SOME FACTORS INFLUENCING ECLOSION AND EARLY POST-EMBRYONIC DEVELOPMENT IN SCHISTOCERCA GREGARIA (FORSKÅL) ORTHOPTERA ACRIDIDAE

By

DAVID EDWARD PADGHAM M.Sc.

Thesis submitted for the degree of Doctor of Philosophy, Imperial College, University of London and the Centre for Overseas Pest Research, College House, London W.8.

ABSTRACT

The physiology and behaviour of the later stages of the developing Schistocerca embryo are considered in relation to the influence of the environmental temperature. Under both field conditions and cycled temperature laboratory conditions hatching of the embryos occurs during the cold period of the day. The exact time of hatching appears to be related to the magnitude of the change in temperature on the final day of development and not to absolute temperature. There is also evidence that under cycled temperature conditions entrainment is established.

Physiological and behavioural parameters have been investigated with a view to finding supporting evidence for entrainment. Embryonic activity was examined in relation to temperature over the last five days of development. There is no correlation between activity and the temperature cycle other than on the day of hatching. There is however a period of quiescence during the penultimate day which reinforces a suggestion of this phenomenon in the hatching experiment data. Respiratory activity does show a proportional relationship to the temperature cycle other than on the penultimate and final day when it follows the same pattern as activity.

Haemolymph sugar levels show a daily fluctuation whilst haemolymph amino acid concentrations appear to remain constant. There is no evidence for a hormonal involvement in the initiation of hatching. There is a steady increase in the levels of brain acetylcholine esterase which reaches a plateau at about the time of hatching. Of the other pharmacologically active substances investigated only serotonin was shown to have any influence on the time of hatching.

In the early first instar the hormonal control of cuticular sclerotization and melanization was examined. Release of the hormone bursicon is from axon terminals posterior to the metathoracic ganglion. The initiation of release is induced by a change in the orientation of the embryonic cuticle at the onset of the intermediate moult, neuronal stimulation being conducted via the prothoracic ganglion to the release site. The hormone is also present in non-melanizing forms of Schistocerca where only sclerotization is induced.

ACKNOWLEDGEMENTS

I would like to thank Dr. R.F. Chapman, in whose division at the Centre for Overseas Pest Research this work was carried out, and Mr. R.G. Davies of Imperial College for their guidance, supervision and continued encouragement with this study. My thanks also to Mrs. G.A. Colquhoun for assistance with the photography and to Dr. P.S. Baker for assistance with the electrophysiology.

TABLE OF CONTENTS Page

LIST OF FIGURES

(* less than 24 hour observations)

LIST OF TABLES

INTRODUCTION

The anatomical development of the embryo of Schistocerca gregaria (Forskål) is well documented (Shulov and Pener 1963, Hunter Jones 1966a). Similarly the hatching behaviour of the pharate first instar, its anatomy and behaviour in the vermiform state and the cuticular changes taking place in the early first instar are also well described (Bernays 1970, 1971, 1972a, 1972b). In the present study the physiological and behavioural aspects of the period of development from the formation of the pharate first instar to the fully hardened, free living first instar are considered with special reference to extrinsic environmental and intrinsic physiological control systems.

The eggs of Schistocerca are laid in a batch referred to as a pod, the number of eggs in any one pod varying from 10 to 140, the average being about 70 eggs (Norris 1952). Thus when considering development in relation to the natural environment one must consider not only the behaviour of the individual insect but its behaviour in relation to the pod as a whole.

Embryonic development at 30-32°C takes 12 - 13 days and for almost the whole of the incubation period the embryos within a single egg pod show some variation in the state of development between one another (Shulov and Pener 1963). This variation becomes most evident at about six days when blastokinesis takes place. At this time the embryos show their first overt movements which lead to a change in position within the egg. The embryo, which was originally lying with its ventral surface towards the ventral surface of the egg, moves round the micropylar end to the dorsal surface so that it comes to lie with its ventral surface adjacent to the dorsal surface of the egg. At about six days after

oviposition all stages of these blastokinetic movements can be seen within eggs from a single pod (Hunter Jones 1966a). Thus after six days the embryos of a single pod can be as much as 17 - 20 hours out of phase with one another. In Locusta migratoria (L), Salzen (1960) has also shown that there is variation in the degree of development between the embryos of a single pod. This variation is most marked between the beginning of katatrepsis at about 5 - $5\frac{1}{2}$ days up until about 10 days when differentiation is virtually complete. In Schistocerca the variation appears to persist, but does not increase from the 17 to 20 hours seen at blastokinesis, up until the end of development (Shulov and Pener 1963, Tyrer 1970).

During the last 48 hours of development the Schistocerca pharate first instar shows little gross morphological change, the principal developments being an increase in the pigmentation of the eyes; the mandibles and tibial spines increase in sclerotization and the areas of cuticular melanization in the first instar show pale grey. However at about this time significant changes do occur in the structure of the egg. Two to three days prior to emergence the thick serosal cuticle starts to be digested, reducing in thickness from 12.5μm at 9 days to 3μm at 12 days (Hunter Jones 1966a). There is a similar decrease in its dry weight from 200μg/egg at 3 days before hatching down to 30μg at hatching (Bernays 1972a). This process of digestion has been shown to be due to the action of an enzyme secreted by appendages on the first abdominal segment, the pleuropodia (Slifer 1937, 1938). By ligaturing pharate first instars in front and behind the pleuropodia Slifer demonstrated that only the half of the body containing the pleuropodia digested

the white serosal cuticle. The pharate first instar itself is protected from this digestive process by the embryonic cuticle through which the pleuropodia protrude. The functional relationship between the digestion of the white serosal cuticle and hatching was demonstrated by Bernays (1972c). In otherwise normal fully developed embryos in which the serosal cuticle was digested to only 62µg/egg the larvae were unable to burst the egg shell. All hatched larvae had serosal cuticles not greater than 50µg/egg. In Locusta migratoria it is suggested that the secretory activity of the pleuropodia is stimulated by a hormone produced by the prothoracic glands (Jones 1956a, 1956b). Larvae in which the pleuropodia are isolated by ligature from functional prothoracic glands do not digest the serosal cuticle.

Also about two to three days before hatching the larvae begin to swallow the remaining extra-embryonic fluid (Bernays 1972c). This process increases the volume of the larva and enables it to gain sufficient purchase on the inside of the egg shell to rupture it at emergence.

Embryonic activity during the last 3 days of incubation has been studied by Bernays (1970). Initially the movements of the pharate first instar are either random jerks of appendages or longitudinal waves of contraction down the body. Close to the time of hatching, however, these movements become both more vigorous and more co-ordinated. The larva exerts characteristic movements termed 'hatching efforts' by Bernays which consist of strong dorso-ventral contractions of the abdomen. These contractions, combined with a general contraction of the body towards the anterior end, lead eventually to the rupture of the egg shell.

The influence of temperature on the rate of embryonic development has been the object of much examination both under field and laboratory conditions. For practical control purposes it is important to know egg incubation periods in the field. To this end Symmons, Green, Robertson and Wardhaugh (1974) carried out an extensive study of the egg development times of Schistocerca throughout its invasion area. In the laboratory, Hunter Jones (1966a) gives a detailed account of embryonic development rates for the complete range of physiologically acceptable incubation temperatures. Within the range of favourable temperatures the rate of development shows a linear relationship with temperature and deviates only at the extremes. Incubation periods range from 60 days at 20°C to 11 days at 35°C, and by linear extrapolation Hunter Jones (1966a) has shown the minimum incubation temperature for Schistocerca to be 15°C. Above 35°C the incubation period is not reduced below 11 days and above 40°C there is a marginal increase in development time and also high mortality.

When eggs are incubated with a fluctuating daily temperature there is some contradiction in the literature as to whether or not the rate of development remains linear with temperature. Hunter Jones (1966a) suggests that the total number of degree days remains constant at 224 ± 12 no matter what the temperature regime (between 15°C and 35°C). However Uvarov (1966) reports that with fluctuating temperatures incubation periods for Schistocerca eggs vary from 151-216 degree days and suggests that the value of temperature summation is uncertain.

Like many Acridids the eggs of Schistocerca are laid in the soil beneath a foam plug. The depth varies but on average the

eggs are about 10 cm down and consequently the emergent larvae must make their way to the surface after hatching from the egg. At the time of emergence the larva is still enveloped by the embryonic cuticle and at this stage is referred to as the vermiform larva. Bernays (1971) describes in detail the process of emergence through the soil. Larval movement is achieved by cycles of longitudinal contraction passing forwards from the end of the abdomen to the head. These vermiform movements are assisted by pulsations of the cervical ampullae which gain the larva an increased purchase on the soil at the anterior end whilst the abdomen is contracted. The ampullae then collapse whilst the head is extended and the cycle is repeated.

Under natural field conditions it is commonly observed that larval emergence from many pods of a gregarious locust oviposition site occurs almost simultaneously (Ashall and Ellis 1962, Ellis and Ashall 1957, Predtechenskii 1935). For Schistocerca there is general agreement that this emergence occurs principally in the period from 2 hours before to about 3 hours after sunrise. Among other Acridids a daily pattern of emergence behaviour is also seen, though times of day vary for different species. Pickford (1976) describes the major emergence of Melanoplus bivittatus Say. in Canada to be during the early afternoon whilst Rubtzov (1935) suggests that emergence of Siberian grasshoppers is during the late morning or early afternoon depending upon the weather conditions. In both these cases soil temperatures are frequently too low to permit hatching and thus the temperature is exerting a direct physiological control on emergence. With Schistocerca the soil temperatures in its invasion area are almost never below the hatching threshold of 20°C, thus suggesting that the control over

hatching exerted by environmental temperatures in this instance is acting indirectly.

Not only will the pods of an oviposition site emerge within hours of one another but 80 - 90% of the larvae of a single pod will emerge within minutes of one another. The general pattern is for about 80% of the larvae of a pod to emerge in the first 5 - 10 minutes, about 10% emerge over the following 10 - 20 minutes and the remaining 10% may then take as much as another day before they emerge. Papillon (1960) describes pods laid by the 'gregaria' phase of Schistocerca containing a small percentage of 'solitaria' forms which occured in the lower third of the pod. She found that these eggs emerged about 20 hours later than the gregaria. Similarly Venter and Potgieter (1967) report that when egg pods of Locustana pardalina (Walker) were divided into top and bottom halves those from the top half invariably emerged before those from the bottom half. There is thus the suggestion of a sequential emergence from the top of the pod downwards. Uvarov (1966) suggests that this synchronised emergence of the larvae of a single pod is due to the mechanical disturbance produced by the first egg hatching stimulating the other eggs with a chain reaction effect.

The synchronous emergence from many egg pods of an oviposition site in itself suggests that there must be some environmental stimulus controlling this unified behaviour. Temperature, moisture and light have all been invoked as the controlling factor in Acridid larval hatching (Uvarov 1966), and Bernays (1970) suggests "that if larvae are ready to hatch then many, if not all, environmental changes will stimulate hatching activity". The time of natural

field emergence of Schistocerca larvae coincides with the coldest time in the daily temperature cycle. At dawn the soil temperature has been falling gradually for about 15 hours and does not tend to rise until the end of the emergence period. Thus cooling might provide the stimulus for hatching and Bernays (1970) has confirmed this in the laboratory. It has also been shown that cooling followed by a slight increase in temperature is an effective stimulus but that an increase in temperature alone was not significantly stimulating. Other effective stimuli are dehydration and mechanical disturbance at the very end of the incubation period (Bernays 1970). However since dehydration and mechanical disturbance under field conditions are very variable phenomena it is unlikely that these play any significant role in the control of synchronised emergence between pods. The effect of a sudden wetting at the end of the incubation period has also been investigated but this was shown to have no significant effect as a hatching stimulus (Bernays 1970).

The stimuli which induce hatching to other insects are generally unknown and in many instances the insects appear to hatch when they are ready to do so (Chapman 1971). There is thus the suggestion that in many instances hatching is provoked by entirely endogenous phenomena. In some instances however environmental factors have been shown to induce hatching. Amongst aquatic insects the eggs are often laid out of water and do not hatch until wetted. With parasitic insects the warmth or vibration caused by the host may often be a hatching stimulus. In a very few cases hatching has been shown to occur only at particular times of the day. The eggs of both the pink bollworm Pectinophora gossypiella

(Saunders) (Minis and Pittendrigh 1968) and the spotted bollworms Earias fabia (Stoll), Earias insulata (Boisduval), and Earias cupreoviridis (Wlk) (Meng, Chang, Du and Ge 1973) all hatch at dawn and in the former case at least this has been demonstrated to be the result of circadian entrainment to the ambient photoperiod. The eggs of Schistocerca also hatch at approximately the time of dawn and the influence of circadian entrainment is considered in the present study.

Having hatched from the egg and dug its way to the surface via the foam plug (Ewer 1977) the vermiform larva is still contained within the embryonic cuticle. Once free from the confines of the soil, however, the larva almost immediately undergoes the intermediate moult. The stimulus for the initiation of this seems to be associated with the sudden cessation of bodily mechanoreceptor stimulation due to emergence from the soil. In response to this stimulation, or lack of it, the larva immediately begins to swallow air which collects in bubbles throughout the gut (Bernays 1972a). Soon after the initiation of air-swallowing the body begins to show waves of posterio-anterior contractions referred to as abdominal pumping (Bernays 1972a). During these waves of contraction the posterior segments of the abdomen come to lie in an increasingly more anterior position with respect to the embryonic cuticle. Spines on the abdominal sternites grip the embryonic cuticle laterally whilst the brustia hold it posteriorly. With each wave of abdominal contraction the tension in the embryonic cuticle increases until finally it splits along the mid-dorsal line of weakness in the thorax. The rupture of the embryonic cuticle is aided by the air swallowing which increases the haemolymph pressure to over 40mm Hg at the time of ecdysis (Bernays 1972a).

With the initial split in the cuticle air swallowing and abdominal pumping continue and the resulting haemolymph pressure and tension on the cuticle lead to progressive emergence of the anterior end of the larva. The legs become withdrawn by their own movements and eventually the abdomen is also freed leaving the cuticle in folds around the brustia. The old cuticle is rubbed or kicked free from the body and the first instar larva then completes the expansion of the new cuticle which had previously been greatly folded beneath the embryonic cuticle (Bernays 1972a).

A degree of sclerotization of the cuticle begins in the last two to three days of embryonic development and at emergence structures such as the mandibles, leg joints and mechanoreceptor sensilla are hard enough to function normally. However the remainder of the cuticle is still soft at the intermediate moult, this being essential for subsequent expansion. Following the moult, sclerotization proceeds rapidly and the larvae are sufficiently rigid to resist mechanical extension after 30 mins (Bernays 1972b). Biochemically, sclerotization can be demonstrated as a resistance to alkaline digestion and this increases sharply within the first hour after the moult. In addition to sclerotization newly emerged gregarious larvae also develop areas of black pigmentation due to melanin deposition, the process taking two to four hours for completion. With solitarious larvae, either in the true sense or of the type described by Papillon (1960), sclerotization proceeds as normal but no pattern of melanized cuticle is developed. This is similarly true for albino gregarious Schistocerca (Hunter-Jones 1957).

