be found.

Dose (µg)	Mean CA volumes n	(mm^3 ± 1 S.D.)	Mean visible CA volumes n	(mm^3 ± 1 S.D.)
0	32	0.388 ± 0.302	32	0.388 ± 0.302
200	45	0.140 ± 0.192	20	0.314 ± 0.165
400	17	0.034 ± 0.053	9	0.064 ± 0.058
800	9	0.086 ± 0.142	4	0.193 ± 0.163

Figure 29

Time response histogram of relative CA degeneration caused by timed applications of precocene II in the 5th instar. CA degeneration is given as the reciprocal values of mean CA volumes; see Fig. 28.



Time of precocene treatment, 0-44h, 5th instar.

Table 20 The CA volumes determined in adultoids treated with 400µg precocene II, topically applied at certain times after the moult to the 5th instar.

Time of Application (hours, 5th)	Sample Size	Mean CA volumes (mm^3 ± 1 S.D.)
0- 4	11	0.00334 ± 0.00572
4- 8	20	0.01899 ± 0.04452
14-18	8	0.00613 ± 0.01134
22-26	4	0.0 ± 0.0

Treatment in the 5th instar with juvenile hormones.

A number of groups of early 5th instar larvae were treated with the three synthetic JH's (JH I, JH II, and JH III), or with the JH - analogue ZR515. The CA of these animals were examined and measured after emergence to the adult (or adultoid) stage.

i) JH I. 10 locusts received 50µg or 100µg JH I topically applied 12 - 16h into the instar. The CA volumes determined 6 - 7 days after emergence to the adult stage were not significantly different from those of the control group (Table 21).

ii) JH II. 9 locusts received topical applications of 50µg or 100µg JH II, as above. The CA volumes were again unchanged (Table 21).

iii) JH III. No data is available on the effects of JH III on the CA volumes. However, as there were almost no morphogenetic effects induced by topical applications of 100µg or 200µg, it is unlikely that any change in CA volumes would be seen.

iv) ZR515. Topical applications of ZR515 that were potent in the morphogenetic juvenilization of the locusts were ineffective in causing any changes in the CA volumes (Table 22). There was no sex difference in the CA volumes of these adultoids and no differences between groups treated at specific intervals 0 - 28h after the moult to the 5th instar (Table 23). These CA volumes were slightly larger than those of the control group, probably because the control group were examined 6 - 10 days after emergence while the treated groups were examined 10 - 13 days after emergence.

D. Surgical allatectomy prior to precocene treatment

20 5th instar larvae were surgically allatectomized, either in air or under insect saline, using a technique reported by Strong (1963). Twelve of these animals were allatectomized within the first 48h of the instar, and were topically treated with 200µg precocene II, shortly after the operation.

Many of the 20 animals did not fully recover from the operation, and 11 died within a few days. The remainder showed considerable recovery, but all died or became trapped in the larval cuticle at the following larval : adult moult.

A group of 7 sham - operated controls were treated with 200µg precocene, after the cervical cuticle was split and resealed. The animals recovered within 24h of the operation, and all survived to the moult. One became

Table 21 The CA volumes determined in adultoids treated with JH I or JH II, topically applied in the 5th instar (12-16 h after the moult).

Treatment	Dose (µg)	Sample Size	Mean CA volume (mm^3 ± 1 S.D.)
Control	0	32	0.338 ± 0.302
JH I	50	5	0.338 ± 0.107
"	100	5	0.338 ± 0.107
JH II	50	5	0.419 ± 0.044
"	100	4	0.441 ± 0.078

Table 22 The CA volumes determined in adultoids treated with all doses of ZR515, topically applied in the early 5th instar.

Treatment	Sample Size	Mean CA volume (mm^3 ± 1 S.D.)
Control	32	0.388 ± 0.107
ZR515	53	0.498 ± 0.249

Table 23 The CA volumes determined in adultoids treated with 85.5µg ZR515, topically applied at certain times after the moult to the 5th instar.

Time of Application (hours, 5th)	Sample Size	Mean CA volume ($mm^3 \pm 1$ S.D.)
0- 4	10	0.586 ± 0.202
6- 8	11	0.607 ± 0.179
8-12	5	0.581 ± 0.320
12-16	5	0.595 ± 0.274
16-20	4	0.499 ± 0.197
20-24	5	0.441 ± 0.110
24-28	5	0.549 ± 0.253

trapped in the larval cuticle, because of the wax seal used in the operation, and 4 adults died within 5 days.

There were no indications of infection or disease in either of these groups and therefore no conclusions about the cause of death could be drawn. There were no differences in the 5th instar durations, either between the allatectomized and sham - operated groups, or between these and normal animals. No attempt was made to repeat these treatments.

E. Combined treatments of precocene II and ZR515

Five groups of 5th instar larvae were treated with various combinations of doses of precocene II and ZR515. Control groups contained animals receiving either compound alone, at the same dose and time. The juvenilization of the animals was scored after their emergence to the adult stage, and the CA volumes determined 10 - 14 days after ecdysis (Table 24).

An estimate of the 'expected' scores resulting from these combined treatments of ZR515 and precocene was made by summing the mean scores of the two corresponding control groups, that had each received one of the two compounds. This data is given in Table 25.

Three of the combined treatment scores were smaller than the expected scores, while the remaining two were approximately within 1S.D. of the expected. There is therefore no evidence to suggest that there was a synergistic relationship between these two exogenously applied compounds, each of which acted independently as a JH - analogue.

The probable non - linearity of the scoring system would tend to introduce an error into the values of the 'expected' score however. The $\log_{10}$ (score) values are given for two combined treatment groups and their controls, but show little improvement in the estimation of, or agreement between, expected and observed values (Table 26).

The estimation of CA volumes within each group showed a dose - dependant relationship that was controlled only by the dose of precocene applied. Two groups treated with 200µg precocene, 20 - 24h into the instar showed no significant CA volume differences, although one group had received twice the dose of ZR515. Another group received only 100µg precocene II, with 85µg ZR515, and showed significantly less CA - degeneration (Table 24).

F. Repeated treatments of precocene II or ZR515.

Four groups of larvae were treated twice in the early 5th instar with combinations of precocene and/or ZR515. The first dose was topically applied

Table 24(a) The mode, timing and doses of combined treatments of precocene II and ZR515, topically applied in the 5th instar. The relevant control groups treated with only one of the two compounds are also shown.

Group Number	Treatment	Dose (µg)	Time of Application (hours, 5th)	Sample Size
1	ZR515	85.5	0-24	17
2	Precocene	200	"	58
3	ZR515 and Precocene	85.5 200	"	13
4	ZR515	85.5	12-24	33
5	Precocene	100	"	10
6	ZR515 and Precocene	85.5 100	"	11
7	ZR515	43	20-24	15
8	"	85.5	"	15
9	Precocene	100	"	18
10	"	200	"	18
11	ZR515 and Precocene	85.5 100	"	11
12	ZR515 and Precocene	43 200	"	15
13	ZR515 and Precocene	85.5 200	"	17

Table 24(b) The degree of juvenilization (morphological score), 5th instar duration and the CA volumes determined in adultoids treated in the 5th instar as specified in Table 24(a).

Group Number (Table 24a)	Morphological Score ± 1 S.D.	5th instar duration (days ± 1 S.D.)	Mean CA volume (mm^3 ± 1 S.D.)
1	2.667 ± 1.237	8.667 ± 1.085	0.3773 ± 0.0718
2	1.560 ± 1.520	8.682 ± 0.883	0.1396 ± 0.1910
3	4.808 ± 0.560	7.154 ± 0.555	0.0939 ± 0.1674
4	2.758 ± 1.112	7.750 ± 0.732	0.5253 ± 0.2361
5	2.900 ± 2.011	8.300 ± 0.823	0.0821 ± 0.0523
6	3.500 ± 1.414	8.091 ± 0.302	0.1682 ± 0.1184
7	2.200 ± 0.978	8.867 ± 0.640	0.2680 ± 0.1579
8	3.600 ± 0.890	7.600 ± 0.548	
9	1.583 ± 1.734	8.555 ± 0.078	0.1348 ± 0.1200
10	1.833 ± 1.499	8.824 ± 0.113	0.1929 ± 0.1557
11	2.682 ± 0.815	8.182 ± 0.603	0.1927 ± 0.0899
12	4.300 ± 0.839	7.933 ± 0.704	0.0377 ± 0.0526
13	4.677 ± 0.900	7.529 ± 0.800	0.0237 ± 0.0449

Table 25 The observed and expected scores of the morphological responses to combined treatments of precocene and ZR515, topically applied at certain times after the moult to the 5th instar. Expected scores were determined by addition of the two scores produced after treatments with each of the two compounds alone.

Group Number (Table 24)	Time of Application (hours, 5th)	ZR 515 Dose (µg)	Precocene Dose (µg)	Expected Score	Observed Morphological Score ± 1 S.D.
3	0-24	85.5	200	4.227	4.808 ± 0.560
6	12-24	85.5	100	5.658	3.500 ± 1.414
11	20-24	85.5	100	5.163	2.682 ± 0.815
12	20-24	43	200	4.033	4.300 ± 0.839
13	20-24	85.5	200	5.433	4.677 ± 0.900

Table 26 The observed and expected morphological score ratios determined after combined treatments of ZR515 and precocene II in the 5th instar. Both the E/O and the (E-O)/E values are given for the normal scores and the $\log_{10}$ scores.

Group Number (Table 24)	Time of Application (hours,5th)	ZR 515 Dose(µg)	Precocene Dose (µg)	Normal scores		$\log_{10}$ scores	
				E/O	(E-O)/E	E/O	(E-O)/E
6	12-24	85.5	100	1.1617	0.3814	1.7979	0.4438
13	20-24	85.5	200	1.1616	0.1391	1.1596	0.1376

0 - 24h into the instar (Day 0), and the second dose was applied two days later, 48 - 72h into the instar (Day 2). The juvenilization of the animals was scored after the following moult, and the CA volumes of those animals treated with precocene II were determined 8 - 10 days after ecdysis (Table 27).

Comparison of these treatments with groups receiving the same total dosage on Day 0, and with groups receiving a single dosage on either Day 0 or Day 2 revealed the relative potency of each treatment.

i) Repeated precocene treatment. A double dosage of 200µg precocene was no more effective than a single 200µg dose applied on Day 0 ($p = 0.3875$. 2-sample t-test), and was significantly more effective than a 200µg dose applied on Day 2 ($p = 0.0312$, 2-sample t-test). Although the repeated dose was not significantly less effective than a 400µg dose applied on Day 0, ($p = 0.6166$, 2-sample t-test), the dose - response curve determined on Day 0 implies that a 400µg dose is considerably less than twice as effective as a 200µg dose (Fig. 22).

There was a slightly greater delay in the final moult following repeated precocene treatment, but this was no greater than the normal variation seen in different groups of larvae.

The CA volumes were lower in the repeated treatment group than in the single 200µg dosage groups, and this is presumed to indicate that some of the CA that survived the first treatment were susceptible to the second treatment; this difference in CA volumes was not statistically significant however ($p = 0.1872$, 2-sample t-test). The CA volumes of the repeated - treatment group were larger than those of the 400µg - dosage group; again this was not significant ($p = 0.2362$, 2-sample t-test).

ii) Repeated ZR515 treatment. A double dose of 43µg ZR515 was significantly more effective than a single 43µg dose applied on Day 0 ($p = 0.0092$, 2-sample t-test), but not significantly more effective than a 43µg dose applied on Day 2 ($p = 0.3059$, 2-sample t-test), or more effective than an 85µg dose applied on Day 0 ($p = 0.0703$, 2-sample t-test). The mean score after repeated treatment (Table 27) was higher than both the mean scores of the latter two groups, however. As was found in the dose - response curve for precocene, an 85µg ZR515 dose was less than twice as effective as a 43µg dose (Fig. 22).

The 5th instar duration following repeated treatment was somewhat shorter than seen in the control group and in some ZR515 treatment groups.

Table 27(a) The mode, timing and doses of repeated (or sequential) treatments of precocene II and ZR515, topically applied in the 5th instar.

Group Number	Treatment	Dose (μg)	Time of Application (hours, 5th)	Sample Size
1	Precocene, repeated	200 "	0-24 48-72	15
2	ZR515, repeated	43 "	0-24 48-72	8
3	Precocene and ZR515	200 43	0-24 48-72	16
4	ZR515 and Precocene	43 200	0-24 48-72	9

Table 27(b) The degree of juvenilization (morphological score), 5th instar duration and the CA volumes determined in adultoids treated in the 5th instar as specified in Table 27(a).

Group Number	Morphological Score ± 1 S.D.	5th instar duration (days ± 1 S.D.)	Mean CA volume (mm^3 ± 1 S.D.)
1	2.000 ± 1.680	9.600 ± 0.828	0.0792 ± 0.1342
2	4.250 ± 0.378	7.875 ± 0.354	
3	3.437 ± 1.300	9.187 ± 0.750	0.0643 ± 0.0925
4	3.111 ± 1.294	8.667 ± 0.707	0.2940 ± 0.1496

It was not significantly different however, and little systematic change in instar durations could be observed after ZR515 treatment.

iii) Asynchronous treatment with precocene and ZR515. Two groups of 5th instar larvae were treated with both precocene II and ZR515. One group received 200µg precocene on Day 0 and 43µg ZR515 on Day 2; the second group received the same doses in reversed order. The morphological scores recorded at the following moult indicated no significant difference between these two groups ($p = 0.4607$, 2-sample t-test), although the group receiving precocene treatment first showed slightly greater juvenilization (Table 27).

The instar durations of each group were within the normal limits shown by various control groups and groups receiving precocene and/or ZR515 treatment.

As found using precocene alone (p. 43), there was a sharp fall in the CA susceptibility between Days 0 and 2, and the CA volumes determined for the two groups were significantly different ($p = 0.0032$, 2-sample t-test). The group receiving precocene on Day 0 and ZR515 on Day 2 showed greater CA degeneration than the other group, and the CA volumes were similar to those found in the group receiving 200µg precocene alone, 0 - 24h into the instar (p. 43).

SECTION 2. DISCUSSION

A. The role of JH and the CA in the development of the locust.

The importance of the corpora allata and the hormone(s) that they produce in the control of insect development has been known for many years (Wigglesworth, 1935, 1970). The CA are the site of juvenile hormone synthesis and release, and the presence of this hormone in the haemolymph, in concert with other hormones, regulates metamorphosis. The exogenous application of JH or JH - analogues to final instar larvae can cause the retention of juvenile characters in the adult stage (Wigglesworth, 1970; Sláma *et al*, 1974) and in some instances can induce a complete supernumerary larval instar. In contrast, premature removal of circulating JH by surgical allatectomy (Wigglesworth, 1935; 1970; Fukuda, 1944; Williams, 1961) or by chemical allatectomy (Bowers *et al*, 1976; Pener *et al*, 1978; Sláma, 1978) in the early instars can result at the next moult in the production of precocious, permanent adults (adultiforms).

Many compounds have been discovered that possess juvenile hormone activity (Sláma *et al*, 1974) and three distinct chemical forms of naturally occurring juvenile hormone have been identified. In locusts all three (JH I, II and III) have been reported to be synthesized in the CA (Pratt and Tobe, 1974; Caruelle *et al*, 1979); which of the three forms is most active is still uncertain (reviewed by Lazarovici and Pener, 1977), and even the quantities and timing of their occurrence are open to question (Baehr *et al*, 1979; Huibregtse - Minderhoud, 1979; C.A.D. de Kort, 1979, personal communication).

In this study the three synthetic juvenile hormones that are known to be active in insects (Bowers *et al*, 1965; Meyer *et al*, 1965) and are reported to be active in *Locusta* (Roussel, 1975, 1976a, 1976b; Lazarovici and Pener, 1977) were all found to be ineffective in inducing the retention of larval characters, even when applied at very high doses. The reasons for this are uncertain but some possible explanations are discussed below.

i) Specific activity of JH I, II and III in *Locusta*. Roussel (1975, 1976a, 1976b) compared the activity of injected JH's on morphogenesis, oöcyte development, pigmentation and cardiac rate in *Locusta*. Although large doses of 50 - 200µg/animal were used, all three JH's were found to have morphogenetic activity. JH I

was found to most closely duplicate those effects caused by implantation of active CA. Lazarovici and Pener (1977) reported similar results after studying the effects of JH's on oocyte development in allatectomized adult females. Moreover, Baehr *et al* (1979) have reported that JH I is the most common JH in the haemolymph of adults. However, Huibregtse - Minderhoud (1979) found that JH III was most common in both adults and larvae, and was present at very much lower concentrations than reported by Baehr *et al*. In this study JH I and II were tested at doses of up to 100µg while JH III was tested at doses of up to 200µg : in all cases the degree of juvenilization was little different from that observed in the control groups.

ii) Breakdown of JH during storage and application. The hormones were purchased in solution in hexane in sealed glass vials and were transferred to teflon - rubber sealed vials. These were stored in a freezer at -20°C throughout their usage. Before application the hormones were diluted in hexane or acetone and were applied to the locusts within 30 minutes of removal from cold storage. The hormones were sealed in vials at all times. It is therefore unlikely that any significant breakdown occurred in storage or application. A sample of JH III proved highly effective in a bioassay on the bean aphid, *Megoura viciae*, after several months storage (J. Hardie, unpublished observations). Although topical applications of a variety of JH - analogues have been used to good effect in this study, Lazarovici and Pener (1977) reported poor responses to topical applications of 70µg JH I in allatectomized adults, but injections of 60µg were more effective. In this study neither method of application of JH III was effective; it remains uncertain whether injections of JH I would also have proved ineffective.

iii) Inactivation of JH after absorbtion. The haemolymph of larval and adult *Locusta* contains powerful JH - esterases that can rapidly inactivate exogenous JH's (Slade and Zibett, 1972; Riddiford and Truman, 1978) and it has been suggested that the lack of circulating JH in the 5th instar is due to the action of these esterases, as well as to the reduced synthesis by the CA (Johnson and Hill, 1973; Joly, 1967; Joly *et al*, 1969). However, two facts argue against the complete control of haemolymph JH - titres by these enzymes. Firstly, numerous JH - analogues and injections of JH (JH I, II or III) are morphogenetically active in the 5th instar

(Sláma *et al*, 1974; Roussel, 1975, 1976a, 1976b). Secondly, the JH released following precocene treatment in the early 5th instar must have been synthesized by the host CA, and was exposed to the haemolymph JH - esterases and to other tissue degenerative enzymes, but was nonetheless found to be morphogenetically active (p. 35).

iv) Binding of JH to non - target tissues. Injections of JH - analogues in water - soluble solutions such as saline or acetone are less effective than either topical applications or injections in oil - based solutions (Sláma *et al*, 1974). Unless the JH - analogue is gradually released into the haemolymph - either by diffusion through the cuticle or by slow partition from the oil carrier - it can become bound non-specifically to fatty tissues, and then be attacked by the JH - esterases before reaching a target tissue (Sláma *et al*, 1974; Peter *et al*, 1979). Two groups received injections of JH III in this study, but the small sample size of each group does not allow any firm conclusions to be drawn about the effectiveness of hexane or oil as a carrier fluid.

v) JH - resistance. The development of resistance to exogenous JH - analogues has been discussed by Schneiderman (1972), Sláma *et al* (1974) and Hammock and Quistad (1976), in the light of great differences in response to JH - treatments. However, although the culture of *Locusta* used in this study has been laboratory reared by the C.O.P.R., London, for many generations, there were little differences in the responses to various JH - analogues when compared to other cultures (Tang, 1976), and it is unlikely that specific resistance to the synthetic JH's used would have developed in the culture.

The JH - mimic known as Law's mixture or Law - Williams' mixture is a mixture of compounds resulting from the reaction of farnesoic acid with hydrogen chloride, some of which have juvenile hormone activity (Law *et al*, 1968). The morphogenetic responses to this mixture tend to be diverse and often more extreme than those observed with more highly purified JH - analogues. Both in this study and in the work of Tang (1976), the number of adultoids produced after Law's mixture treatments that could not fully emerge from their larval cuticle was higher than observed with ZR515. Also, although I lack data to support this, I believe that the melanization of adultoids following Law's mixture treatments was more intense and more frequent than seen after other treatments.

Some severely affected adultoids and supernumerary larvae induced by Law's mixture also displayed crinkled or granular cuticular areas that were never observed after other forms of juvenilization. It is likely that some of these effects are due to toxic impurities within this mixture of compounds.

The morphological forms of the adultoids that successfully emerged from the 5th instar were similar to those reported by Tang (1976) and were in general similar to those induced by CA implantation (Tang, 1976) or following treatment with JH - analogues (Sláma *et al*, 1974; Tang, 1976). However, the doses required in this study to elicit these effects were much higher than those reported by Tang. He reported that 56.6% of larvae treated with 80µg could not complete their normal life cycle: about 3% of the sample were supernumerary larvae, 18% died before or during ecdysis and the remainder showed varying degrees of juvenilization. In this study the lowest dose of 107µg induced only slight juvenilization in some animals, while doses of 1069µg were required to produce responses similar to those of Tang (1976). Treatment at the two higher doses produced almost exclusively supernumerary larvae, but required liberal applications of the undiluted analogue mixture.

The reduction in the 5th instar duration apparent in the higher dosage groups was not seen within the low dosage group. Although an advance of the moult is expected after JH - treatments due to the induction of prothoracotropic hormone release (Sláma *et al*, 1974), the most severely affected adultoids produced by 107µg Law's mixture emerged last, while normal adults emerged first. The highest dosage group did exhibit the expected advance in the moult, however. No reasons are apparent for these observations, except that evidence of secondary toxic effects of Law's mixture are present, and that the groups tested did not all originate from one batch of locusts, but were selected over the course of several weeks. It may be that differences in instar durations that are observed in normal, untreated larvae (Table 7) confused the results obtained after JH - treatments.

ZR515 proved to be particularly effective as a JH - analogue and although the doses used were again higher than needed by Tang (1976), the morphogenetic effects were similar. Injection of ZR515 in a carrier of acetone and insect saline proved to be of very low morphogenetic activity. However, many such treated animals had difficulty in emerging from the cuticle at the next moult. It is tempting to suggest that without the protection of an oil - based carrier fluid, the JH - analogue

was vunerable to, and inactivated by, the JH - esterases present in the haemolymph, with the result that the majority of the compound was metabolized before causing exoskeletal juvenilization. Sláma *et al* (1974) report that internal developmental defects caused by JH - treatment can prevent complete ecdysis, and it may be that these internal effects resulted from the ZR515 injection, causing the unsuccessful ecdysis reported here and by Tang (1976).

There was no critical period within the first 72h of the 5th instar where the response to ZR515 was particularly high. Most insect species require treatment within the first third of the instar to be affected by exogenous JH, but some time differences in the responses of different organs have been reported (Joly, 1956; Sláma *et al*, 1974; Staal, 1975). No animals were treated more than 3 days after ecdysis, however, when the likely reduction in the activity of JH treatment would be observed.

There was a significant reduction in the 5th instar duration of those pronounced adultoids induced by ZR515; no significant changes in duration could be seen within separate dosage groups, however. Each dosage group was formed from a number of cages of 5 to 10 animals that emerged to the 5th instar together, and there were often greater differences in average instar durations between cages than were observed within each cage. Thus the expected negative correlation between morphogenetic response and instar duration could only be found amongst the entire group of 95 animals, without consideration of the dosage used.

The supernumerary larvae produced by Law's mixture and ZR515 were often severely crippled and only a minority survived or were maintained long enough to attempt an additional moult. None of those that did attempt such a moult survived, but all showed the normal adult colouration in the new cuticle, and did not attempt a second supernumerary stage, as has been observed in some insects (Wigglesworth, 1970).

The CA volumes estimated in adults, adultoids and supernumerary larvae induced by treatments with the synthetic JH's I and II or with the JH - analogue ZR515 showed no significant differences from the control groups CA volumes. There is evidence of a negative feedback system governing JH - synthesis in the cockroach (Tobe and Stay, 1979), however, and Wajc (1973) has shown that implantation of active CA prevented growth of the host's glands. The limited series of experiments using combinations of precocene II and ZR515 treatments indicated that the morphogenetic action of ZR515 is independent of the CA, and is additive in effect to any circulating JH. In other words, the morpho-

genetic activity of JH's or JH - analogues in the haemolymph are direct and are not influenced by either the source of the JH or by the presence of the CA.

B. The scoring of adult juvenilization and morphometric studies of JH - induced adultoids.

The variation in morphogenetic responses to a given form and dose of JH or JH - analogue was high in all experiments and did not decrease appreciably until very large doses were used, when a high proportion of supernumerary larvae were produced. For this reason it was never possible to estimate the dose used from the morphogenetic response observed and scored in any individual. The function of an accurate score is therefore limited to the standardization of the recording of such responses by grading a limited number of parameters. Ideally such a scoring system would be linearly correlated with the morphogenetic responses, and would allow reliable identification of the various stages of juvenilization evident, such that comparisons of results from different treatments or from different laboratories could be made. A new system was developed during this study, as the system used by Tang (1976), although adapted from the system developed by Bull (Jacobson *et al*, 1972), was felt to be too crude at the lower end of the scale and weighted mortalities too heavily. The system developed is suspected to be non - linear, however, and tends to over - weight the small, initial signs of juvenilization, so that even the control groups have a positive score. The system is additive, and while at both ends of the scale the minimum steps are integers (0, 1, 2, 5 and 6), the central region of the scale includes half-units (2.5, 3.5 and 4.5; Table 28). The lower end of the scale also includes only the earliest signs of juvenilization, while the central region of the scale includes many forms of response, each adultoid displaying different combinations of juvenile characters.

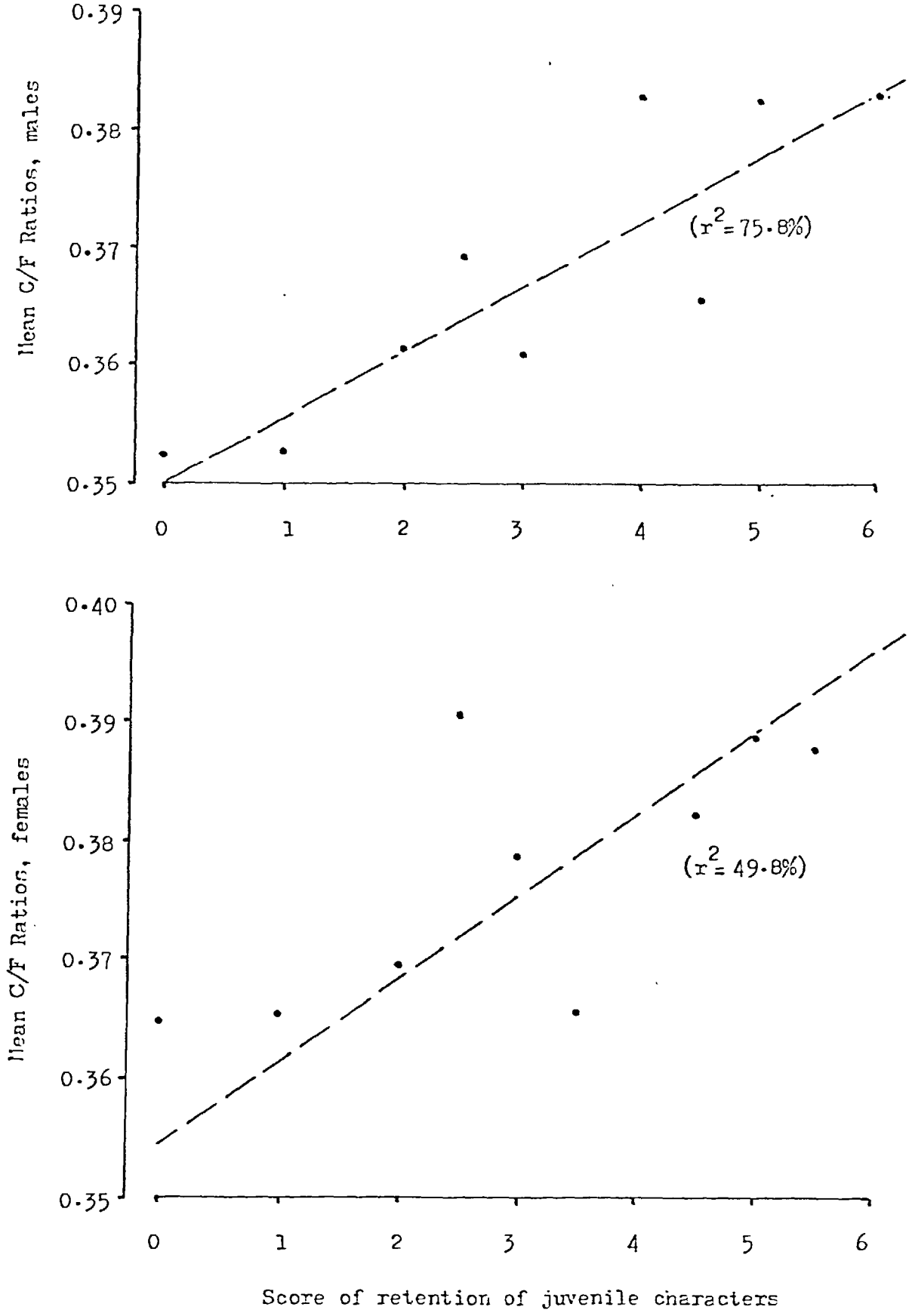
Although no data is provided, I suspect that more definative scoring of this middle range of characters would give a closer relationship between the scores and the morphometric values given in Tables 11 and 14. For example, the morphometric ratio C/F is shown to be positively correlated with the score of juvenile characters (males; $n = 123$, $r = 0.486$, $p<0.001$). When the mean C/F ratios for each score group are plotted (Figure 30), it can be seen that the relationship between C/F and score approximates to a straight line ($n = 9$, $r = 0.87$). Transformation of the score to the natural log ($\log_e$) or $\log_{10}$ does not improve either the correlation coefficients or the fit of a straight regression line. More accurate scoring of the morphogenetic responses would tend to de-

Table 28 The scoring of juvenile characteristics observed in adultoid locusts. Two systems were used, one developed during this study and one adopted from Tang (1976). Characters were recorded as being slight (+), medium (++), or pronounced (+++).

Juvenile characters observed				Juvenilization score	
Wing deformation	Pronotum deformation	Larval colouration	Extra moult	Miall's system	Tang's system
				0.0	0
	+			1.0	1
	++			2.0	1
+				1.0	1
+	+			2.0	1
+	+	+		2.5	3
+	++			2.5	1
+	++	+		3.0	3
++				2.0	1
++	+			2.5	1
++	+	+		3.0	3
++	++			3.0	1
++	++	+		3.5	3
++	+++			3.5	2
++	+++	+		4.0	3
+++				3.0	2
+++	+			3.5	2
+++	+	+		3.5	3
+++	++			3.5	2
+++	++	+		4.0	3
+++	+++			4.0	2
+++	+++	+		4.5	3
+++	+++	++		5.0	3
+++	+++	+++		5.0	3
+++	+++	+++	+	6.0	3

Figure 30

Linear regression and r^2 values of the mean C/F ratios determined at each score of juvenilization after treatment with 200µg precocene II applied 0-44h into the 5th instar. C/F ratios were determined separately for males (above) and females (below).


0.39
0.38
0.37
0.36
0.35
Mean C/F Ratios, males
$(r^2 = 75.8\%)$
0
1
2
3
4
5
6
0.40
0.39
0.38
0.37
0.36
0.35
Mean C/F Ratios, females
$(r^2 = 49.8\%)$
0
1
2
3
4
5
6
Score of retention of juvenile characters

crease the variation seen in the central region of the graph and improve the correlation between C/F ratios and scores.

The reversal of the above analysis i.e. an analysis of the scores recorded within a particular range of C/F ratio measurements was also attempted. The C/F ratios were rounded up or down to the nearest 0.01, so that each group had a range of C/F values of ± 0.005. As expected, there was a positive correlation between the C/F ratios and the mean scores of each group, although the relationship was not linear. At low C/F values (representing normal or only slightly adultoid animals) the mean score increased only slowly; at the higher values of C/F there was a sharp increase in the mean score. The relationship was slightly improved by taking the $\log_e$ or $\log_{10}$ values of the score. This emphasizes the earlier suggestion that the initial morphogenetic responses to JH were over - weighted at the lower end of the scale.

The morphometric parameters determined in ZR515 treated animals were consistent with the known changes in morphology induced by JH or CA - implantation (Joly, 1956, 1962). Although a number of these changes in morphology are equivalent to the shift in phase morphology from gregarious to solitary forms, a distinction should be drawn between the two processes. For example the most critical morphometric parameters of phase status, the E/F and C/F ratios (Dirsh, 1953), show a marked difference between normal, gregarious, larvae and adults (p. 41). Both JH - induced adultoids and solitary adults show ratios that approach those of the larvae (p. 34 ; Gunn and Hunter-Jones, 1952; Joly, 1956, 1962), and one might consider that juvenilization was morphometrically equivalent to phase shifting. Further changes such as the arching of the pronotal shield and the retention of the prothoracic glands are also features of both processes. However, the more severe stages of juvenilization, such as the appearance of larval colouration in the adultoid cuticle, and the induction of a supernumerary moult are features that cannot be caused by reduced breeding densities.

The variation in these morphometric parameters was such that although the C/F ratio was positively correlated with the score of juvenilization (♂, $n = 123$, $r = 0.488$; ♀, $n = 122$, $r = 0.401$; $p<0.001$), the r^2 values that represent the percentage of data agreeing with the regression equations, were only 24% and 16% respectively. Thus it is not possible to access the score and hence the degree of juvenilization of an individual from morphometric values alone. Dirsh (1953) reached

similar conclusions in a study of phase polymorphism in *Locusta*.