Melanization as a biochemical process is independent of sclerotization (Andersen 1974, Goodwin 1952, Cottrell 1964, Hackman and Goldberg 1971) and is less fully understood than sclerotization. Evidence suggests that as with sclerotization tyrosine is the initial substrate (Hackman 1967). However catechol, rather than N-acetyl dopamine may be the immediate precursor to melanin (Jones and Sinclair 1958, Hackman and Goldberg 1971) or alternatively the distribution of enzymes and phenolic material in the exocuticle may be having a controlling influence on melanin formation (Malek 1957). Hackman and Goldberg (1971) suggest that in Lucilia suprina Weid. the production of melanin is due to the presence of an activator in the cuticle which converts a prophenoloxidase in the haemolymph to an enzyme with a high activity on tyrosine and dopamine which appears to be responsible for the formation of melanin.

The initiation of cuticular melanization and sclerotization after an ecdysis are controlled by the hormone bursicon. This was first reported in the blowfly Sarcophaga bullata Parker by Fraenkel and Hsiao (1962, 1963, 1965) but has since been demonstrated in the cockroach Periplaneta americana (L) (Mills, Mathur and Guerra 1965), in the locust Locusta migratoria migratorioides (R. and F.) (Vincent 1971, 1972), in the tobacco hornworm Manduca sexta (Johannson) (Truman 1973), in the cockroach Leucophaea maderae (Fabricius) (Srivastava and Hopkins 1975) and in the cabbage white butterfly Pieris brassicae L. (Post 1972, Post and DeJong 1973). The site of release of the hormone varies from one insect to another but is universally associated with the nervous system. Similarly the time of release and its relative

activity in the haemolymph at different times after ecdysis vary from one insect to another. These variations may be related either to the mode of release or to the mode of response to the hormone since interchanges of haemolymph from insects of different taxonomic orders appear to induce the normal mode of tanning in all the recipient insects examined. The hormone itself would thus appear to be analogous throughout the above mentioned insects.

In Schistocerca there is evidence for a blood-borne factor inducing cuticular darkening, which is present immediately after the intermediate moult (Bernays 1972b). However the nature of this melanization inducing factor has not been further examined by previous workers.

The nature of the control exerted by bursicon is not fully understood but evidence points to it being involved in membrane permeability changes to sclerotizing and melanizing metabolites. Seligman, Friedman and Fraenkel (1969) have demonstrated that when dopadecarboxylase is inhibited in newly emerged adult Sarcophaga there is an accumulation of dihydroxyphenylalamine (DOPA). However when bursicon release is also inhibited there is no accumulation of DOPA. They conclude that one of the actions of bursicon is to stimulate tyrosine oxidation. Mills, Lake and Alworth (1967) demonstrated that bursicon has no direct effect on the metabolism of N-acetyl dopamine from tyrosine. However Mills and Whitehead (1970) suggest that whilst there is no direct biochemical involvement the action of bursicon is to render the haemocyte cell membrane permeable to tyrosine. Thus the substrate in the serum is brought into contact with the enzyme system in the haemocyte. They further suggest that bursicon may render the

epidermal basement membrane permeable to dopamine so enhancing its uptake. In Pieris, Post (1972) and Post and de Jong (1973) have also demonstrated that the rate of tyrosine metabolism in stabilised haemocyte suspensions is proportional to bursicon concentration. Vandenberg and Mills (1974) suggest that bursicon might produce the changes in membrane permeability mentioned above via stimulation of adenyl cyclase. Additionally dopamine may act as its own adenyl cyclase stimulator in the epidermal cell basement membrane as it has been shown to do in mammalian systems (Voorhees, unpublished data from Vandenberg and Mills 1974). As well as its role in sclerotization there is also evidence that bursicon may stimulate endocuticle deposition in newly emerged adults of Sarcophaga (Fogal and Fraenkel 1969).

As already mentioned, the present study considers aspects of the physiology and behaviour of the pharate first instar in relation to hatching. In particular, consideration is given to the questions of how synchronisation with environmental stimuli arises, how this influences the physiology of the developing embryo and how entrainment with the environment manifests itself at hatching. In addition, one specific aspect of early post-embryonic development, the control of cuticular sclerotization and melanization in newly emerged first instar larvae of Schistocerca, is also considered.

MATERIALS AND METHODS

1. Location of the research.

All of the field work was carried out at the research station of the Saudi Arabian Ministry of Agriculture in Jeddah. Some of the laboratory work associated with emergence was also carried out in Jeddah with the remainder of the research being carried out at the Centre for Overseas Pest Research (COPR) in London.

2. Experimental animal.

Embryo, pharate first instar and newly hatched first instar Schistocerca gregaria (Forskål) were used as experimental animals in this study.

3. Rearing.

In Jeddah the locusts were used from a stock culture recently captured from the field. They were reared under crowded conditions in wood and glass cages kept out of doors at the ambient shade temperature, humidity and photoperiod. A diet of sorghum and maize leaves was provided daily. At COPR the locusts were reared under crowded conditions with cycled room temperatures of 8 hours at 28°C and 16 hours at 22°C though temperatures within a cage may rise to 32-34°C due to radiant heat from the light bulbs during the 12 hour photoperiod. The relative humidity was 40% ± 5%. Females oviposited into moist sand contained in aluminium tubes beneath the floor of the cage. These tubes were changed daily and the egg pods incubated for 12 days at 31°C. When egg pods were to be used for hatching experiments they were removed from the aluminium tubes after 4-5 days and placed in damp sand in small plastic pots. Since each aluminium tube frequently contained two or more egg pods this transfer enabled the hatching time of pods to be recorded individually in every case and also increased the uniformity of

incubation. In an aluminium tube a pod could be laid adjacent to the side of the tube or centrally. Thus egg pods adjacent to the wall of the oviposition tube experience any change in temperature before those in the more insulated centre. Since much of the work concerned the reaction of pharate first instars to changes in temperature the small plastic pots provided a more uniform replication of conditions for each pod.

4. The time of emergence.

i) First instar emergence under field conditions.

Fifty mature adult locusts were placed in a two square-yard cage open to the sandy soil at the Jeddah Research Station. The insects were allowed to oviposit on two consecutive days. The egg pods were left in position so that they incubated at the ambient temperature and on the morning when hatching was expected observations were started at 04.00 h. in order to record the precise emergence times. Records of the ambient air and soil temperature were also taken for the period of incubation. Soil moisture was measured by taking four 50 g. soil samples at a depth of 8 to 10 cm. at four times during each day for the last five days of the incubation period. The sampling times were 06.00 h., 12.00 h., 18.00 h. and 24.00 h. The samples were then dried to constant weight in an oven at 105°C., reweighed and their percentage moisture calculated.

ii) Emergence times under laboratory conditions.

For constant temperature studies egg pods in small plastic pots filled with damp sand were incubated at a constant 31°C in an incubator and their emergence times recorded. When a cycled temperature regime was required the egg pods were incubated in an

Astall dual temperature incubator for 12 hours per day at each of two temperatures with lights on during the warmer of the two periods. The standard incubation cycle was 11.00 h. to 23.00 h. at 28°C and 23.00 h. to 11.00 h. at 33°C each day.

5. The behaviour and physiology of the developing pharate first instar.

i) Activity recordings.

Embryonic activity was recorded using time-lapse photography of dechorionated embryos.. Eggs 6-7 days old were immersed briefly in a 7% sodium hypochlorite solution to dissolve the chorion and then washed briefly in distilled water. This procedure rendered the egg shells transparent. The eggs were then mounted in a sealed glass chamber containing a small piece of moist filter paper. The sealed unit containing the eggs was mounted in front of a Bolex cine camera geared to take one exposure every 2 min. The eggs were given constant illumination from a 60 W. light bulb, this requiring a 2 sec. exposure at f8 with Kodachrome 40 colour movie film. Each film lasted approximately 5 days during which time the temperature was either cycled to give 12 hours at 33°C and 12 hours at 28°C or kept at a constant 33°C.

ii) Respiration recordings.

The determinations were carried out using a micro-Warburg constant volume respirometer as shown in Fig 1. A single egg buried in sterile sand was placed in the left hand chamber, a role of filter paper moistened with 10% potassium hydroxide solution being wedged into the neck of the chamber to absorb carbon dioxide. The right hand chamber acts as an equilibration blank and contains only sand and potassium hydroxide filter paper. The system is

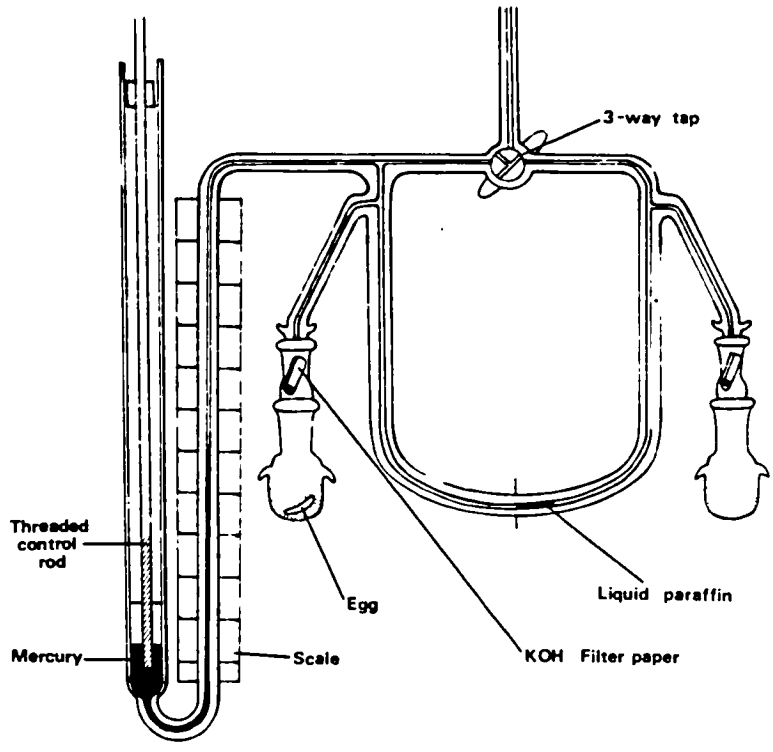


Fig. 1. Diagram of the micro-Warburg constant volume respirometer used for the oxygen consumption determinations.

open to the air only via the three way tap and this is closed when the equipment is in use. As embryonic respiration proceeds oxygen is consumed in the left hand chamber and this has the effect of drawing a small column of liquid paraffin along the bottom of the 'U' tube towards the left hand chamber. The movement of the liquid paraffin can be reversed by decreasing the volume of the left hand side of the equipment by an amount equal to the oxygen consumed. This is done by screwing a threaded rod into the column of mercury so pushing the column up the graduated stem. Thus if the liquid paraffin starts exactly in line with a fixed mark it can be returned to this fixed mark after, for example, one hour by raising the mercury column through a measurable distance. The oxygen consumed by the embryo in that hour is thus equal to the volume of new space occupied by the raised column of mercury. The mercury column is calibrated by filling a known length with mercury and then weighing the mercury.

Thus if x mm of mercury weigh y grams,

$$\text{Then} \quad 1 \text{ mm} \equiv \left(\frac{y}{13.5939} \right) \Big/ x \times 1000 \ \mu l$$

As respiration proceeds the mercury column is raised every hour to return the paraffin column to the zero mark. When the mercury column cannot be raised further the system is opened to the air via the three way tap, the mercury column dropped to the bottom of the scale, the system closed again and the liquid paraffin adjusted to zero. The respirometers are mounted on perspex back plates and the whole system, up to the level of the top of the back plates, was immersed in a water bath. The temperature was either cycled, 12 hours at 30°C/12 hours at 35°C or kept at a constant 35°C.

iii) Haemolymph sugar (Anthrone positive material) determinations.

For the quantitative analysis of total blood sugar a modified anthrone method was used (Roe 1955). 10µl of blood from 2-5 embryos, depending on their age, was diluted in 50µl of distilled water. To this was added 20µl of 10% $ZnSO_4.7H_2O$ and 2µl of NaOH, mixing after each addition. A further 50µl of distilled water was then added, the reagents mixed and then centrifuged at 3500 rev/min for 15 min. A 20µl aliquot of the supernatant was added to 1000µl of Anthrone reagent. and the reagents mixed and placed in a boiling water bath for 15 min. After cooling for 15 min the optical density was read at 620nm in a Pye Unicam SP 1800 spectrophotometer.

For each set of determinations a standard curve was drawn using a standard trehalose solution.

iv) Haemolymph amino acid determinations.

The method used was that of Rosen (1957).

Reagents.

(1) Stock solution of 0.01 M NaCN.

(2) Acetate buffer. 16.2g sodium acetate plus 5 ml glacial acetic acid, made up to 75 ml with distilled water.

(3) Acetate cyanide. 1 ml of solution (1) made up to 50 ml with solution (2).

(4) Ninhydrin. 3% in 2-methoxyethanol.

(5) Isopropyl alcohol : water, 1 : 1.

Either 5µl of blood from 2-5 larvae or 5µl of amino acid standard were added to 100 µl of distilled water, the sample mixed and then centrifuged at 3500 rev/min for 10 min. A 20µl aliquot was taken and added to 100µl of acetate cyanide reagent. The reagents were mixed, 100µl of ninhydrin added, mixed again, and

then placed in a boiling water bath for 15 min. After removing from the water bath 2000µl of isopropanol:water was added and the reagents allowed to mix and cool for 10 min. The optical density was read at 570nm on a Pye Unicam SP 1800 spectrophotometer. For each set of determinations a standard curve was drawn using a standard serine solution.

v) Vermiform larvae.

In the experiments, relating to both the induction of hatching by pharmacological agents and the control of melanization, vermiform larvae were used. Under natural hatching conditions the vermiform stage is passed through very rapidly and since hatching of the eggs of a whole pod is highly synchronised all the vermiform larvae emerge within minutes of one another. For experimental purposes it was not convenient to have all of the larvae of a pod emerging at once and therefore a procedure was adopted which provided for a greater duration of the availability of vermiform larvae. Egg pods were incubated for 11 days at 31°C in their aluminium laying tubes and the eggs were then divided up and transferred to damp sand in petri dishes. The petri dishes of eggs were then stored at room temperature and the vermiform (i.e. pharate first instar) larvae emerged singly over the next two days.

vi) Vermiform larva and blowfly adult bioassay for a potential hatching hormone.

In an attempt to find evidence for the existence of a hatching hormone, haemolymph from emerged vermiform larvae was injected into unhatched but fully developed pharate first instar locust larvae. The recipient pharate first instars, still within the chorion, were then reburied in damp sand and the time of their emergence was

recorded. As a control, similar vermiform larvae were injected with haemolymph from adult locusts and the latency between injection and hatching was again recorded.

In order to inject into fully developed pharate first instars the chorion must be ruptured with the consequent loss of the remaining amniotic fluid and changes in the hydrostatic pressure within the egg. Since this seemed to be a disturbing influence in itself an alternative recipient insect was tested. The activity of a pharate first instar emerging from the egg is not identical to that of a pharate holometabolous adult emerging from the pupa but it was felt that there is sufficient similarity between the two processes to be worthwhile testing such pupae as an alternative recipient. Also, since blowflies have been used to assay for bursicon activity from other insects there is some evidence to suggest that insect hormones are not necessarily species specific. Thus pharate adult Calliphora were used as a recipient for vermiform locust haemolymph. These have the advantage of being completely dry within the puparium and a small window can be cut in the puparial case without any noticeable disturbance to the pharate adult. The pharate adult Calliphora were injected in the same way as the vermiform locust larvae, the small window of puparial wall replaced, and the latency between injection and emergence recorded.

vii) Removal and reinjection of blood.