C. The effects of precocene II as an 'anti-JH' on the development of the locust.

Since the discovery of the two potent forms of the plant chromene, precocene I and II (Bowers *et al*, 1976), there have been many reports of their action as 'anti - JH' or 'anti - CA' compounds. Bowers *et al* (1976) reported the induction of precocious metamorphosis in the milkweed bug, *Oncopeltus fasciatus*, after treatment in the 2nd instar with precocene I or II, although precocene II was about 10 times more active than precocene I. They also reported sterilizing effects of precocene II in various *Hemiptera*, *Coleoptera* and *Diptera* and the induction of diapause in a *Coleopteran*. They could not induce precocious metamorphosis in *Holometabola*, however. In 1977 Pratt and Bowers reported that precocene II could inhibit JH biosynthesis by *Periplaneta americana* CA *in vitro*, while morphogenetic effects have also been reported in *Rhodnius prolixus* (Tarrant and Cupp, 1978) and in the aphid *Acyrthosiphon pisum* (MacKauer *et al*, 1979). Pener *et al* (1978) reported the morphogenetic effects of precocene II on larval *Locusta migratoria*, and demonstrated its apparently specific anti - CA activity. This has been confirmed and further studied by Němec *et al*, (1978), Brooks *et al*, (1979) and Schooneveld (1979); Pedersen (1978) has reported the effects of precocene I on *Locusta*, but could not induce greater than 80% precocious adultiform larvae among the survivors. There is little evidence of any effects in other insect species, however, and many species appear unaffected unless extremely high, toxic, doses of precocene are employed.

While there is now clear evidence from a few insect species that precocenes have morphogenetic effects equivalent to surgical allatectomy, and therefore have the reversed effects of JH - treatments, the label 'anti - juvenile hormone' is inaccurate. The compounds are not naturally occurring insect compounds, but are derived from the plant, *Ageratum houstonianium*, and cannot be described as hormones (Sláma, 1978). They also do not act *per se* as 'anti - juvenile' compounds but as anti - CA compounds and indirectly cause symptoms consistent with reduced JH levels. Many workers have recognized these facts (Bowers, 1977; Němec *et al*, 1978; Sláma, 1978; Muller *et al*, 1979; Schooneveld, 1979), but the term 'anti - juvenile hormone' still appears widely. Even the term 'anti - JH' while more accurately describing the compound as one displaying effects opposite to JH, is misleading, and I feel the terms 'anti - CA' or 'antiallatotiopin' are the most accurate labels, particularly when discussing the effects of precocenes in *Locusta*.

Topical applications of precocene are highly effective against the CA in all larval stages of *Locusta* (Němec *et al*, 1978; Pedersen, 1978; Pener *et al*, 1978) and are effective in causing the degeneration of the CA even when no morphogenetic effects can be induced. For example Pener *et al* (1978) have reported the degeneration of the CA when larvae were treated towards the end of the 4th instar; the morphologically normal adults resulting from this treatment showed no egg production by the females or yellowing of the males. Němec *et al* (1978) however reported that treatment of newly emerged adult females was ineffective in inhibiting egg production, while Pedersen (1978) reported a delayed effect on CA transplanted from adults treated shortly after emergence into normal larval females. Recent work by Pratt *et al* (1980) indicated that precocene is only effective in cells that are actively synthesising JH and they suggest that the cytotoxic effects are due to the production of a mixture of highly reactive dihydrodiols, synthesised from precocene by the normal JH - biosynthetic pathways, that degrade many proteins within the cell. They postulate that precocene would only be active in those stages of the locust life cycle involved in JH synthesis : these are classically thought to be the early instars (1 - 4) and in the mature adults (Sláma *et al*, 1974). The work reported here confirms that precocene II is highly effective morphogenetically and also as an anti-allatotiopin when applied topically or injected in oil in the early 4th instar. The morphogenetic data suggest a strong dose - response relationship but high doses were required to produce almost exclusively precocious adultiforms. At no dose was the response complete, however, and mortalities were considerably lower in all groups that those reported by Pener *et al* (1978) and by Pedersen (1978). Although the oil injections caused high mortalities in both control and treatment groups the 100µg treatment group survivors showed similar morphogenetic responses to those of the 100µg topical application group. It appears that unlike JH-treatments, injection of precocene in an oil based fluid does not significantly increase its activity. While no detailed study of the time - response relationship was made, one group was treated topically with 200µg precocene, 24 - 48h into the 4th instar. This group showed a considerably larger proportion of Types 0 and 1 adultiforms than were induced by treatments 0 - 24h after the moult. No data is included from these trials, but they indicate a rapid decline in the morphogenetic responses during the 4th instar; Pedersen (1978) and Pener *et al* (1978) also reported such a decline during the 4th instar.

The morphometric measures taken show changes induced by precocene

that are opposite to those induced by JH treatments. There were obvious changes in the wing and head capsule sizes, the former increasing and the latter decreasing from normal larval dimensions. The hind femur was not changed from normal 5th instar proportions, however, and the ratios E/F, C/F and F/C were primarily influenced by the E and C values. The morphological changes in the premature adultiform larvae were consistent in most respects with a change from normal larval towards normal adult characteristics. This was further confirmed by the advanced development of the flight musculature, to the extent that Type 3 adultiforms could display vigorous wing beating. The locust can therefore considerably 'reprogramme' its development within the course of a single instar, as recognized by Němec *et al* (1978). Treatment must occur within one or two days of the start of the instar however (Pedersen, 1978; Pener *et al*, 1978), since precocene treatment after this stage causes CA degeneration after the developmental sequence has been determined by the early presense of JH in the haemolymph.

Pener *et al* (1978) reported a delayed moult to the adultiform stage and Němec *et al* (1978) reported up to a doubling of the 2nd and 3rd instar durations after precocene treatment. The instar durations recorded here did increase and were positively correlated with both the dosage and the extent of precocious development induced. The instars were only increased by 1 or 2 days, however, from the normal 6 and 10 days of the 4th and 5th instars. The limited number of morphologically normal, permanent, 5th instar larvae induced at doses above 50µg were also reported by Bowers *et al* (1976) in *Oncopeltus fasciatus*. They found that 'less than 10%' of the sample showed arrested development after treatment at unspecified near toxic-doses of precocene. From the single specimen examined here it seems that the precocene was still active against the CA, which could not be identified during dissection, although the normal morphogenetic sequence was maintained. The prothoracic glands or its secretions must have been inhibited in the 5th instar however, so that the animals remained as permanent larvae.

The corpora allata of the adultiform larvae were examined and measured after preservation in Bouin's fixative approximately 17 - 23 days after emergence. The corpora allata show considerable increase in volume during the 5th instar and early adult life (Johnson, 1973), thus the period of several weeks before fixing should have increased the reliability of identification of the CA in the subsequent dissection. Although the CA could only be found in 89% of the control group through tissue degeneration because of poor preservation, the CA in groups

treated with above 50µg precocene had completely degenerated in most specimens. There was a clear relationship between the degree of precocious development and the mean CA volumes and the CA could not be identified in any of the Type 2/3 adultiforms examined. These results agree with those of Pener *et al* (1978). Schooneveld (1979) has reported detailed histological studies of the breakdown of the CA cells and suggests that the process is complete with 24 - 48h of treatment with precocene II. Some animals showed evidence of precocious development without complete degradation of the CA however; it is suggested that some individuals may have received only sufficient precocene to partially block JH synthesis or release without causing the full destruction of the CA. The variation in effectiveness of a standard dose of precocene within a group of larvae is analogous to the variation in response to JH treatments, and is presumed to be a function of the mode of application, variations in the exact developmental status of the larvae and individual differences in receptivity to either exogenous JH or to reduced JH levels (p. 48).

It is evident therefore that precocene II not only acts as an antiallototropic agent in the early 4th instar of *Locusta migratoria*, but that it is apparently specific in its action on CA cells. This is probably due to the metabolism of precocene by JH - biosynthetic enzymes and would therefore only be active in JH producing cells within the CA. All the effects of precocene can be explained by the destruction of this site of JH synthesis, with the possible exceptions of the reported toxicity at high doses and the limited induction of permanent, normal larvae.

D. The JH - mimicing effects of precocene II in the development of the locust.

While there have been many reports on the antiallatotropic action of precocene in early larval and adult insects, there has been only one report of its effects in the final larval stage of *Locusta* (Pener *et al* 1978). Treatment at the end of the 4th instar or in the first day of the 5th instar was reported to be morphogenetically inactive, although the normal adults produced showed no egg production or cuticular yellowing; dissections confirmed that the CA had degenerated in these animals. Other workers have assumed that precocene would be ineffective in the final instars, but there are no tests reported in the literature.

Having used precocene II to induce precocious adultiform larvae

for use in the behavioural sections of this project, it was felt necessary to study the behaviour of animals treated with precocene in the early 5th instar. It was thought that such experiments would indicate whether precocene had any effects, other than the morphogenetic effects resulting from the degeneration of the CA, that might influence the larval behaviour in the 5th instar. However, during this work it became clear that precocene treatment within the first 24h of the 5th instar caused the retention of juvenile characters in the adult stage of some individuals, and was acting as a JH - mimic or analogue. The dose - response curve determined after treatments 0 - 24h into the instar indicated that these effects were equivalent to a JH - analogue of greater activity than the Law's mixture and of about half the activity of the potent analogue ZR515. To further study these unexpected results a number of closely timed treatments were applied, and the resulting adultoids were examined and compared to adultoids resulting from treatment with ZR515.

These tests confirmed that precocene II could indeed induce adultoid characters and was acting as a powerful JH - mimic within a critical period 8 - 20h after ecdysis to the 5th instar. At the peak of this time - dependant response curve precocene caused the marked juvenilization of the majority of the sample, and induced a complete supernumerary larval instar in nearly half of the group. Dissections revealed that the extent of juvenilization was related to the degeneration of the CA, although the relationship was not as close as had been seen after the 'anti - JH' treatment in the 4th instar. In other words, the mean CA volumes fell with the increasing score of juvenile characters, but even some fully supernumerary larvae were found to have CA that were normal or only slightly reduced in volume. There was a great variation in the extent of juvenilization seen within any group, and even treatment 72h after ecdysis could produce occasional retention of juvenile characters.

The mean CA volumes determined in adultoids treated within the first 44h of the instar indicated a slight difference in the time response relationship for the morphogenetic and the antiallatotropic action of precocene in the 5th instar. Treatments within the first 4 - 6h of the instar resulted in almost complete degeneration of the CA but little juvenilization. However, treatment at the second peak of antiallatotropic activity, 8 - 16h into the instar resulted in almost complete CA degeneration but was coupled with the pronounced juvenilization of the adultoids. The series of experiments involving combined, repeated or serial treatments of precocene and ZR515 indicated the mechanism involved in

these effects.

Firstly the juvenilization caused by precocene was independent of the existing levels of JH or JH analogue in the haemolymph. The scoring system was not suited to calculating the additive effects of two forms of treatment, but as far as could be estimated, the juvenilizing effects of precocene and ZR515 were independent and purely additive. No synergistic effects could be seen. Secondly, the order in which the treatments of precocene and ZR515 were applied did not affect the previously determined activity of either compound. While precocene had been seen to show a rapid decline in morphogenetic activity over the first 72h of the instar, ZR515 showed less decline. Thus, treatment of precocene on Day 0 and ZR515 on Day 2 was more effective than the treatment of ZR515 first and precocene second. Likewise, the antiallatotropic activity of precocene was unaffected by the presence or timing of ZR515 treatments. Finally, the juvenilization caused by the precocene treatments was dependent on the presence of healthy CA : repeated precocene treatments were only slightly more effective than a single dose. It is postulated that the initial treatment caused the breakdown of the CA in most animals and simultaneously caused the juvenilization of these animals. The second precocene dose, applied at a time when considerable juvenilization could still be expected, probably caused the degeneration of some of the remaining CA, and also some juvenilization of these animals. It did not cause enough juvenilization to indicate that animals affected by the first treatment were in any way affected by the second.

These results, combined with the reported effects of precocene on CA cells (Schoonveld, 1978; Pratt *et al*, 1980), indicate that the CA may be stimulated to synthesise JH during the 5th instar, and that this JH is released during the breakdown of the CA cells. The evidence that JH must be synthesised during the instar, rather than stored in the CA from the previous instar, is two-fold. Firstly, Johnson (1973) reported that CA transplanted from 5th instar larvae into other receptive larvae showed positive activity when taken from all stages of the instar except for the first day, when reduced activity was observed. Secondly, the CA degeneration reported here to occur within the first 4 - 6h of the instar does not cause significant juvenilization, whereas later treatments are highly juvenilizing. From the data presented here it is not possible to determine whether the CA of older animals (>72h into the 5th instar) can synthesize JH or not. While there is strong evidence that the response to JH declines after the first third of the instar, the response to ZR515

observed here did not decline during the first 72h of the instar. Therefore the decline in morphogenetic response to precocene applied later than 24h into the instar is probably mainly due to reduced synthesis and release of JH by the CA, rather than to lack of response by the target tissues. It is also unclear as to whether the CA normally synthesize JH in the 5th instar, or whether the precocene treatments or CA transplantation triggered abnormal synthesis. What is clear is that the CA do have the potential to synthesize JH at some period 6 - 40h into the 5th instar, but that during the normal developmental sequence either this synthesis is inhibited, or the release of stored hormone is presented, or any released hormone is inactivated before it can affect the target tissues.

The morphometric studies of these precocene - induced adultoids showed exact conformation to those made on ZR515 - induced aultoids. Similarly, a reduced 5th instar duration was evident, but the data was as variable as that recorded after JH - analogue treatments. Again, the naturally large variation between the mean instar durations of groups of larvae treated over periods of several weeks was probably as great or greater than the changes caused by these treatments.

It can be assumed that not only were the JH - effects of precocene treatments the result of a raised JH - titre in the haemolymph, but also that ZR515 caused identical effects to those caused by the endogenously synthesized JH.

SECTION 3 MATERIALS and METHODS

Animals, hormones and mode of application.

The locusts used in the behavioural trials were the same animals that were described in the previous section (Section 2).

The treadwheels.

Four wheels were employed, which were simplified versions of the design described previously (p. 7). Each wheel was made of an expanded polystyrene disc surrounded by a cardboard rim covered by a renewable layer of masking tape. The rim had a circumference of 500mm and was 16mm wide. The axle of the wheel was fashioned by cementing two bisected syringe microfilters onto either side of the polystyrene disc; hypodermic needles were then attached and supported in V-groove bearings made from PTFE sheeting. When correctly balanced the wheels were extremely light and almost frictionless. No locust showed any difficulty in starting or stopping the wheels. The wheels were supported vertically in balsa-wood frames, and a small metal flag attached to each wheel passed between two opposed infra-red light switches mounted on these frames (Figure 31). The switches were coupled so that the wheel had to complete a full revolution before activating a counter (Figure 32). Each frame also bore a vertically mounted needle supporting a counter-balanced arm pivoting on the needle with a PTFE bearing. The arm was restrained until the end nearest the wheel surface could move approximately 2cm in all directions, and balanced approximately 1cm above the wheel surface. A locust could be clipped to the arm by a small wire clip secured to the pronotum and held in a minature crocodile clip mounted on the arm.

Each wheel was surrounded by a U-shaped white wall, 1m deep, 0.75m wide and 1m high. The bottom 15cm of the arena and the floor were matt black; a matt black stripe 5cm wide and 1m high was positioned vertically on the wall in front of the locust. The ceiling was white and the open side of the wall, behind the locust, was covered by a white muslin curtain. The arena was lit by a 75 watt flourescent tube mounted horizontally above and behind the wheel; each 5ft tube was used to light two adjacent arenas.

The room was maintained at 28°C ($\pm$ 1°C) with uncontrolled humidity. Although the relative humidity ranged from 40 - 60%, the change within any 3h period was less than 2%.

Figure 31

Arrangement of treadwheels used for measuring the distance walked by Locusta larvae. (a) Small spring clip holding (b) larva with wire clip attached to pronotum. (c) Bearings and restraining supports for (d) counter-balanced arm. (e) Wheel with (f) attached metal flag passing through (g) opposed infra-red light switches.

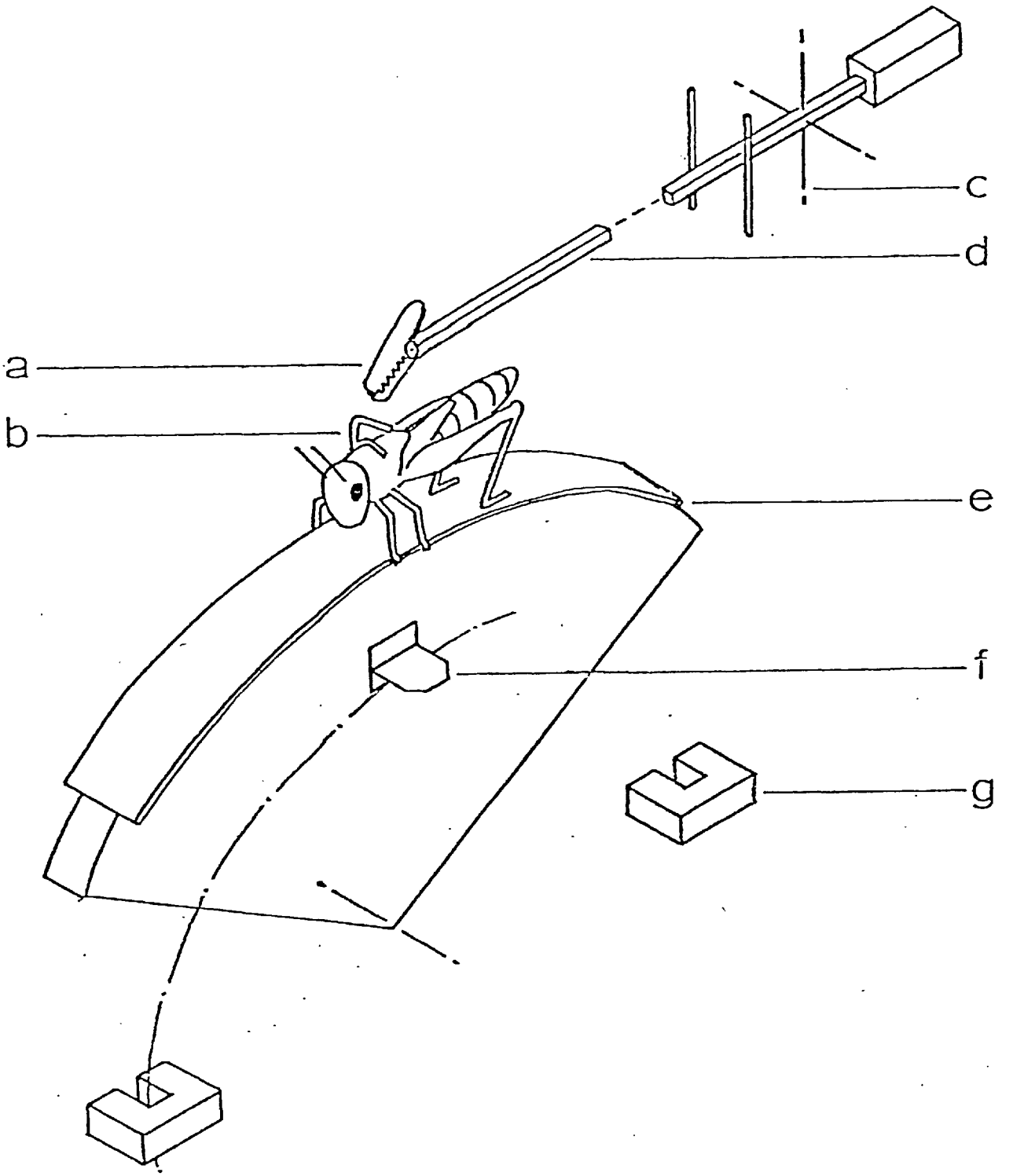
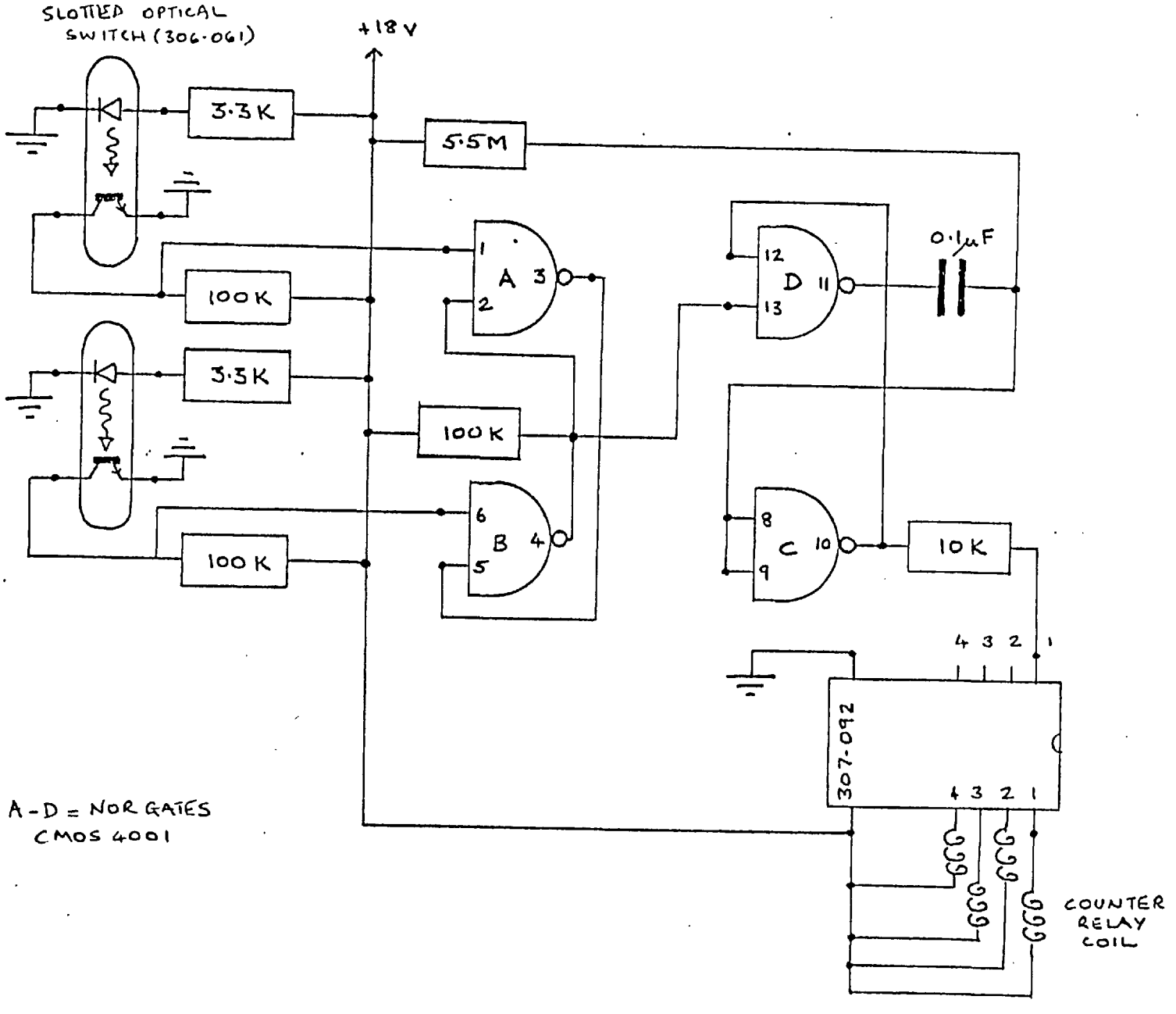

a
b
c
d
e
f
g

Figure 32

Circuit diagram of the electronics used in recording each revolution of the treadwheel. The signals from the two light switches are used to switch 4 NOR logic gates, driving one of a bank of 4 solenoid driven counters. This system ensures that the metal flag attached to the wheel must pass through both light switches before a wheel revolution is recorded.


SLOTTED OPTICAL SWITCH (306-061)
+18 V
3·3 K
5·5 M
100 K
3·3 K
100 K
100 K
10 K
0·1 μF
A
B
C
D
307-092
COUNTER RELAY COIL
A-D = NOR GATES CMOS 4001

Data collection.

Each wheel was connected to an electrically-driven counter that could be read from outside the room, to minimize disturbance to the animals. Each revolution of the wheel was counted and represented 500mm walked; small to and fro movements of the wheel could not be recorded, however. The counters were read immediately after the locusts were positioned on the wheels and at 1h intervals thereafter. Small adjustments were made *pro rata* to allow for readings that were not made exactly on the hour. The final reading was always made within 1 - 2 minutes of the correct time, giving an overall accuracy of 0.5 - 1.0%.

Locust pretreatment

The experimental procedure was changed during the course of the study to allow the behavioural responses of larger numbers of precocene-induced adultiform larvae to be investigated. The two procedures used will be described separately.

i) Pretreatment of JH - treated locusts. All animals receiving JH treatment, including larvae treated with precocene during the 5th instar, were treated at known times after ecdysis to the 5th instar. These animals were fed exclusively on a diet of wheat seedlings until Day 3, when their marching behaviour was monitored on the treadwheels. Following these tests the locusts were maintained on a mixed diet until the moult to the adult stage. The morphological juvenilization of most animals was then recorded individually; some animals could not be identified after the moult, however, as the paint-spot code used was occasionally lost before or during the moult.

The locusts were tested in two 3h sessions making it possible to test up to 8 locusts each day; no locusts were ever tested twice. The sessions ran from 1200h - 1500h (Session 1) and from 1500h - 1800h (Session 2); a number of trials occured at intermediate times and these are considered separately during analysis.

Three hours prior to the start of the behavioural tests, 5 animals of the correct age were selected and transferred to a foodless cage. Small metal clips were then attached and the locusts were left undisturbed until the start of the session. The clips were positioned across the dorsal surface of the pronotum and secured with either a 40 : 60 resin-wax mixture (Steel, 1971) or a rapid bonding cyanoacrylate superglue. At the start of a session

the locusts were clipped to the counterbalanced arms of the treadwheels and allowed to walk undisturbed for 3 hours. At the end of the session the locusts were marked with a series of spots on the hind femur, using a non-toxic enamel paint. The wire clips were removed and any small tears made in the cuticle were immediately sealed with beeswax.

ii) Pretreatment of 'anti - JH' treated locusts. All animals were treated in the early 4th instar (Table 15) and tested on Day 3 of the 5th instar. A number of adults were also tested, after treatment with acetone in the 4th or 5th instars. All animals were fed on a mixture of wheat seedlings and bamboo leaves from the time of selection in the 4th instar until death. Those tested on the treadwheels were provided with abundent supplies of wheat seedlings on the day before the trial.

Two hours before the start of each behavioural trial 4 healthy locusts of the correct age were selected and transferred to a foodless cage. The wire clips were attached with a cyanoacrylate superglue, and the animals were left undisturbed until the start of the session. They were then positioned on the treadwheels and allowed to walk for 2h. Five two hour sessions were employed allowing a maximum of 20 locusts to be tested each day. The first session started at 1000h, and these animals were selected before the introduction of fresh food. The final session ran from 1800h to 2000h and overlapped the 'lights-off' time of the separate culture room by 1h.

After each session the clips were removed and the locusts were left undisturbed and without food for a further 30 - 60 mins., until their jumping responses were measured.

Quantification of the escape jump responses.

Some of the precocene-induced 5th instar adultiforms and a number of normal adults and 5th instar larvae were made to exhibit a series of escape jumps, following their sessions on the treadwheels. Each animal was placed singly at the centre of a concentric grid marked out on the floor of the 28^{o}C culture room. On release, these animals either spontaneously jumped away from the handler, or were made to jump away from a slowly approaching finger. Normal adults were prevented from flying by joining the wing tips with wax or superglue. After each jump the

distance covered was measured to the nearest 5cm while the locust was recaptured and returned to the starting point. Ten consecutive jumps were recorded for each locust, but small hops of less than 15cm were ignored. Although adults and precocious adultiforms could not fly, the wing movements could be heard, and records were kept of those animals displaying wing flapping during the course of their ten jumps. Records were not kept on the occurrence of wing movements during individual jumps, however.

Measurement of kicking activity.

While many normal locusts can be seen to kick their hind legs at other locusts approaching from behind, the frequency of these kicks was thought to be higher amongst precocious adultiform larvae than amongst normal larvae or adults. Also, precocious adultiform larvae exhibited these defensive, kicking type movements in the absence of observable stimulii. The frequency of these kicks and the time spent in kicking were therefore recorded under crowded and isolated conditions. All adults and adultiforms were observed 10 days after ecdysis; normal 5th instar larvae were observed on Day 3, the most active stage of the instar (p. 21). All animals were supplied with abundent quantities of food prior to the trials and only healthy and intact animals were selected.

i) Kicking behaviour under crowded conditions. Ten animals of a type (i.e. all larvae, or all adults) were selected and introduced into a cage furnished with twigs but without food. The same arrangement of cage and twigs was used in each trial. Three of the 10 locusts were marked with enamel paint and observations began 10 minutes after introduction. Each marked animal was observed for 3 five minute sessions, in rotation over a 45 minute period. The time spent in kicking, either triggered by other locusts on occurring spontaneously, was recorded by stopwatch and converted to the percentage of time spent in kicking by each animal over the 15 minutes of observation.

ii) Kicking behaviour under isolated conditions. The observations of the activity of 10 animals in one cage indicated that the behaviour of each individual was heavily influenced by the behaviour of the remaining 9 animals. Much of the movement observed seemed to be related to the positioning of the group of animals on the twigs, and with movement over the floor of the foodless cage. Animals were therefore selected and introduced singly into cages containing a

small quantity of wheat seedlings but provided with no twigs. All animals were well fed before the start of observation. The perspex cages were separated from each other by 10 - 20cm and no cage was adjacent to any cage containing more than one locust. The animals were left 10 minutes before observations began; observations were made on pairs of cages for 5 minute periods. Thus, the first pair of cages were studied 10 - 15 minutes after isolation and the last pair were studied 45 - 55 minutes after isolation. Both the numbers of kicking movements and the time spent in kicking were recorded for each animal.

Measurement of respiratory rates.

A number of animals were observed for 1 minute periods while at rest in crowded cages. All animals were well fed and healthy. Observations in which the locust became agitated or moved from its position were disregarded.

Measurement of food intake and faecal production.

20 healthy locusts were selected according to age, weighed, and transferred to a clean cage containing a number of twigs and 50g of fresh wheat-seedlings. A control cage containing 50g of wheat-seedlings but no locusts was also employed. After 6h the locusts were removed and the cage contents were sorted into uneaten food and faecal pellets. Both were weighed and returned, and the locusts were reintroduced to the cages. After 18hr the uneaten food and faecal pellets were again weighed, and then dried to constant weight. The quantities of food eaten in 6 and 18hr were determined by comparison with the fresh and dry weights of the control wheat, to eliminate weight changes due to desiccation of the seedlings. A control group of twenty adults was found to eat all of the 50g wheat within 18h, so the experiment was repeated for this group only, using 140g of wheat seedlings.

SECTION 3 RESULTS

Sex differences in the behaviour of the locust.

The possibility of a sex-related difference in the behaviour of the normal larvae, normal adults and all treated animals was tested statistically at every opportunity. There were no sex differences in the escape-responses of these animals (p. 73), nor in the timing and frequency of their kicking behaviour (p. 74). There was no sex difference in the respiratory rates of the locusts observed (p. 75), and although there is a sex difference in the quantities of food eaten and of faeces produced, these are related to clear differences in body weights (p. 76).

Of the 21 groups of animals tested on the treadwheels, after a variety of treatments of JH, JH - analogues and precocene, only one group showed a statistically significant sex difference in the distance walked in 2 or 3h. In this group of eleven 5th instar larvae receiving 213µg ZR515 injected in an acetone : saline mix (Table 34), the females walked significantly further per hour than the males ($n = 4 : 7$, $p < 0.05$, 1 - way analysis of variance). Various combinations of treatment groups were also analysed but in no other instance was there a significant sex difference in walking behaviour.

The following sections are therefore given regardless of the sex of the animals involved. However, in almost all groups the numbers of males and females were approximately equal, thus minimizing the possible influence of sex on the behaviour of the groups.

Characterization of treadwheel performance.

i) Differences in behaviour exhibited on each treadwheel. All four treadwheels were used throughout these experiments and the distribution of trials on each wheel was approximately even in all treatment and control groups.

No significant differences could be seen in the distance walked in the 5th instar animals tested on each of the wheels. The five groups analysed consisted of 2 control groups treated with acetone in the early 4th and 5th instars respectively and 3 groups treated with different doses of Law's mixture, 0 - 24h into the 5th instar. Each group's behaviour was analysed using a 1 - way analysis of variance (Table 29).

Table 29 The results of analysis of variance tests performed on the distance walked by five groups of larvae tested on the four treadwheels used.

Group	Sample Size	Degrees of freedom	F	p
Controls treated 0-24h, 4th instar	41	3:36	0.78	>0.05
Controls treated 0-24h, 5th instar	22	3:18	2.50	>0.05
107µg Law's mixture	29	3:25	0.26	>0.05
1069µg " "	31	3:27	0.89	>0.05
10690µg " "	32	3:28	2.17	>0.05

All further analysis of the distances walked on the treadwheels includes data from all four wheels, without reference to which wheels were employed or the numbers tested on each wheel.

ii) Differences in behaviour exhibited through the week. Analysis of the same five groups as used above revealed no significant differences between animals receiving the same treatment but tested on different days of the week. However when all the data from four of these groups was combined for each day of the week and examined regardless of treatment (n = 113), a significant drop in the distance walked was observed in animals tested on Sundays, as compared to the rest of the week. The fifth group was not included since this data was collected over 2h sessions. More detailed analysis of this phenomenum was not possible since after selection of the data by both dosage and day of the week, the sample sizes became too small for statistical tests to be applied.

iii) Differences in behaviour exhibited throughout the day. Analysis of the same four groups indicated an apparent difference in the behaviour that was related to the session in which the animals were tested. Only animals tested within sessions 1 and 2 (p. 63) were included, and the sample sizes were very uneven (Table 30). Combination of the four groups (n = 113) revealed a significant difference between the behaviour exhibited in each session, with a fall in walking behaviour in the second session (1500h - 1800h). However, the control group of animals tested in 5 two hour sessions revealed no significant differences, and no indication of a systematic change in behaviour during these 5 sessions. Furthermore, when the combined data (n = 113) was subdivided into animals tested on weekdays or Sundays no significant differences could then be observed in either group.

The walking behaviour of JH treated 5th instar larvae.

All animals considered in this section were treated 0 - 24h into the 5th instar unless otherwise stated; all animals were tested on the treadwheels on Day 3 of the same instar (72 - 96h after ecdysis). All the groups receiving JH or JH - analogue treatments and the relevant control groups were tested on the wheels for 3h. Those animals treated with precocene II acting as a JH - mimic were tested for only 2h. Therefore comparisons of the behaviour of these groups can only be made or data collected over the first 2h of any experiment (p. 71).

Table 30 The results of tests performed on the distance walked by five groups of larvae tested during either two 3h sessions per day or five 2h sessions per day.

a) An analysis of variance on the control group treated 0-24h, 4th instar, and tested in five 2h sessions.

Total sample size	Sample size within each session					F	p
	1	2	3	4	5		
41	8	8	4	13	8	0.58	> 0.05

b) Two sample t-test and Mann-Whitney U-tests on animals tested in two 3h sessions.

Group	Sample Size	Sample size in each session		Test	p
		1	2		
Combined data	95	54	41	t-test	0.013
Controls	17	15	2	U-test	0.031
107µg	26	15	11	"	>0.05
1069µg	27	14	13	"	>0.05
10690µg	25	10	15	"	>0.05

i) JH III. Because of the very low morphogenetic activity of the 3 synthetic JH's used, only one group was tested on the treadwheels. 43 animals were topically treated with 100µg JH-III and their walking behaviour was compared with that of a group of 27 animals topically treated with acetone. There were no significant differences between the total distance walked by either group (n = 27 : 43, p = 0.2179, 2 sample t-test), although the JH - treated group walked an average 34% further in 3h (130.085 ± 96.0m and 96.969 ± 115.0m). Both groups showed very high standard deviations about the mean. Such large variations in the behaviour of apparently similar animals have been observed in all groups tested and make it difficult to draw firm conclusions about behavioural changes induced by the various treatments used (p. 79).

The distance walked in each of the 3 hours by both groups was almost equal, although both walked slightly further in the middle hour than in the first or last hour.

ii) Law's mixture. Three groups were tested on the treadwheels after treatment with the JH - analogue, Law's mixture. The distance walked in each hour for 3h was analysed by (a) dosage, (b) the resultant juvenilization and (c) by the duration of the 5th instar.

Both control and treatment groups displayed almost uniform walking over the 3h, with slightly greater distances walked in the middle hour of each session. No significant differences could be seen between the treated and control groups in the pattern of development of this behaviour, nor in the variation in the distance walked observed in each group.

(a) There was a marked reduction in intensity of walking behaviour displayed in the highest dosage group, and the correlation coefficient calculated between the dosage and the distance walked in 3h was statistically significant ($r = -0.193$, $n = 114$, $p_r < 0.05$). However, an analysis of variance on the distances walked after 1, 2 and 3h indicated no significant differences at any dose (Table 31). A 2 sample t-test did indicate a significant reduction in the distance walked by the highest dosage group after 2 and 3h, from the distances walked by the control group (n = 22 : 32, $p \leq 0.0177$), thus indicating the differences in the power of some statistical tests. The

Table 31 The cumulative distance walked after 1, 2 and 3h by groups treated with Law's mixture in the early 5th instar. Animals were tested on Day 3 of the same instar and the distances walked are given in metres (± 1 S.D.). An analysis of variance was performed on the data for each hour and is summarized below.

Dosage of Law's mixture (µg)	Sample size	Cumulative distance walked (m ± 1 S.D.) 1h	2h	3h
0 (controls)	22	38.6 ± 33.35	110.5 ± 71.50	185.5 ± 116.0
107	29	48.4 ± 47.45	109.0 ± 107.0	151.5 ± 135.0
1069	31	46.2 ± 39.60	108.0 ± 83.5	153.5 ± 113.0
10690	32	37.2 ± 30.50	80.5 ± 66.5	113.0 ± 93.0
Analysis of variance:				
Degrees of freedom		3 : 110	3 : 110	3 : 110
F		0.58	0.90	1.83
p		>0.05	>0.05	>0.05

Table 32 The distance walked in 3h by morphologically defined groups of locusts treated with 0-10690 µg Law's mixture. The groups were defined by the score of juvenilization recorded after the following moult using the system of Tang (1976).

Morphological Score group (Tang,1976)	Sample size	Distance walked in 3h (m. ± 1 S.D.)
0	42	162.5 ± 122.0
1.0	29	176.0 ± 129.5
2.0	11	119.5 ± 96.0
3.0	27	112.5 ± 86.0

Table 33 The results of an analysis of variance performed between the 5th instar durations and the distance walked on the treadwheels in 4 groups treated with Law's mixture.

Dose of Law's mixture (µg)	Sample size	Degrees of freedom	F	p
0 (controls)	22	7:14	0.52	> 0.05
107	29	6:22	0.34	>0.05
1069	31	4:26	2.04	>0.05
10690	32	8:23	0.75	>0.05

data indicated a fall in the intensity of walking behaviour between animals tested in the 1st and 2nd sessions (p. 68), but animals tested in both sessions showed similar dose-related changes in behaviour.

(b) Analysis of the distance walked by Law's mixture adultoids classified into morphologically defined groups, using the scoring system of Tang (1976), revealed a negative correlation between the behaviour exhibited in the 5th instar and the juvenilization apparent in the adultoid stage ($r = -0.195$, $n = 114$, $p_r < 0.05$). The highest morphological group (score = 3.0) showed considerably reduced walking behavior (Table 32), although this reduction was just outside the level of statistical significance ($n = 42:27$, $p = 0.0504$, 2 sample t-test).

(c) No relationship could be seen between the duration of the 5th instar and the distance walked by either the control group or the treated groups (Table 33).

iii) ZR515 Four groups were treated with the potent JH - analogue ZR515 before their walking behaviour was monitored on the treadwheels. The treatments that each group received and their relevant control groups are given in Table 34.

The group treated with 427µg ZR515 applied topically on Day 0 was comparable in the form of treatment with those groups treated with Law's mixture. Fifteen animals were tested on the treadwheels and showed a marked increase in the mean distance walked in 3h (Table 34). This 84% increase above the control level was not statistically significant, however ($n = 15 : 27$, $p = 0.0647$, 2 sample t-test), due to the high variation in behaviour evident in both groups. The standard deviation of the distance walked in each group was almost equal to the mean. As before the animals in each group showed slightly greater walking in the middle hour of the 3h session.

The group treated with 427µg ZR515 applied topically on Day 3 showed a similar increase in the mean distance walked to the group treated on Day 0. There was no appreciable change in the behaviour of the two control groups which might be expected after treatment just 3h prior to the start of the behavioural trial. Again the increase shown by the ZR515 treated group, while representing a mean increase of 65% above the control level, was not quite statistically significant ($n = 27 : 16$, $p = 0.0625$, 2 sample t-test).

Table 34 The cumulative distances walked by groups treated with the JH-analogue ZR515 and by the control groups for each form of treatment. Distances are given in metres (± 1 S.D.). All animals were tested on Day 3 (72-96h) of the 5th instar.

Mode of Application	Dose of ZR515 (µg)	Day of Treatment 5th instar	Sample size	Cumulative distance walked over 3h		
				1h	2h	3h
Topical	0 (controls)	Day 0	27	29.334 ± 37.65	70.260 ± 79.0	105.335 ± 116.0
"	427	"	15	62.335 ± 58.00	136.035 ± 124.5	193.835 ± 172.5
"	0 (controls)	Day 3	16	36.969 ± 45.60	81.25 ± 93.0	115.875 ± 130.5
"	427	"	27	55.315 ± 38.00	130.26 ± 70.5	191.465 ± 110.0
Injected	0 (controls)	Day 0	12	54.915 ± 47.45	116.04 ± 102.0	168.585 ± 141.5
"	43	"	8	17.687 ± 14.85	75.625 ± 65.5	115.625 ± 96.0
"	213	"	11	55.225 ± 27.65	164.135 ± 51.0	239.865 ± 67.0

The same pattern in the development of the walking behaviour over the 3h, that has been described above, was observed in both groups.

Three small groups received either injections of ZR515 or of insect saline on Day 0, in a total injected volume of 2.5µℓ of a 1 : 10 acetone : saline mix (Table 34). Both the control group and the high dosage group showed marked increases in the mean distance walked when compared to groups treated topically (Table 34). The low dosage group did not show an increase in the mean distance walked, however, and remained close to the mean levels observed in the topical control groups. Neither of the two treated groups' behaviour was significantly different from that of the control group ($p \geq 0.1413$, 2 sample t-tests), but the high dosage group walked significantly greater distances than the low dosage group ($n = 8 : 11$, $p = 0.0095$, 2 sample t-test), and walked 42% further than the control group. All three groups showed slightly higher mean walking behaviour in the middle hour of the 3h sessions.

iv) Precocene II. 35 animals were treated with 200µg precocene II, topically applied 0 - 24h into the 5th instar. These animals reacted morphogenetically as if treated with a JH - mimic of considerable potency (p. 58). The animals were tested on the treadwheels in 2h sessions and their behaviour was therefore compared with that of a separate control group also tested over 2h, and with the 2h data from one group treated with 427µg ZR515 on Day 0 (Table 35). The control group of 20 animals walked markedly further over the 2h than the control groups considered previously (Table 34). As before, they showed slightly greater walking in the second hour than in the first.

The precocene II treated animals walked almost the same distance in 2h as this control group, although this represented an increase of between 47 - 70% above the distance covered by the two topical treatment control groups shown in Table 34. The precocene II treated animals did not walk a significantly different distance than either the control group or the ZR515 treated group ($n = 35 : 20 : 15$, $p \geq 0.6402$, 2 sample t-tests; Table 35). They showed the same slight increase in walking during their second hour on the treadwheels.

The walking behaviour of 'anti - JH' treated locusts.

A large number ($n>150$) of larvae were treated with precocene II in the 4th instar to induce the morphologically distinct 5th instar types

Table 35 The cumulative distances walked by the groups treated with precocene or ZR515 on Day 0 of the 5th instar and by a control group treated with acetone alone. Both the control and precocene treated groups were tested for 2h; the ZR515 group was tested for 3h but only the data from the first 2h are included. Distances are given in metres (± 1 S.D.)

Treatment, Day 0 5th instar	Sample size	Cumulative distance walked	
		1h	2h
0 (controls)	20	49.25 ± 46.10	114.625 ± 86.0
200µg precocene II	35	55.77 ± 41.85	119.355 ± 83.0
427µg ZR515	15	62.335 ± 58.00	136.035 ± 124.5

described previously (p. 28). These precocious adultiform larvae were tested on the treadwheels in 2h sessions, and their behaviour was compared to both normal larval and normal adult walking behaviour. The data from these experiments has been analysed by both the dose of precocene employed and by the morphological form of the animals at the time of the trials.

i) Internal treatments. Injections of 100µg precocene proved to be morphogenetically effective (p. 39), but caused higher mortality rates than topical applications. The control group suffered 75% mortalities coincident with the expected moult to the adult stage, while mortalities in topically treated control groups was approximately 10% at the same stage. For this reason, although some control and treated animals were tested on the wheels, it was considered inappropriate to attempt any analysis of their behaviour.

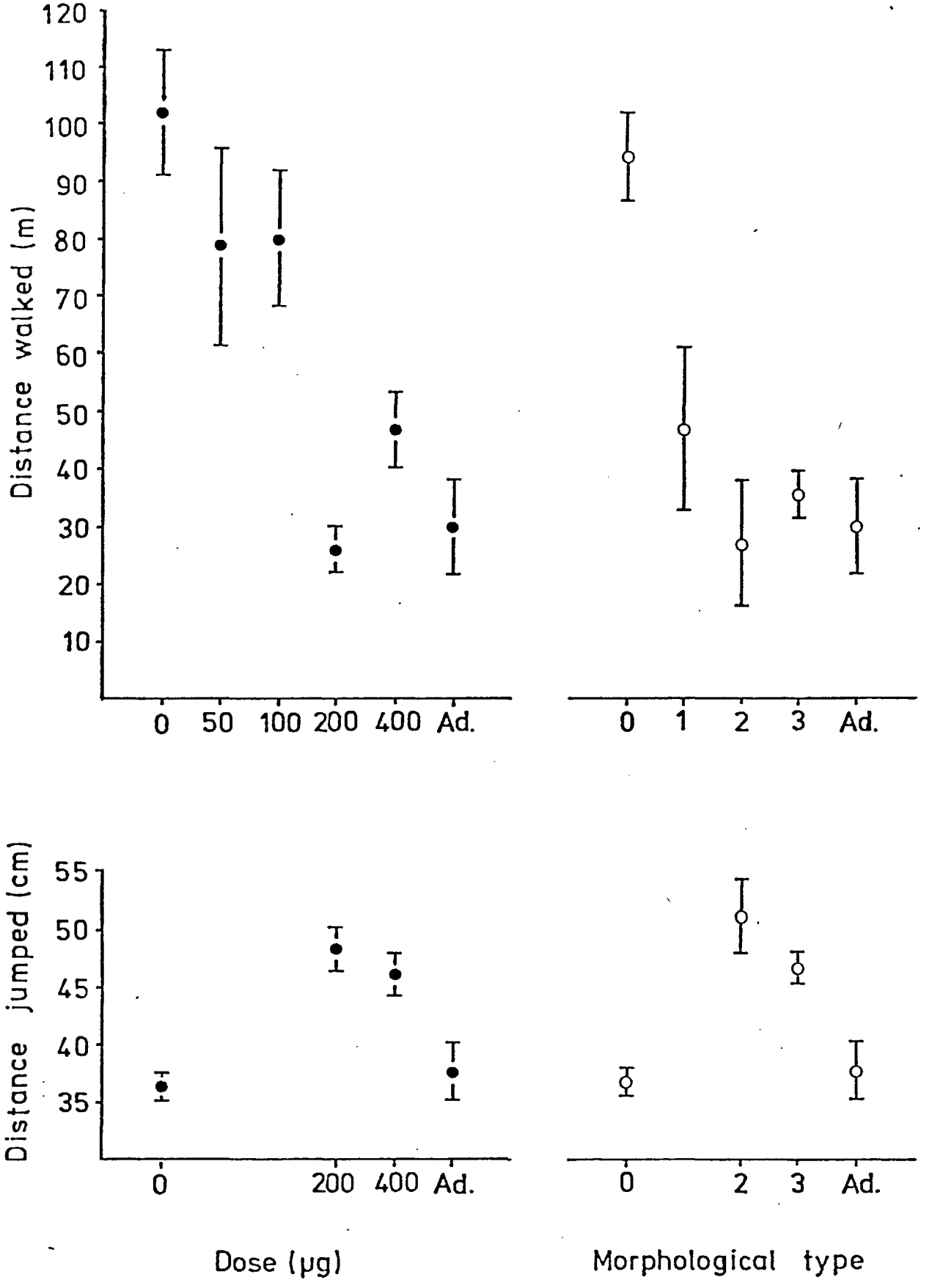
ii) Topical treatments. There was a sharp fall between the distance walked by normal Day 3 larvae and Day 3 adults (Figure 33; $n = 41 : 30$, $p<0.0001$, 2 sample t-test). While the adults initially attempted to fly free of the treadwheels, the restrained arm returned them to the wheel surface, and the majority abandoned attempts to fly after only a few seconds.

(a) There was a dose-related drop in the distance walked by the groups of adultiforms of mixed morphological types. The animals treated with 50 or 100µg precocene II did not walk significantly different distances from the control larvae ($p \geqslant 0.1703$, 2 sample t-tests) but walked significantly further than the control adults ($p \leqslant 0.0221$, 2 sample t-tests). Both the 200 and 400µg groups walked significantly less than the control larvae ($p < 0.0001$, 2 sample t-tests), while neither group significantly differed from the control adult group ($n \geqslant 0.1167$, 2 sample t-tests).

(b) This dose - related change was thought to be due to the changing proportions of each morphological type within each group, and analysis of the behaviour exhibited within each morphological group supported this (Figure 33). All type 0 animals, including both control larvae and treated but morphologically unaffected animals, showed considerably greater walking behaviour than the type 1 - 3 adultiforms and the normal

Figure 33

Distances walked and jumped by normal and adultiform animals. All figures are given as means $\pm$ 1 S.E. Above, distances walked (m) on treadwheels over 2h. Left, groups defined by precocene dosage : 0 and Ad, control larvae and adults; 50-400, doses of precocene II (µg) applied in the 4th instar. Right, groups defined morphologically. Below, jump lengths (cm) of dose-defined groups (left) and of morphologically-defined groups (right).


Distance walked (m)
120
110
100
90
80
70
60
50
40
30
20
10
0
50
100
200
400
Ad.
0
1
2
3
Ad.
Distance jumped (cm)
55
50
45
40
35
0
200
400
Ad.
0
2
3
Ad.
Dose (µg)
Morphological type

adults. There were no significant differences between the behaviour of these last 4 groups (types 1 - 3 and Ad; $p \geq 0.3055$, 2 sample t-tests); in other words all the morphologically identifiable adultiform larvae showed walking behaviour that was similar to normal adult levels. By contrast, all 4 groups showed significantly lower walking behaviour than the type 0 group ($p \leq 0.0070$, 2 sample t-tests). The type 0 group including unaffected, treated, animals did not significantly differ from the larval control group alone ($n = 68 : 41$, $p = 0.5638$, 2 sample t-test); the type 0 data excluding these 41 control larvae was not examined, however. Finally the type 2 group's walking behaviour, which was lower than that of the type 1 or 3 groups', was not significantly different from either ($p \geq 0.2724$, 2 sample t-tests).

(c) Analysis of the distance walked by these morphological types (0 - 3) also showed a dose - dependent change. Animals treated with 200µg precocene II walked considerably less than morphologically similar animals produced at other doses. Although most of these differences were not statistically significant ($p > 0.05$, Mann-Whitney U - tests), many were close to this significance level, and the type 3 animals produced by 200µg precocene walked significantly less than those type 3 animals produced by 100 or 400µg ($p < 0.05$ Mann-Whitney U - tests). Increased sample sizes might have indicated a true difference in the behaviour of these apparently similar adultiforms that is related to the dose of precocene applied in the previous instar.

The jumping behaviour of precocious adultiform larvae.

Some of the animals considered in the previous sections were made to exhibit escape jumps and 10 such jumps were measured for each individual. The average of the best (longest) five jumps made by each animal was taken as a measure of its physical and/or excitatory state. From Figure 33 it can be seen that precocious adultiform larvae (types 2 and 3) exhibited much longer jumps than the normal 5th instar larvae ($p < 0.005$, Mann-Whitney U - tests). However, during these tests it was noticeable that the most advanced adultiform larvae and the normal adults tended to make small leaps, coupled with wing movements, that could easily be distinguished from the hopper's escape jumps. It should be stressed that although the mean distances jumped by the normal larvae and adults were almost identical

Figure 34

Cumulative frequency distributions of all jump lengths for normal 5th instar larvae (——→), normal adults (-----), type 2 (—·—) and type 3 adultiforms (—··—).

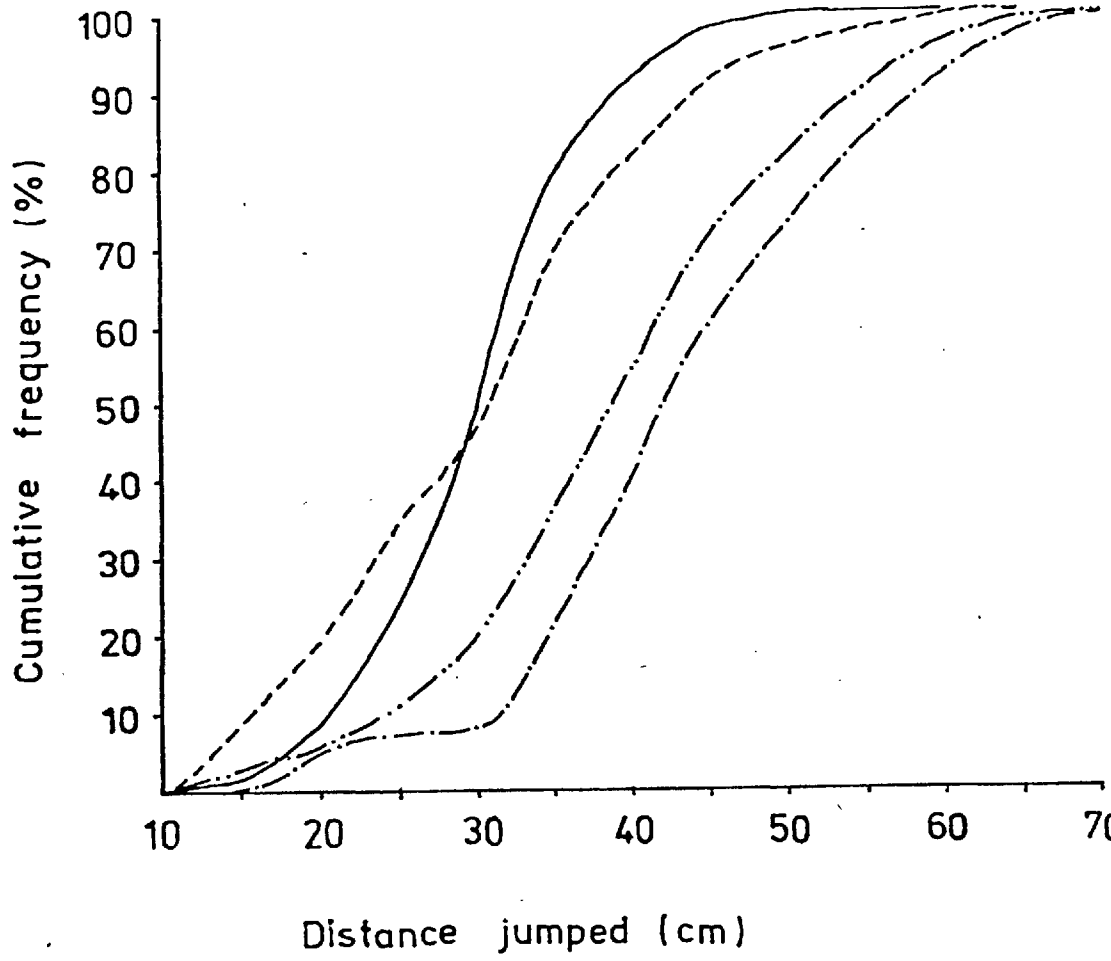

Cumulative frequency (%)
100
90
80
70
60
50
40
30
20
10
10
20
30
40
50
60
70
Distance jumped (cm)

Table 36 The numbers of adultiform larvae observed to combine wing beating with the adoption of the typical adult flight posture. The animals were held by the hind legs and had air gently blown onto the head; wing beating and flight posture were graded as being absent (-), slight (+), medium/strong (++) or strong (+++).

		Flight posture		
		-	+	++
Wing beating	-	4		
	+	3	1	
	++	1	1	1
	+++		3	3

(Figure 33), the two forms of behaviour were very distinct.

A typical larval jump was long and low, with the animal travelling vertically an estimated 60% of the horizontal distance; the larvae landed upright with the legs outspread. The typical adult jump was apparently intended as the prelude to flight, with the animal travelling at least as far vertically as horizontally. The tethered wings could be heard to beat from the start of the jump and these animals usually 'crash - landed', with the legs close to the body. The adultiform larvae were seen to display both forms of jump, although the short 'take-off' jumps were not always associated with audible wing movements. It was noticeable that advanced adultiforms could display both forms of jump, often with an equal frequency of occurrance.

From the frequency distributions of all 10 jumps made by each animal within the groups (Figure 34), it can be seen that the control larvae were grouped closely around the median of a 30cm jump. Very few longer (>40cm) or shorter (<20cm) jumps were made. By contrast, the adultiform larvae showed a larger distribution of jump lengths, with nearly 20% of the jumps being greater than 50cm. There is also a distinct plateau at the lower end of the distribution curve where nearly 10% of the adultiform jumps were of 30cm or less. This plateau was present in curves determined from adultiforms grouped either by dosage or by morphology, and represented part of a series of short jumps that were apparently attempts at flight.

Normal adults rarely displayed larval-type jumps and the frequency distribution (Figure 34) shows a large percentage of short jumps. There is a slight plateau at the higher end, however, representing a few (<5%) long, larval-type jumps of lengths equal to those made by the adultiform larvae.

A number of Type 2/3 adultiform larvae were held by the hind legs while air was gently blown onto the head: this position usually initiated wing beating and the adoption of the flight posture (Kutsch, 1971) in normal adults. The distribution of the occurance of wing beating and the adoption of the flight posture is indicated in Table 36. There was a strong correlation between the strengths of both responses.

The kicking behaviour of precocious adultiform larvae.

Cages of precocious adultiform larvae showed more general excitability and spontaneous movements than cages of normal locusts. This was particularly evident in the frequency of their kicking movements, which among normal

Table 37 The % time spent in kicking behaviour and the number of kicking movements recorded in Day 3, 5th instar larvae, in Day 10 adultiform larvae and Day 10 adults. Observations were made over 15 minutes for crowded animals (a) and over 5 minutes for isolated animals (b).

a) crowded conditions

Group	Sample size	% time in kicking (mean ± 1 S.D.)	No. of kicking movements (mean ± 1 S.D.)
Larvae	12	1.2264 ± 1.36	No data available
Adultiforms	15	5.6162 ± 3.54	
Adults	9	2.1886 ± 1.30	

b) isolated conditions

Larvae	12	0.04167 ± 0.0793	0.5000 ± 1.00
Adultiforms	16	0.58125 ± 0.6870	4.8125 ± 6.47
Adults	14	0.09048 ± 0.1760	0.8571 ± 1.75

animals was almost always the result of contact between two animals. The adultiform larvae were often seen to kick even when clearly separated from other animals, however; observations of the frequency of this behaviour were made and the results are indicated in Table 37.

i) Crowded conditions. The majority of the kicking behaviour in crowded cages was related to brief periods of movement amongst most of the animals : as one locust changed position the others also began to move, with some kicking occurring as they spaced themselves out over the twigs. These periods of activity usually lasted 10 - 30s and occurred about every 1 - 2 minutes. However, under identical conditions, the adultiform larvae spent more than twice as much time in kicking than either normal larvae or adults. This increase was statistically significant ($n = 9 - 15$, $p \leqslant 0.0031$, 2 sample t-tests), while the larval and adult behaviours did not significantly differ ($n = 12 : 9$, $p = 0.1199$, 2 sample t-tests).

ii) Isolated conditions. When isolated all locusts remained stationary for longer periods than when crowded. However, some movement was seen, and occasionally one or two kicking movements were made by normal larvae or adults. The adultiform larvae tended to make longer burst of kicking, of 3 - 6 kicks, and these bursts occurred more frequently than in normal animals. Thus, both the time in kicking and the number of kicks made were significantly greater amongst the adultiform larvae ($n = 12 - 16$, $p \leqslant 0.0312$, 2 sample t-tests), while the normal larval and adult behaviours did not differ ($n = 12 : 14$, $p \geqslant 0.3634$, 2 sample t-tests).

The respiratory rates of precocious adultiform larvae.

Normal larvae and adults displayed very shallow abdominal motions during resting that were involved in respiratory movements of air through the trachae. This motion was not regular and increased with or after increasing activity; there was also an abdominal motion that was presumed to be associated with movements of the gut contents and it was difficult to distinguish between the two. The adultiform larvae showed striking abdominal movements, however, that involved contraction and flexion of the abdomen. These movements tended to be more regular than the respiratory motions of normal animals and were at a much slower rate (Table 38; $n = 15-24$, $p \leqslant 0.0008$, 2 sample t-tests). The respiratory movements of normal or adultiform animals cannot be observed during locomotion and only records from animals that remained stationary for the duration of the 1 minute observation period were included.

Table 38 The rate of respiratory movements recorded in resting 5th instar larvae, adultiform larvae and adults. Observations were made over 1 minute and the rates are given as movements/ minute ± 1 S.D.

Group	Sample size	Respiratory rate (mean ± 1 S.D.)
Larvae	15	51.067 ± 12.50
Adultiforms	24	26.625 ± 9.72
Adults	15	45.000 ± 17.40

Food intake and faecal production in precocious adultiform larvae.

The quantities of wheat seedlings eaten over 6 and 18h by cages of 20 animals were transformed into figures of the fresh weight of food eaten per hour per animal and the fresh weight of food eaten per hour per gram body weight. Both figures were corrected for the desiccation of seedlings during that period (Table 39). Estimates calculated over the 6h and 18h changed little, although the rate of feeding of the normal larvae fell by approximately 10% over the period. Each group contained equal numbers of both sexes, since there is a clear sex difference in body weights.

The normal Day 3 larvae and adults ate more than the Day 3 adultiform larvae, while the weights eaten per gram body weight were similar for the larvae and adultiforms. The adults ate significantly more than either the larvae or the adultiforms (120 and 156% more, respectively), but by body weight they consumed only 44 and 54% more.

The Day 10 adults and adultiform larvae ate considerably less than their respective Day 3 groups, while their body weights remained almost the same (+ 9% and -8% respectively). No dry weights of the animals were taken.

The dry weight of faeces produced per hour remained constant over 18h. The ratios of faecal weights from larvae, adults and adultiform larvae were similar to the ratios of their food intake (Table 39). The Day 3 adults excreted considerably more than the larvae or adultiforms, although by body weight the larvae and adults excreted similar quantities. By Day 10 the quantities produced by the adults and adultiforms had fallen to 65% and 36% of the Day 3 levels.

Table 39 The fresh weight of wheat seedlings eaten per hour (a) and the dry weight of faeces produced per hour (b) by normal larvae, adultiform larvae and normal adults. The rates of feeding and excretion are given as weights per animal and weights per gram body weight. Each group contained 20 animals and the experiments ran for 18h.

(a) Food intake

Group	Weight (mg) per hour per animal	Rate relative to larval rates	Weight (mg) per hour per gram body wt.	Rate relative to larval rates
Day 3 Larvae	94.658	1.000	81.077	1.000
" Adultiforms	82.287	0.869	75.840	0.935
" Adults	210.88	2.228	117.161	1.445
Day 10 Adultiforms	22.404	0.237	22.348	0.276
" Adults	101.512	1.072	51.489	0.635

(b) Faecal production

Day 3 Larvae	8.6659	1.000	7.4225	1.000
" Adultiforms	6.3555	0.7334	5.8576	0.7892
" Adults	14.053	1.6216	7.8070	1.0518
Day 10 Adultiforms	2.1428	0.2473	2.1375	0.2880
" Adults	10.072	1.1623	5.1088	0.6883

SECTION 3 DISCUSSION

A. The walking behaviour of JH - treated 5th instar larvae.

The walking behaviour exhibited on the four treadwheels was quantitatively and qualitatively similar to that exhibited on the more complex wheel used previously (see Section 1). Although there were major differences both in the design of the wheels and in the experimental procedures, the total distances walked by the animals, which have previously been considered as valid measures of their marching behaviour (p. 19), were similar. The most important differences between these experimental systems were that:-

i) In the first system the locust was held stationary (by the ventral thoracic sternites) while the wheel could move vertically and laterally; in the second system the wheel was held stationary and the locust was allowed limited vertical and lateral movements (while held by the dorsal pronotal shield).

ii) The data collection was reduced from an 'on-line' record of each millimetre of forward movement to the recording of the total number of complete revolutions of the wheel made in each hour.

iii) The lighting in the first system was from a point source positioned directly above the wheel and powered by DC batteries; in the second system an AC - powered fluorescent tube was positioned horizontally above and behind the wheel.

iv) The pretreatment of the locusts was changed slightly, and there was considerably less handling of the locusts immediately before the trials in the second system.

The second experimental system was thus simpler and allowed the testing of many more animals without any appreciable change in the locusts' behaviour. It is also possible to state that the behaviour was therefore similar to that observed in the field and in unrestrained laboratory experiments. The only facet of the behaviour that could not be studied in detail in the second system was the temporal pattern of development of the marching behaviour within the sessions. It has been shown that there is a steady increase in both the speed of walking and the time spent in walking, such that the distance walked in each 1000s period increased during the first 1 - 1.5h (p. 13 ; Moorhouse *et al*, 1978). This high

level of activity was maintained for a further 1h before it gradually declined over the remainder of the session. All groups tested on the second set of treadwheels walked farthest in the middle hour of the 3h sessions; likewise, those animals tested for only 2h walked further in the 2nd hour than in the 1st. As far as can be estimated, therefore, the development of the walking activity in the two systems was similar.