Vermiform larvae were anaesthetized with carbon dioxide and kept immobile on a cold stage mounted on a binocular microscope. Haemolymph from donor insects of various ages was injected into the dorsal abdomen.

The hypodermic needles used for the blood transfers were prepared from 50µl Drummond Microcaps drawn to a point on an SRI electrode puller. Each needle was calibrated to either approximately 1 or 2µl by comparison with a measured sample drawn into a similar needle.

viii) The injection of pharmacologically active chemicals and extracts.

Schistocerca pharate first instars were prepared for injection three to four days before hatching was expected. The preparation procedure involved the removal of egg pods from their original oviposition tubes in which they were laid, breaking up of the pods into single eggs and the washing of these eggs to remove the majority of the adhering sand and froth. The single eggs were then immersed in 7% sodium hypochlorite solution to dissolve the chorion and render the egg shell transparent. This procedure is necessary for the accurate injection of drug solutions. The cleared eggs were then transferred to moist filter paper in petri dishes and their incubation continued until the pharate first instars were judged, by both anatomical development and known behavioural responses to temperature change, to be within hours of hatching.

Using micro glass syringe needles drawn to a point of approximately 0.05mm, 1µl of the following solutions were injected:-

(a) Corpora cardiaca homogenate.

20 pairs of glands were dissected from mid-fifth Schistocerca hoppers and placed in 200µl of Ringer solution. The glands were homogenised and the homogenate centrifuged at 3000 rev/min for 5 minutes. The supernatant was then used for injection.

(b) Dibutyryl cyclic AMP.

0.1 mg/µl made up in Ringer solution.

(c) Theophylene.

5µg/µl made up in Ringer solution.

(d) Ecdysone.

1 ng/µl, 5 ng/µl, 10 ng/µl, 100 ng/µl made up in Ringer solution.

(e) 20-hydroxyecdysone.

1 ng/µl, 5 ng/µl, 10 ng/µl, 100 ng/µl, made up in Ringer solution.

In each case the recipient pharate first instar was anaesthetised with carbon dioxide beforehand and 1µl of solution injected into the abdominal haemocoele.

(f) 5-hydroxytryptamine. (Serotonin)

80 µg/ml made up in Ringer Solution.

In view of the results of Cymborowski (1970a) which suggested a haemolymph - brain barrier (see also Treherne 1974) to 5-hydroxytryptamine, injections were made into the head of the pharate first instar. The exact position of the needle tip within the head must vary with each individual injection but it is suggested that this mode of injection results in a range in the extent of damage to the brain peritoneum. In some instances it is probable that no damage was caused to the peritoneum whilst at the other extreme damage to the brain was sufficiently great to result in the inability to hatch and eventual death of the insect. However, intermediate to these two extremes it is suggested that in many cases slight damage to the peritoneum resulted

allowing access of 5-hydroxytryptamine to the brain without causing mortality. The rationale for this mode of injection is, however, hypothetical, there being no direct evidence for the existence of a blood-brain barrier to 5-hydroxytryptamine in the Schistocerca pharate first instar.

(g) Reserpine.

80 µg/ml made up in Ringer solution.

Since reserpine is suggested to cause disruption of circadian rhythms (Cymborowski and Muszynska 1974) this was not injected immediately prior to the time of expected hatching but approximately 24 hours beforehand. Injections were made into the abdominal haemocoele. The pharate first instars were then returned to the cycled temperature incubator and their hatching times recorded.

ix) Ringer solutions.

NaCl	129.0 mM
KCl	8.6 mM
$CaCl_2$	2.0 mM
$MgCl_2$	8.5 mM
NaH_2PO_4	4.3 mM
$NaHCO_3$	10.2 mM
Glucose	34.0 mM

The above standard Ringer was converted to a high potassium Ringer by increasing the concentration of KCl to 70 mM and reducing the concentration of NaCl to 67.6 mM.

x) Estimation of brain acetylcholine esterase activity.

Estimations of brain acetylcholine esterase activity were made at ten stages of development:-

(1) Approximately $4\frac{1}{2}$ days before hatching was expected.

(2) Approximately $3\frac{1}{2}$ days before hatching was expected.

(3) Approximately $2\frac{1}{2}$ days before hatching was expected.

(4) Approximately $1\frac{1}{2}$ days before hatching was expected.

(5) Approximately $\frac{1}{2}$ day before hatching was expected.

(6) Approximately 6 hours before hatching was expected.

(7) Approximately 4 hours before hatching was expected.

(8) Approximately 1 hour before hatching was expected.

(9) Within 10 to 30 minutes after hatching.

(10) $3\frac{1}{2}$ to $4\frac{1}{2}$ hours after hatching.

With the exception of stage (9) 40 insects were used in each sample, the brains being dissected out in Ringer solution, dried briefly on lint free tissue paper and placed into a pre-weight micro-beaker containing 200µl of Ringer. With stage (9) only 20 larval brains were collected and these were placed into 100µl of Ringer. During the dissections the beakers were sealed with parafilm to prevent evaporation. After dissection of all the brains in a group the micro-beaker was reweighed so as to give the weight of the brains. The weighed brains, together with the 200µl of Ringer, were then homogenised in a microhomogeniser and the homogenate centrifuged at 3000 rev/min for 10 minutes. The supernatant was pipetted off and used for the determinations. The method of determination of acetylcholine esterase was that of Ellman, Courtney, Andres and Featherstone (1961). The reagents used were:-

(1) 0.25 m mol/l 5,5-dithiobis (nitrobenzoic) acid in 50 m mol/l phosphate buffer at pH 7.2 as the buffer-chromogen.

(2) 5 m mol/l acetylthiocholine iodide as the substrate.

The reaction is given below:-

Acetylthiocholine $\xrightarrow{\text{acetylcholine esterase}}$ Thiocholine + acetate.

Thiocholine + Dithiobis (nitrobenzoate) $\longrightarrow$ 2-nitro-5-mercaptobenzoate.

The reagents at 25°C are pipetted directly into a spectrophotometer cuvette.

Buffer-chromogen	3000µl
Sample	20µl
Substrate	100µl

After mixing by invertion, the cuvette is placed in the spectrophotometer and read at 405 nm against an empty cuvette. The time taken for an absorbance increase of 0.100 is then recorded with a stop-watch.

The acetylcholine esterase activity was calculated on both a whole brain and on a per unit wet weight of brain basis. The activity per 20µl aliquot of whole brain is given as:-

$A = \frac{1}{t}$ t = time for an absobance increase of 0.100 (seconds)

The activity per 20µl aliquot per unit wet weight of brain is given as:-

$A = \frac{1}{t \times \frac{wt}{d}}$ t = time for an absorbance increase of 0.100

wt = total wet weight of brains. (grams)

d = dilution of brains. (millilitres)

6. Hardening and darkening of the first instar.

i) Vermiform larvae bioassay for the melanisation hormone.

Bernays (1972b) has shown that vermiform larvae injected with blood from newly ecdysed first instar larvae develop the normal pattern of melanization characteristic of the first instar. Vermiform larvae injected with blood from first instar larvae over four hours old, with Ringer or not injected, do not darken. Vermiform larvae were therefore used to assay the melanizing activity of the haemolymph and various tissue extracts. After injection the vermiform larvae were buried in sand to prevent ecdysis from the embryonic cuticle (and hence normal melanization) and kept at room temperature for three hours. The larvae were then scored on a 0 to 5 scale of darkening: (0) No melanization. (1) Very slight melanization, often of the cuticle just above the dorsal vessel in the abdomen. (2) Slight melanization, usually as in (1) but spreading down either side of the abdomen. (3) Moderate melanization. As in (2) but with the sternites of the abdomen and the thoracic wing buds showing a degree of darkening. (4) Almost the normal melanization pattern. Usually the thorax and abdomen are as dark as normal but the head is slightly less pigmented. (5) The vermiform larva attains the full melanization pattern of a gregarious first instar larva both in area and intensity.

It should be noted that whilst vermiform larvae were used in the bioassay because they do not melanize spontaneously, there is a condition in which these larvae may do so without shedding the embryonic cuticle. The larvae used in the bioassay not only failed to split the embryonic cuticle but also did not displace it horizontally in the manner characteristic of the initial stages of ecdysis (Bernays 1972c).

ii) Ligatures.

In an attempt to locate the release site of the factor inducing melanization, ligatures were applied in the following positions: (a) Around the neck, separating the suboesophageal and prothoracic ganglia. (b) Between the prothoracic and mesothoracic legs. This separates the prothoracic and mesothoracic ganglia. (c) Immediately in front of the mesothoracic legs. This cuts across the ventral nervous system at the posterior end of the metathoracic ganglion. (d) Diagonally between the metathoracic legs. This cuts the ventral connectives between the metathoracic and first abdominal ganglion at approximately one third of the distance from the metathoracic ganglion. (e) Across the end of the abdomen so as to isolate the terminal abdominal ganglion.

The ligature positions described above are shown in Fig 2. Histological examination of many of the ligatured larvae confirmed the ligature positions and showed not only that the haemocoele was occluded but that the ventral cord was severed as well.

During the course of experimentation it was noted that slight differences in the time at which ligatures were applied during ecdysis could have a fundamental effect on the result. For this reason ligatures were applied at four different stages as given below.

Stage 1. Immediately after emergence from the soil but before air swallowing.

Stage 2. From the time of air swallowing until the moment the first split occurs in the embryonic cuticle.

Stage 3. An extremely short period of two to three seconds after splitting when the first hump of the thorax appears through the split in the cuticle.

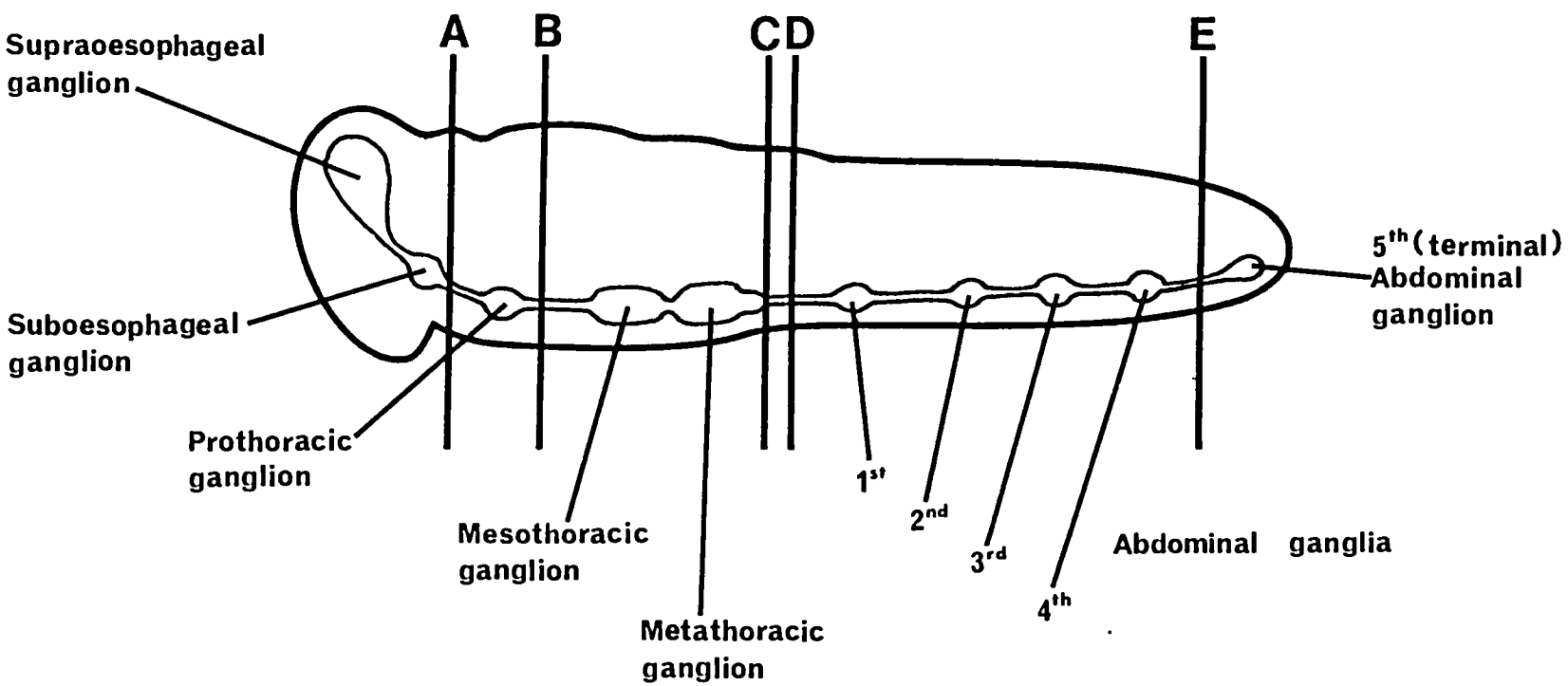


Fig. 2. Diagram of the positions, relative to the ganglia of the ventral cord, of the five ligatures used to locate the release site and neural control of the melanization hormone.

Stage 4. From the end of stage 3 onwards.

It should be noted that the characteristics of each stage provide a convenient time scale but they are not necessarily directly associated with internal physiological changes. Thus whilst the horizontal displacement of the cuticle at the beginning of eclosion is associated with hormone release, further internal changes will proceed even if the external behaviour described in stages 2-4 is inhibited.

iii) Interruption of the ventral nerve cord.

It was found impossible to cut the ventral connectives in vermiform larva without killing them. However, it was found possible to sever the ventral cord using a modification of a method used by Fraenkel and Hsiao (1965). Newly emerged vermiform larvae were held loosely in a small arterial clamp and allowed to swallow air and to begin the abdominal contractions leading to splitting of the cuticle. At the precise moment the larvae split the cuticle the clamp is closed so as to pinch only the ventral surface of the body but still leave a continuous haemocoele between the anterior and posterior ends. Histological examination showed that this procedure severs the nerve cord.

iv) Electrocoagulation of areas of the ventral nerve cord.

Vermiform larvae about to emerge were cooled on a cold plate to inhibit activity. Parts of the ventral nerve cord of the larvae were then electrocoagulated using a Martin Electron-60 electrocoagulation system. The larvae were then reburied in sand to recover and emerge. Those larvae which emerged and shed the embryonic cuticle were kept for 8 hours and then scored for melanization.

v) Preparation of extracts.

Extracts of parts of insects for bioassay were prepared using the high potassium Ringer of Maddrell and Gee (1974). This makes possible the collection of a hormone extract which does not involve homogenizing the tissue to be tested. This is advantageous since suspensions of homogenised cellular material of any origin, even when dilute, can induce darkening, possibly as a result of a wound reaction to foreign cellular material.

Fifty larvae were used for each extraction and each was cut into three pieces. The body was sectioned (a) between the prothoracic and mesothoracic segments and (b) between the second and third abdominal segments. The 50 anterior, middle and posterior pieces were placed, without further dissection, in 200µl of high potassium Ringer. This preparation took approximately 1 hour; after the last insect had been dissected the tissue was allowed to extract for a further 30 min. and then centrifuged at 3000 rev/min for 5 min. The three supernatants were injected into vermiform larvae for bioassay and scored for melanization after five hours.

vi) Histology.

(1) Acetaldehyde fuchsin and paraldehyde fuchsin staining.

Complete nervous systems of embryo, vermiform and first instar larvae were fixed in Bouin's fluid and prepared for histological staining in the usual way. The sectioned larvae were then stained with either paraldehyde fuchsin (Highnam 1962, Ewen 1962) or acetaldehyde fuchsin (Kasim 1973).

(2) Mallory stained sections.

For the examination of the cuticle of ligatured first instar larvae Mallory's triple stain was used. The larvae were fixed by cutting off the tip of the head and abdomen and immersing in Bouin's fluid under vacuum for 24 hours. 8μm paraffin sections were then prepared and stained in the normal way.

vii) Electron microscopy.

The region of the central nervous system from the posterior end of the metathoracic ganglion to half way between this ganglion and the first abdomonal ganglion was examined in an electron microscope in both vermiform and first instar larvae. The material was dissected out under 2.5% glutaraldehyde solution buffered with 0.05 M cacodylate containing 0.25 M sucrose. After fixation for 2 hours in three changes of glutaraldehyde the tissues were transferred to 1% osmium tetroxide in buffer for one hour. The material was then dehydrated through an ethanol series and embedded in Araldite in the usual way. 70 nm sections, collected on Formvar coated grids, were double stained with 2% aqueous potassium permanganate and Reynolds lead citrate. The material was examined in an AEI EM 6G electron microscope.

viii) The blowfly bioassay for the melanization hormone.

The method adopted was that of Vincent (1972). A culture of the blowfly Calliphora erythrocephala Meig. was reared from the late larval stage of the third instar to the pupal stage at room temperature. Adults were then ligatured with hair around the neck immediately they had emerged from the puparium. At this stage the ptilinum is still expanded and, as Vincent points out, this has three advantages. Firstly it provides a check on the tightness of the

ligature since a loose ligature will allow the ptilinum to collapse back into the body. Secondly, the expanded ptilinum reduces the blood volume of the thorax and abdomen behind the ligature, thus making the test more sensitive. Finally the reduced blood volume in the abdomen almost eliminates bleeding after injection.

The ligatured blowfly were allowed to stand for two hours before being used for the bioassay and any flies showing darkening at this stage were rejected. The flies were then injected with 2µl of blood. They were incubated for three hours at room temperature and then scored on a 0 to 4 scale of darkening identical to that of Vincent (1972) : (0) No darkening. (1) Slight darkening; approximately 30% of the dorsal surface of the abdomen. (2) Darkening of patches of the thorax in addition to areas of the abdomen. (2.5) Abdomen entirely dark, thorax less so. (3) Abdomen and thorax almost totally dark. (3.5) Interference colours present at the tip of the abdomen. (4) Fully dark.

ix) Cuticle digestion.

As a measure of cuticular tanning, the cuticles of Schistocerca larvae at various stages before and after the embryonic ecdysis were digested in weak alkali. The amount of protein going into solution gives a measure of the extent to which the cuticle is digested and is thus inversely proportional to the extent of tanning in the cuticle (Karlson, Kalliope and Marmaras 1969).

Cuticle samples were prepared by cutting off the head and terminal segments of the abdomen and removing the gut. The legs were then removed leaving a cylinder of body wall. This was then cut open down one side, opened out and scraped with a microspatula so as to remove the majority of the internal organ systems. The

remaining pieces of larval body wall were then homogenised in water and the homogenate was left standing for 24 hours to extract water soluble material. Since the epidermis cannot easily be separated from the cuticle this initial extraction in water removes much of the more soluble epidermal protein so that cuticular protein is the principal component remaining. The suspension was centrifuged at 3000 rev/min and the supernatant discarded. The residue was washed three times in water, centrifuging and discarding the supernatant each time. The washed residue was freeze dried. 28mg of residue was suspended in 25ml of 0.16% Na_2SO_4 to digest for 16 hours. The suspension was centrifuged at 3000 rev/min and the supernatant collected and dialysed for 24 hours. The dialysed solution was then reduced from approximately 30ml to exactly 5 ml by evaporation in an air current. The solution was then estimated for protein by the Folin and Ciocalteau method (Lowery, Rosenborough, Farr and Randall 1951). Extracts were prepared from batches of 80 larvae from each of the following 6 times:

(1) Approximately 14 hours before hatching was expected.

(2) Vermiform larvae (0 time).

(3) 0.5 hours after hatching.

(4) 1 hour after hatching.

(5) 2 hours after hatching.

(6) 4 hours after hatching.

x) Non-melanizing larvae.

On emergence from the embryonic cuticle most gregarious first instar larvae of Schistocerca are pale yellowish-green in colour except for the eyes, mandibles and the semi-lunar crescent of the metathoracic femoro-tibial joint. These larvae progressively darken over the next two to three hours until they are almost completely

black. However in a typical egg pod of gregarious Schistocerca a small number of the eggs at the bottom of the pod produce larvae which do not darken but remain green. These are slightly smaller than typical gregarious larvae and their initial colouration is more of a whitish-green. Frequently the eyes and mandibles are less pigmented and the semi-lunar crescent may be completely colourless. The green larvae change colour only slightly and are finally pale green. The characteristics of such larvae are not, however, totally reliable. Thus when such larvae were used as blood donors they were always kept for some hours and the presence or absence of melanization recorded as a check against the results in the recipient larvae.

Albino Schistocerca, kept as a stock culture at COPR, are morphologically identical to normal gregarious Schistocerca except for the absence of black pigmentation. They show a Mendelian pattern of inheritance in which albanism is recessive (Hunter-Jones, 1957).

7. Terminology.

Within the text a number of technical terms are used about which there may be some ambiguity of meaning. For clarity therefore the definitions of a number of words, as they are to be understood in the present text, are given below.

Embryo. The period of development within the egg up until apolysis, i.e., the separation of the embryonic cuticle from the epidermis.

Pharate first instar. The period of development within the egg from the time of apolysis until the time of the intermediate moult.

Hatching. In many insects the act of hatching brings the first instar larva directly into the environment which is its natural habitat. In Acridids, however, there is a transitional period between hatching and emergence into their natural environment at the surface of the soil whilst the vermiform larva digs up through the froth tube. Thus hatching and emergence are distinct from one another, the former being the act of rupturing the chorion of the egg whilst the latter is the appearance of the vermiform larva at the surface of the soil.

In the present study adherence to this distinction raises difficulties in so far as there are instances in which stimuli are given to fully developed eggs which initiate hatching but what is actually recorded is emergence. This distinction is of little practical significance in the present study since direct observations (Bernays 1970) have shown that digging through the soil takes only one to five minutes and this time is insignificant in relation to the average latencies of three to five hours which were recorded. However the distinction has been made by Uvarov (1966) and is of practical relevance in such Acridids as Acrida pellucida Klug in which it is reported that there is a delay between hatching and emergence whilst the vermiform larvae accumulate in the froth tube (Hafez and Ibrahim 1958). Thus despite the frequent use of the terms hatching and emergence interchangeably (Clark, Ashall, Waloff and Chinnick 1969, Ashall and Ellis 1962, Ellis and Ashall 1957, Duck 1944, Pickford 1967, 1977) the distinction has

been made and there are occasions on which this is of practical value. The distinction between hatching and emergence as defined above is therefore made in the following account even though in many instances emergence and hatching are intimately associated, the former being a natural progression from the latter.

Intermediate moult. The ecdysis of the embryonic cuticle which, under natural conditions, occurs immediately after the vermiform larva has dug to the surface of the soil after hatching.

Vermiform larva. This is a specific stage at the end of the pharate period of the first instar between hatching and the intermediate moult. Under natural conditions the insect is in this form only whilst digging through the soil immediately after hatching.

Hatching effort. This is a simultaneous contraction of all the abdominal segments of a pharate first instar which results in a visible narrowing of the posterior end of the body and is compensated for by an anterior enlargement. It is during such a movement that the egg shell ruptures, (Bernays, 1972c). This characteristic muscular activity may be repeated many times over a period of hours prior to hatching and this is referred to as the 'hatching effort period'.

Gregarious larva. A vermiform or later stage larva showing the physiological, behavioural and anatomical characteristics of a locust subjected to crowding during its development.

Such larvae typically show increased physiological fitness (Albrecht 1962), greater activity and a darker colouration than solitarious larvae.

Solitarious larva. A vermiform or later stage larva showing the physiological, behavioural and anatomical characteristics of a locust subjected to isolation during its development. Such larvae are typically less physiologically fit, less active and of greener colouration than gregarious larvae.

Circadian rhythm. An endogenous oscillation with a natural period close to but not necessarily equal to that of the solar day (24 hours).

Endogenous rhythm (or oscillation). A periodic system which is part of the temporal organisation of the organism. It is self sustaining, i.e. it 'free-runs' in the absence of temporal cues such as the daily cycles of light and temperature.

Exogenous rhythm. A rhythm of activity which is a direct response to the environmental cycle of light or temperature. In the absence of these variables the rhythm does not persist.

Diurnal. Occurring during the day or light period of the cycle, i.e. the opposite of nocturnal. An alternative usage of the term denoting a daily (24 hour) cycle is not intended to be understood by this term and the word "daily" is used to denote 24 hour periodicity.

Entrainment. The coupling of a self sustained oscillation, i.e. a circadian oscillation, to a forcing oscillation, i.e. the environmental cycles of light and temperature, so that both have the same frequency.

Gate. The 'allowed zone' of the cycle, dictated by the circadian clock, during which an organism may perform a 'once only' activity, e.g. hatching, moulting or pupal eclosion. If a particular organism is not at the 'correct' morphogenetic stage to utilize one gate it must wait approximately 24 hours (depending upon the duration of the gate) until the next gate (Pittendrigh 1966).

RESULTS

1. THE TIMING OF EMERGENCE: EXTRINSIC FACTORS

A. Natural emergence times.

Initial experiments on the time of emergence were carried out under semi-natural conditions in the grounds of the research station at Jeddah. (See Methods 4i, p 45). On the first day approximately 30 adult pairs were placed in the oviposition cage open to recently watered soil and left for 24 hours. Oviposition occurred on that evening and again on the following morning. On this following day the adults were removed from the first cage and placed in a second cage in another part of the prepared oviposition site together with approximately 30 more adult pairs. These were then allowed to oviposit over the following 48 hours. After 48 hours in the second cage all the adults were removed and returned to the breeding cages. At the two oviposition sites the top $\frac{1}{2}$ inch of soil was carefully removed so as to expose the foam tubes above each of the egg pods which had been laid. Each egg pod was then marked with a matchstick pushed into the soil adjacent to it. A total of nine pods were laid at the first oviposition site and 23 at the second.

The pods were not disturbed in any way so that their development was as similar to that of a naturally occurring population as possible. Both oviposition sites were exposed directly to the heat of the sun during the day. The oviposition in the first cage was used as an indicator of when the pods in the second cage would be due to hatch. After 17 days incubation the egg pods in the first cage were seen to have emerged at some time overnight so that observation of the pods in the second cage were then started at 04.00 h on the two following mornings. No insects had emerged prior to this and the emergence times of these pods are given in Table 1. The soil temperature at a depth of 8-10 cm,

photoperiod and emergence period for the two days of observation are given in Fig 3. Emergence only occurred in the period from 05.00 h to 06.30 h on each day. No emergence was observed at any other time. The results seem to confirm the observations of other field workers (Ellis and Ashall 1957, Ashall and Ellis 1962, Roffey, personal communication) that emergence of Schistocerca larvae from the egg pod occurs only in the period around dawn.

Egg pod number	Date	Emergence time
1.	7.11.72	05.15 - 05.30
2.	"	06.00 - 06.15
3.	"	06.00 - 06.15
4.	"	05.20 - 05.40
5.	"	06.10 - 06.30
6.	"	05.05 - 05.20
7.	"	05.15 - 05.30
8.	"	05.50 - 06.10
9.	"	05.45 - 06.00
10.	8.11.72	05.20 - 05.35
11.	"	05.40 - 06.00
12.	"	06.10 - 06.30
13.	"	05.55 - 06.15
14.	"	06.00 - 06.15
15.	"	05.30 - 05.45
16.	"	05.20 - 05.30
17.	"	06.15 - 06.30
18.	"	05.50 - 06.10
19.	"	05.20 - 05.35
20.	"	06.00 - 06.15
21.	"	05.25 - 05.45
22.	"	05.55 - 06.15
23.	"	05.10 - 05.30

Table 1. The emergence times of egg pods incubated under semi-natural conditions in the soil at the Jeddah Research Station.

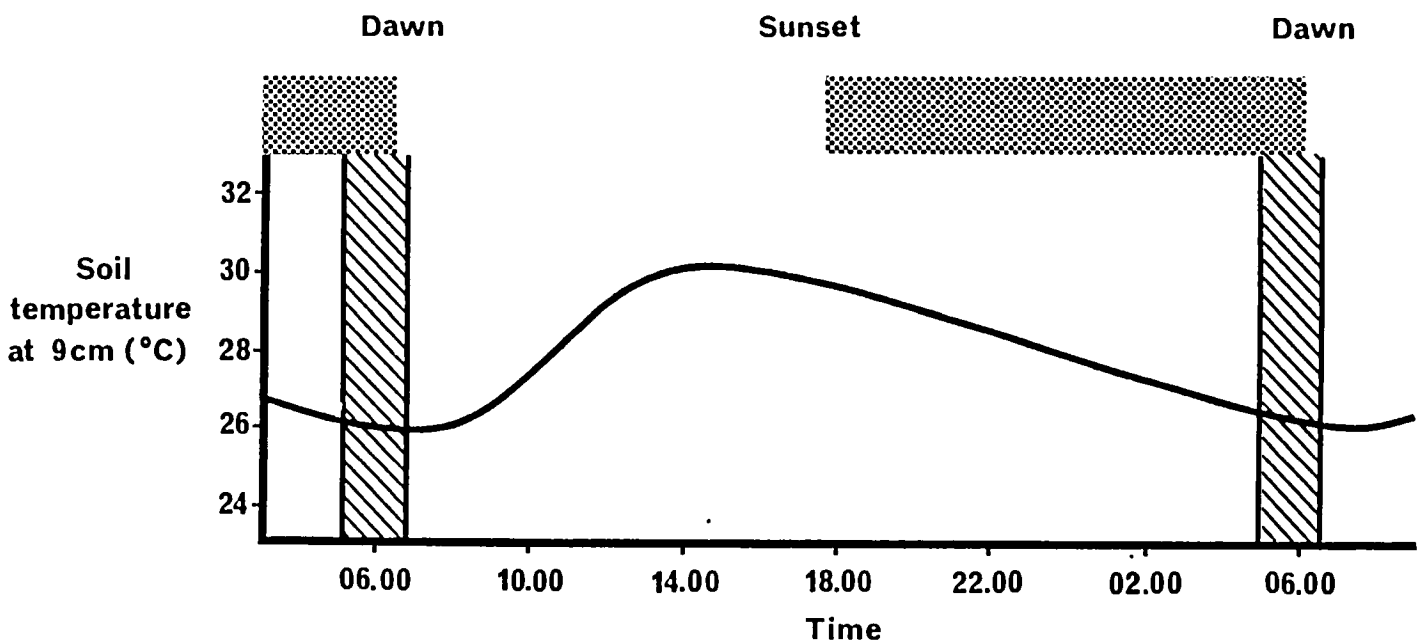


Fig. 3. The distribution of emergence under field conditions (////) in relation to photoperiod and soil temperature.