From the literature (Goldsworthy, 1976; Kammer and Heinrich, 1978; Goldsworthy *et al*, 1979) it is evident that the adipokinetic hormone released during long flights of adult locusts is responsible for the change from carbohydrate to lipid metabolism, and in the mobilization of these lipid fuel reserves. This change in fuel metabolism occurs over the first 30 minutes of flight and is coincident with a change from rapid, trivial flight to slower, migratory flight that can be sustained for several hours. No data has been reported on the possible influence of adipokinetic hormone on the fuel metabolism in walking larvae, although the changing activity levels observed both over 3h and over the instar could be related to such a system. However, in the flying adults, adipokinetic hormone release is coupled with a reduction in flight speed to allow sustained activity: in the larvae the changes observed involve an increase in walking speeds, and the most sustained walking behaviour (on Days 4 - 6; p. 12) occurs at high average speeds. JH treatments did not apparently cause any change in the pattern of development of walking behaviour of Day 3 larvae, and there have been no reports on the influence of JH on the fuel metabolism of adult locusts.

Locusts have been reported to exhibit a diurnal rhythm to their locomotor and general activity levels (Bernays, 1979), and there were indications that greater distances were covered in the earlier 3h sessions (1200 - 1500h) than in the later 3h sessions (1500 - 1800h). However, the variation in the intensity of walking behaviour exhibited by individuals within each group proved to be so large that no significant differences could be found within most of the groups tested. The low levels of walking activity recorded on Sundays were suspected to be related to the relative quiet of the building on that day. These differences in behaviour may have influenced the statistical analysis of the diurnal rhythms, since separate analysis of the groups tested on weekdays or on Sundays revealed no such diurnal changes. While all efforts were made to avoid disturbance to the animals during the trials, the experimental room was not soundproofed and the noises of people talking, movements in the corridor and various machine noises may have acted to stimulate walking behaviour during the main working days (Monday - Saturday). Detailed analysis of these diurnal

and weekly changes in behaviour could not be made because of the small and uneven sample sizes of the subgroups considered. However, since the distribution of trials for each treatment tended to be roughly even throughout the day and the week, these differences have been disregarded in all later analyses.

There has been a great deal of work published in recent years on the hormonal control of migratory flight in insects, reviewed by Truman and Riddiford (1974), Goldsworthy (1976), Truman (1978), and most recently by Rankin (1979). While there is good evidence to suggest that the CA (and therefore JH) influence the flight behaviour of various insect species, the evidence amongst locusts was confusing. After consideration of the conflicting ideas reported in the literature (see Introduction), Goldsworthy (1976) and Rankin and Rankin (1979) have concluded that the effects of the CA and JH on flight of adult *Locusta* are not simple, but that allatectomy leads to an initial reduction in flight, possibly mediated by changes in the flight muscle development and the processes of aging in this species.

There is very little data published from experiments on larval behaviour to support or refute these ideas. Truman and Riddiford (1974; Truman, 1978) have briefly reviewed the subject and have concluded that the falling JH titres seen during the last part of the larval instars are responsible for the changing behavioural patters exhibited and for the expression of premetamorphic behaviour in various hemi- and holometabolous insects. However, there are no data on the changing intensity of a larval behaviour such as the marching behaviour studied here.

The behaviour of the 5th instar larvae treated with JH III, ZR515 and precocene II (acting as a JH - mimic) has revealed a large increase in the average distance walked from the control groups' levels. Briefly, the JH III treated group walked 34% further than their controls; the two groups treated typically with ZR515 (425µg applied on Day 0 and Day 3, respectively) showed increases of 84% and 65% above their control groups; one group injected with 213µg ZR515 walked 42% further than the control group. A group treated topically with 200µg precocene II walked between 47 and 70% further than the above control groups, but did not differ from its own control group which displayed particularly high walking behaviour. Because of the large variation in the behaviour within each group none of these differences were statistically significant. Furthermore, one group injected

with 43µg ZR515 showed very low walking behaviour. This form of treatment had low morphogenetic activity, however, and there were no signs of toxicity : there are, therefore, no apparent reasons for such reduced behaviour. All animals treated with Law's mixture showed a marked reduction in walking behaviour, and the highest dosage group showed a statistically significant reduction from the control levels. It is suspected from the studies of the morphological effects of this treatment that Law's mixture contains a number of toxic compounds, in addition to its JH - analogues (Law *et al*, 1966). These toxic effects may have acted to reduce walking behaviour more than the component JH - analogues could have increased it.

It is therefore proposed that exogenous JH or JH - analogues, or treatment resulting in the release of endogenous JH, can enhance the locomotor activity of the mid - 5th instar larvae. At this stage the larvae are at the peak of the walking activity curve determined over the course of the instar, and the enhanced walking behaviour reaches levels higher than are normally evident at any stage of the instar. The effects are not clearly dose - responsive but are related to the extent of juvenilization observed at the following moult. There is some evidence that the effects are independant of changes in the development of the locust, and therefore these effects may be due to direct or indirect effects of JH on the endocrine, nervous or muscular systems.

The effects of other possible hormonal influences on larval and adult behaviour, namely the corpora cardiaca hormone(s) and the prothoracic gland hormones (ecdysones), have been studied in some detail (reviews: Goldsworthy, 1976; Truman, 1978; Rankin and Rankin, 1979). No comparable studies were undertaken here, however. It is apparent that the larval behaviour of many insects, including *Locusta*, is influenced by these hormones and by JH, such that the balance of hormones may control the general form and intensity of the expressed behaviour. Bernays (1980) has recently reported that neurosecretions from the corpora cardiaca are involved in the fall in locomotor activity following feeding in *Locusta*. As observed here, JH can enhance the average walking behaviour of a group of larvae, but by no means is there a rigid relationship between the (assumed) haemolymph titre of JH and the intensity of marching. It is therefore unlikely that any of the hormones present can alone greatly influence the behaviour of the locust, but they must act in concert and in combination with developmental processes to control the exhibited behaviour.

B. The rationale behind the use of 5th instar *Locusta*

At the start of this project 5th instar *Locusta migratoria* larvae were selected as experimental animals since the behaviour and experimental procedures had already been extensively studied. The details of hormonal action in both larvae and adults had been studied; the rearing procedures were uncomplicated and the insects are robust in this final instar. The 5th instar was also chosen because the JH titres were known to be about zero; exogenous JH would therefore be more effective than at other stages when the haemolymph JH titre was already high.

However, having undertaken these studies it is apparent that the 5th instar was not the ideal choice. As discussed, a major difficulty in this work is in distinguishing between the direct behavioural effects of JH and the JH - induced differentiation towards a supernumerary larval form. It may have proved more useful to study the 4th instar behaviour, when exogenous JH treatments would have only minor effects on the locusts' morphology while still raising the haemolymph JH titre. This approach has been useful in adult insects, where the JH produced by the CA can be supplimented by JH treatments or CA implantation (review: Goldsworthy, 1976), or reduced by allatectomy or precocene treatments (Rankin, 1979). These treatments can have only limited morphological effects in the adults, and can be reversed by, respectively, allatectomy and CA re-implantation.

In the 5th instar the endocrine system, nervous system and muscular system are all subject to considerable developmental changes. However, the treatment of 425µg ZR515, applied on Day 3 only 3h before the behavioural trials, was found to be effective in enhancing the walking behaviour of these 5th instar larvae. It is unlikely that major differentiation could occur in this time, to cause this change, but it is not possible to rule out indirect hormonal effects on the behaviour, for example mediated by the neurosecretory system.

Precocene could also have been used effectively in the 4th instar to reduce JH titres, if treatment occurred at the start of the instar, so that the CA would degenerate before the behavioural trials in the mid - instar. However, when this technique was attempted in the 5th instar, to study the effects of precocene itself on the mid - instar behaviour, the JH - effects of this form of treatment became apparent. Care would be needed to ensure that there is no comparable JH release following precocene treatment in the early 4th instar. It would also

become difficult to distinguish the effects of a reduced JH titre on the behaviour from the effects on behaviour of the development towards a premature adultiform stage that such treatments induce. It is therefore necessary to compare the effects of these treatments at more than one stage of the larval development, to determine to what extent the behaviour is governed by the hormonal titres or by the changes in growth that these hormones produce.

C. The behaviour of precocious adultiform larvae

The previous section has considered the changes in 5th instar walking behaviour induced by the presence of JH before or during the behavioural trial. This section aims to discuss the behaviour of precocious 5th instar adultiform larvae produced by precocene treatment in the 4th instar. The adultiform larval stage does not represent a normal 5th instar larva that can be considered to have received 'negative' or 'anti - JH' treatments: it is not the opposite of those animals treated with JH or JH analogues. Rather, it is a developmental stage intermediate between the larval and adult stages and must therefore be considered in relation to the normal and adult behaviours.

From the data recorded from the treadwheels, normal adults walked much less than normal 5th instar larvae. By the relatively coarse measure used here, of the distance walked in 2h, precocene treatment apparently shifts the 5th instar behaviour from the larval to the adult pattern. The drop in the distance walked by the high - dosage groups was due to the increasing proportion of adultiforms within each group : when the groups were redefined by morphological type, the type 1 and 2/3 adultiforms walked distances similar to those of the adults. The type 0 animals walked distances similar to those of the normal larvae, which they resembled morphologically. The very small distances walked by the type 2 animals may have been an artifact due to their being distinguished from the type 3 animals only by their lack of wing flapping during handling. Although intended to distinguish between two developmental stages of adultiforms, this criterion may have selected only the less excitable animals from what may be a single developmental stage.

The reduced walking exhibited by the adultiforms (types 1 - 3) is not explicable as a lack of leg musculature or as a neuromuscular defect as the jumping responses were strong. However, from observations of their activity under crowded and isolated conditions, and of their jumping

behaviour, it may be related to a reduction in the continuity of their walking behaviour (p. 84).

A study of the escape responses of the locust was attempted by measurements of the horizontal distance travelled during a jump. This criterion alone could not effectively distinguish between the responses of normal 5th instar larvae and normal adults that were restricted from flight. Both apparently covered approximately equal distances during their jumps. However, in performing these experiments it was clear that the function of the responses was very different, and that while larvae typically escaped by long jumps, the adults attempted to escape by flight.

The responses of the adultiform larvae ranged from a typical larval-type jump to an adult-type 'take-off', although the wings of these adultiforms were not sufficiently developed for flight to occur. The criterion of the average distance covered during the jumps was therefore concealing an important difference in the behaviour of the animals, and frequency distributions were employed to present these differences.

The larval jumps were Normally distributed about their average value and there was no reason to suspect that the infrequent short jumps they made were anything other than short, larval type jumps. By contrast, the adults displayed a large number of short jumps coupled with wing movements, with less than 5% of their responses being of the long, low, larval type. The typical adult response was therefore to fly, although they could and occasionally did, display the larval form of escape jump. When the adults did attempt such a larval jump, without wing movements, they covered distances of greater than 50% more than the normal larvae.

All the adultiform larvae displayed the typical larval jumping response. The type 0 animals displayed only this type of jump, and covered distances similar to normal 5th instar larvae, which they morphologically resembled. The type 2 and 3 adultiforms displayed longer, larval-type jumps and could cover distances similar to those of the longest jumps made by the much larger adults. However, these type 2 and 3 adultiforms also performed a large percentage (up to 50% in some individuals) of the typical adult-like jump. These were short, steep and accompanied by audible wing movements in some type 3 adultiforms; the animals often 'crash-landed' after such a 'take-off' jump.

Furthermore, the brief test of flight posture adoption indicated that

these adultiform larvae could express the adult behaviour pattern, although some individuals showed wing beating without the adoption of the flight posture. Kutsch (1971) has reported the adoption of flight posture in 2nd - 5th instar *Schistocerca gregaria*, but few signs of such flight posture could be seen in the normal mid - 5th instar *Locusta* used in this study. Thus, the central nervous system of the adults and adultiforms does not exhibit wholly larval or adult behaviour, but has the ability to display characteristics of both. While normal adults only occasionally display larval jumping behaviour, the precocious adultiforms readily and separately display both larval and adult jumping behaviours. This possibility of a varied behavioural repectaire was not exposed by the relatively coarse measurement of behaviour recorded by the treadwheels. Furthermore, the greatly increased kicking behaviour observed in these adultiform larvae, and their highly excitable state when in the stock cages, indicated an enhanced activity level that was not supported by the treadwheel data. The ventilatory cycle of these adultiforms was considerably slower but more pronounced than that of normal animals, and later neurophysiological data supported the suggestion of an increase in motor activity levels (p.100). It is therefore suggested that the reduced walking behaviour recorded on the treadwheels may be due to frequent disruption of the walking bouts, rather than due to inactivity. Thus, in these experiments, the parameter of the total distance walked in 2h may not accurately reflect the behaviour of the adultiform larvae. Unfortunately the detailed description of the marching behaviour possible in the first section of this study (p. 10) could not be repeated for the adultiforms. I would suspect that such a study would show frequently interupted bouts of walking as different behaviours such as biting, peering and grooming were expressed, to such an extent that the long walking bouts associated with the greatest walking behaviour (Moorhouse *et al*, 1978) could not develop.

Finally the intake of food was considerably lower in adultiform larvae than in normal larvae or adults, although by body weight the larvae and adultiforms consumed almost equal quantities. By the 10th day after ecdysis feeding had fallen to very low levels. Presumably the initial high feeding rates were related to somatic growth, while later feeding, in the adultiforms and adults, was related only to the maturation of tissues and to metabolic requirements. These studies were not accurate enough to estimate metabolic rates by an analysis of intake of food and output of faeces.

SECTION 4 MATERIALS and METHODS

Animals.

All the animals used during the experiments described below were obtained from crowded cultures maintained by the C.O.P.R., London, or were raised from egg pods from these cultures, or were bred from adults raised by either of the above methods. They were kept under conditions described previously (p. 7), at densities of 5 - 20 animals per cage. All animals were provided with abundant supplies of fresh wheat seedlings up to the moment of removal for experimentation, on the 3rd day after the moult (72 - 96h after ecdysis). Three types of animal were used in these experiments:

a) normal 5th instar larvae;
b) precocious 5th instar adultiform larvae produced by treatment 0 - 24h into the 4th instar with 200µg precocene II, topically applied in acetone. Only type 2/3 adultiforms were used (p. 28);
c) normal adults.

Unless specified, both sexes were used in these experiments.

Experiments.

The experiments fell into two main categories:

i) Neurophysiological studies. Three studies of the motor innervation of the metathoracic leg were undertaken using extracellular recording techniques.

a) The spikes of four neurones known to terminate on the extensor tibialis muscle were identified and characterized.

b) The spontaneous activity levels of the slow extensor tibialis motor neurone (SETi) and the dorsal unpaired median extensor tibialis motor neurone (DUMETi) were monitored over 5 minute periods in 5 or 6 animals from each group. The average rates of activity per second were plotted and statistical analyses of the average rates in normal and adultiform animals were performed.

The activity of all neurones in nerve 3Nv5 with spike heights of greater than 15µV was also recorded from a further 5 animals in each group.

c) The activities of the fast extensor tibialis motor neurone

(FETi) and DUMETi, both recorded in nerve 3Nv5, were compared in 5 animals from each group during air stimulation of the anal cercii. The stimulus was first presented 5 minutes after preparation and at least 1 minute separated each of the 3 stimulus presentations.

Peri-stimulus time histograms of the average activity of both neurones were prepared for each group of locusts. No statistical analyses were attempted.

ii) Myological studies. The tension developed in the extensor tibialis muscle during electrical stimulation of the nerve 3Nv5 was monitored. Responses to stimulation at 0.5, 1.0 and 2.0Hz were observed and the frequency at which tetanus developed was determined in each animal. The average muscle tensions developed by the 6 animals in each group were compared statistically.

Dissections and preparations.

The locusts were pinned to a wax block without an anaethetic; care was taken to ensure that the pins did not enter the metathoracic legs, and the wings if present, were trimmed. The body was then opened along the dorsal midline and the gut removed to expose the nervous system. Fatty tissues were eased away from the metathoracic ganglion and the large trachea running adjacent to the nerve 3Nv5 was removed. The body cavity was then flooded with saline (Usherwood and Machili, 1968), and this fluid was replaced as necessary during the dissection. The nerves could then be lifted onto the wire electrode hooks for electrical stimulation or cut and drawn into a suction electrode for recording.

When determining the muscle tension the metathoracic femur-coxa-trochanter joints were immobilized with a drop of molten beeswax, and the locust was decapitated. The femural cuticle was then severed close to the femur-tibia joint, and the tibia removed just beyond this joint. The entire joint capsule was then held in a tension-sensitive transducer and the transducer was carefully positioned to lie in line with the immobile femur. A standard tension was maintained by drawing the transducer back until a gap of 1mm separated the distal and proximal portions of the femural cuticle.

Experimental procedure

i) Recording neuronal activity levels. All neurophysiological

records were made using a glass suction recording electrode and a silver/silver chloride wire indifferent electrode. The glass electrodes were drawn from 1mm diameter tubing and the tip was trimmed until the internal diameter was approximately equal to the nerve's diameter. The electrode was held by a rubber seal into a small perspex block allowing a 1mm connecting plug and a flexible syringe-powered suction line to be attached. A silver/silver chloride wire ran down the electrode to within 1cm of the tip, and the electrode was back-filled with saline. Both recording and indifferent electrodes were held in a Zeiss dual micromanipulator; the nerve was usually cut approximately 2mm from the ganglion and either the distal or proximal end drawn up into the suction electrode. Some *en passent* recordings were made with a suction electrode large enough to accommodate a loop of the uncut nerve; these preparations tended to leak and the nerve often disengaged from the electrode after several minutes.

The dissections were made under a Wild stereo dissecting microscope and lit by a Fibrox fibre optic light source. The equipment was housed in a large Faraday cage and supported on a concrete based table.

ii) Neuronal signal storage. The signal from the electrodes was amplified by either one or two Palmer CFP 8121 preamplifiers; usually a single amplifier was used but in some experiments they were used serially. The AC - coupled and high-frequency filtered output was displayed on a Telequipment twin beam storage oscilloscope. Three methods of permanent recording were employed:-

a) Traces displayed on the oscilloscope were photographed.

b) The signal could be recorded on direct-print light sensitive paper using a Bell and Howell BHL 5000 SC UV-light recorder.

c) The rate of spiking of a neurone or population of neurones could be determined using a Haer amplitude analyser and rate/interval analyser. The multiplexed output from the amplitude analyser was displayed on the oscilloscope and the window was adjusted to select the desired spike heights. The synchronous output pulses were fed into the rate analyser, and the stepped, linear output voltage, representing the number of spikes per second, was displayed on a Servoscribe flat-bed

pen recorder. This system had a maximum frequency response of 50Hz; most records were well below this level, however. The paper record was then calibrated and the data transferred to a permanent computer file using a Ferranti Cetec X-Y digitizer. These files therefore contained a record of the neuronal activity for each second over the duration of the 5 minute recording, allowing the numerical description of each recording and the production of average activity rates from all the individuals in each group.

iii) Recording muscle tensions. An isometric Washington Type D tension-sensitive transducer was used to determine the tension developed by the extensor tibialis muscle of the metathoracic leg. The transducer was linked to a vertically suspended pair of forceps by a fine wire, and was held by one side of the dual micromanipulators. The forceps could be locked onto the muscle apodeme with a threaded screw. The output from the transducer was amplified and displayed on the oscilloscope as well as recorded by the UV-light recorder.

There was a standing 5V DC component to the transducer output, however, that could not be eliminated with the equipment available. It was therefore only possible to record the AC - coupled output. Thus, the standard method of calibration - of adding small weights to the transducer arm and recording the DC output - could not be used for this system. Instead an approximate calibration was undertaken by dropping small weights from a fixed height onto the transducer arm; the peak output voltages were measured and the transducer calibrated in units of force (Newtons). This estimate was then used to calibrate the recordings taken from the muscle preparations.

iv) Stimulii. The following forms of stimulation could be employed in these experiments:

a) Two silver/silver chloride electrodes were implanted in the extensor tibialis muscle and a Grass SD9 stimulator was used to provide sub- and supra - threshold stimulii during the identification of nerve branches carrying the motor axons. The stimulus pulse was also recorded to indicate the time of the nerve stimulation.

b) A glass pippette was positioned 2cm from the anal cercii and linked to a pipette teat by flexible tubing. Relatively constant puffs of air could be directed at the cercii by single, rapid, compressions of this teat. A microphone was positioned beyond the cercii and the signal used to indicate the time of stimulation. Since there was some delay involved, a standard 10ms delay was assumed in preparing peri-stimulus time histograms of the responses.

c) The motor nerve carrying the axon responsible for the rapid extension of the metathoracic leg, 3Nv5 (Hoyle, 1953, 1954) was supported clear of the body fluids by two silver/silver chloride wire hooks and stimulated electrically as before. The stimulus pulse was recorded to identify the timing of stimulus presentations.

SECTION 4 RESULTS

Neurophysiological experiments.

i) Identification of the motor neurones. The four motor neurones known to terminate in the metathoracic extensor tibialis were identified as follows:-

a) The fast excitatory neurone (FETi) ran in nerve 3Nv5 and was easily identified by either electrical stimulation in the muscle or by disturbing the preparation by touch or by blowing air over the cercii. It was the first neurone to be activated by a steadily increasing stimulus voltage to the muscle, and also the largest spike recorded.

b) The dorsal unpaired median neurone (DUMETi) sends a process down the nerve 3Nv5 on each side of the ganglion (Hoyle, 1978a), and was evident as a regularly firing unit in that nerve. Hoyle (1978a) has demonstrated that the DUMETi axon has the second largest spike in nerve 3Nv5, and one could therefore identify the neurone by its spike height. The spike height was usually about 0.3 - 0.5 times that of FETi and a larger stimulus voltage was required to activate the unit.

c) The axon from the slow excitatory neurone (SETi) leaves the ganglion in nerve 3Nv3, passing through the fine branch 3Nv3b before it joins nerve 3Nv5 in the coxa. It was identified in branch 3Nv3b by anti-dromic stimulation in the muscle and was the only unit in that branch to be consistently activated by such electrical stimulation.

d) In some preparations a much smaller spike could also be seen in branch 3Nv3b at high stimulus voltages and this was originally thought to be from the common inhibitory neurone (CI). Hoyle (1955) stated that two slow axons ran through this branch and labelled them S_1 and S_2. However, in later work (Hoyle, 1978a) he stated that only S_1 (SETi) ran in 3Nv3b, and that S_2, now known as the common inhibitor, ran in branch 3Nv3c. Recording from branch 3Nv3c confirmed these points, although the identification of branch 3Nv3c was occasionally difficult because of variations in the branching pattern of

the nerve. CI was observed to be the largest unit in 3Nv3c and readily activated by stimulating electrodes placed in the extensor tibialis muscle.

In the following experiments the 3 neurones examined in detail were those most easily identified in all preparations. The activity of FETi and DUMETi were recorded from nerve 3Nv5, and the activity of SETi from 3Nv3b.

ii) Spontaneous motor neurone activity.

a) The total activity in nerve 3Nv5 was monitored by recording all electrical events above 15μV; this value was chosen to distinguish between the majority of neuronal activity and signal noise. The rate of activity above this level was variable and in most recordings fell gradually during the 5 minute recording period. The average rates of activity in each second were determined from the six animals in each group (adults, adultiforms and larvae) and are given in Figures 35 - 37. There were no obvious differences in the shape of the curves determined in the adultiforms and larvae (Figures 36 and 37), while the curve determined in the adults (Figure 35) showed numerous peaks of activity, particularly in the last third of the record. These peaks were mainly due to the activity of one animal. The rate of activity over each minute of the 5 minute recording was determined for each of the 6 animals in each group; the thirty (5 x 6) values determined were then grouped together and these were compared between the three types of animal. The activity was highest in the adultiform larvae and the 30 rates per minute values were significantly greater in this group than in the normal larvae ($p = 0.0118$, 2 sample t-test). The differences between the activity rates of the adults and adultiforms, and of the adults and larvae were not statistically significant, however ($n = 30$, $p \geq 0.0887$, 2 sample t-tests).

The rate of activity over each minute and each half minute of the averaged curves (Figures 35 - 37), for each group of 6 animals, was also compared. These 5 or 10 values determined will be referred to as the average rates of activity per minute or half minute. In each case the differences between the average

Figures 35-37

The average rate of spontaneous neuronal activity recorded from nerve 3Nv5 above a threshold level of 15μV. Each figure is the average from 6 individuals in which the activity rate was determined every second for 300s (5 minutes). Fig. 35, average activity recorded in normal adults; Fig. 36, average activity recorded in adultiform larvae; Fig. 37, average activity recorded in normal larvae. Abscissae, time in seconds; ordinates, rate of activity in Hz.

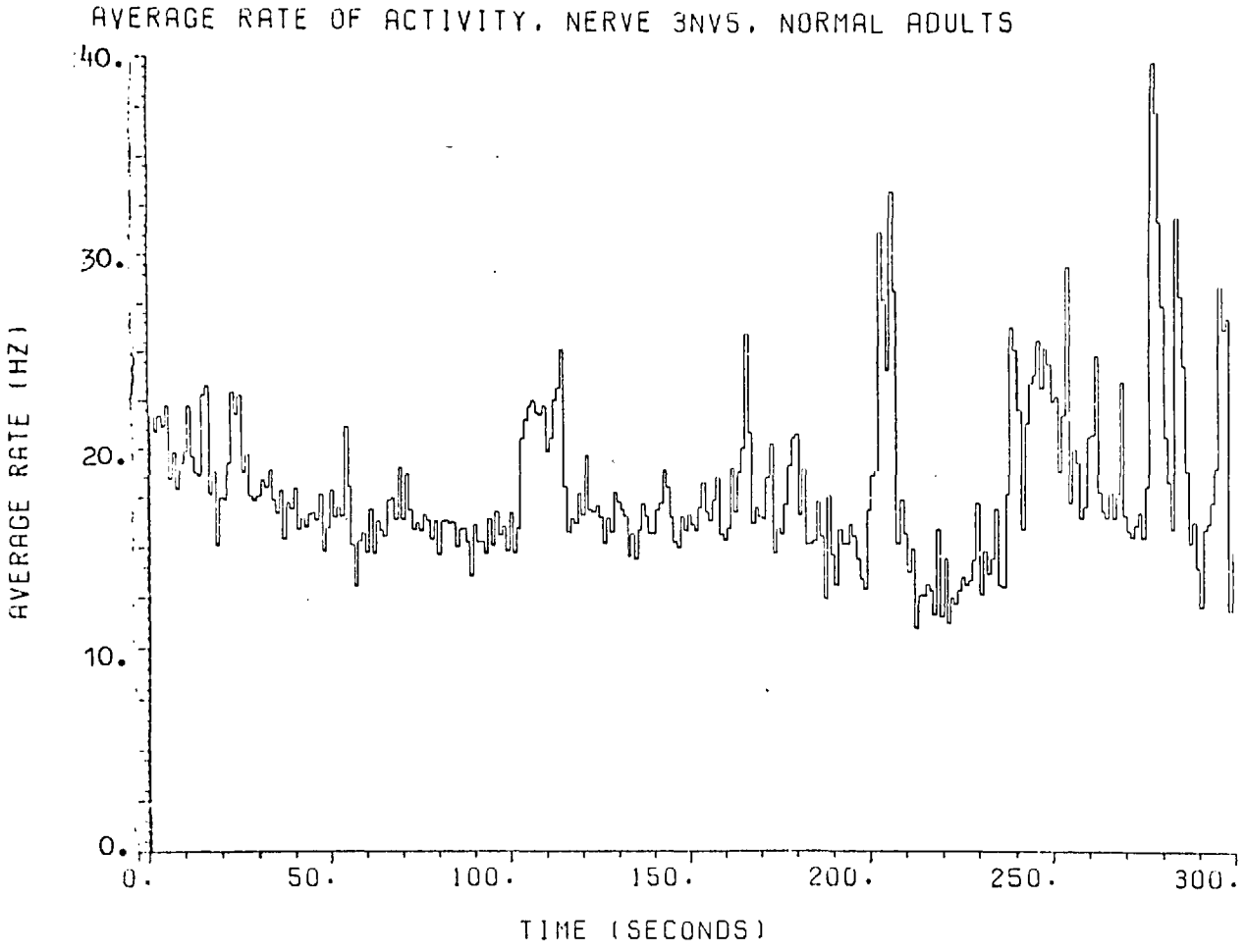

AVERAGE RATE OF ACTIVITY, NERVE 3NVS, NORMAL ADULTS
AVERAGE RATE (HZ)
40.
30.
20.
10.
0.
0.
50.
100.
150.
200.
250.
300.
TIME (SECONDS)

Fig. 35.

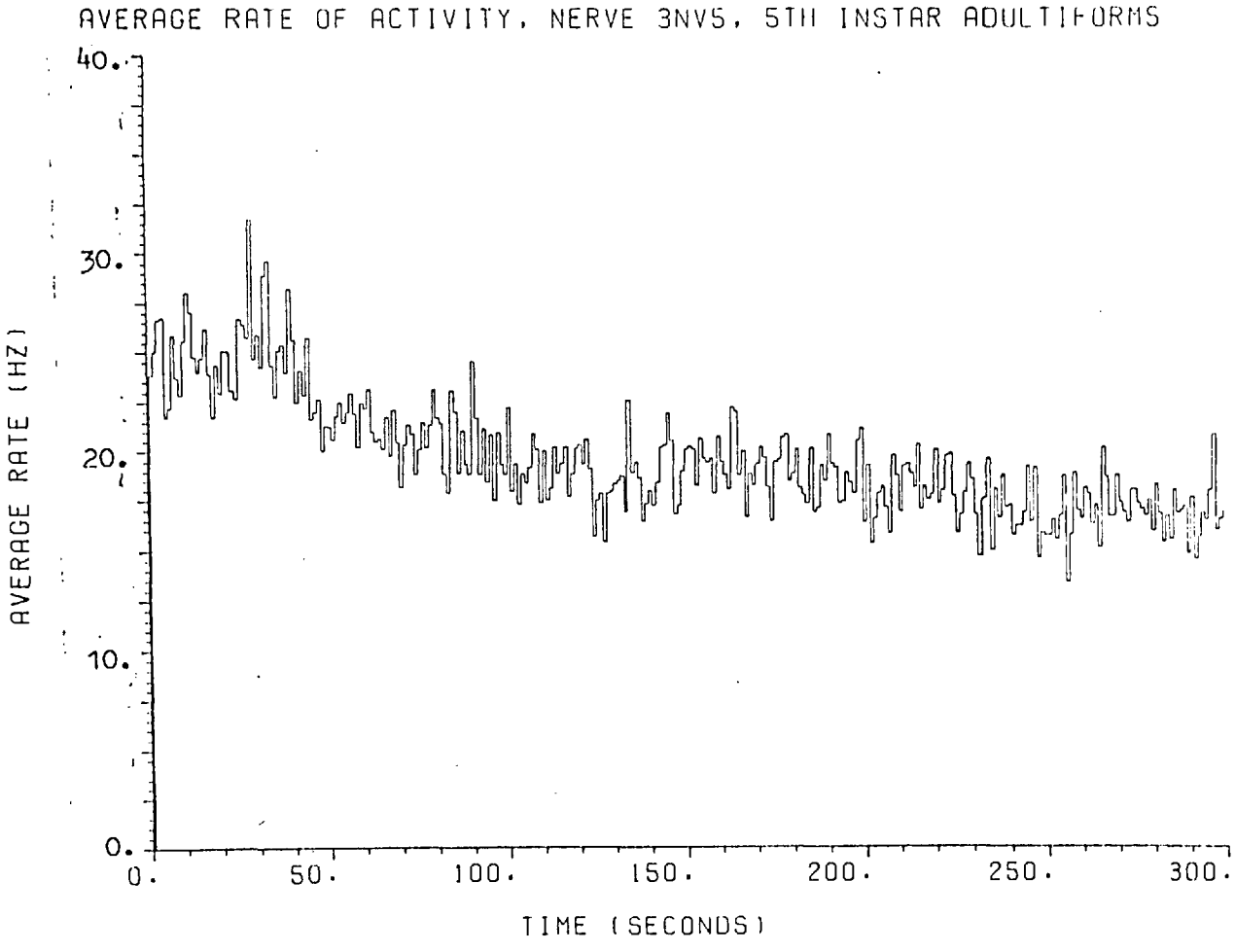

AVERAGE RATE OF ACTIVITY, NERVE 3NV5, 5TH INSTAR ADULTIFORMS
AVERAGE RATE (HZ)
40.
30.
20.
10.
0.
0.
50.
100.
150.
200.
250.
300.
TIME (SECONDS)

Fig. 36.