B. The time of emergence in relation to the time of oviposition.

Since earlier work has suggested that the embryonic development of Schistocerca requires a relatively constant number of degree days (Hunter Jones 1966a) it could be argued that emergence within a limited period of each day simply reflects the fact that oviposition also occurs within a limited daily period. This possibility was examined under laboratory conditions at COPR. Egg pods were collected from standard rearing and breeding cages (Hunter Jones 1966b) over a period of 11 days, the exact time of the end of oviposition being recorded in each case. These pods were then incubated either with a fluctuating daily temperature of 12h 29°C / 12h 33°C or at a constant temperature of 31.5°C. The emergence times of these pods were then recorded and the data is given in Figs 4 - 8.

Oviposition occurs largely within two periods of the day, in the late morning and again in the evening (Fig 4). The probability that this distribution is random is less than 0.001, (χ^2 analysis). Hatching on the other hand shows a single daily peak when the eggs are given a cycled incubation temperature (Fig 5) and the probability of this being a random distribution is again less than 0.001, (χ^2 analysis). When the eggs are given a constant incubation temperature (Fig 6) the distribution is not significantly different from a random distribution, (χ^2 analysis).

The relationship between oviposition time and emergence time is given in Fig 7. Fig 7a is for egg pods incubated at a constant temperature and Fig 7b is for egg pods incubated with a cycled temperature. The coefficient of linear correlation is weak for both groups, ($r = -0.2370$ for the cycled temperature group and

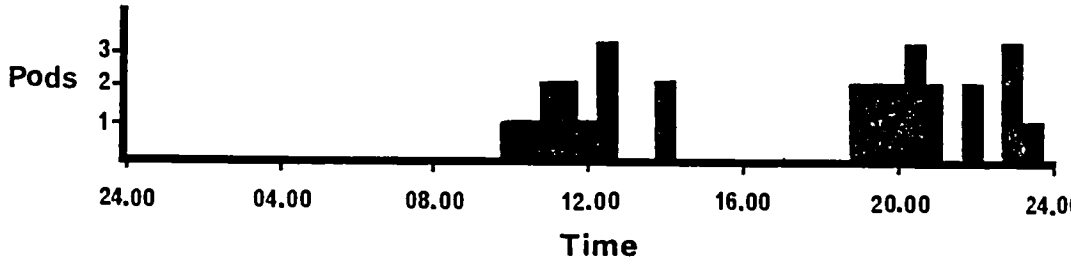


Fig. 4a. The distribution of oviposition times of eggs subsequently given cycled temperature incubation.

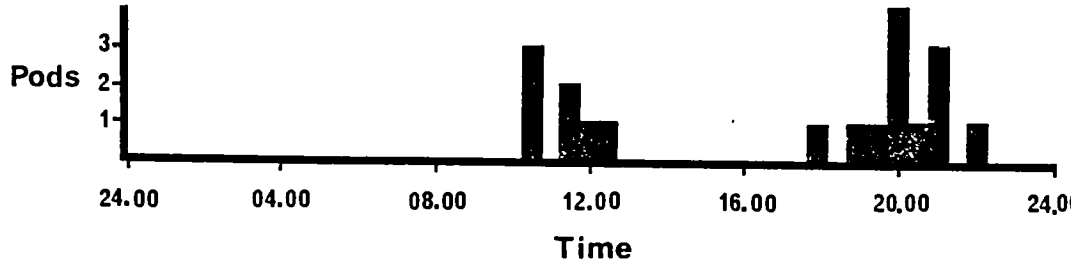


Fig. 4b. The distribution of oviposition times of eggs subsequently given constant temperature incubation.

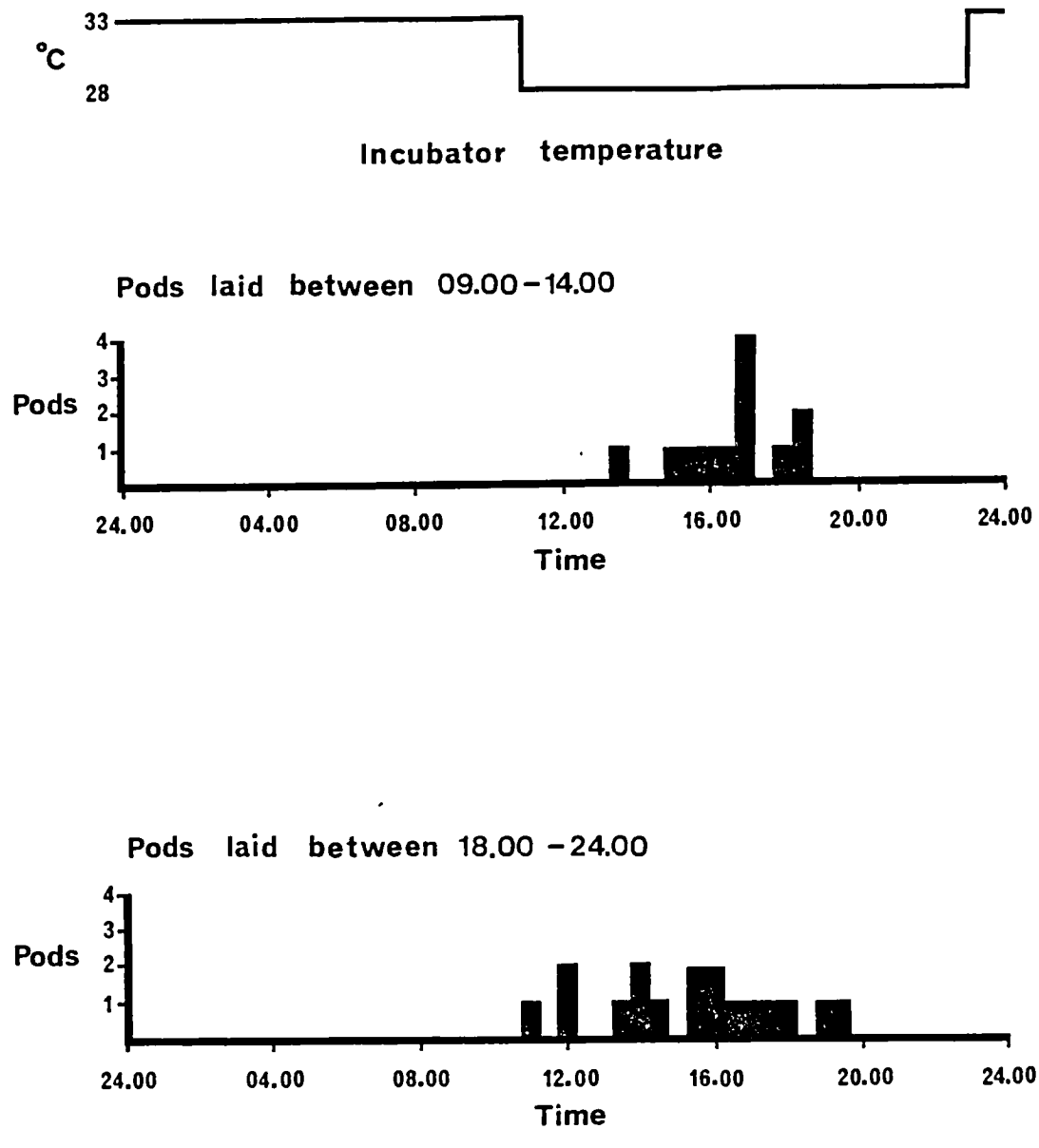


Fig. 5. The emergence times during the day of egg pods given a cycled temperature incubation.

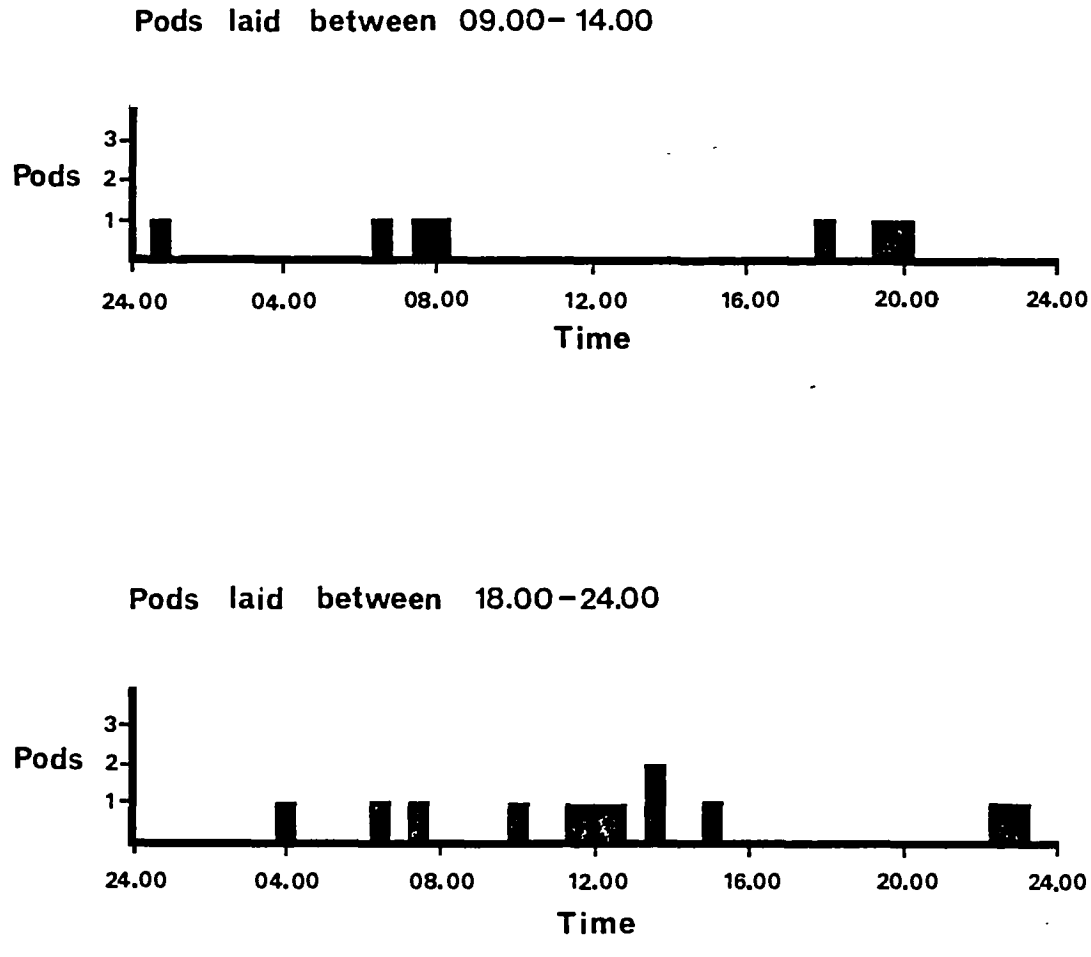


Fig. 6. The emergence times during the day of egg pods given a constant temperature incubation.

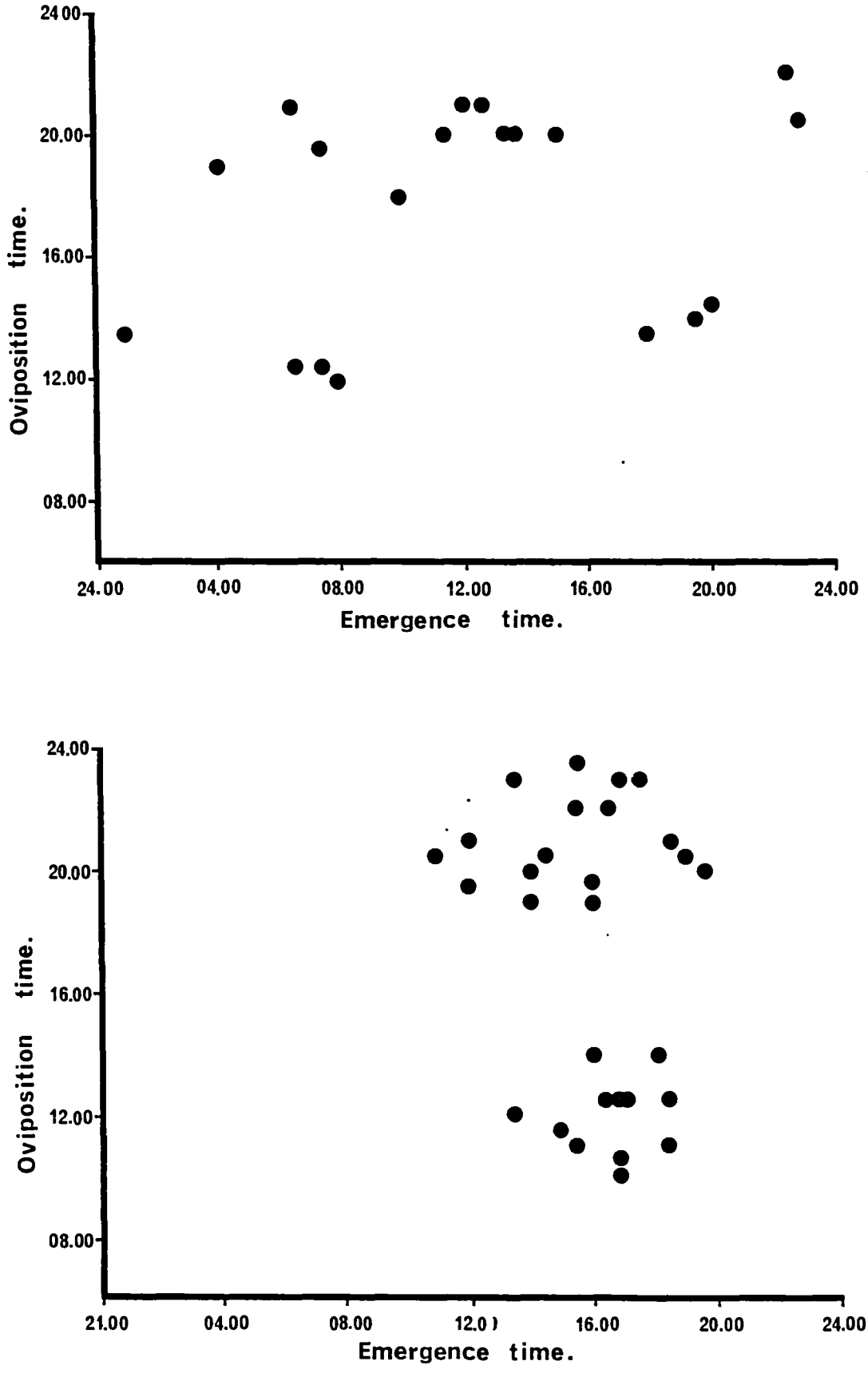


Fig. 7. Diagram illustrating the absence of a correlation between oviposition time and emergence time for egg pods incubated with either a constant incubation temperature (upper figure) or a cycled incubation temperature (lower figure).

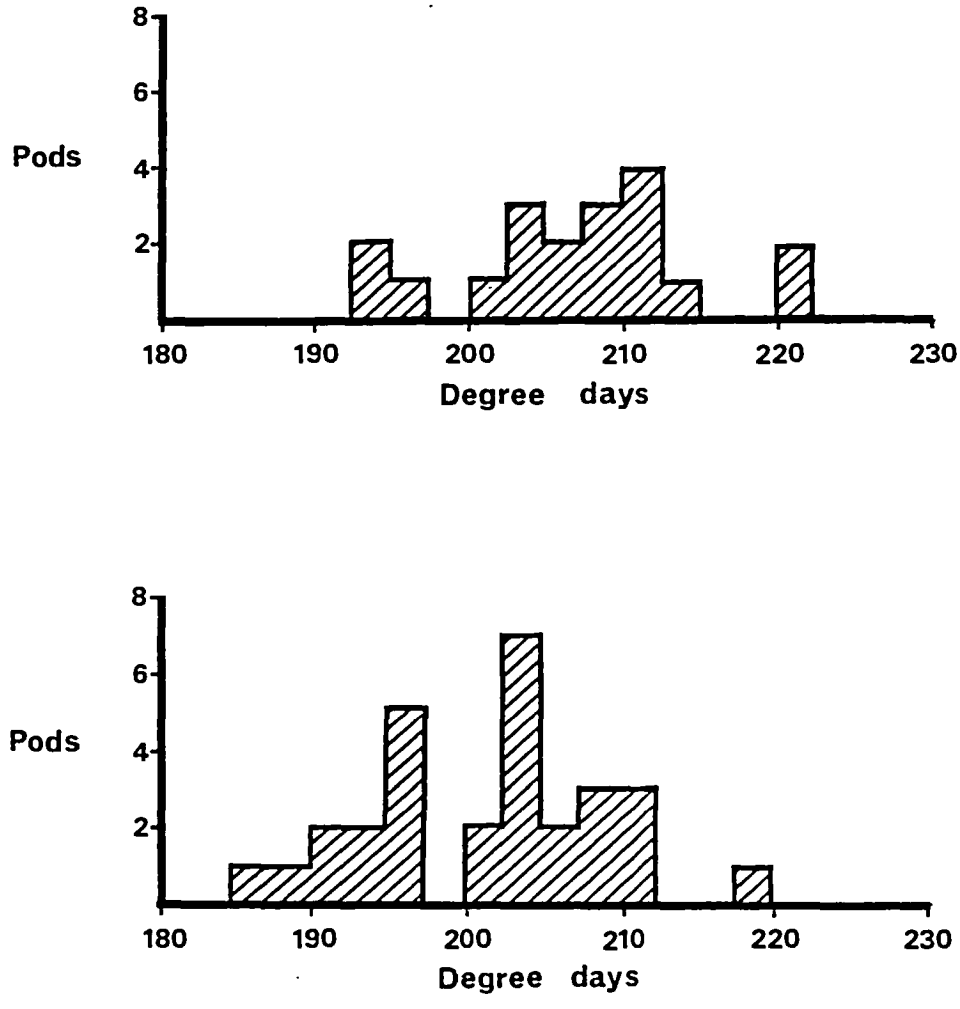


Fig. 8. The duration of incubation of egg pods given a constant incubation temperature (upper figure) or a cycled incubation temperature (lower figure).

r = 0.2044 for the constant temperature group). The probability of getting these values, given that there is no correlation, is greater than 0.1. Thus under constant temperature the duration of incubation is largely a function of the incubation temperature (Hunter-Jones 1966a) and the emergence times show no correlation with the oviposition times. Given a cycled incubation temperature the same is true. However in this instance a second factor, in addition to the incubation temperature, is having a limited influence on the duration of incubation. The pods emerge only in the first two thirds of the cold period each day. This phenomenon of a behavioural pattern being confined to a limited period of a daily temperature or photoperiod cycle is termed 'gating' (Pittendrigh 1966). This gated emergence of the larvae appears to reduce the incubation time slightly (Fig 8), the means of 201.5 degree days in cycled temperature and 206.9 degree days at constant temperature being significantly different ($0.05 > p > 0.01$). The influence of a cycled incubation temperature in establishing this behaviour and the magnitude of the temperature changes which punctuate the rhythm are considered in more detail in subsequent experiments.

With both cycled and constant temperature the distribution of emergence times differs from the distribution of oviposition times. In addition to this, the variation in incubation times themselves are sufficient to discount any correlation between oviposition and emergence time. Thus the restricted daily period of emergence shown by natural populations of locusts is not a function of synchronised oviposition.

C. The relationship between temperature and emergence time.

The synchronised emergence of a large number of pods within a limited period of each day suggests that they must be responding to some environmental stimulation and previous workers have suggested temperature, light and soil moisture as possible influential factors in Acridid hatching, (Uvarov 1966). The first of these, temperature, has been considered in relation to hatching from three viewpoints. Since environmental conditions vary from one locality to another and from one time of year to another the emergence times of Schistocerca egg pods were first examined in relation to various temperature cycles both of differing magnitude of change and of different mean temperature. Secondly, as already mentioned in relation to the previous duration of incubation experiments, a cycled temperature incubation leads to a gating of emergence. This was investigated more fully. Finally, since a drop in temperature appears to stimulate hatching, this phenomenon was investigated with a view to determining its relative influence when in competition with the gated emergence behaviour.

i) The time of emergence in relation to the change in temperature experienced by the embryo immediately prior to emergence.

a) Field experiments.

In the compound of the research station at Jeddah in November the soil temperature at 8-10 cm shows a slight daily fluctuation as shown in Fig 9. At the time of hatching the cycle is approaching its minimum having fallen about 5°C from the previous days maximum. Thus if temperature is involved it is a cooling rather than a warming of the eggs which initiates hatching. Two possible alternatives to account for this hatching at dawn are:-

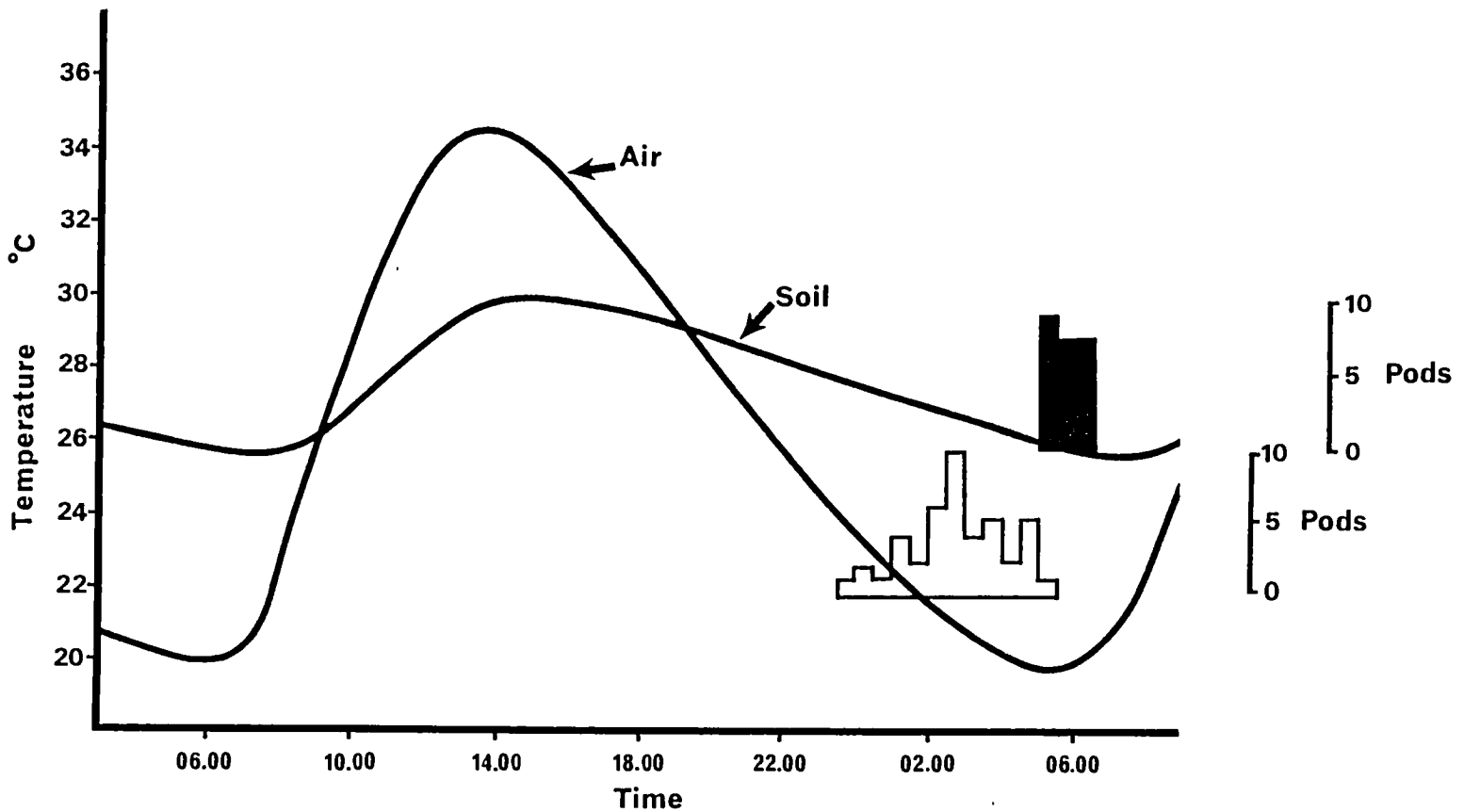


Fig. 9. The distribution of emergence times of egg pods incubated at the ambient soil temperature (solid block) compared with egg pods incubated at the ambient air temperature (open block).

1. The eggs hatch at approximately the minimum temperature which they regularly experience during incubation.
2. The eggs hatch after a fixed 'quantity of cooling' in terms of degree hours above the developmental zero.

To test these two alternatives eggs laid in standard tubes in cages were incubated at the ambient air temperature. Thus these eggs, unlike those deep in the soil, experienced a drop in temperature much earlier in the night because they were in much closer contact with the air. Thus whilst they still experienced a minimum temperature at dawn they were subjected to a much more rapid drop during the night. The results of this experiment are shown in Fig 9.

There is considerably more variation in the emergence times of egg pods incubated at the air temperature compared with those in the soil (variance ratio significant at 1% level). Also, comparing the mean emergence times for the two groups it can be seen that those given air incubation hatch about 3.5 hours earlier than those in the soil. Thus whilst those pods in the soil tended to emerge close to the minimum in the temperature cycle those in the air start to emerge about 5-6 hours before the minimum. Assuming that both the air and soil incubated groups are responding in the same way to the ambient temperature cycle it would appear that it is not the minimum point in the temperature cycle which is stimulating hatching. It seems rather that the egg pods are responding to a magnitude or rate of fall in temperature which happens to be coincident with the minimum in the soil temperature cycle.

Comparing the emergence times of the two groups in relation to the cooling which either has experienced is difficult since the ways in which insect perceive temperature and temperature changes

are not well understood. Being poikilothermic the body temperature of an insect, in this case a pharate first instar, is generally the same as the ambient temperature. Thus ambient temperature will have a direct influence on metabolic processes. In Schistocerca the rate of embryonic development shows a linear relationship with temperature between 15°C and 35°C (Hunter-Jones 1966a). Therefore if temperature is acting directly via some metabolic process associated with falling temperature the relevant temperatures in terms of degree hours for each of the two groups would be represented by the area under the temperature profile graph with the base-line at 15°C. This is shown for the air and soil incubated groups in Fig 10.

The two areas beneath the cooling period profile are similar, 169 degree hours for air and 195 degree hours for soil. However with only these two results it is difficult to know whether or not the closeness between the two quantities is meaningful. Additionally the time period over which cooling has been measured may have no practical significance. The cooling period may not be recognised as such by the pharate first instar until a drop of one or two or more degrees has been experienced and if this were the case the length of the 'recognised' cooling period would be changed by different amounts in the two groups. For example if the area under the cooling profile were measured after a 0.5°C drop from the maximum the two areas of degree hours would be almost exactly the same. Alternatively if it were measured after a 1°C drop from the maximum the situation would be reversed with the air incubation degree hours being greater than the soil. Thus no satisfactory conclusions can be drawn on this basis.

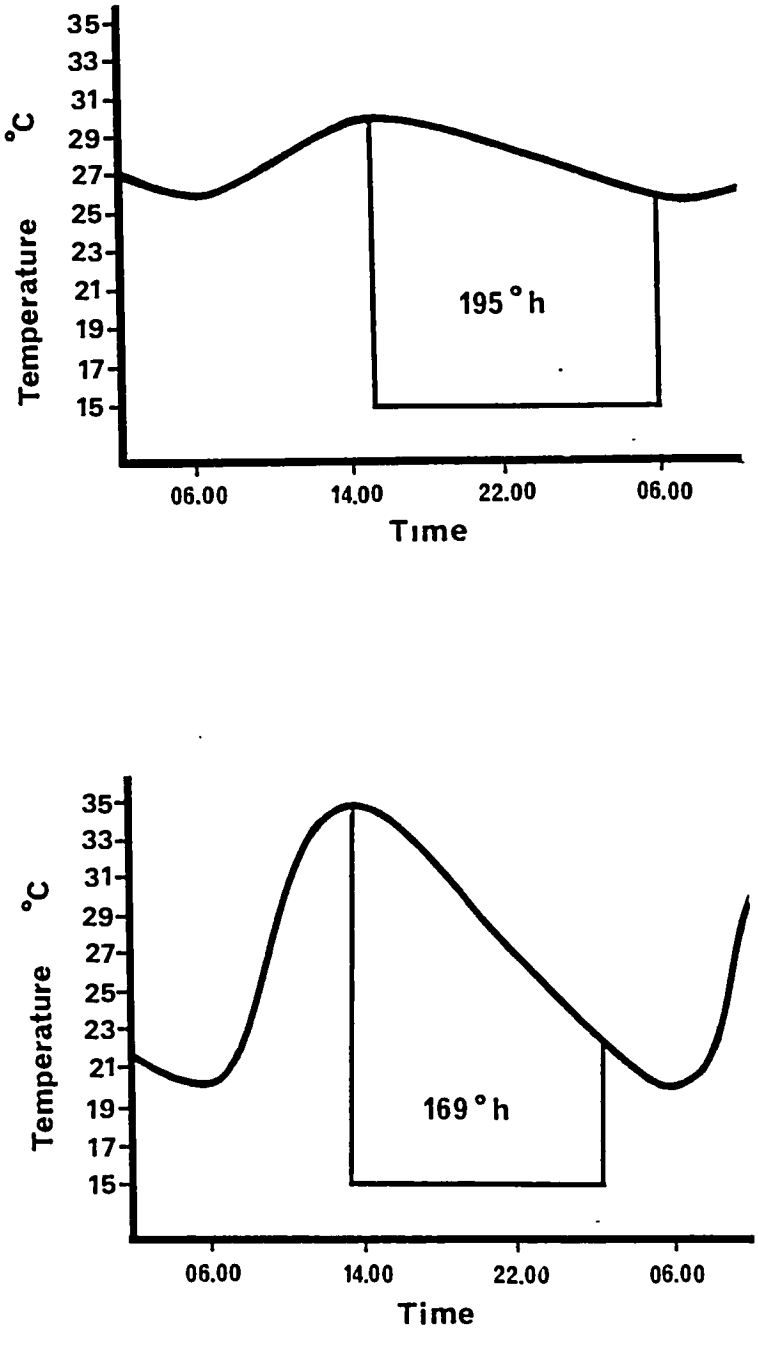


Fig. 10. A quantitative estimation of the cooling period in degree hours before the mean emergence time for egg pods incubated at either the ambient soil temperature (upper figure) or at the ambient air temperature (lower figure).