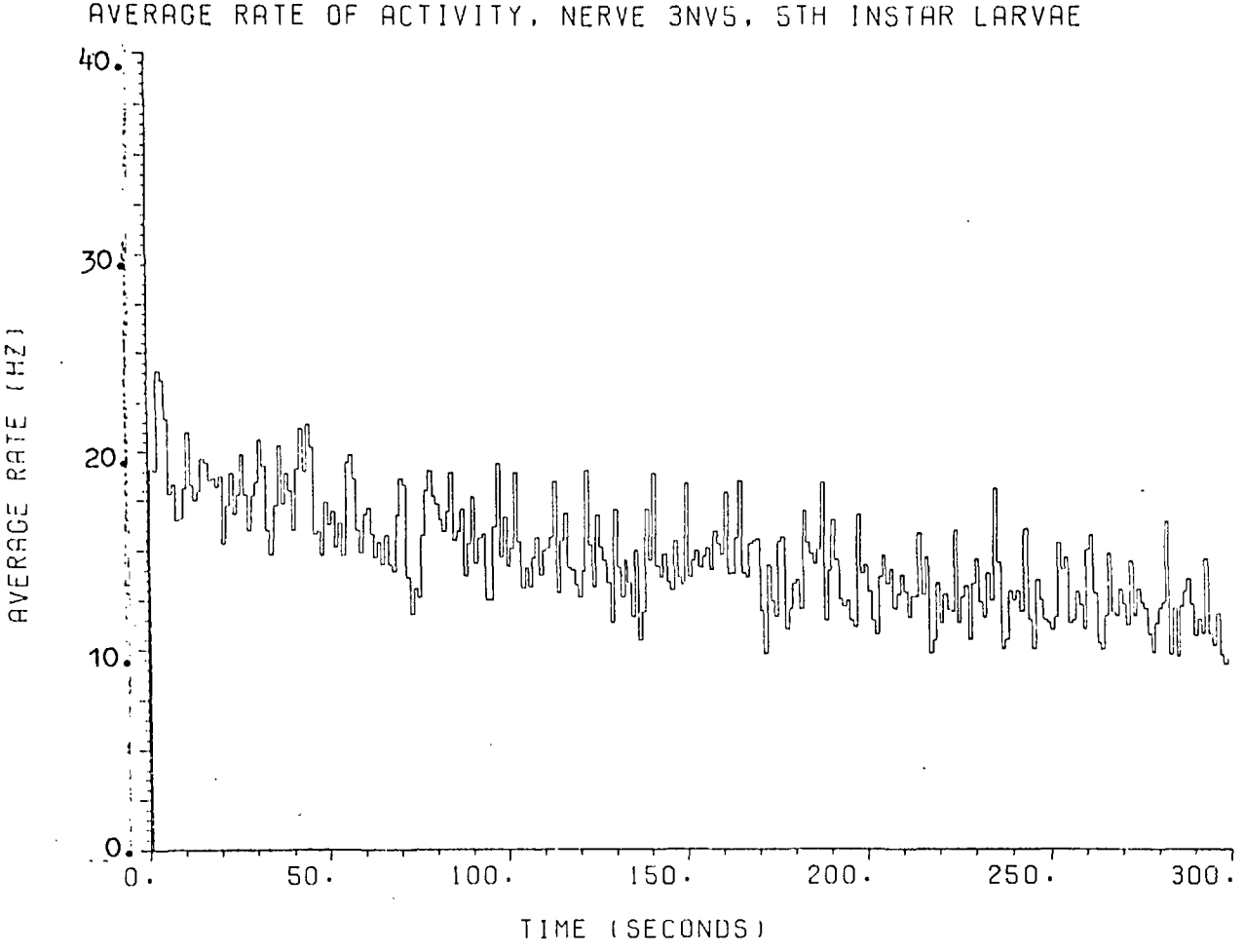

AVERAGE RATE OF ACTIVITY, NERVE 3NV5, 5TH INSTAR LARVAE
AVERAGE RATE (HZ)
40.
30.
20.
10.
0.
0.
50.
100.
150.
200.
250.
300.
TIME (SECONDS)

Fig. 37.

activity of the larvae and adultiforms, and of the larvae and adults, were significant ($p \leq 0.0355$, 2 sample t-tests), while the differences between the adults and the adultiforms were not significant ($p \geq 0.1383$, 2 sample t-tests).

b) The spontaneous activity of the FETi neurone was extremely low and no recordings of the rate per second over a 5 minute period were attempted. However, an indication of the activity is given in Figure 44, where the average activity of this neurone over the three 500ms periods prior to stimulation of the cercii was determined. The number of events in each 100ms bin was low and corresponded to an average frequency of 0.5 - 1.0Hz. In studying the individual's records it should be noted that at least two (and at most four) of the five individuals in each group showed no spontaneous activity of FETi during these three 500ms pre-stimulus periods. There were no significant differences between the spontaneous activity levels within the three groups ($p \geq 0.2713$, 2 sample t-tests).

c) The spontaneous activity of the DUMETi neurone was recorded from 5 animals in each group and was analysed in the same way as described in section (a) above. The curves of the average activity per second over 300s are given in Figures 38 - 40. The activity of this neurone was seen to increase gradually over the first 100 - 150s of recording in most preparations and then to remain constant or to decline slowly during the remainder of the recording. This is evident in Figures 38 - 40 as a shallow U - shaped curve. Each recording began some 10 - 15 minutes after the start of the preparation, and about 2 - 5 minutes after the nerve was first cut. The activity in some individuals' records periodically increased - often to the upper recording limit of 50Hz - and this is seen as brief peaks in the averaged curves (Figures 38 - 40). These bursts were coincident with wing, leg and antenna movements in some preparations and are thought to be the result of attempts at movement away from the recording situation.

The basic activity level was considerably higher in the adultiform larvae than in the adults, but there were also differences, although smaller, between the activity levels of

Figures 38-40.

The average rate of spontaneous activity in the DUMETi neurone recorded from nerve 3Nv5. Each figure is the average from 5 individuals, as before (Figs. 35-37). Fig. 38, average activity recorded in normal adults; Fig. 39, average activity recorded in adultiform larvae; Fig. 40, average activity recorded in normal larvae. Abscissae, time in seconds; ordinates, rate of activity in Hz.

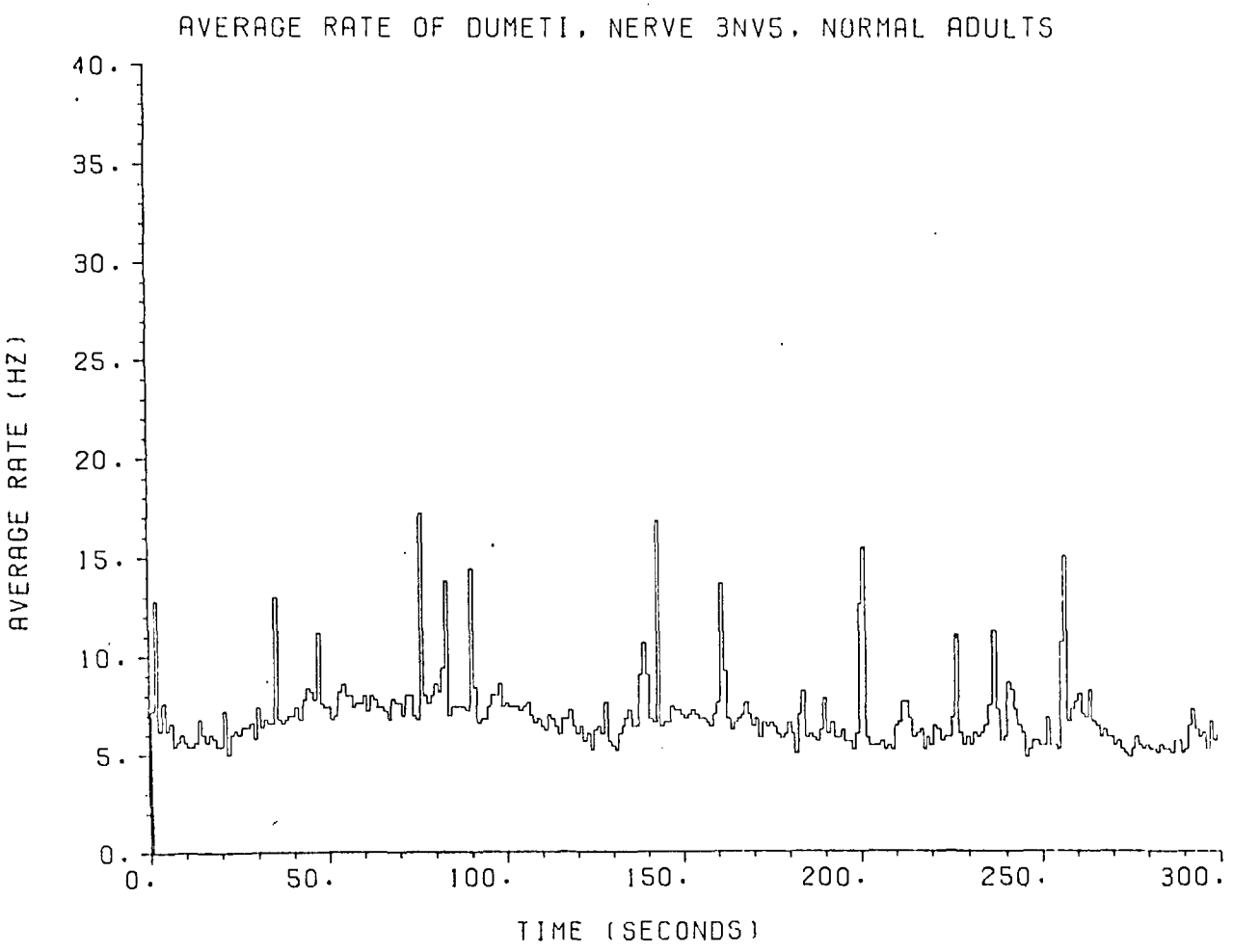

AVERAGE RATE OF DUMETI, NERVE 3NV5, NORMAL ADULTS
AVERAGE RATE (HZ)
40.
35.
30.
25.
20.
15.
10.
5.
0.
0.
50.
100.
150.
200.
250.
300.
TIME (SECONDS)

Fig. 38.

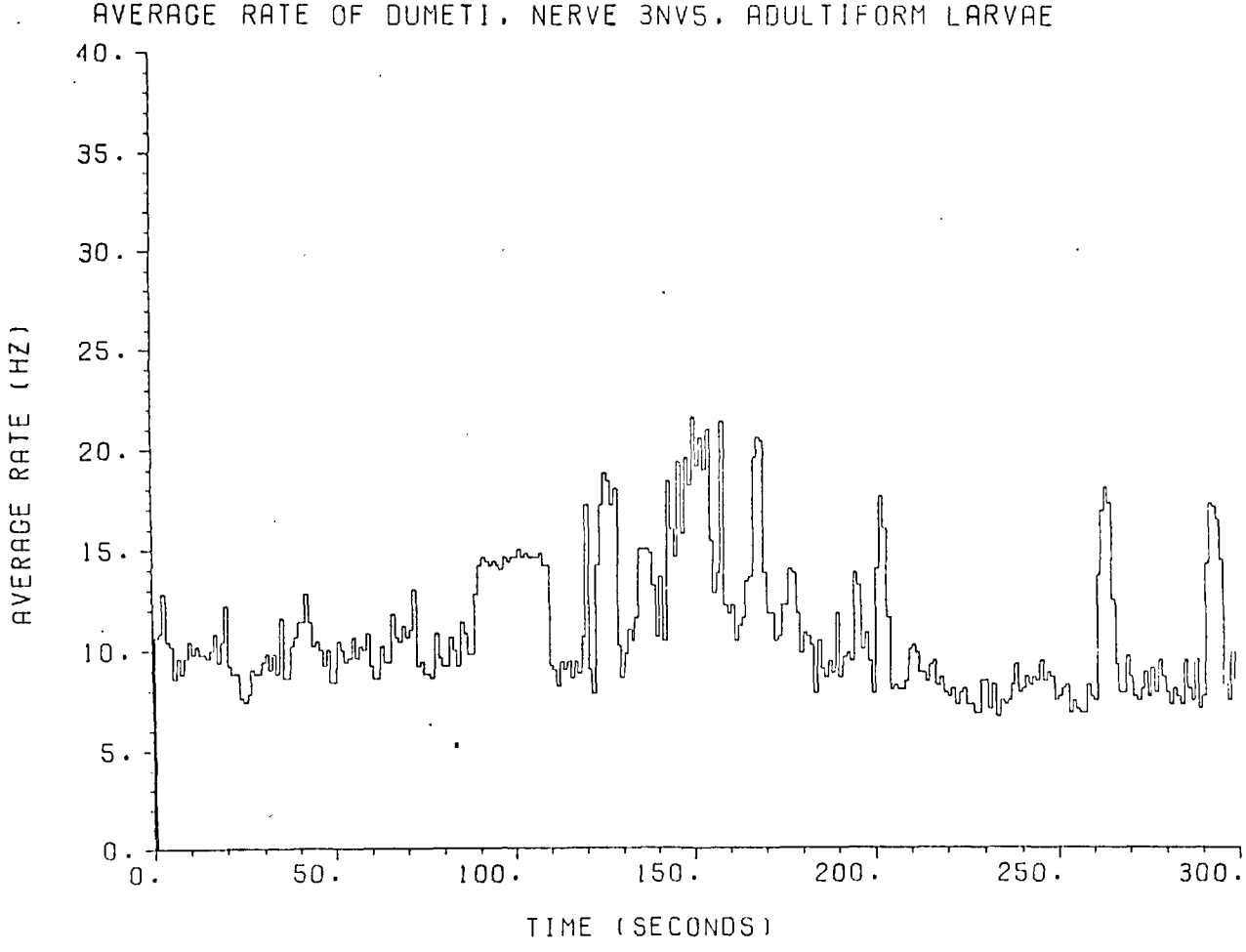

AVERAGE RATE OF DUMETI, NERVE 3NV5, ADULTIFORM LARVAE
AVERAGE RATE (HZ)
40.
35.
30.
25.
20.
15.
10.
5.
0.
0.
50.
100.
150.
200.
250.
300.
TIME (SECONDS)

Fig. 39.

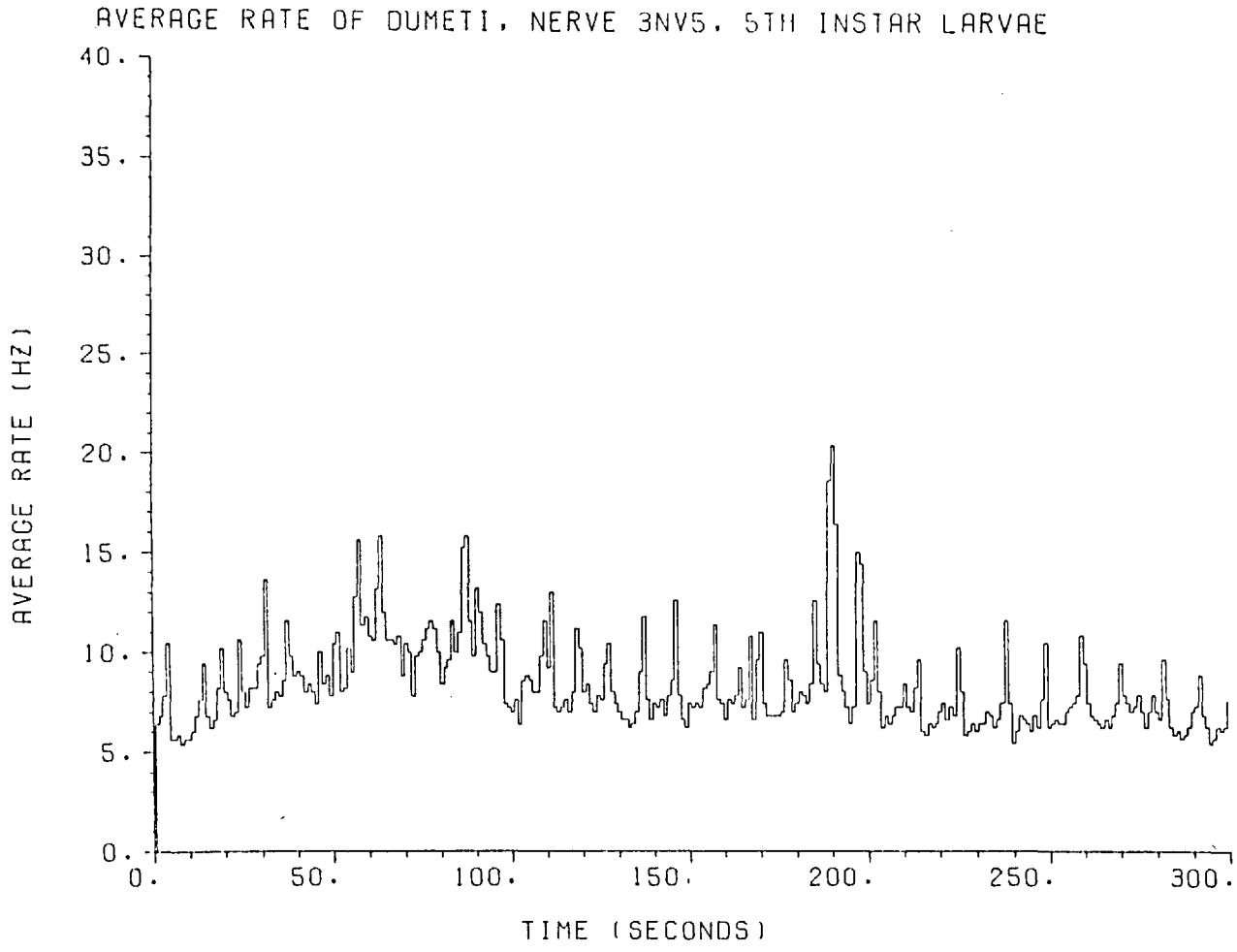

AVERAGE RATE OF DUMETI, NERVE 3NV5, 5TH INSTAR LARVAE
AVERAGE RATE (HZ)
40.
35.
30.
25.
20.
15.
10.
5.
0.
0.
50.
100.
150.
200.
250.
300.
TIME (SECONDS)

Fig. 40.

the adults and the larvae. Statistical analyses of the average activity rates per half minute revealed that the spontaneous activity of the DUMETi neurone in the adultiforms was significantly higher than that of the adults and of the larvae ($n = 10$, $p \leq 0.0105$, 2 sample t-tests); the larval average activity rate per half minute was also significantly higher than the adult rate ($n = 10$, $p = 0.0080$, 2 sample t-test). Similar analysis of the average activity rates per minute revealed that the differences in average activity rates between the 3 groups was not so significant, however ($n = 5$; $p = 0.0173$, 0.0402 and 0.0690, 2 sample t-tests). Furthermore, analysis of the individual one minute activity rates determined over the 5 minutes of each recording from the 5 animals in each group reduced these probability levels until none of the differences were statistically significant ($n = 25$, $p = 0.0536$, 0.2291 and 0.2313, 2 sample t-tests).

d) The spontaneous activity of the SETi neurone was recorded from 6 animals in each group and analysed in the same way as described in sections (a) and (c) above; the average activity per second for each group is indicated in Figures 41 - 43. The activity of this neurone was less variable than observed for DUMETi and FETi; it was observed to be a regularly firing unit that showed few signs of being responsive to slight external stimulii. Although brief bursts of higher activity were seen in one or two preparations and appear as peaks in the averaged curves (Figures 41 - 43), the activity in most preparations declined gradually and consistently over the 5 minute recording. The average activity level was highest in the adults, although the larvae showed almost as great activity; the adultiform larvae showed considerably lower activity, however. Using the same statistical analyses as before it was evident from the averaged half minute activity rates that both larval and adult rates were significantly greater than the adultiform rate ($n = 10$, $p \leq 0.0187$, 2 sample t-tests),while the adult rate was not significantly greater than the larval rate ($n = 10$, $p = 0.0812$, 2 sample t-test). As before, the average activity rates per minute were less clearly separated and only the adult and adultiform rates differed significantly ($n = 5$, $p = 0.0130$, 2 sample t-test). Analysis of the individual one minute activity

Figures 41-43.

The average rate of spontaneous activity in the SETi neurone recorded from nerve 3Nv3b. Each figure is the average from 6 individuals, as before (Figs. 35-40). Fig. 41, average activity recorded in normal adults; Fig. 42, average activity recorded in adultiform larvae; Fig. 43, average activity recorded in normal larvae. Abscissae, time in seconds; ordinates, rate of activity in Hz.

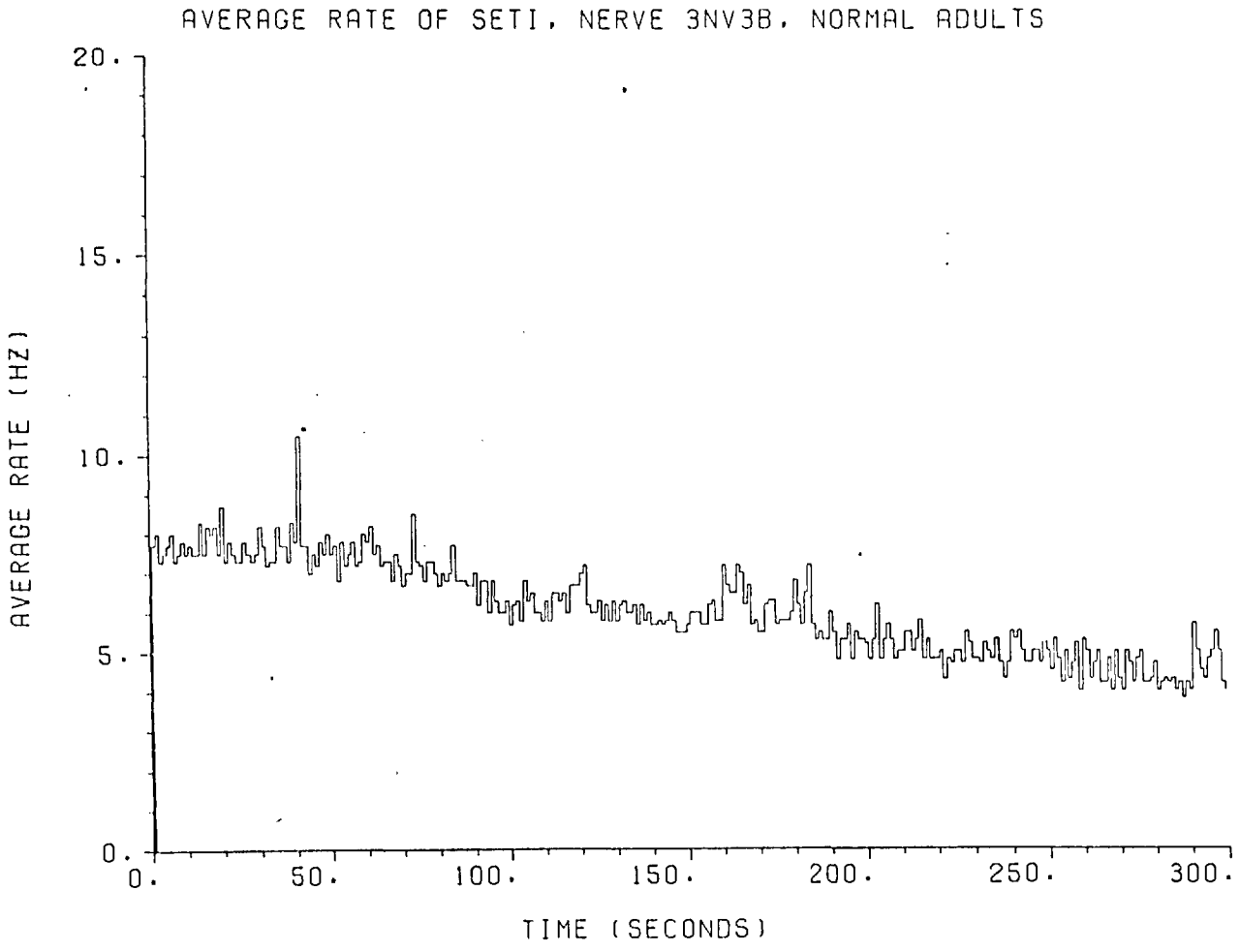

AVERAGE RATE OF SETI, NERVE 3NV3B, NORMAL ADULTS
AVERAGE RATE (HZ)
20.
15.
10.
5.
0.
0.
50.
100.
150.
200.
250.
300.
TIME (SECONDS)

Fig. 41.

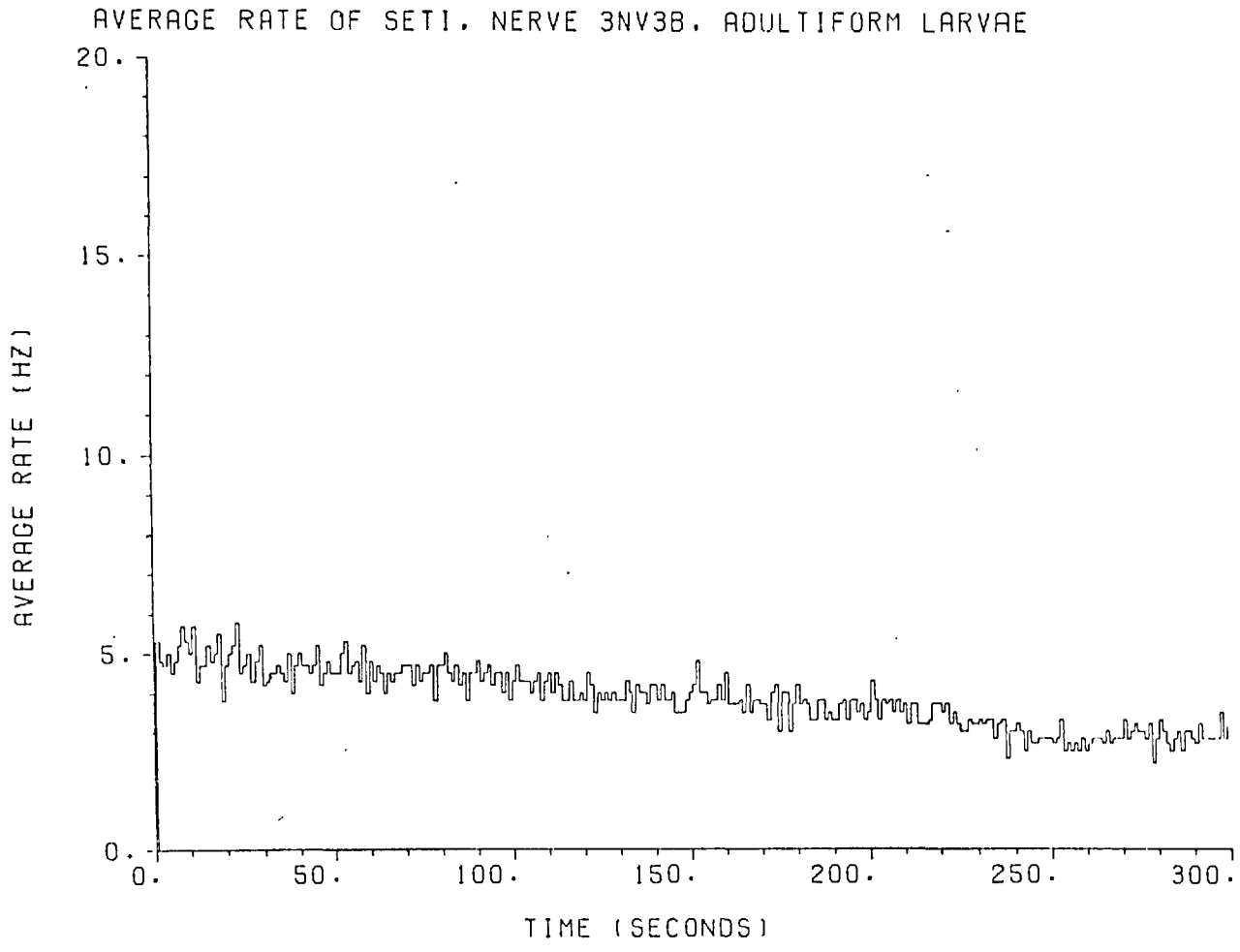

AVERAGE RATE OF SETI. NERVE 3NV3B. ADULTIFORM LARVAE
AVERAGE RATE (HZ)
20.
15.
10.
5.
0.
0.
50.
100.
150.
200.
250.
300.
TIME (SECONDS)

Fig. 42.

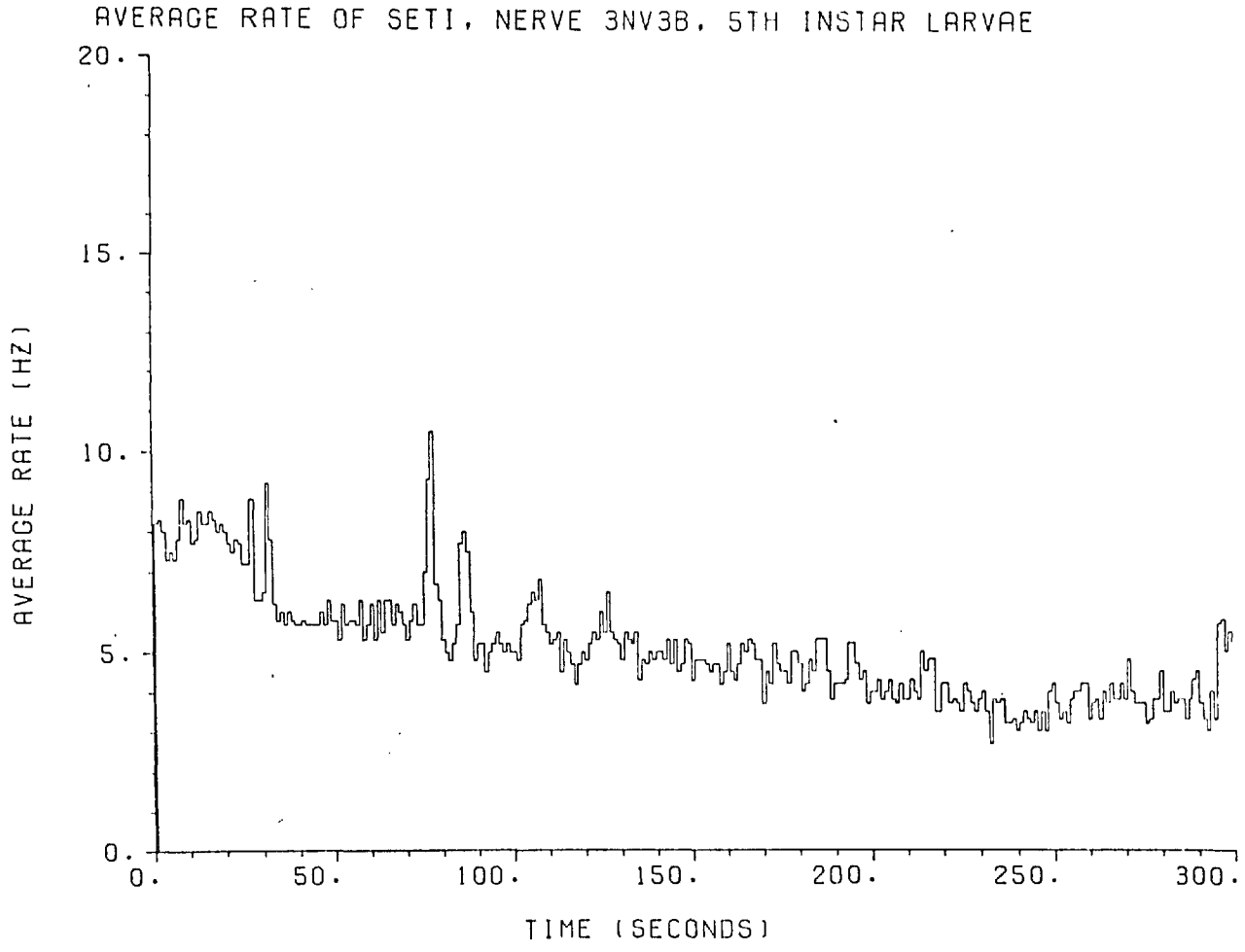

AVERAGE RATE OF SETI, NERVE 3NV3B, 5TH INSTAR LARVAE
AVERAGE RATE (HZ)
20.
15.
10.
5.
0.
0.
50.
100.
150.
200.
250.
300.
TIME (SECONDS)

Fig. 43.

rates for the 6 animals in each group indicated that the spontaneous activity of the SETi neurone in the adults was significantly greater than in the adultiforms ($n = 30$, $p = 0.0031$, 2 sample t-test) but not significantly different from that in the larvae ($n = 30$, $p = 0.2142$, 2 sample t-test); the larval activity was not significantly greater than that of the adultiforms however ($n = 30$, $p = 0.0839$, 2 sample t-test).

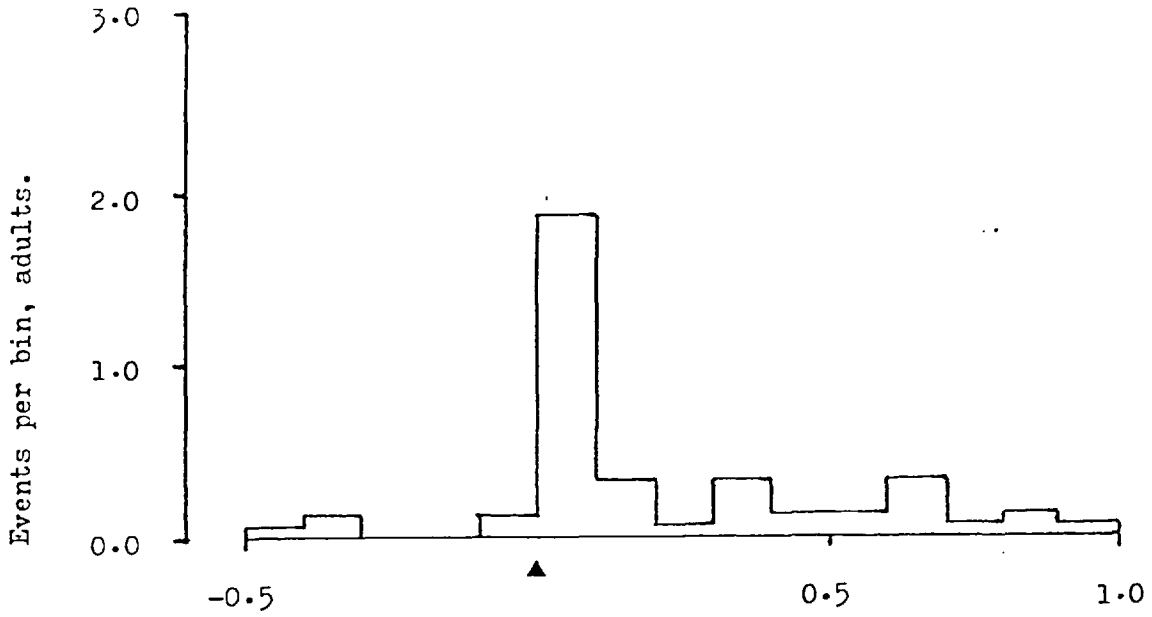
iii) Motor neurone responses to stimulation of the anal cercii.
High speed paper records of the activity of the FETi and DUMETi neurones were examined to prepare the peri-stimulus time histograms given in Figures 44 and 45. Each histogram is the average of 3 responses from 5 animals (i.e. the average of 15 recordings). It should be stressed that individual responses within each group varied considerably from these average patterns.

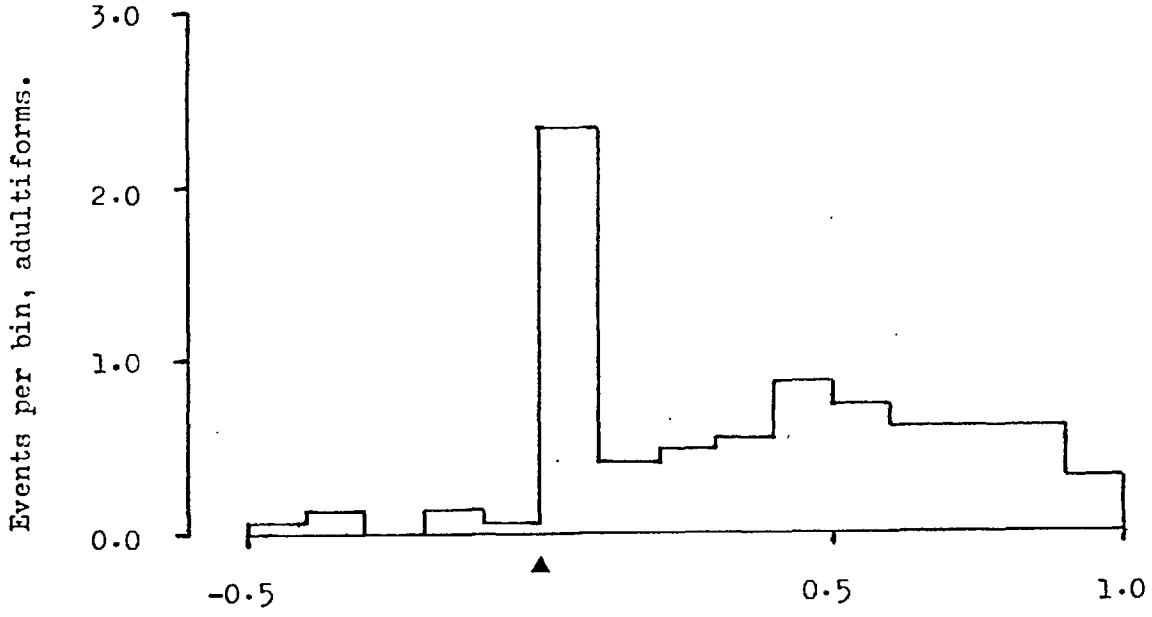
a) The stimulated activity of the FETi neurone is indicated in Figure 44. As discussed previously, this neurone showed very low levels of spontaneous activity. On application of a single jet of air to the anal cercii the unit fired once or twice, but almost immediately returned to a low level of activity. Only in the larvae did the activity almost immediatly reach pre-stimulus levels, however.

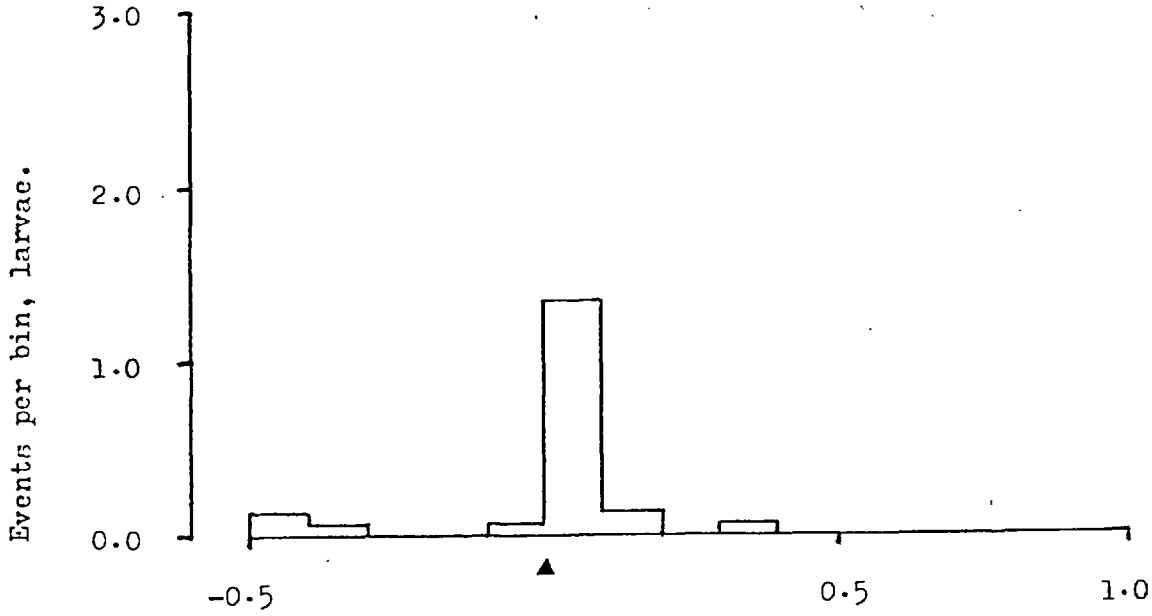
In the adults the average response was 1.87 spikes within the first 100ms following stimulation; this response ranged from 1 to 4 spikes in the individual records. The activity then fell to about twice that of the pre-stimulus level, and returned to the pre-stimulus level within 750ms. The adultiform larvae showed similar pre-stimulus activity but responded with an average 2.33 spikes within the 100ms post-stimulus (range: 2 to 4). The activity was then maintained at between 3 and 6 times the pre-stimulus level for approximately 750ms. There appeared to be a decline in activity after this, but even 1s after stimulation the activity was about twice that of the pre-stimulus levels. The normal larvae showed the lowest pre-stimulus activity, although this was not significantly less than that of the adults or adultiforms (see Section ii (b), above). There was an average 1.33 spikes in the 100ms following stimulation (range: 0 - 2), but the activity rapidly declined and appeared to be at,

Figure 44.

The peri-stimulus time histograms prepared from the average activity of the FETi neurone recorded in nerve 3Nv5. Each histogram was prepared from the average of 3 responses from 5 animals. The timing of the air stimulation to the anal cercii is indicated by the black triangles; each histogram bin was 100ms wide. Upper, the average activity recorded in adults; middle, the average activity recorded in adultiform larvae; lower, the average activity recorded in normal larvae. Abscissae, peri-stimulus time intervals in seconds; ordinates, the average number of events per 100ms bin.


3.0
2.0
1.0
0.0
Events per bin, adults.
-0.5
0.5
1.0


3.0
2.0
1.0
0.0
Events per bin, adultiforms.
-0.5
0.5
1.0


3.0
2.0
1.0
0.0
Events per bin, larvae.
-0.5
0.5
1.0

or below, the pre-stimulus level for the majority of the 1s of post-stimulus recording.

b) The stimulated activity of the DUMETi neurone is indicated in Figure 45. The averaged records from adult and larval groups were similar and will be described together. The pre-stimulus level of activity was higher than that observed in the FETi neurone, as described above (Section ii). Immediately after stimulation there was a rise in activity to about 2.3 - 3.2 times the pre-stimulus levels. This enhanced activity declined gradually until a level just above the pre-stimulus level was reached some 700 - 1000ms after stimulation. The adultiform larvae showed slightly lower activity in the pre-stimulus period than the adults and larvae; this was not supported by the more detailed study of spontaneous activity levels described above (section ii (c)). After stimulation there was an increase in activity of about 3.4 times the pre-stimulus level. This enhanced activity was maintained for some 300 - 400ms after which it declined gradually. However, the activity level remained at more than twice the pre-stimulus level for the period 400 - 1000ms after stimulation, at which point the recordings were terminated.

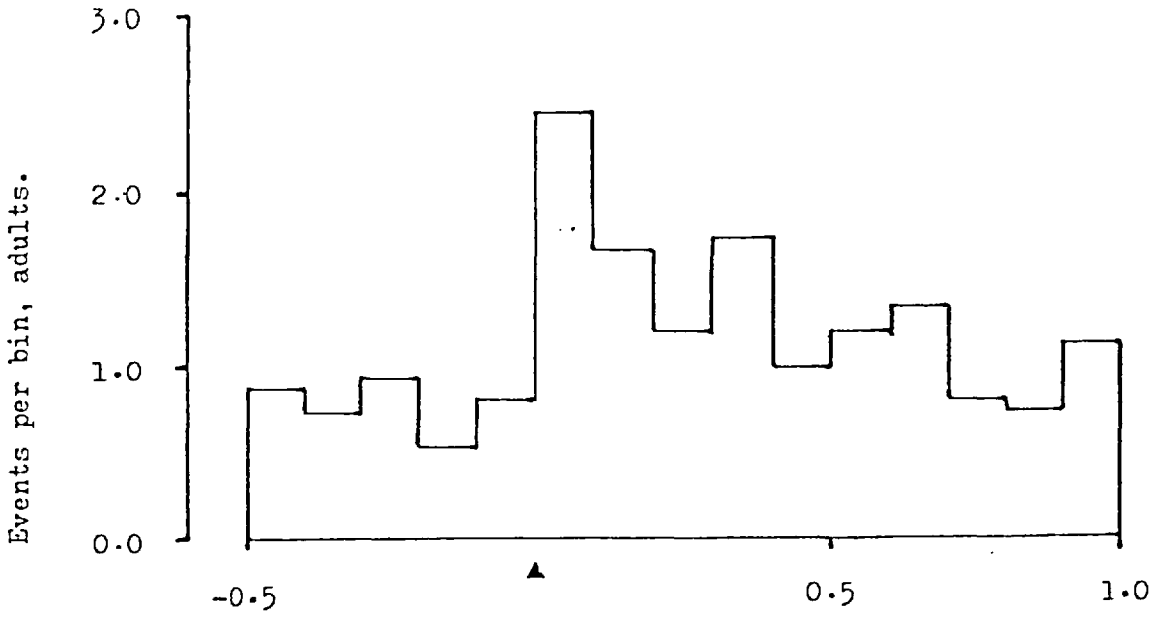
Myological experiments.

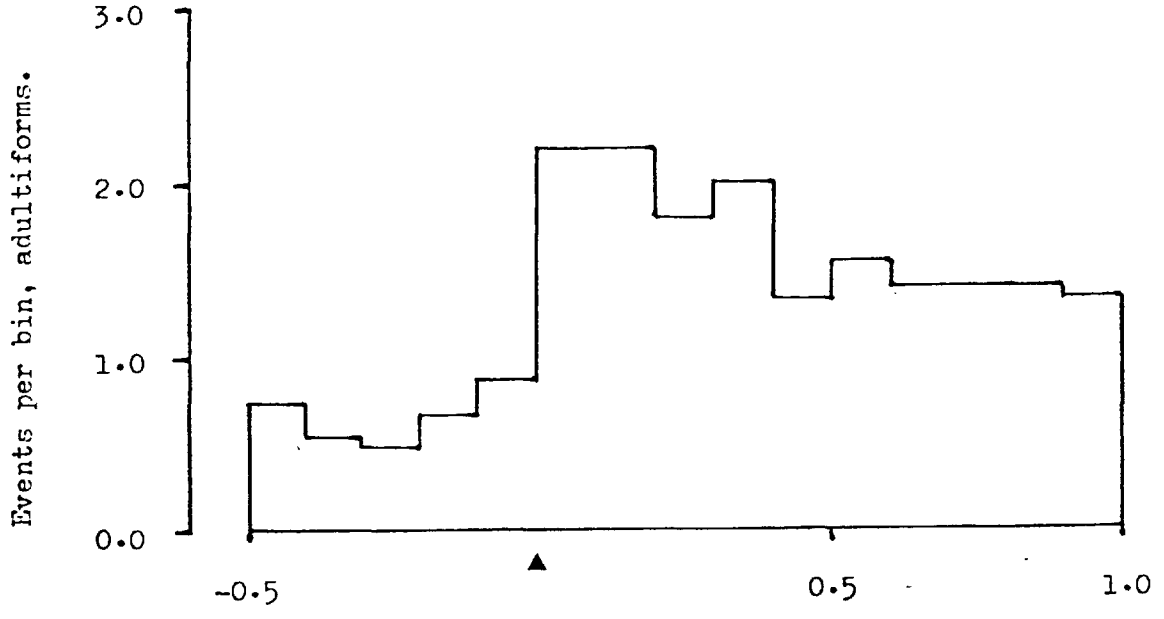
i) The tension developed by the extensor tibialis. The motor nerve 3Nv5 was stimulated electrically at 3 frequencies and the responses from each individual were averaged. Only males were used in these experiments as there was a clear sex difference in the size and weight of the meta-thoracic leg. There was a slight, but statistically insignificant, fall in the tension developed by the muscle as the frequency of stimulation was increased from 0.5Hz to 2.0Hz. The adult muscles developed much greater tensions than either the adultiforms or the normal larvae (Table 40), and the normal larvae developed much greater tensions that the adultiform larvae. These differences were all highly significant ($p < 0.0001$, 2 sample t-tests).

ii) The onset of tetanus. The frequency of stimulation at which tetanus developed was determined for each individual. There were no significant differences in these frequencies between the three groups (Table 40; $p \geqslant 0.1726$, 2 sample t-tests).

Figure 45.

The peri-stimulus time histograms prepared from the average activity of the DUMETi neurone recorded in nerve 3Nv5. Each histogram was prepared from the average of 3 responses from 5 animals. The timing of the air stimulation to the anal cercii is indicated by the black triangles; each histogram bin was 100ms wide. Upper, the average activity recorded in adults; middle, the average activity recorded in adultiform larvae; lower, the average activity recorded in normal larvae. Abscissae : peri-stimulus time intervals in seconds; ordinates, the average number of events per 100ms bin.


Events per bin, adults.
3.0
2.0
1.0
0.0
-0.5
0.5
1.0


Events per bin, adultiforms.
3.0
2.0
1.0
0.0
-0.5
0.5
1.0

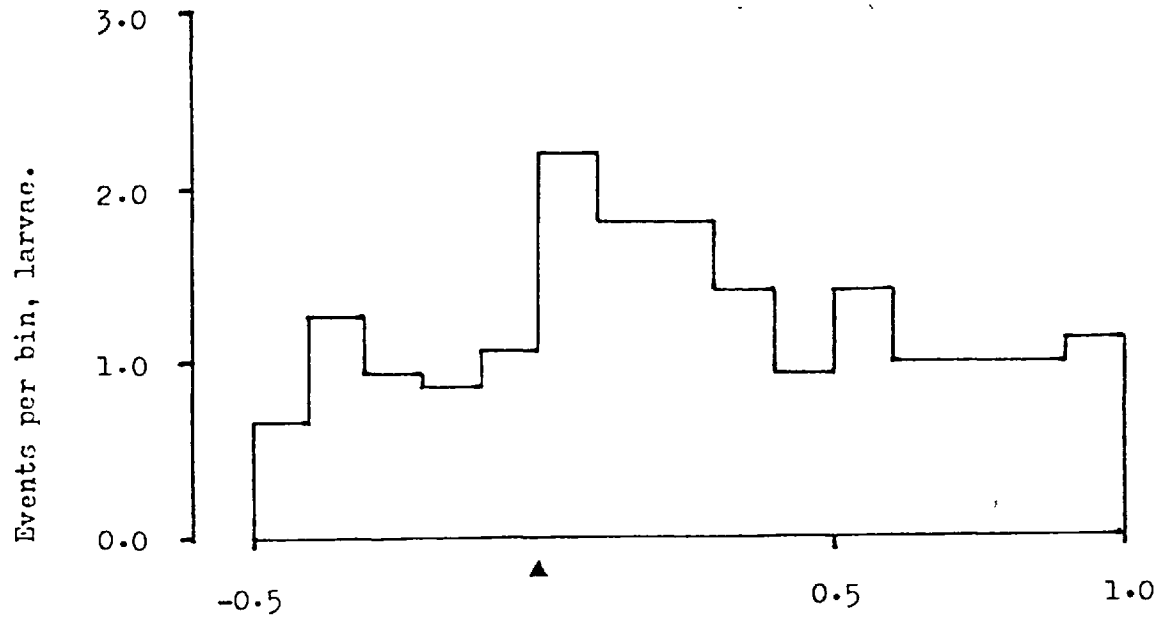

Events per bin, larvae.
3.0
2.0
1.0
0.0
-0.5
0.5
1.0

Table 40 The mean tension developed by the metathoracic extensor tibialis muscle during electrical stimulation of the motor nerve, 3NV5. Each figure is the average of more than 100 responses made by 6 animals*. The tensions are given in Newtons ± 1 S.D., although the conversion to these units was only approximate. The mean frequencies (± 1 S.D.) at which tetanic responses developed are also given.

Group	Sample size*	Mean tension (10^{-6} Newtons ± 1 S.D.)	Mean tetanus-onset frequency (Hz ± 1 S.D.)
Adults	127	5.7122 ± 0.5803	19.167 ± 6.65
Adultiforms	163	1.7864 ± 0.5138	21.000 ± 4.18
Larvae	128	2.6385 ± 1.1039	24.167 ± 4.92

Table 41 The body weights, metathoracic leg weights and femur lengths, and two ratios of these dimensions, for normal adults, adultiform larvae and normal larvae. All measurements were taken on Day 3-4 of the instar from well-fed animals, and are given as the means ± 1 S.D.

Group	Sample size	Body weight (g)	Leg weight (mg)	Femur length (mm)	Body/leg weight ratio	Leg weight/ femur length ratio (mg/mm)
Adults ♂	4	1.7885 ± 0.126	128.92 ± 17.79	24.625 ± 0.854	13.971 ± 1.058	5.227 ± 0.593
♀	4	2.1527 ± 0.301	144.30 ± 10.11	25.750 ± 1.190	14.875 ± 1.237	5.613 ± 0.470
Adultiforms ♂	6	0.7805 ± 0.084	51.14 ± 3.76	17.333 ± 0.408	15.247 ± 0.864	2.951 ± 0.222
♀	5	1.0335 ± 0.073	58.24 ± 9.33	18.200 ± 0.274	18.004 ± 3.218	3.204 ± 0.538
Larvae ♂	5	1.0374 ± 0.068	106.92 ± 8.10	16.700 ± 0.975	9.712 ± 0.309	6.398 ± 0.172
♀	4	1.1079 ± 0.071	84.97 ± 25.50	17.625 ± 0.854	13.829 ± 3.594	5.613 ± 0.472

iii) Muscle volume and weight. The body weights, metathoracic leg weights and femur lengths were measured. The data are given in Table 41, and show clear differences in body and leg weights and in the body/leg weight and leg weight/length ratios. Although the sample sizes were rather small it is evident that the adultiform larvae weigh considerably less that the similarly aged normal larvae; this was supported by the data on body weights used to prepare Table 39. The dimensions of the metathoracic leg are similar in these two groups but the adultiform leg weighs between 30 - 50% less. It must be assumed that at least part of this weight difference is related to the size and volume of the extensor tibialis muscle. The body/leg weight ratio was adopted to indicate the 'lifting power' of the animals and showed that the adultiform larvae have the most disadvantageous body to leg weight ratios. Similarly the femur length to leg weight ratio was adopted to indicate the potential power output due to the leg and muscle proportions, but the adultiform larvae were again shown to have the most disadvantageous ratios.

SECTION 4 DISCUSSION

It has been demonstrated that the 5th instar adultiform larvae induced by treatment with precocene II in the 4th instar behave differently from normal 5th instar larvae. The differences noted are that: their general excitability and activity within the stock cages is greater than that of normal larvae or adults; they can display considerably longer escape jumps; their ventilatory movements are extreme; and the frequency and duration of their kicking behaviour is higher than in normal animals. It has also been demonstrated that their feeding rate is considerably lower than in normal animals, and that their marching behaviour when described in terms of the distance walked per unit time, is considerably reduced from the high level seen in normal larvae. It has been argued (p. 84) that the reduced feeding rates may be related to the lack of growth in somatic or reproductive organs, but that the reduced walking behaviour may be the result of increased interactions of walking and non-walking behaviours. It was suspected that the neurophysiological studies undertaken on the motor control of the hind leg would support the proposition that the adultiform larvae display enhanced central or peripheral nervous or neuromuscular activity.

An indication of the level of neuronal activity was made by recording all events above the level of signal noise in the large motor nerve 3Nv5. This nerve carries two of the motor axons to the extensor tibialis muscle (Hoyle, 1978a) but also carries a number of smaller efferent and afferent fibres. The use of the individual activity rates or the average rates of activity per minute and per half minute during analysis caused changes in the levels of statistical significance of the observed differences between groups. This was due both to the inclusion of the differences between individuals into analysis, and to the changes in the sample sizes when considering the average half minute data instead of the average one minute data.

It was apparent that the adultiform larvae showed greater neuronal activity in nerve 3Nv5 than either the larvae or adults. There was also slightly greater activity in the adults than in the larvae. If one assumes that the activity in nerve 3Nv5 is representative of the CNS as a whole, then these differences would agree with the observed behaviour differences. However, Haskell and Moorhouse (1963) have demonstrated that haemolymph treatments that enhance the CNS activity

of *Schistocerca gregaria* inhibited the activity in nerve 3Nv5. The assumption that the activity in the two regions is comparable is therefore suspect.

The spontaneous activity of the FETi neurone was too low to show significant differences between the groups, but the activity of this neurone after stimulation of the cercii was significantly greater in the adultiform larvae than in the normal animals. The initial response within 100ms of stimulation was about 30% greater than in the adults and about 75% greater than in the normal larvae. The response was also of considerably longer duration, with a marked increase in activity from prestimulus levels even 1s after stimulation. This neurone is responsible for the rapid kicking movement of the hind leg (Hoyle, 1955, 1978a) and the increase in adultiform neural activity to, and after, stimulation agrees with the greatly increased kicking behaviour of these animals.

The DUMETi neurone sends projections into the extensor tibialis muscle on both sides of the body. Its function is uncertain but the axon is known to terminate only within the extensor tibialis (Hoyle, 1978a) and at least one function is to influence the rate of an intrinsic rhythm present in the muscle during periods of inactivity (Evans and O'Shea, 1978; Hoyle, 1978b). The spontaneous activity of this neurone in the adultiform larvae was found to be significantly greater than in the normal animals, while the activity in the larvae was also greater than that in the adults. However, these activity rates were variable with time, and also varied between individuals in each group, to the extent that the differences were not statistically significant ($p > 0.05$). The activity of this neurone after stimulation of the cercii was greatest in the adultiform larvae, and as seen in FETi, remained higher than the pre-stimulus levels for at least 1s after stimulation.

While the FETi neurone controls rapid movements of the hind leg, such as kicking and jumping, a wide range of leg movements can be observed in animals in which nerve 3Nv5 has been sectioned. The SETi neurone in nerve 3Nv3b is involved in these slow contractions of the extensor tibialis and in the control of muscle tonus, and terminates in most fibres within this muscle (Hoyle, 1955). The spontaneous activity in this neurone was found to be highest in the adults and lowest in the adultiform larvae. It is unlikely that the lower activity in these adultiform larvae is directly related to the reduced walking behaviour,

however, since the rate of firing of the SETi neurone was significantly higher in the adults than in the adultiforms, while their walking behaviour was similar. Observations of the locomotor activity of the adultiforms in the stock cages showed similar or more rapid walking than in normal animals. It should also be noted that the usual position adopted by perching or resting locusts, with the hind leg folded closely to the body such that the femur and tibia are parallel and in contact (joint angle = 0^{o}), was less often adopted by the adultiform larvae. These animals were frequently observed to perch or rest with the femur/tibia joint at an angle of 30 - 60^{o}. This position was characterized by the head being lower than the abdomen, which was held in the air; in normal animals the abdomen was in contact with the substrate, similar to the post-prandial rest position (Ellis, 1951). Since the SETi neurone is the excilatory motor neurone controlling the metathoracic leg posture, it is therefore surprising that the spontaneous activity was found to be lower in the six adultiform larvae studied than in the normal animals. The reasons for this are uncertain; however, no attempts were made to study the stimulated activity of this neurone, nor to study the activity of the common inhibitor, CI. Such a study might indicate that the stimulated response of SETi or the activity of CI are more representative of the exhibited 'slow' muscle behaviour than is the spontaneous activity of SETi.

The route of the CI axons from the metathoracic ganglion have been studied by various workers. While Hoyle's earlier reports (Hoyle, 1955) stated that CI ran in branch 3Nv3b, he later recorded its activity in 3Nv3c (Hoyle, 1978b). Pearson and Bergman (1969) and Burrows (1973) reported branches of CI in 3Nv3b and 3c, and Burns and Usherwood (1979) suggest that CI axon, may be found in nerves 3Nv3a, 3b, 3c, 3Nv4 and 3Nv5. However, in this study stimulation of the muscle elicited activity of one large unit in each of nerves 3Nv3b and 3c. As stated earlier (p. 91), it was assumed that the only consistantly activated unit in 3Nv3c was CI. The recordings of SETi were unquestionably the activity of only one unit, and do not combine the activities of CI and SETi.

Finally, the brief study of the tension developed by the extensor tibialis muscle during electrical stimulation of the motor nerve 3Nv5 indicated that the strength of the muscle was related to the overall weight of the leg. The ratio of the average, male, adult/adultiform/larval leg weights was 1 : 0.397 : 0.829; the ratio of the average tensions recorded was 1: 0.313 : 0.462. Allowing for the discrepencies

introduced in assuming that the total leg weight approximates to the extensor tibialis weight, and assuming that the muscle weight differences thus indicated are related only to the muscle volumes, it appears that the differences in the evoked muscle tensions can be explained by differences in the size of the muscles, without postulating any significant changes in the function of the muscle fibres or of the neuromuscular junctions in the adultiform larvae. Further, while these adultiforms have an approximate 20% weight advantage, one can also state that their ratios of body to leg weight and femur length to leg weight are disadvantageous, and that they develop lower tensions in the muscle after a single electrical stimulation. The greater jump lengths exhibited by the intact animals must be partly due to either greater excitation by the FETi neurone or to some undetected mechanical advantage, such as the role of resilin in the jumping movement. The marked increase in the activity of the FETi neurone after stimulation of the cercii lends weight to the simplest explanation : the adultiform larvae show greater neuronal activity and must be considered to show greater motor output from the CNS; this, combined with their slightly smaller body weights, allows these animals to exhibit considerably longer escape jumps.

The larval stages of the locust have been demonstrated to be dependent upon the presence of haemolymph JH to maintain the normal larval developmental sequence. Premature removal of the source of JH by precocene treatment reprogrammes the animal to the adult pattern; supplimenting the normal absence of JH in the last instar by JH analogue treatments maintains the larval developmental programme into the adult stage. Both these changes occur with the course of a single instar. What has become evident from these studies of the adultiform behaviour and electrophysiology is that considerable reprogramming of the CNS also occurs within the course of the 4th instar, following precocene treatment. The adultiform larvae show strong wing beating, can adopt the typical flight posture, and will attempt copulation (Pratt, 1979, personal communication). These adult behaviour patterns are not evident in the normal 5th instar larvae, although Kutsch (1971) has shown that the developing motor programme for flight behaviour is present in the 5th instar of *Schistocerca gregaria*.

While in most respects the observed morphological, behavioural and electrophysiological changes are consistent with a shift from the

larval to adult patterns, there are exceptions (p. 97). The jump responses of adultiform larvae show both larval and adult behaviour patterns that are expressed with more equal frequency than in the adults. Secondly, the morphology and muscle development of their metathoracic leg is reduced. The extensor tibialis muscle increases dramatically from the larvae to adult, but the adultiform larvae had an overall leg weight considerably lower than that of the normal larvae. Beenakkers and Van Den Broek (1975) have suggested that the flight muscles of *Locusta* only develop in the absence of JH, but report that CA implantation has little influence on the growth of 5th instar larvae, and only a slight effect on the metabolic pattern of the leg muscles. They conclude that high JH titres interfere with the initiation of adult muscle development but do not influence processes already in progress. Conversely, the results of the precocene treatments reported here suggest that the premature absence of JH initiates some adult muscle and neuronal development, but does not initiate adult development of the leg muscles. The neuronal development has considerable similarities to the normal adult development, including the expression of motor programmes involved in flight and in reproduction, but has some abnormalities as discussed above.

These data argue against the proposition of Hodkova (1979) that the insect brain 'counts' instars. He suggests that the neuroendocrine system in the brain of *Pyrrhocoris apterus* is programmed as early as the 3rd instar to inactivate the CA in the final (5th) instar. Such a programmed development would suggest that it could not easily be brought forward by an instar, as occurs after precocene treatment, while still allowing the consistency of morphological and neuronal development observed here.

SUMMARY

A.i) The marching behaviour of 5th instar larvae of *Locusta migratoria migratorioides* (R & F) was monitored using a treadwheel designed and built by Moorhouse *et al* (1978). This wheel was counterbalanced and gimballed such that the locust was held stationary in space while still able to perform many of the movements associated with normal walking behaviour. Data on the rotation of the wheel was stored on magnetic tape. Under these constant conditions and with an unchanging visual field the locusts exhibited walking behaviour that appeared similar to that recorded by Moorhouse *et al* (1978), and similar to that observed in the field and in less restrained laboratory experiments.

ii) Analysis of the magnetic tape data was accomplished by defining walking behaviour in terms of the speed of movement of the treadwheel. A minimum starting speed of 4mm/s and a minimum speed of walking of 2mm/s were selected. These definitions introduced an error that discriminated against data recorded during the poorest walking behaviour, possibly up to a 50% rejection level, while rejecting 5% or less of the data recorded during the best walking behaviour.

iii) The recorded walking behaviour was described in terms of the distance walked, the time spent in walking, the number of walking bouts and the speed of walking. Each parameter was determined over ten 1000s periods so that the behaviour was monitored over a total of 10^4 seconds on each day of the 5th instar. These values were compared within each 10^4s session for each age group and also between the 11 age groups used. Each age group represented one day of the instar, except for Days 0a and 0b, which each represented a 12h period within the first 24h.

iv) The changes observed in the walking behaviour during the 5th instar can be summarized as follows:

a) There was a rapid increase in the walking behaviour during the first 48h of the instar, until the maximal speed of walking, time spent in walking and distance walked were displayed on Days 3 and 4 (72 - 120h after ecdysis). The walking behaviour then declined slowly, with some variation in intensity between the age groups, until the penultimate day (Day 8); there was a sharp fall to the lowest levels of walking behaviour recorded on the final day, Day 9.

b) During each 10^4s session there was a gradual increase in the walking behaviour until a peak was reached, after which there was a decline in activity. In the early instar the peak in walking behaviour occurred after some 3000 - 6000s. The peak was observed to appear later in successive age groups during the mid-instar and on Days 5 and 6 the peak was not reached within the course of the recording session. However, during the late instar the peak was again reached within the 10^4s and on Day 8 had returned to a point 4000 - 6000s into the session.

c) The measurement of the distance walked over 10^4s was representative of the major changes observed in the other three parameters throughout the course of the instar. The measurement of the distance walked per 1000s was also considered to be a valid description of the walking behaviour within each session, throughout the instar. It was most closely related to the changing time spent in walking, while the speed of walking was more constant, both within the sessions and over the course of the instar. The relationship between the distance walked and the number of walking bouts was unclear, however.

B.i) A series of four identical treadwheels were developed to allow the recording of the distance walked per hour by large groups of locusts. These wheels were of a simple design and remained fixed in space while the locusts moved above them on counterbalanced arms. The exhibited behaviour was nonetheless similar to that described above. The walking behaviour of normal Day 3, 5th instar larvae was determined with these wheels and compared with the behaviour of JH - treated larvae. The subsequent morphological development of these JH - treated animals was scored as an indication of the JH titre in the haemolymph during their behavioural trials on the treadwheels.

ii) Synthetic juvenile hormones JH I, II and III proved to be of low morphogenetic activity when applied in the early 5th instar. No dose-response relationship was evident even when using high doses and there were no other indications of their activity in *Locusta*.

The Law's mixture was found to be a potent JH analogue, producing all of the morphogenetic effects associated with a high JH titre in the 5th instar. These effects included the production of typically juvenilized adultoids or fully supernumerary larvae. However, there were

high mortalities, a higher frequency of incomplete ecdysis and heavier melanization in these animals than was seen after treatment with another JH - analogue, ZR515. There was an increase in the 5th instar duration that was associated with the extent of juvenilization within each dosage group, but the instar duration was negatively correlated with dosage when the data from all groups was combined.

The JH - analogue ZR515 was found to have potent JH - effects when applied topically, inducing a variety of adultoid and supernumerary larval forms. These animals were studied morphometrically. The 5th instar duration was negatively correlated with the adultoid morphology over the 95 animals treated, but this was not evident within each dosage group.

iii) Precocene II was seen to act as an antiallatotropic agent when applied in the early 4th or 5th instars. In the 4th isntar this resulted in the loss of circulating JH and induced the precocious development to permanent 5th instar adultiform larvae. Within the first 18h of the 5th instar, precocene was seen to act as a potent antiallatotropin but also to cause pronounced juvenilization in the adult stage. The adultoids produced were morphologically indistinguishable from ZR515 - induced adultoids, as confirmed by detailed morphometric studies. It was postulated that, during a critical period of 8 - 18h into the 5th instar, the CA may be capable of synthesis and storage of JH, and that this was released during the precocene-triggered degeneration of the CA cells. The JH - effects of precocene were dose-responsive and were estimated to be of about half the activity of ZR515. The 5th instar duration was advanced in some groups but delayed in others. Within each group there was a negative relationship between instar duration and juvenilization, as indicated previously with ZR515, but this was not supported when the combined data from all dosage groups was examined.

iv) The marching behaviour of the JH - treated animals was determined on Day 3 of the 5th instar, i.e. between 0 and 3 days after JH treatment. It was found that the synthetic JH III, which was of low morphogenetic activity, caused 34% increase in the walking behaviour but this was not statistically significant. Treatment with Law's mixture caused a dose - responsive fall in marching behaviour that was postulated to be due to

the toxic effects of this form of treatment. Both the morphogenetically potent JH - analogues, precocene II and ZR515, were found to cause an increase in walking behaviour, of up to an 85% increase in the mean distance walked in 3h. However, the walking behaviour was highly variable in all groups and few of the differences in mean walking distance were statistically significant. ZR515 was found to enhance the mean walking behaviour by 65% when applied only 3h prior to the trial; this suggested that the change in behaviour was not mediated by growth or differentiation.