Hatching in response to a drop in temperature may not be the result of the stimulation of a metabolic process which is proportional to temperature. It is known that insects respond to both increases and decreases in temperature by an increase in activity (Kennedy 1939). Also in dipausing pupae and eggs the temperatures required to break diapause often fall below the developmental threshold (Truman 1971, Bodine 1929) and with the diapausing eggs of Chesias legatella Schiffermüller, the rate of diapause development has been shown to increase proportionally with the reduction in temperature below the developmental threshold down to -20°C (Wall 1974). Thus in these instances the reaction to the change in temperature must be mediated via a system capable of producing a metabolically positive response from what would normally be considered to be a negative temperature stimulation. Thus the response of an insect to a temperature change need not be proportional to the quantity of heat it is receiving in terms of degree hours above the developmental threshold but may only be related to the temperature change. Comparing the hatching times of the air incubated pods with those of the soil incubated pods it can be seen that the group experiencing the greater change in temperature is also the group with the earliest hatching time, i.e., the air incubated group. This gives some support to the hypothesis of the cold stimulus acting via cold receptor neurones (Kerkut and Taylor 1958) which in turn lead to the stimulation of activity.

b) Laboratory experiments.

The field experiments on emergence times in soil and air temperature cycles suggest that the time of emergence in response to a cold stimulus is influenced by the magnitude of that stimulus. To investigate this further, egg pods were incubated under artificial

conditions in the laboratory. However the incubator has the disadvantage of producing a temperature cycle with a square wave profile rather than the approximately sine wave form of the natural daily temperature fluctuation. Thus there is no minimum in the cycle which is the equivalent of dawn and no maximum which is the equivalent of mid-day. The first experiment was therefore to examine exactly when egg pods would emerge under these conditions.

Egg pods were incubated for 12 days with a temperature cycle of 12 hours at 33°C and 12 hours at 28°C. The distribution of emergence times is shown in Fig 11. As a control to this experiment egg pods were also incubated for 12 days at a constant 31°C. The emergence times of these pods are also shown in Fig 11.

The results show that when egg pods are incubated in a daily temperature cycle they show a distinct synchrony of emergence with a mean emergence time approximately $4\frac{1}{2}$ hours after the drop in temperature. The egg pods incubated at constant temperature emerged over a 29 hour period.

Having established that the emergence of pods incubated with a fluctuating daily temperature occurs within the period approximately 3-5 hours after the cold drop additional experiments relating to the magnitude of the drop were carried out. Egg pods were incubated on three different cycles with the same magnitude of change, i.e., 23/28°C, 28/33°C, and 33/38°C. Also a cycle each of greater and smaller magnitude, i.e., 25/35°C, and 30/32°C. The emergence times from each of these experiments are shown in Fig 12 and table 2.

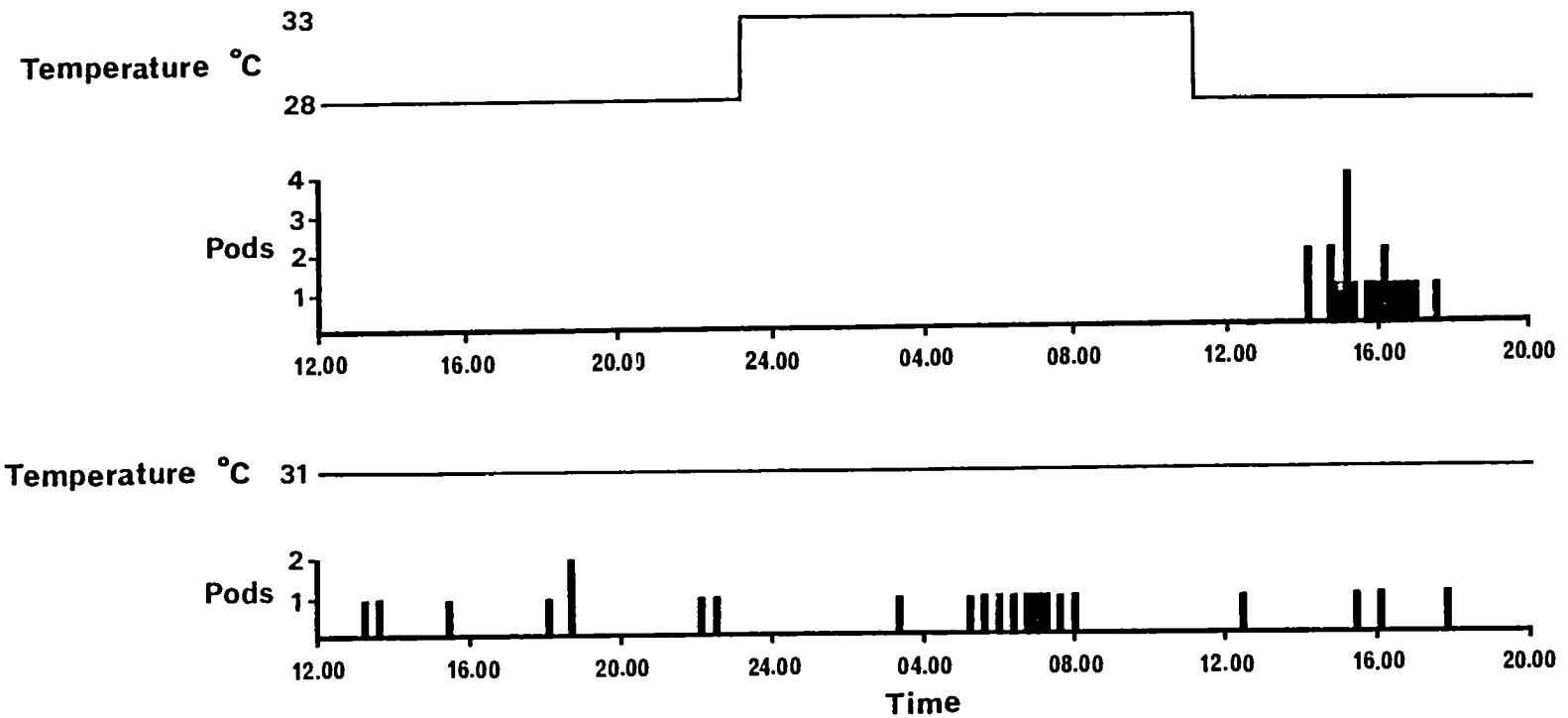


Fig. 11. The distribution of emergence times of egg pods given either a cycled incubation temperature (upper figure) or a constant incubation temperature (lower figure).

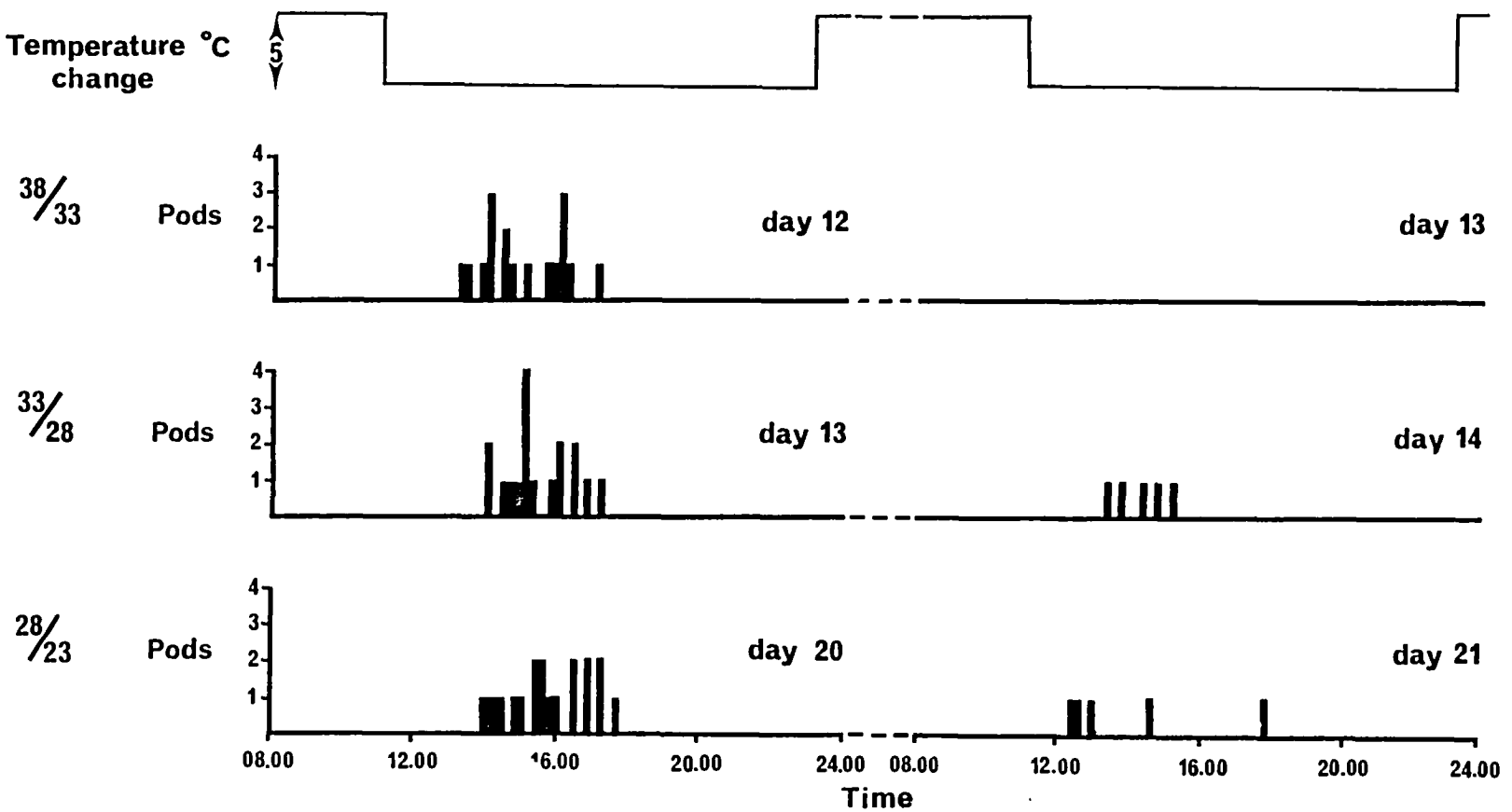


Fig. 12a. The distribution of emergence times of three groups of egg pods given three different 5°C incubation temperature cycles.

(continued)

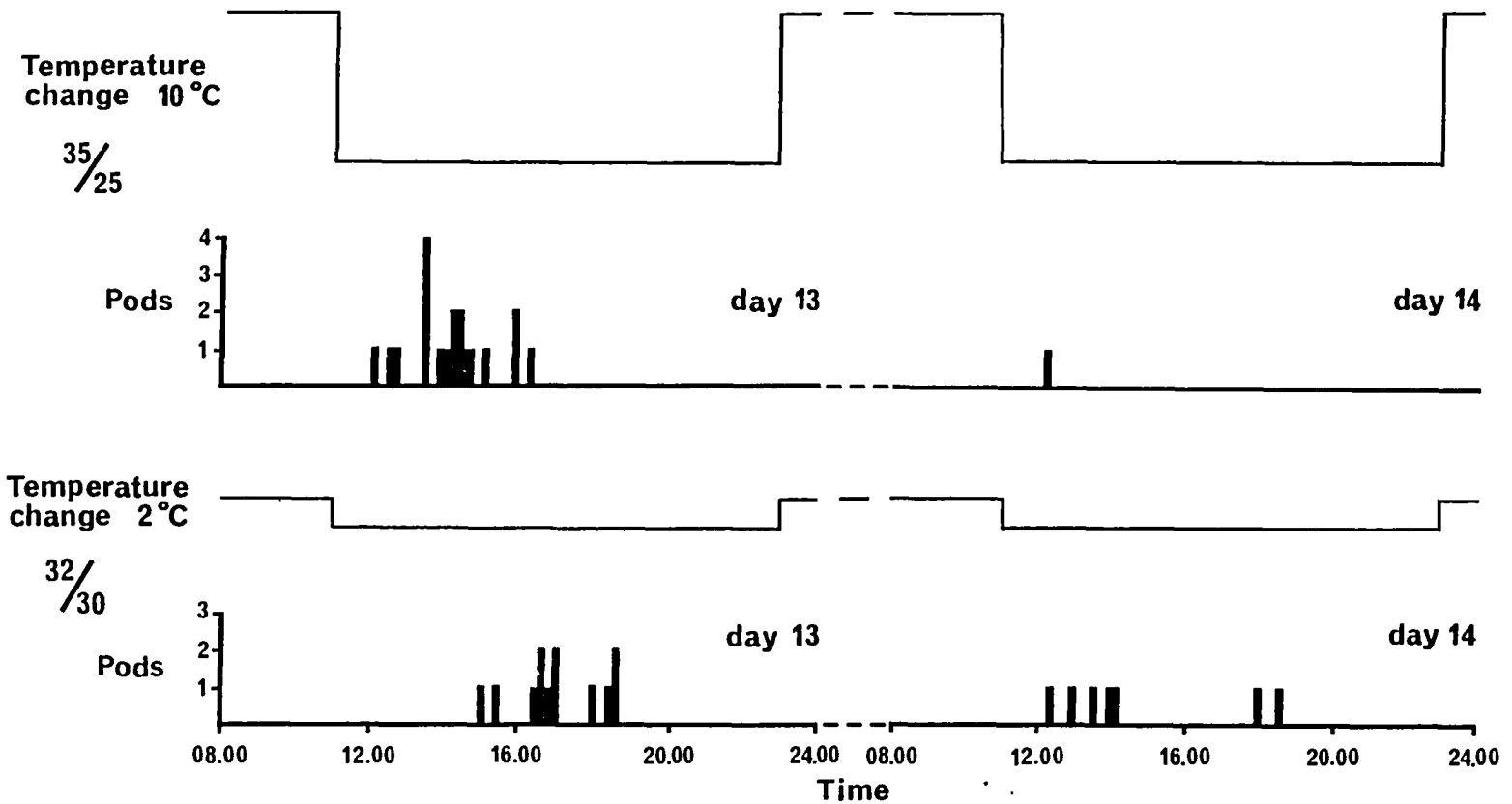


Fig. 12b. (continued) The distribution of emergence times of two groups of egg pods given either a 10°C incubation temperature cycle (upper figure) or a 2°C incubation temperature cycle (lower figure).

Temp cycle	Emergence mean Day 1	Standard deviation	Lag from stimulus	Emergence mean Day 2	Lag from stimulus
38/33°C	15.00h	1h 8 min	4h 0 min	-	-
33/28°C	15.30h	57 min	4h 30 min	14.24h	3h 24 min
28/23°C	15.54h	1h 4 min	4h 54 min	14.11h	3h 11 min
35/25°C	14.09h	1h 8 min	3h 9 min	12.20h	1h 20 min
32/30°C	17.10h	1h 5 min	6h 10 min	14.11h	3h 11 min

Table 2. The time of emergence in relation to the magnitude of the temperature change in the incubation cycle.

With different temperature cycles of the same magnitude of change, in this case 5°C, there is very little difference in the time during the day when these pods emerge. The mean emergence times for the three cycles are:-

38/33°C - 4 h. after the cold drop,

33/28°C - 4 h. 30 min. after the cold drop,

28/23°C - 4 h. 54 min. after the cold drop.

However with a 10°C change in temperature the egg pods emerge earlier whilst with a 2°C change the emergence period is later than that for the 5°C change.