C.i) The precocious adultiform larvae produced by treatment of early 4th instar larvae with precocene II were found to be shifted from the normal 5th instar morphology towards that of the adults. This was confirmed by morphometric studies and by thoracic dissections. The hind, jumping, leg was of a similar size to that of normal larvae but weighed considerably less; the adultiform body weight was about 20% lower than that of the normal larvae.

ii) The behaviour of these adultiform larvae was studied in relation to normal larval and adult behaviour. The walking behaviour (as determined with the treadwheels) was reduced in the adultiform larvae from the high levels of the larvae towards the low adult levels. This change was statistically significant and was correlated with the increasing adult developmental status of the animals. The escape - response jumps of the adultiform larvae were of greater average length than in normal larvae but included frequent short jumps that were analogous to the adult 'take-off jumps'. Adults were found to occasionally display larval jumping patterns, but never at such high frequencies as observed in the adultiform larvae.

The tension developed by the extensor tibialis muscle during electrical stimulation of the motor nerve 3Nv5 was determined for each group of animals. It was found to be related to the average leg weight for each group, and was lowest in the adultiform larvae.

Finally the ventilation and feeding rates were monitored in the crowded stock cages, and the kicking behaviour was studied under crowded and isolated conditions. It was seen that the adultiform larvae were highly excitable and displayed extreme ventilatory and kicking movements. Their reduced marching behaviour recorded on the treadwheels was therefore suggested to be due to the increased competition between different behaviours.

iii) The spontaneous activity rates of the motor neurones DUMETi and SETi were monitored extra-cellularly in groups of normal larvae, adultiform larvae and normal adults. The gross spontaneous activity in nerve 3Nv5 was also determined in these groups. The activity of the FETi and DUMETi neurones was determined in similar groups, in response to stimulation of the anal cercii. These studies confirmed that the adultiform larvae had greater neuronal activity levels in nerve 3Nv5 than normal adults and this would partly explain their enhanced jumping, kicking and ventilatory behaviours. The activity of the 'slow' motor neurone, SETi, was however found to be lowest in the adultiforms. No attempts were made to record the responses of SETi to stimulation, nor to record the activity of the inhibitory neurone, CI.

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ADDENDUM

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The flicker fusion frequencies of six laboratory insects, and the response of the compound eye to mains fluorescent 'ripple'

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ABSTRACT. The ERG response of the compound eye to single, brief, light pulses, to sustained stimulation for 2 s, and the dark adapted flicker-fusion frequency (FFF) under stroboscopic light was measured in six species: *Locusta migratoria* (FFF range: 40–90 Hz), *Periplaneta americana* (25–60 Hz), *Saturnia pavonia* (65–85 Hz), *Antheraea pernyi* (25–70 Hz), *Glossina morsitans* (85–205 Hz) and *Drosophila hydei* (60–100 Hz). The first four species have typical 'slow-eyed', monophasic ERG responses; the two flies typical 'fast-eyed', biphasic responses. The FFF proved to be dependent on the state of light adaptation, being 40–70% higher than the above figures after only 2 min exposure to as little as 300 lx. Adult male *Glossina*, but not *Locusta* nymphs, showed a clear 100 Hz ERG ripple in response to single-phase, mains fluorescent lighting. To three-phase fluorescent lighting no 300 Hz ERG ripple was detected, but the 100 Hz component was still evident.

Introduction

Because of the nature of both fluorescent tubes and incandescent tungsten-wire lamps, these lights emit a flicker at a frequency of twice that of the alternating current (AC) power source driving them. Although the light may not actually be extinguished between peaks, a substantial sinusoidal ripple in the intensity is produced. This flicker is therefore normally present in rooms used for rearing insects or for observing their behaviour, and may affect that behaviour.

Renner (1957) described a reduction in the gathering behaviour of the honeybee (*Apis mellifera*) in a bee flight room illuminated by fluorescent tubes. Van Praagh (1972) described a bee flight room that can be illuminated with either a 100 or 300 Hz ripple, and has shown (Van Praagh, 1975) that honeybees reared entirely under 300 Hz conditions display higher wing-stroke frequencies, slower flying speeds and reduced obstacle-avoidance behaviour, when tested under a 100 Hz light-ripple.

Van Praagh (1975) also demonstrated that the honeybee has a flicker-fusion frequency (FFF) of above 100 Hz but below 300 Hz. The FFF is the maximum frequency of flickering light that the eye can resolve as discontinuous, and can be determined by a study of either the optomotor responses or the electroretinogram (ERG) responses of the insect (Fig. 1).

Autrum (Autrum, 1950; Autrum & Stoecker, 1950; Autrum & Gallwitz, 1951) cites FFFs ranging from 180 Hz to over 300 Hz for the honeybee, dragonfly and blowfly, but of below 60 Hz for the cricket and stick-insect. He designated these as fast and slow eyes, respectively, and both he and Ruck (1958a, b) have described various parameters of the ERG typical of each type.

In Britain, Europe and America the mains electrical supply has either a 50 or 60 Hz AC

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cycle, producing a light ripple of 100 or 120 Hz. These are both considerably lower than the FFFs typical of the fast eyes, and will be present in all experimental situations illuminated by standard, mains-driven lights.

With these problems in mind, this paper describes an ERG-based study of the FFF of several common laboratory insects.

Materials and Methods

Insects

The work was undertaken in two stages: first, a preliminary study involving adults of *Locusta migratoria migratoroides* (R & F), *Periplaneta americana* (L), *Glossina morsitans morsitans* (Westwood), *Drosophila hydei* Sturtevant, *Saturnia pavonia* L. and *Antheraea pernyi* Guérin Méneville; and second: a more detailed examination of fifth instar *Locusta migratoria* nymphs and adult *Glossina morsitans*. The locusts were obtained from the Centre for Overseas Pest Research, London; the tsetse flies were raised at the Imperial College Field Station from pupae reared by the Tsetse Research Laboratory, Langford, Bristol. Although some changes in the detail of the techniques and equipment were made between the two stages, the methods were basically similar.

Stimulation

Each insect was anaesthetized for *c.* 2 min with CO_2, and then fixed securely by the head and legs to a small metal platform, with black dissecting wax. For recording, this platform was held within a light-proof Faraday cage. Once the electrodes were in place the insect was left in total darkness until stimulation started. The flickering light stimulus was provided by a Flash–Tac transistorized stroboscope (Electronic Applications Ltd) for frequencies up to 250 Hz. For FFF measurements of over 250 Hz a Dawes Strobotorch 1202D was used. The light was focused on to a 5 mm fibre optic (Barr & Stroud) and further focused within the Faraday cage to produce a well-defined spot of *c.* 2 mm diameter on the insect's compound eye. Accurate measurements of the intensity of this spot were not possible, as the photometers available were not designed to record flickering light. However, using a Lunasix Mark III, values of between 5 and 22 lx were recorded over the range of frequencies used.

Recording

The recording electrode was inserted tangentially in the dorsal hemisphere of the eye, with the tip lying just beneath the cornea. The indifferent electrode was positioned ipsilaterally in the rear of the head or in the thorax. The electrodes were drawn from glass capillary tubing to a tip diameter of *c.* 20 μm. The recording electrode was filled with 2 M potassium citrate, while the indifferent electrode was filled with insect Ringer's solution. The signal was recorded on a Tektronix 564 B twin-beam storage oscilloscope, after impedance matching with a voltage follower (W.P. Instruments, type V.F.1). A trace of the stimulus was provided on the second oscilloscope beam from a photodiode placed near the stroboscope. Permanent records were made on film but in most trials the trace was temporarily stored on the oscilloscope screen. This was particularly important when determining FFFs, as it was often desirable to overlay a number of successive responses, so that any ripple-response present could be identified in the background noise.

Experiments

Each experiment consisted of three sections. First the FFF was determined by studying the ERG while increasing the stimulus frequency in steps of 5 or 10 Hz, and recording the highest frequency at which a ripple was seen (Fig. 1). The duration of stimulation was governed by the levels of background noise, but was generally 2–10 s for any one stroboscope frequency. The ERG waveform was also studied, in response to: (a) a single, brief pulse (40 μs) of light, and (b) a 2-s period of effectively sustained stimulation (see Fig. 3), provided by the stroboscope run at its maximum frequency (250 Hz).

While most references to FFFs in the literature concern the dark-adapted values, it is the light-adapted values that are of practical importance in behavioural experiments, as light tends to raise the FFF above

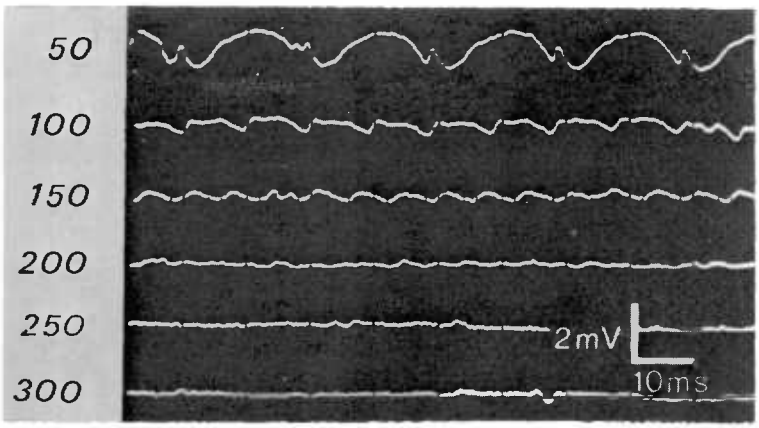


FIG. 1. Reduction and extinction of ripple-responses in male *Glossina* with increasing stimulus frequency. (The flicker-fusion frequency of this individual was 240 Hz). Stimulus frequencies are given on the left.

the dark-adapted levels (Bullock & Horridge, 1965). For this reason the FFF and ERG waveform were studied both after 30 min dark-adaptation and during a subsequent 20 min of light-adaptation. During this latter period the insect's head was illuminated by a ripple-free DC light (from a battery-powered torch), with the beam focused on to a 7-mm light-guide (Barr & Stroud). This beam had an intensity of *c.* 300 lx at the insect, though, because its light was continuous, this measurement is not directly comparable with that of the strobed stimulus light (above). The FFF was measured at intervals during the light-adaptation period by simultaneously stimulating the insect via the flickering (stroboscope) and DC (torch) light-guides. By this method it was possible to determine the increase in FFF as light-adaptation occurred.

Finally the equipment was transferred to an experimental room that provided both 100 and 300 Hz fluorescent lighting, and the ERGs of a number of insects were measured under these conditions, to determine if a ripple-response occurred to mains flicker.

Because of the limited number of trials per species, most results are quoted as ranges with the mean and sample size given only where appropriate.

Results and Discussion

ERG waveform

The responses of the six species were determined, after 90 min dark-adaptation, to a single brief pulse of light. The responses of *Locusta, Periplaneta* and *Saturnia* were of the 'slow' type. They were all similar, with little variation in form or speed. All displayed a sharply falling cornea-negative wave, slowly returning to the resting potential, often after a short plateau on the return slope. The response had a latent period of 10–18 ms, and a duration of 250–320 ms (Fig. 2a). The response of *Antheraea* was of a similar form and duration, but no plateau was seen and an initial, brief, cornea-positive component of low amplitude occurred occasionally. All showed a sustained, ripple-free, cornea-negative potential in response to sustained stimulation (Fig. 3a). When stimulation ceased the potential did not return immediately to the resting level, but dropped sharply towards it initially and then gradually travelled the remaining distance.

The responses of *Glossina* and *Drosophila* were of the 'fast' type and were similar to each other, with a fast diphasic response, comprised of a 4–6 ms latent period followed by a positive and a negative component. The potential then slowly returned to the resting level, within 200–300 ms. Although the diphasic components were usually of equal magnitudes, some responses were more complex, with more than one cornea-positive component (Fig. 2b), while others had reduced or absent positive components. The flies' responses to sustained stimulation were also similar (Fig. 3b), with a positive on-response, a sustained negative component (containing

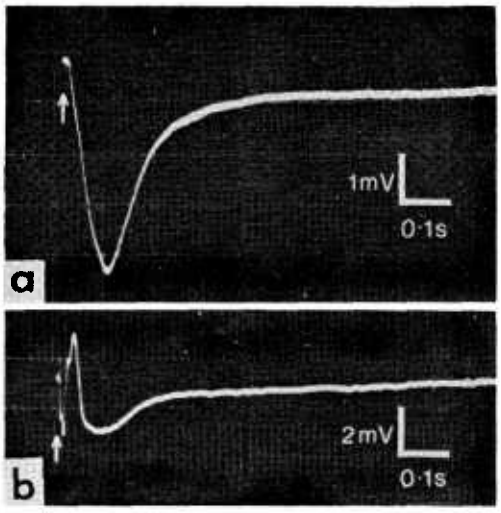


FIG. 2. ERG responses of (a) *Locusta* and (b) *Glossina* to brief (40 μs) light pulses (indicated by white arrows).

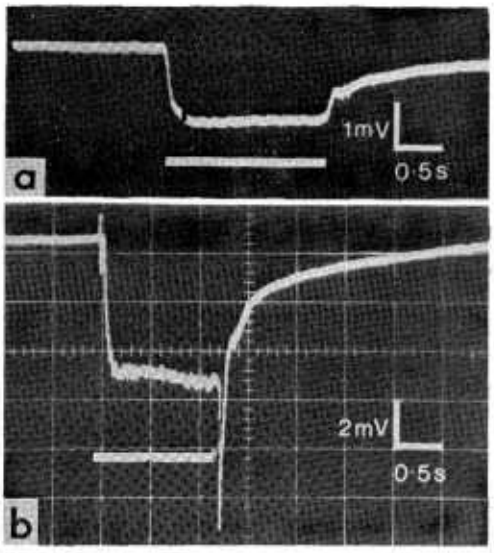


FIG. 3. ERG responses of (a) *Locusta* and (b) *Glossina* to short (2 s) periods of sustained stimulation (indicated by white bars; see text).

the ripple-response in some individuals, but not visible in Fig. 3b) and a fast negative off-response.

These responses are all similar to those found by others (Autrum, 1950; Ruck, 1958a, b, 1961; Yinon, 1970), and tend to fall within Autrum's classification, so that the locust, cockroach and moths could be described as slow-eyed, and the flies as fast-eyed (Table 1).

FFFs of dark-adapted insects

The FFFs (determined after 90 min dark adaptation, and to the nearest 5 Hz) showed large overlaps between the six species (Table 2), although the nocturnal or less active species of each order (*Periplaneta, Anthereae* and *Drosophila*) tended to have the lower fusion frequencies. This also fits with Autrum's (1950) classification.

In general the FFFs are comparable with those already reported for the same or related species (see Bullock & Horridge, 1965; Ruck, 1958a). However, those of the Diptera are lower than have previously been reported (e.g. Ruck, 1961). One reason for this may be the relatively low intensity of the stimulating light, since in contrast to the 5–22 lx used here, Ruck (1961) used up to 12,000 lx, which would not only provide a much stronger stimulus, but would also tend to raise the FFF through light-adaptation (see below).

FFF changes during light adaptation

Working only with the fifth instar *Locusta* and adult *Glossina*, it was found that exposure of the dark-adapted insects to even short periods of light resulted in an increase of the FFF. This increase was studied by determining the FFF of individuals as rapidly as possible (to minimize the change caused by the stimulus itself) after 30 min dark-adaptation, and then at frequent intervals during 20 min light exposure, at approximately 300 lx.

Locusta migratoria. The dark-adapted FFFs agree closely with those determined above, with a mean of 49.5 Hz (range 40–80 Hz, $n = 10$). After light adaptation this had increased to a mean of 85.0 Hz (range 55–100 Hz, $n = 10$). The rate of FFF-increase was initially high, and the process nearly complete within 2 min (Fig. 4).

Glossina morsitans. Because the FFFs of the males were over 250 Hz, the higher range stroboscope had to be used (Methods). The light this provided appeared to be comparable in both colour and intensity to that of the original machine, both having Xenon gas discharge tubes as their light source, but no precise measurements could be made.

It was suspected that the lower FFFs found in the unsexed tsetse flies in Table 2 were those of the females, as later trials with

TABLE 1. ERG response characteristics of the adults of six insect species to single light pulses, after a total of 90 min dark adaptation

Species	*n*	Mono/ biphasic	Latency (ms) Range	Latency (ms) Mean	Duration (ms) Range	Duration (ms) Mean	Amplitude (mV) Range	Amplitude (mV) Mean
Locusta	17	Mono	6–18	10.5	200–450	277.7	6.8–1.2	3.8
Periplaneta	14	Mono	11–25	17.5	180–600	290.0	4.7–0.7	2.4
Saturnia	3	Mono	11–18	14.3	170–320	245.0	7.4–0.2	4.2
Antheraea	4	Mono/bi	13–25	18.0	210–420	295.0	8.4–0.2	4.0
Drosophila	11	Biphasic	3–8	4.8	90–250	173.3	1.5–0.2	0.6
Glossina	14	Biphasic	4–10	6.7	150–350	268.2	3.0–0.9	1.9

n = no. of individuals tested (sex and age unknown). Amplitude refers to major, cornea-negative component only.

TABLE 2. Flicker-fusion frequencies of the adults of six insect species after a total of 90 min dark adaptation

Species	*n*	Flicker-fusion frequency Range	Flicker-fusion frequency Mean
Locusta	16	40–90	66.3
Periplaneta	14	25–60	41.8
Saturnua	3	65–85	76.7
Antheraea	4	25–70	42.5
Drosophila	8	60–100	81.9
Glossina	14	85–205	116.4

n = no. of individuals tested (sex and age unknown).

sexed flies showed a marked sex difference. Only five individuals of each sex were tested, but they showed only a slight overlap of their FFF ranges at each determination, and the mean frequencies were clearly separated (Fig. 4). The males had dark-adapted FFFs ranging from 183 to 233 Hz (mean 212.5 Hz, $n = 5$); the females from 150 to 190 Hz (mean 176.0 Hz, $n = 5$). Both ranges are considerably higher than those determined above (Table 2), but agree more closely with FFFs found in other Diptera (Autrum, 1950; Ruck, 1961). The reasons for this increase are unknown, but are not connected with the change in stimulus-

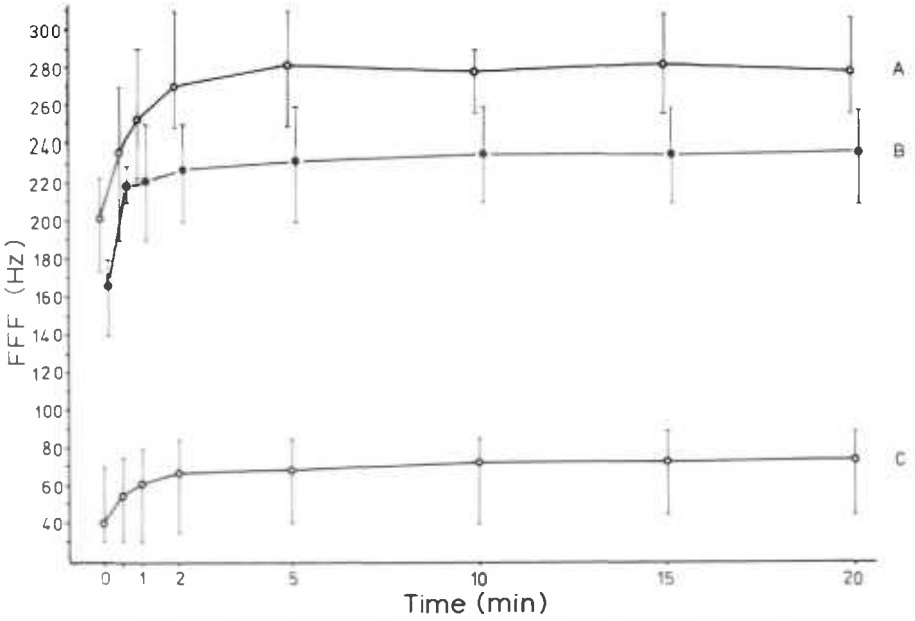


FIG. 4. Increase in flicker-fusion frequency during exposure to light of 300 lx; FFF against time after light-on. Circles indicate mean values; bars, ranges. (A) *Glossina* (male, $n = 5$); (B) *Glossina* (female, $n = 5$); (C) *Locusta* nymphs (sex unknown, $n = 10$).

source, as this was itself necessitated by the high FFFs recorded with the original light-source.

The FFFs of both sexes increased rapidly when the insects were light-exposed, reaching 285–308 Hz (mean 290.0 Hz, $n = 5$) in the males, and 220–270 Hz (mean 248.0 Hz, $n = 5$) in the females. As in the locust the process was almost complete within 2 min (Fig. 4). The sex difference in FFF cannot be attributed to changing experimental conditions, as the two sexes were used in overlapping sessions of trials.

FFF increases under varying light intensities

The effect of light intensity on the FFF was tested in one locust nymph, and in one male and one female tsetse fly. Each was dark-adapted for 30 min and its FFF determined as usual; further FFF determinations were then made during a 20-min light-exposure, as before, to light of a known intensity. This entire process (dark-adaptation and subsequent light-exposure) was repeated five times using different light intensities, with stimulus and adapting lights being reduced together by neutral density filters.

The results (Fig. 5) show that light intensity had two effects on the change in FFF during light-adaptation. First, as the intensity was increased, the overall change within the 20-min adaptation period also increased. In the locust this rise was 15 Hz under *c.* 0.6 lx, and 50 Hz under 300 lx, representing increases of 33% and 111%, respectively. In the male tsetse the rise was 30 Hz (14%) under 8 lx and 60 Hz (26%) under 300 lx. In the female it was 30 Hz (17%) and 80 Hz (42%), under the same intensities. Second, as the intensity increased, the speed of the adaptation process was also increased, and it seems probable that at 70 lx and below, adaptation was not complete within the 20 min allowed. It is likely that these phenomena are partly, but not wholly, due to the reduced intensity of the stimulating light, reducing the ripple-response magnitude of the ERG.

ERG responses under 100 Hz and 300 Hz fluorescent lights

A total of six male *Glossina*, with light-adapted FFFs ranging from 270 to 300 Hz,

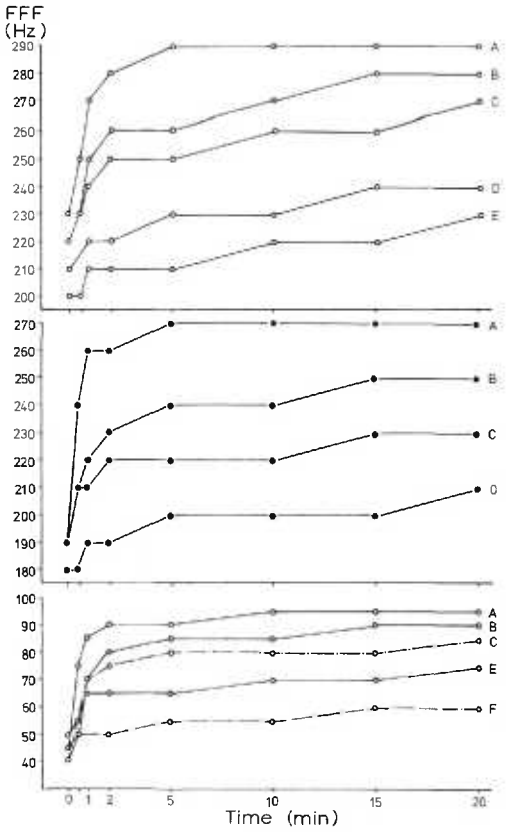


FIG. 5. Increase in flicker fusion frequency during exposure to light of varying intensities; FFF against time after light-on. Top: *Glossina* (male, $n = 1$); middle: *Glossina* (female, $n = 1$), bottom: *Locusta* nymphs (sex unknown, $n = 1$). Light intensities: (A) 300 lx; (B) 70 lx; (C) 25 lx; (D) 7.5 lx; (E) 3 lx; (F) 0.6 lx.

were tested without dark-adaptation, under both 100 and 300 Hz conditions. Because the former was achieved by cutting out two-phases of a three-phase lighting system, the light intensity at the insect's eye was reduced from 180 lx (at 300 Hz) to 70 lx (at 100 Hz).

The single-phase lighting had an intensity ripple of ±56% of the mean intensity. All six flies gave strong 100 Hz ERG ripple-responses to this (Fig. 6a). Because the fluorescent tubes used on the three phases were not perfectly matched, in colour or intensity, a low amplitude 100 Hz component of ±6% of the mean intensity overlaid the 300 Hz ripple emitted (Fig. 6b). The ERGs also showed this 100 Hz component but apparently did not respond to

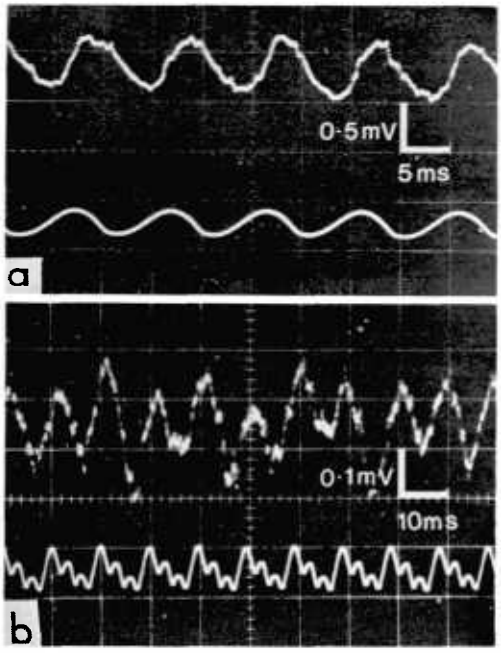


FIG. 6. Ripple responses of male *Glossina* to fluorescent lighting at (a) 100 Hz and (b) 300 Hz. Top trace: ERG response; bottom trace: photocell response.

the 300 Hz component (Fig. 6b). Two individuals with high light-adapted FFFs (290 and 300 Hz) were left under the 300 Hz lighting for over 1 h, but still no 300 Hz components were detected in their response. The possibility cannot be excluded, however, that there was a small 300 Hz component to these responses hidden in the noise. This was high owing to the difficulty of shielding the apparatus in this room.

Two locust nymphs (with light-adapted FFFs of 85 and 90 Hz) were similarly tested, but neither showed any ripple-responses to the 100 Hz lighting. The 90 Hz individual was left for over 1 h under the fluorescent lighting, but still no ripple was detectable.

Conclusions

From these findings it seems probable that those insects classified by Antrum (1950) as 'fast-eyed', e.g. honeybees, dragonflies, house-flies and most diurnal, fast-flying insects, can resolve mains-driven lighting as flickering. From the work of Renner (1957) and Van Praagh (1975), it is also likely that this ability is reflected in some behavioural changes, either when studied under 100 Hz lighting or as a result of being reared under such lighting systems. Although the 'slow-eyed' insects, such as locusts, stick-insects, cockroaches and moths are generally thought to have FFFs of 40–60 Hz (Bullock & Horridge, 1965), these values are considerably raised by light-adaptation. It therefore seems likely that at least some individuals can resolve the flicker of mains-driven lights, if allowed even 2 min exposure to relatively dim conditions (300 lx). It also seems apparent that if three-phase lighting is adopted to avoid these problems, care must be taken to ensure that each phase is of equal intensity and colour, since tsetse flies, at least, can resolve even a 6% 100 Hz ripple.

Acknowledgments

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THE MORPHOLOGICAL AND BEHAVIOURAL EFFECTS OF PRECOCENE II ON *LOCUSTA*

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(*Received* 8 *February* 1980)

Abstract The precocious adultiform larvae produced by topical applications of precocene II to early 4th instar *Locusta* were found to be intermediate between normal 5th instar larvae and normal adults on behavioural as well as morphological criteria. Precocious adultiforms walked significantly smaller distances in 2 hr than normal larvae, but slightly more than normal adults. The adultiforms jumped significantly greater distances without wing movements than normal larvae, but they also made frequent, short, adult-type jumps with wing movements. Most advanced adultiforms made jumps of both types whereas normal adults made only a small percentage of the larval-type jumps. Apparently the central nervous organisation of the adultiforms provides equally and separately for both larval and adult behaviour patterns, rather than compounding the two.

Increasing doses of precocene above 100 μg produced more advanced adultiforms, and there was some indication that morphologically similar types produced by different doses may differ in their behaviour.

Key Word Index: Precocene II, *Locusta*, morphology, behaviour

INTRODUCTION

ALTHOUGH some confusion surrounds the topic, it seems that the changes seen in the locomotor activity levels of larval and adult locusts are at least in part related to the levels of the circulating hormones (review: TRUMAN and RIDDIFORD, 1974).

CARLISLE and ELLIS (1963) found that injections of prothoracic gland homogenates reduced the marching behaviour of gregarious, 4th instar *Locusta*, and this agrees with the observation of HASKELL and MOORHOUSE (1963) that active prothoracic gland homogenates reduced the motor neurone output associated with walking in locusts.

ODHIAMBO (1966) showed that allatectomy of both immature and mature male *Schistocerca* produced persistent locomotor inactivity; the implantation of active corpora allata (CA) reversed this effect. STRONG (1968), however, did not find any obvious reduction in the locomotor activity of young male *Locusta* adults following allatectomy, and assumed that the reduced activity levels reported by ODHIAMBO (1966) were due to the reduced sexual behaviour of *Schistocerca* following allatectomy. WAJC and PENER (1971) subsequently reported significantly reduced flight activity in allatectomized *Locusta*, measured on a roundabout where sexual behaviour was excluded.

In order to study further the influence of hormones on marching behaviour, a series of experiments were undertaken using precocene II to effectively allatectomize *Locusta* larvae. The morphogenetic effects of precocene II on locusts have been described by PENER *et al.* (1978), following the discovery by BOWERS *et al.* (1976) that certain plant chromenes, and in particular precocene II (6,7-dimethoxy-2, 2-dimethylchromene), have 'anti-juvenile hormone' effects in several insect orders.

PENER *et al.* (1978) reported that precocene causes all the effects expected in *Locusta*, with atrophy of the CA and a resultant loss of any circulating juvenile hormone (JH). They found that topical treatment with 100 μg, applied in the 4th instar and within 24 hr of ecdysis, was most effective in producing exclusively permanent, precocious 5th instar adultiforms, and with the lowest mortalities. Responsiveness dropped rapidly during the instar and by the 4th day even 400 μg was ineffective in producing adultiforms. These higher doses were toxic when applied in the responsive period. Other effects noticed were delayed moulting and anti-gonadotrophic effects in morphologically normal adults. There is no evidence to suggest that precocene II affects any other organs in *Locusta*.

The highly selective action of precocene II on the CA therefore makes it a powerful tool for chemical allatectomy in *Locusta*. In the work described here topical treatments of 50–400 μg were applied at the most responsive period in the 4th instar. The marching and jumping behaviour of the 5th instar larvae or adultiforms was subsequently monitored, using treadwheels and forced escape jumps. Morphological measurements and dissections were used to characterize further the effects produced.

MATERIALS AND METHODS

Locusts

Batches of mid-third and mid-fourth instar larvae of *Locusta migratoria migratoriodes* (R & F) were provided from crowded cultures by the Centre for Overseas Pest Research, London. They were kept in cylindrical perspex cages (20 × 35 cm) provided with

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Fig. 1. Arrangement of treadwheels used for measuring the distance walked by *Locusta*. (a) Small spring clip holding: (b) larval locust with wire clip attached to pronotum; (c) Bearings and restraining supports for (d) counter-balanced arm; (e) Wheel with (f) attached metal flag passing through (g) opposed infra-red light switches.

perforated zinc lids and furnished with twigs. The room was maintained at 28°C (±0.5°C) under a 12:12 LD regime with 'lights on' at 7 am. All animals were fed on a mixture of glasshouse-grown wheat seedlings and bamboo leaves grown locally, provided in abundance at 8 am daily. At the same time all animals that had moulted to the 4th instar were removed, treated and then kept in cages containing approx. 25 animals each. All locusts in each cage had received the same treatment.

The treadwheel

Four identical wheels were used, based on the design of Moorhouse *et al.* (1978). Each consisted of a disc of polystyrene surrounded by a rim of card 16 mm wide covered with masking tape, and with a circumference of 500 mm. The wheels were supported in balsawood frames, and a small metal flag attached to the wheel passed through two opposed infra-red light switches. Thus the wheel had to complete a full revolution before the counter was activated. The wheels were carefully balanced, and ran on bearings of PTFE sheeting (Fig. 1). The locust was held above the wheel by a counter-balanced balsa wood arm which was restrained from excessive movements in any direction, and also ran on PTFE bearings.

The wheel was surrounded by a U-shaped white wall, 1 m deep, 0.75 m wide and 1 m high. The arena floor was matt black, the ceiling white, and a matt black stripe 50 mm wide and 1 m high was positioned vertically in front of the wheel. The opening in the wall, behind the wheel, was covered by a curtain of white muslin. The arena was lit by a 75 W fluorescent tube mounted horizontally above and behind the wheel, each 5 ft tube being used to light two adjacent arenas.

Behavioural quantification

All animals were tested 72–96 hr after ecdysis. Using the 4 treadwheels in 5 sessions it was possible to test 20 animals a day. The control and test groups had an approximately even spread of trials throughout the 5 sessions.

Two hours before the start of each session, four animals of the correct age were selected from a given precocene dosage group and put in a separate cage, deprived of food. Only those precocious adultiforms were used that appeared healthy and showed no atypical features. At the time of this selection a small wire clip was fixed across the dorsal surface of the pronotum, using a cyanoacrylate ester glue with very rapid bonding properties (Fig. 1).

The locusts were then left undisturbed for 2 hr before being clipped into position above the four treadwheels. They remained on these wheels for 2 hr, and the distance walked in each hour was recorded. The wire clips were then removed and the animals left for a further 30–60 min, still without food. They were individually stimulated by an approaching finger to jump repeatedly across a concentrically marked grid. The first ten jumps were measured, while small hops of less than 15 cm were ignored. The wings of the adults were immobilized with glue or wax to make flight impossible. Attempts to fly could still be recognized by the sound of restricted flapping movements.

Morphometrics

A number of the treated locusts were killed and various measurements taken in order to quantify the morphological changes seen in the precocious adultiforms (Table 2). Only two classes of adultiforms were defined as there were no morphological differences visible between type 2 and type 3 adultiforms as defined later (see Results). Type 1 therefore corresponded to groups 1 and 2 of Pener *et al.* (1978), while type 2/3 corresponded to Pener's groups 3 and 4. A number of normal 5th instar larvae and normal adults were also measured. The measurements taken were chosen to describe the major morphological differences visible, and corresponded to the measurements used by Dirsh (1953). All measurements were taken to the nearest 0.5 mm. Dirsh (1953) recommends the use of calipers for most measurements, but the differences seen between normal and adultiform animals were large enough to allow less accurate measurements.

Treatments

Precocene II (99%) was obtained from Aldrich Chemical Co. Ltd. All animals were treated within 24 hr of ecdysis. A given dose of precocene II was applied by microsyringe in 5 μl acetone, such that the liquid film spread beneath the wing-buds and across the dorsal surface of the abdomen. Controls received acetone alone.

Table 1 shows the various treatments used and the numbers tested on the treadwheels. Animals shown as having received treatment while adult were derived from the control cages of 5th instar larvae which had received a dose of acetone in the 4th instar, or from stock cages of 5th instar larvae and had so received acetone on the first day of adulthood. No animals were

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Table 1. Time and mode of application of precocene II, numbers of animals used and duration of instar

Time of application	Dose of precocene (μg)	Total No. treated	No. tested on wheels	Median length 4th	Instar (days) 5th
0–24 hr 4th instar	0	105	41	6	9
	50	25	12	6	10
	100	53	23	7	10
	200	122	69	7	10
	400	51	42	8	11
0–24 hr Adult	0	(see text)	30		

used twice, however, and no differences were found between the two groups.

RESULTS

Treatment with four doses of precocene on Day 0 (0–24 hr after ecdysis) of the 4th instar produced a graded series of morphological and behavioural changes in the 5th instar.

Morphology

The morphological changes are indicated in Fig. 2 and Table 3. Doses of 50 μg were apparently ineffective in all respects, with no significant differences in duration of the 4th and 5th instars, no morphological effects and no significant behavioural differences.

Doses of 100 μg were noticeably more effective, producing a mixture of normal 5th instar larvae and precocious adultiforms. There was a slight delay in the moult to the 5th instar, and those animals moulting to the 6th (adult) instar were also delayed (Table 1). A limited number of apparently normal 5th instar larvae did not display the expected moult to the adult stage; this was typical of 1–2% of all groups treated with 100 μg or more.

Doses of 200 μg were found to be highly effective, producing a majority of fully precocious adultiforms in the 5th instar [corresponding to types 3 and 4 in Fig. 1 of PENER *et al.* (1978)]. The highest dose of 400 μg was only slightly more effective, producing almost exclusively the most severely affected adultiforms after a delayed moult. Mortalities in all groups were similar to those of the control group, and were generally lower than those of PENER *et al.* (1978) where total mortalities in the 200–400 μg dosage groups were 57–87%.

The animals were classified into five types as follows:

Type 0 morphologically normal 5th instar larvae;
1 larve of almost normal appearance, but displaying some degree of the grey colouration typical of adults, and occasionally slightly enlarged wing-buds;
2 adultiforms with strong adult colouration and well developed wings; the wings were manually movable but did not flap during handling or jumping;
3 adultiforms morphologically indistinguishable from those of type 2, but seen to flap their wings during handling;
A morphologically normal adults.

The morphological measurements summarized in Table 2 show a graded series of changes in the size of the head capsule and the body. Wing lengths were also greatly affected. The hind legs, however, were relatively close to expected 5th instar dimensions and showed no overall pattern of change. The two ratios commonly used in studies of phase-polymorphism (E/F and C/F) showed a graded shift from larval values to adult values, with the precocious adultiforms inbetween.

Table 2. Morphological measurements of normal fifth instar larvae, precocious adultiforms and normal adults. (All measurements in mm ± 1 S.D.)

Dimension	Sex	Normal fifth instar larvae ♀n = 15 ♂n = 11	Type 1 adultiforms ♂n = 11	Type 2/3 adultiforms ♀n = 40 ♂n = 30	Normal adults ♀n = 16 ♂n = 10
Elytron (E)	♀	11.13 ± 0.55		16.85 ± 2.17	48.47 ± 1.85
	♂	11.73 ± 0.68	13.21 ± 0.91	16.72 ± 2.66	53.45 ± 0.96
Hind femur (F)	♀	17.00 ± 0.46		17.00 ± 0.47	22.72 ± 0.73
	♂	17.82 ± 0.46	17.43 ± 0.67	17.55 ± 0.46	23.90 ± 0.57
Head width (C)	♀	7.07 ± 0.26		6.50 ± 0.30	7.84 ± 0.30
	♂	7.41 ± 0.20	7.00 ± 0.29	6.95 ± 0.27	8.50 ± 0.00
E/F ratio	♀	0.655 ± 0.025		0.991 ± 0.127	2.135 ± 0.088
	♂	0.658 ± 0.029	0.756 ± 0.058	0.952 ± 0.151	2.238 ± 0.067
C/F ratio	♀	0.416 ± 0.016		0.383 ± 0.019	0.345 ± 0.001
	♂	0.416 ± 0.013	0.397 ± 0.015	0.397 ± 0.013	0.356 ± 0.009

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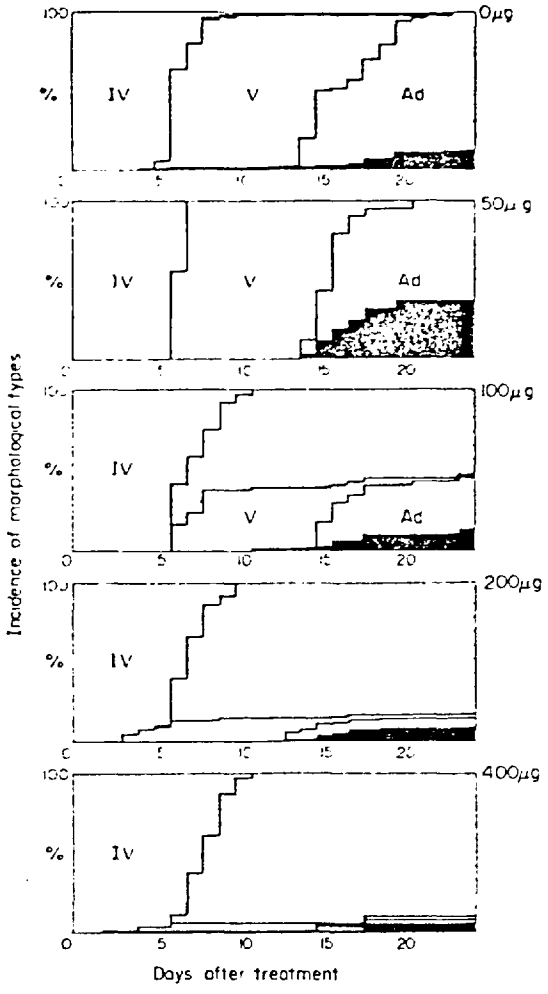


Fig. 2. Percentage incidence of morphological types following treatment with precocene II on Day 0. Doses are given on the right. IV and V, normal 4th and 5th instar larvae: Ad, normal adults. Cross-hatched areas, precocious adultiforms: black areas, mortalities.

From a limited number of thoracic dissections, it was possible to relate the overall development of the flight musculature to the external appearance of the adultiforms. Animals of types 0 and 1 had very reduced muscle blocks identical to those of normal 5th instar larvae, while type 2 and 3 adultiforms had apparently fully developed flight muscles, although the thorax was smaller than that of a normal adult.

Behaviour

Analysis of the treadwheel results from the control group of 41 larvae indicated that there were no significant differences in the distance walked in two hours that might have been related to the wheels used ($n = 10$–11; $P > 0.05$, 1-way analysis of variance). Moreover, the experimental design was such that approximately equal numbers of animals were used from each wheel, within each treatment group, to reduce the effects of any behavioural differences related to the four wheels. The following results are therefore presented regardless of the wheels used.

Table 3. % frequency distribution of morphological types (0–3) produced in the 5th instar by topical applications of precocene II at the start of the 4th instar

Dose (μg)	Sample size (n)	Morphological type (see text) 0	1	2	3
0	41	100	0	0	0
50	12	100	0	0	0
100	22	31.82	13.64	0	54.55
200	69	10.15	13.04	7.25	59.42
400	42	2.38	4.76	4.76	80.95

There were no sex differences in behaviour in the control ($P = 0.59$, Mann-Whitney U-test) or test groups ($P = 0.11$–0.38, Mann-Whitney U-tests). Approximately equal numbers of both sexes were used in all groups, and the results are given regardless of sex.

Analysis of the distance walked by the 41 control larvae indicated no significant differences in the behaviour of animals tested over the five 2 hr sessions used each day ($n = 8$–13; $P > 0.05$, 1-way analysis of variance). The locusts in each treatment group were used to give as even a distribution as possible over the 5 sessions, and this possible source of variation is ignored hereafter.

Distance walked. Topical treatment with 100–400 μg at the start of the 4th instar resulted in adultiforms that showed reduced marching behaviour in the 5th instar. Because of the mixed morphological effects produced within each dosage group, analysis of the marching

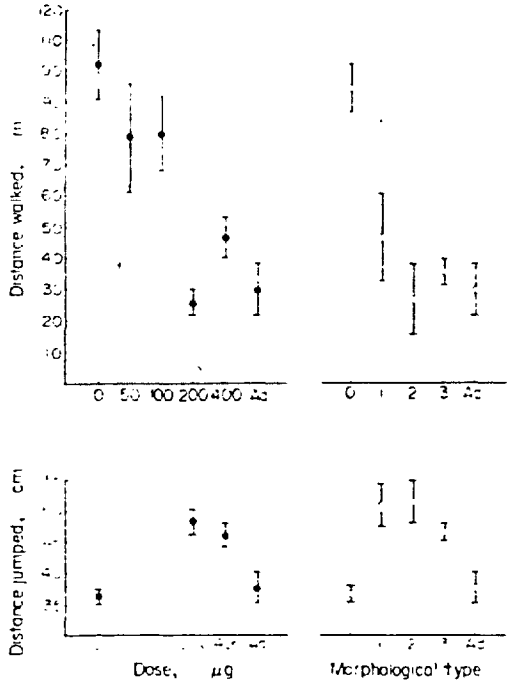


Fig. 3. Distances walked and jumped by normal and adultiform *Locusta*. All figures given as means ± S.E. Above, distances walked (m) on treadwheels over 2 hr. Left, groups defined by precocene dosage: 0 and Ad, control larvae and adults; 50–400, doses of precocene II (μg) applied in the 4th instar. Right, groups defined morphologically. Below, jump lengths (cm) of dose-defined groups (left) and of morphologically-defined groups (right).

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behaviour was made on morphologically defined groups (types 0–3 and Ad.), as well as by dosage. The results were similar: increasing doses of precocene resulted morphologically in increasingly affected adultiforms that displayed a graded reduction in walking (Fig. 3).

The control larvae were not significantly different from the 50 μg and the 100 μg groups. All walked significantly more than the control adults ($P \leq 0.0001$, 0.0221 and 0.0013 respectively). Both the 200 μg and 400 μg groups walked distances similar to those of the control adults, but the 200 μg group walked significantly less than the 400 μg group ($P = 0.0080$). Both these treated groups walked significantly less than the control larvae ($P < 0.0001$), the 50 μg and 100 μg groups ($P = 0.0002$ and 0.0194). Analysis in terms of the morphologically-defined groups revealed that the type 0 adultiforms did not differ significantly from the control larvae. Types 1, 2 and 3 walked significantly less than the controls and the type 0 group ($P \leq 0.0070$), and were similar to the control adults. The type 2 adultiforms walked less than the other adultiforms at all doses but these differences were not statistically significant ($P \geq 0.1211$). All the above significance levels are from 2-sample *t*-tests.

However, analysis of the distances walked within these morphological types also appeared to show a dose-related change. All morphological types produced by 200 μg walked considerably less than morphologically similar animals produced at other doses. Although most of these differences are not significant ($P > 0.05$, Mann-Whitney U-tests), many are nearly so, and the type 3 animals produced by 200 μg walked significantly less than the type 3 animals produced by 100 or 400 μg ($P < 0.005$, Mann-Whitney U-tests).

Distance jumped. After each locust had been made to jump ten times across a marked surface the average of its five best jumps was taken as a measure of that individual's physical and/or excitory state. From Fig. 3 it can be seen that the precocious adultiforms (types 2 and 3) exhibited much longer jumps than the normal 5th instar larvae ($P < 0.005$, Mann-Whitney U-tests).

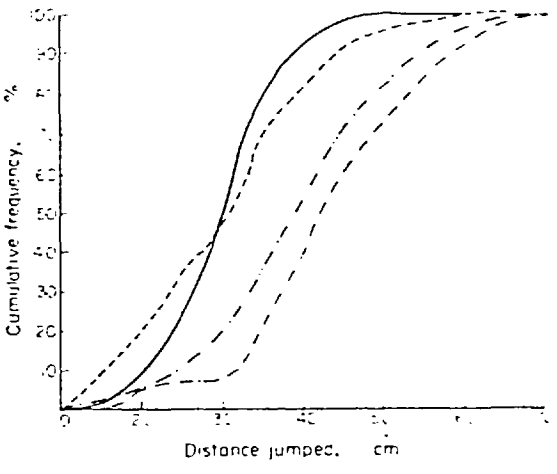


Fig. 4. Cumulative frequency distributions of all jump lengths for normal 5th instar larvae (——), normal adults (– – –), type 2 (— · —) and type 3 adultiforms (— · · —).

However, during these trials, it was noticeable that the most advanced adultiforms and normal adults tended to make small leaps, coupled with wing movements, that could be easily distinguished from the hoppers' escape jumps. From the frequency distributions of all jumps (Fig. 4), it can be seen that the control larvae were grouped closely around the median of a 30 cm jump. Very few longer (> 40 cm) or shorter (> 20 cm) jumps were seen. By contrast, the adultiforms show a larger distribution of jump lengths, with nearly 20% of the jumps being greater than 50 cm. There is also a distinct plateau at the lower end of the distribution curve, particularly in the type 2 group, where nearly 10% of the adultiform jumps were of 30 cm or less. This represented part of a series of short jumps that were apparently attempts at flight.

Normal adults rarely displayed larval jumps, and the frequency distribution shows a large percentage of short jumps. There is a slight plateau at the higher end, however, representing a few (< 5%) long, larval jumps of lengths equal to those made by the adultiforms.

DISCUSSION

Morphogenetically, precocene treatment in the early 4th instar of *Locusta* acts as an anti-JH (PENER *et al.*, 1978). The number and forms of adultiform larvae produced in this work were similar to those produced by PENER *et al.*, but they did not report the low percentage of permanent, morphologically normal, 5th instar larvae found here; BOWERS *et al.* (1976) produced similarly suspended development in less than 10% of 4th and 5th instar *Oncopeltus fasciatus*, using unspecified, near-toxic doses of precocene II. The morphometric measurements confirm that the 5th instar adultiform larvae produced were consistently shifted in their development towards the normal adult form. The two morphometric ratios that were used, E/F and C/F, showed a graded transition from larval to adult values, with no indication of any phase-shifting towards solitarious morphology. From the gross muscle appearance, the internal development was consistent with the external morphology, and active, well-developed flight muscles were seen in the most precocious adultiforms.

Behaviourally, normal adults walked much less than normal 5th instar larvae. By the relatively coarse measure used here, of the distance walked in 2 hr, precocene treatment apparently shifts the 5th instar behaviour from the larval to the adult pattern. The drop in the distance walked by the high-dosage groups was due to the increasing proportion of adultiforms within each group. When the groups were redefined by morphological type, the type 1 and 2/3 adultiforms walked distances similar to those of the adults. The very small distances walked by the type 2 animals may be an artifact due to their being distinguished from the type 3 animals only by the lack of wing flapping on handling, since this criterion would select less excitable animals. The reduced walking exhibited by the adultiforms on the treadwheels is not explicable as a lack of leg musculature, as the jumping responses were strong.

The escape responses in *Locusta* are not rigid. Normal larvae can only exhibit larval jumps, since the

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wing-buds are immobile: their infrequent short jumps cannot be classified into 'leaps' or 'attempted take-offs'. Normal adults display almost exclusively short, near-vertical, take-off jumps; less than 5% of the jumps are of the low, larval pattern. The few low, larval jumps exhibited by these adults were over distances similar to those of the precocious adultiforms.

All the adultiforms displayed larval jumping patterns, with jump lengths considerably greater than those made by the normal larvae, although their hind legs were morphologically indistinguishable. The type 2 and 3 adultiforms also made a number of the short, adult jumps, and these were accompanied by wing flapping in many of the type 3 animals. The same individuals were seen to display both types of escape response within the few minutes of the trials. The frequency of these adult-like jumps is not evident in Fig. 3 because of the sampling error introduced by choosing only the best five jumps made by each individual. Examination of the frequency distributions of all jumps made within each group reveals the non-Normal distribution of jump lengths.

The central nervous system of the adults and adultiforms is therefore not wholly larval of adult, but has the ability to display characteristics of both. While normal adults only occasionally display larval jumping behaviour, the precocious adultiforms readily and separately display both larval and adult jumping behaviours. This possibility was not exposed in the relatively coarse measurement of their behaviour recorded by the treadwheels.

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PRECOCENE II HAS JUVENILE-HORMONE EFFECTS IN 5TH INSTAR *LOCUSTA MIGRATORIA*

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Abstract—Precocene II can cause juvenilization of 5th instar *Locusta migratoria*. The adultoids and supernumerary larvae produced following precocene treatment are morphologically indistinguishable from adultoids produced by treatment with a JH-analogue, while the CA are markedly reduced in size or absent. The time-response curves for the induction of juvenile characters and for the degeneration of the CA suggest that the CA may be active during the early 5th instar. The CA are necessary for juvenilization to occur in response to precocene, and the effect is probably the result of synthesis or release of JH during the breakdown of the glands.

Key Words Index: Precocene, *Locusta*, juvenilization, CA-degeneration

INTRODUCTION

PRECOCENE has been reported to induce precocious metamorphosis in several insect species (BOWERS *et al.*, 1976). Such a morphogenetic effect has been confirmed for *Locusta*, and in this species precocene has an apparently specific anti-allatotropic action (NEMEC *et al.*, 1978; PENER *et al.*, 1978; PEDERSEN, 1979; MIALL, 1980). There is no evidence to suggest that precocene affects any other organs in this insect. Treatment of *Locusta* larvae causes rapid, irreversible breakdown of the corpora allata (CA), followed by a delayed moult to a precocious adultiform, as is expected from an insect caused to be prematurely devoid of juvenile hormone (JH). Precocene also attacks the CA of adult *Locusta* (PENER *et al.*, 1978; PEDERSEN, 1979), but is claimed to be ineffective during the fifth instar (PENER *et al.*, 1978), when the CA are presumed to have a low level of activity.

However, in using precocene to effect chemical allatectomy of *Locusta* larvae (MIALL, 1980), it became apparent that precocene II is effective in the fifth instar. Topical treatments at specific intervals throughout the fifth instar induced the marked retention of juvenile characteristics at the next moult, and this paper is an attempt to explain this unexpected observation.

MATERIALS AND METHODS

Mid-fourth instar *Locusta migratoria migratorioides* (R & F) were obtained from crowded cultures reared at the C.O.P.R., London and were kept in crowded conditions at 28°C (±1°C) with a 12:12 hr light regime. Fifth instar larvae were selected at known intervals after ecdysis and were kept in groups of 10–25, in cylindrical, 11 l. perspex cages. Abundant supplies of wheat seedlings supplemented by fresh grass were provided daily.

Precocene II (99%, Aldrich Chemical Co.) was applied topically using a microsyringe in 5 μl acetone to the dorsal surface of the abdomen; ZR515 (Zoecon), a potent JH-analogue in *Locusta* (TANG, 1976) was applied topically in 5 μl acetone; control groups received 5 μl acetone alone.

The degree of juvenilization of the emergent adultoids was estimated using an additive system in which pronotal deformation, wing deformation and larval colouration were scored. A normal adult was scored as 0.0, a full supernumerary larva as 5.0, and one attempting an additional moult (usually unsuccessfully, as 6.0, the maximum possible score). Morphological measurements based on those used by DIRSH (1953) were made on the adultoids. All adultoids were dissected 6–10 days after emergence, and the CA volumes were determined after measurement of the length and width of the glands. The glands were assumed to be ovoid; volume = $4/3\pi\, a \cdot b^2$.

Mortality in all treatment and control groups was negligible, and the results have been calculated excluding the few fatalities that did occur.

RESULTS

Juvenilization of larvae following precocene or ZR515 treatment

Treatment with either precocene or ZR515 early in the 5th instar produced a proportion of individuals displaying pronounced juvenile characters. A dose–response curves was determined by scoring the degree of juvenilization (i.e. the morphological abnormalities) displayed by the adultoids in response to these chemicals (Fig. 1). All dosages were applied 0–24 hr into the 5th instar. The CA volumes of the precocene induced adultoids were compared with those of control adults

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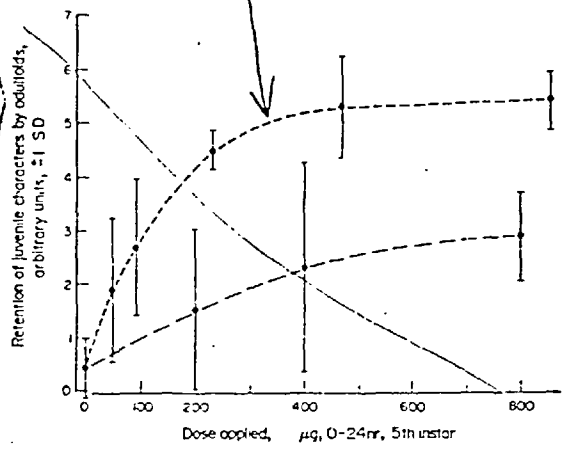


Fig. 1. Dose–response curves of extent of juvenile characteristics displayed by adultoids resulting from treatment with precocene (●—●) or ZR515 (○---○), applied topically 0–24 hr into the 5th instar. Axis: dosage of precocene ZR515. Ordinate: mean score of juvenile characters ± 1 S.D. ($n \geq 17$, except for 800 µg precocene, 214 and 855 µg ZR515, where $n = 5$–9).

6–10 days after the moult; the glands of the adultoids were significantly smaller than those of the control group (controls: 0.388 mm^3 ± 0.302, $n = 32$; 200 µg precocene: 0.140 mm^3 ± 0.192, $n = 45$; $P < 0.001$, 2-sample t-test) but there was no significant difference between the gland volumes of animals treated with 200, 400 or 800 µg precocene. The adultoid gland volumes were smaller than those of early 5th instar larvae, however, and were also significantly negatively correlated with the score of juvenile characteristics displayed (Table 1).

Morphologically the adultoids produced by precocene treatment could not be distinguished from those produced by the JH-analogue ZR515. The ratio of head width to hind femur length shows a strong negative correlation with the degree of juvenilization displayed and the ratios for adultoids produced by precocene and ZR515 show almost complete overlap (Table 2). This also applies to the body, femur, head and pronotum lengths, and to two other ratios of measurements.

Establishment of a critical period for precocene treatment

By careful timing of the application of a standard dose of 200 g precocene, it was possible to determine a critical period when treatment was most effective (Fig. 2). Treatment at the peak of this period, 14–16 hr into the 5th instar, produced a large proportion of individuals with marked juvenile characteristics and greatly reduced CA. On dissection, the CA were only visible in 14% of all animals treated at this time. The activity of precocene, both as an anti-allatotropin and as a JH-mimic, then fell gradually over the first half of the 5th instar, and by day 3 (72–96 hr), doses of 200 µg were scarcely effective.

Double treatments using precocene and ZR515

Five groups of larvae, 0–12 hr, 12–24 hr and 20–24 hr into the 5th instar were treated with various combined dosages of precocene and ZR515. The adultoids produced were no more numerous or more markedly affected than expected from the purely additive effects of single treatments of precocene and ZR515; there was no enhancement of the exogenous JH-analogue by the precocene.

Table 1. CA volumes of morphologically defined groups of precocene-induced adultoids.

Score of juvenile characters	Sample size (n)	C.A. volumes ($\times 10^{-2}$ mm) ±1 S.D.
Controls	32	38.780 ± 30.24
1	85	21.316 ± 18.00
3	5	7.840 ± 7.43
5	14	7.525 ± 11.80
6	8	6.891 ± 11.60

All animals were treated 0–24 hr into the 5th instar with 200 µg precocene applied topically in acetone. CA volumes were determined 6–10 days post-emergence. Scores of 1.0 indicate low juvenilization, scores of 5.0 and 6.0 indicate supernumerary larvae. The control group (0.0) received acetone treatment alone.

Table 2. Morphometric comparison of precocene- and ZR515- induced adultoids: ratio of head capsule width (C) to hind femur length (F), ±1 S.D.; females only. (Scores as in Table 1.)

Score of juvenile characters	ZR515-induced adultoids n	C/F ratio ±1 S.D.	Precocene-induced adultoids n	C F ratio ±1 S.D.
Controls	22	0.3630 ± 0.1013		See left column
1	6	0.3671 ± 0.0098	37	0.3653 ± 0.0116
3	6	0.3752 ± 0.0157	2	0.3787 ± 0.0129
5	9	0.3869 ± 0.0133	7	0.3889 ± 0.0067

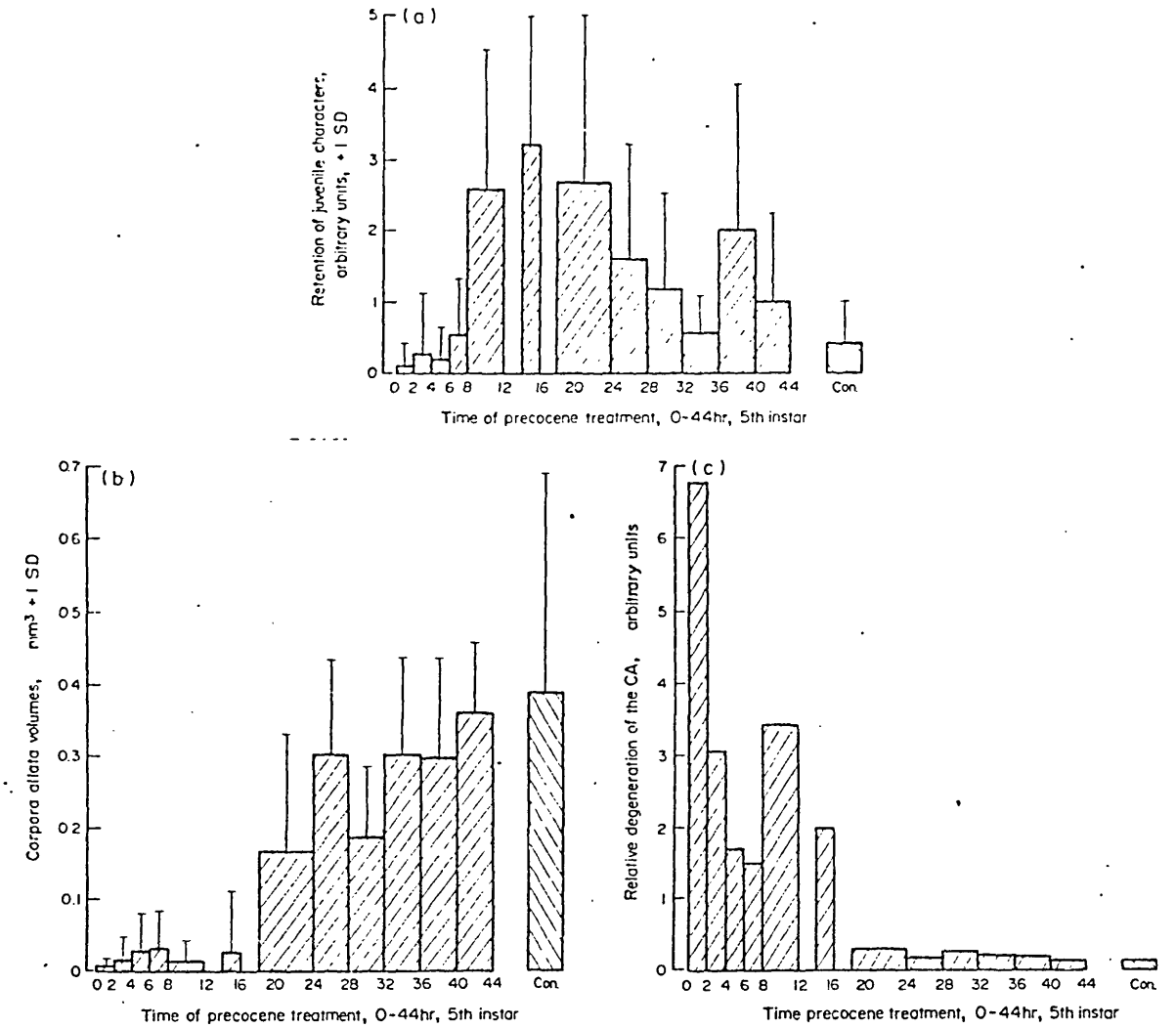


Fig. 2. Time–response curves of juvenile character score, CA volume and relative CA degeneration after topical applications of 200 μg precocene during the early 5th instar ($n \geq 9$ in all groups). Axis: time of application (hr) after moulting to the 5th instar. CON = Control groups. Ordinates: (a) mean score of juvenile characters displayed by the adultoids + 1 S.D.; (b) mean CA volumes (mm^3 + 1 S.D.) each group 6–10 days after emergence to the adultoid form; (c) relative degeneration of the CA (≡ reciprocal of CA volume), arbitrary units.

A group of 5th instar larvae were treated at 0–24 hr with 200 μg precocene, and then treated again with 200 μg precocene, 48–72 hr into the instar. This sequential treatment with precocene resulted in adultoids that were not significantly different in number or morphological deformity from those produced by a single 200 μg dose of precocene at 0–24 hr (2-sample t-test, $n = 15$; $P = 0.3682$). However, larvae treated at 0–24 hr with 200 μg precocene, followed by 43 μg ZR515, 48–72 hr into the instar, showed high juvenile-character scores; the target tissues are therefore still able to respond to JH, or its analogues, 48–72 hr into the 5th instar.

DISCUSSION

Precocene II, applied topically in the early 4th instar, has potent anti-allatotropic activity in *Locusta* (Pener *et al.*, 1978; Miall, 1980), and is also active against adult CA (Pener *et al.*, 1978; Pedersen, 1979). Our evidence demonstrates that in the early 5th instar precocene II mimics JH-activity, but is still active in causing degeneration of the CA. The adultoids or supernumerary larvae produced by precocene treatment display all the characteristics typical of JH-induced adultoids and may occasionally attempt an additional moult. They are morphologically similar to adultoids produced by ZR515.

The time-response curve determined for the period 0–44 hr into the 5th instar indicates that the anti-allatotropic action of precocene has two maxima, at 0–4 hr and 8–16 hr. However, the first peak (0–4 hr) produces only low juvenilization scores, while the second, 8–16 hr into the instar, is associated with pronounced juvenilization. The possibility of there being some synergistic relationship between precocene and JH has been eliminated, by combined treatments of precocene and ZR515.

It is suggested that JH is released during the degeneration of the CA following precocene treatment; only small quantities are released 0–4 hr into the instar, but at 8–16 hr the quantities released are suffi-

cient to exert clear morphogenetic action. BAEHR *et al.* (1979) report a short burst of JH in the haemolymph in the first 5 hr of the 5th instar, but no JH is detectable in the haemolymph after Day 0. JOHNSON and HILL (1973) demonstrated positive activity in the *Galleria* bioassay, from CA taken at all stages throughout the 5th instar.

The JH-release indicated here, following precocene-treatment in the early 5th instar, cannot occur in the normal developmental sequence, since our evidence and TANG'S (1974) demonstrates that the target tissues are receptive to exogenous JH during this period. It is apparent, however, that the CA have the potential for both synthesis and release of JH during the 5th instar, as induced by precocene treatment. Thus it is likely that the effective absence of JH during this instar is normally maintained by a cessation in synthesis and release, rather than by the action of potent JH-esterases, or other mechanisms removing JH from the haemolymph.

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