Looking at these data in the same way as the field date (see Fig 10) the areas below the temperature profile between the cold drop and the mean emergence time are given in Fig 13. The degree hours beneath the cooling profile range from 31.5 to 92.4. These values are all much smaller than either of the two field results which were 169 and 195 degree hours. Thus the data argue against hatching being the direct result of some metabolic process which was proportional to the quantity of heat received by the pharate first instar after the onset of the cold period.

The emergence times for each of the above groups in relation to the magnitude of temperature change, regardless of absolute temperatures, are given in Fig 14. The distributions of emergence times for the pods in each group are represented by horizontal bars, the end points being the times of emergence of the first and last pods. Two additional groups have been included, one with no temperature change and a second group with a 33/28°C change. These data are from subsequent experiments concerned with other aspects of hatching but are included here also since they are relevant to the present considerations.

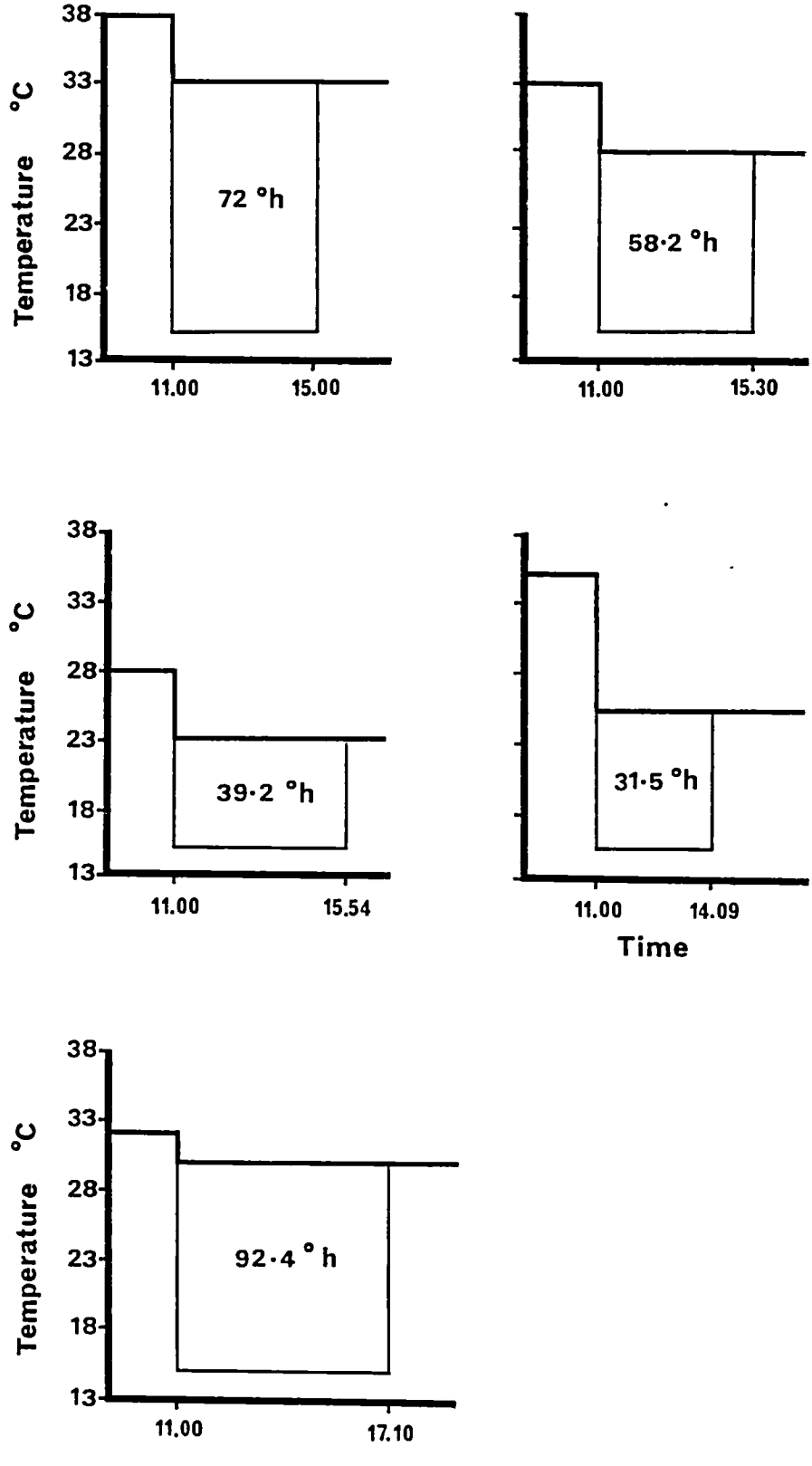


Fig. 13. A quantitative estimation of the cooling period between the onset of the cold drop and the mean emergence time for the five different incubation temperature cycles shown in Fig. 12.

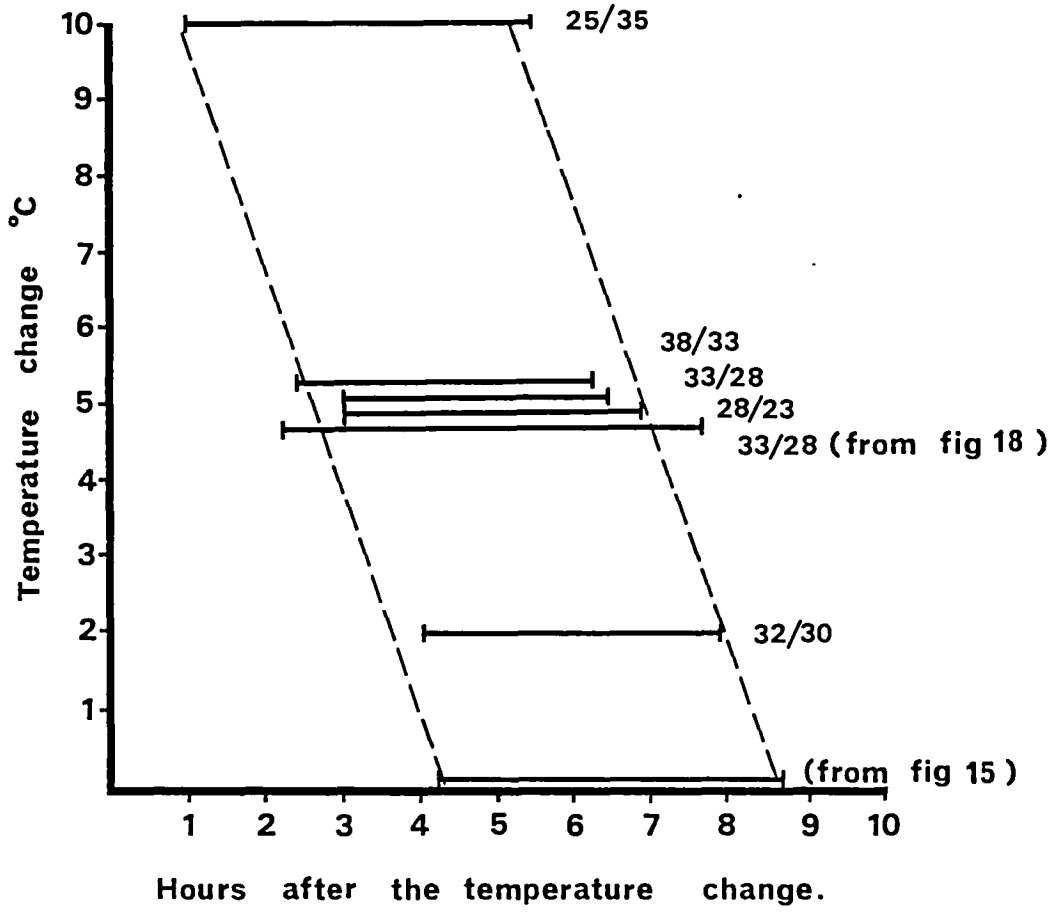


Fig. 14. The relationship between the timing and duration of the emergence period with the magnitude of the preceding drop in temperature. (Data from Figs. 12, 15 and 18).

Fig 14 shows that there is a strong correlation between the magnitude of the stimulating temperature change and the time of emergence ($r = -0.8190$). Those pods given the greater cold drop emerge earlier than those given a smaller or no drop in temperature. It is also noteworthy that the duration of the emergence period is almost always approximately four hours, the correlation between the magnitude of the drop and the end of emergence also being strong ($r = -0.7817$). The field data cannot be correlated quantitatively with the data in Fig 14 since the temperature drop under field conditions was gradual. However, qualitatively the picture is the same, the pods receiving the greater temperature drop emerge before those receiving the smaller reduction.

Whilst the majority of pods emerged on the day predicted by their incubation temperature a few often emerged on the following day as shown in Fig 12. These pods also emerge in the cold period and show a degree of synchrony but in each case the mean emergence time relative to the beginning of the temperature drop is earlier than that of pods of the previous day. The emergence of egg pods incubated with a fluctuating daily temperature shows a 'gated' behaviour pattern which has been mentioned earlier in relation to its influence on the duration of incubation. In this instance the phenomenon shows itself again but in addition the data suggests that those pods which perhaps just missed the emergence gate of the first day respond more rapidly on the second day.

ii) The influence of the time of the cold stimulus on the time of emergence.

Having confirmed the work of Bernays (1970) that a drop in temperature is a stimulating influence on hatching two further

groups of experiments were carried out. The first considered what would happen if the egg pods were given no cold stimulus whilst the second considered what would happen if a cold stimulus were given before it would normally occur. In both cases experiments were carried out under both field and laboratory conditions. The earlier field observations on natural emergence times were undertaken in November 1972 whilst the subsequent field experiments were carried out during April and May 1973 when environmental temperatures were higher and incubation periods correspondingly shorter.

a) The effect of no cold stimulus.

The initial experiment was carried out at the field station in Jeddah. In this experiment two groups of 10 egg pods were incubated at the fluctuating ambient air temperature (max 39°C min 27°C, mean 33°C) for 12 days (09.00h. day 1 - 09.00h. day 12). Group 1 was then transferred to an incubator at 32.5°C to determine how long the eggs would remain unhatched without the cold stimulus whilst group 2 acted as controls, remaining at the ambient temperature. The emergence times of the individual pods in each group were recorded and are given in Table 3.

The results show that the eggs kept in the incubator, and thus experiencing no reduction in temperature on the last day, emerged at almost the same time as those incubated at the ambient temperature. This therefore suggests that the developing embryos become synchronised with the environmental temperature and that this sets a biological clock within the animal. Thus on the last day hatching occurs at the 'correct' time even though there is no cold drop to signal the approach of the emergence gate.

INCUBATOR		CONTROLS	
Pod No	Emergence time	Pod No	Emergence time
1	02.45h Day 13	11	03.10h Day 13
2	01.15h "	12	02.40h "
3	00.20h "	13	02.20h "
4	03.40h "	14	04.20h "
5	03.55h "	15	02.25h "
6	01.20h "	16	01.40h "
7	02.05h "	17	02.35h "
8	00.50h "	18	03.55h "
9	01.25h "	19	01.10h "
10	02.25h "	20	02.20h "
Mean	02.00h	Mean	02.40h

Table 3. The emergence times of egg pods incubated under either completely ambient conditions (control group) or with the last day at constant temperature (incubator group).

This experiment was repeated under incubator conditions. Eggs were incubated on a 12 h 33°C / 12 h 28°C cycle for 11 days and then the temperature was kept constant at 33°C for the whole of the final day. The emergence times were recorded and are shown in Fig 15. Data from Fig 11, which give the emergence times of pods given a cycled temperature for the whole of the incubation period is presented alongside for comparison. The results confirmed those from the Jeddah work and again suggest that the developing embryos become entrained to the temperature cycle sufficiently to hatch at the "correct" time even when this cycle is absent on the last day.

Since entrainment is established by the last day of incubation further experiments were carried out to determine the maximum period of constant temperature possible at the end of incubation before entrained emergence is not seen. Egg pods were incubated for either ten, nine or eight days with the cycled temperature, the remainder of the incubation period being at constant temperature. The emergence times for these egg pods were recorded and are shown in Fig 16. Variance ratio analysis (F-test) of the data indicates that the standard deviations of groups 1 and 2 are not significantly different. However comparing group 1 with groups 3, 4 and 5 gives a steady increase in the significance level of the difference, these being 2.5%, 1% and 0.1% respectively. From Fig 16 it appears that entrainment to the rhythm is either established at some stage in the last two to three days of development in a 12-13 day incubation or alternatively is established at some earlier stage in embryonic development and then is progressively lost with increasing time at constant temperature.

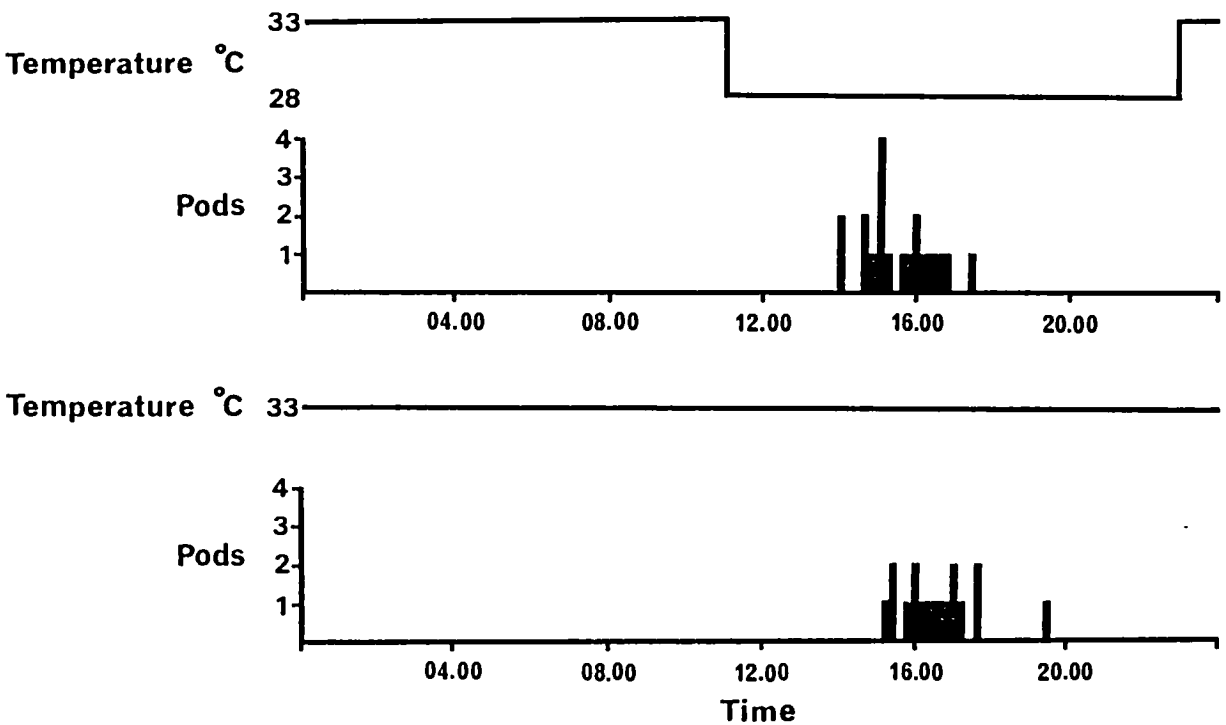
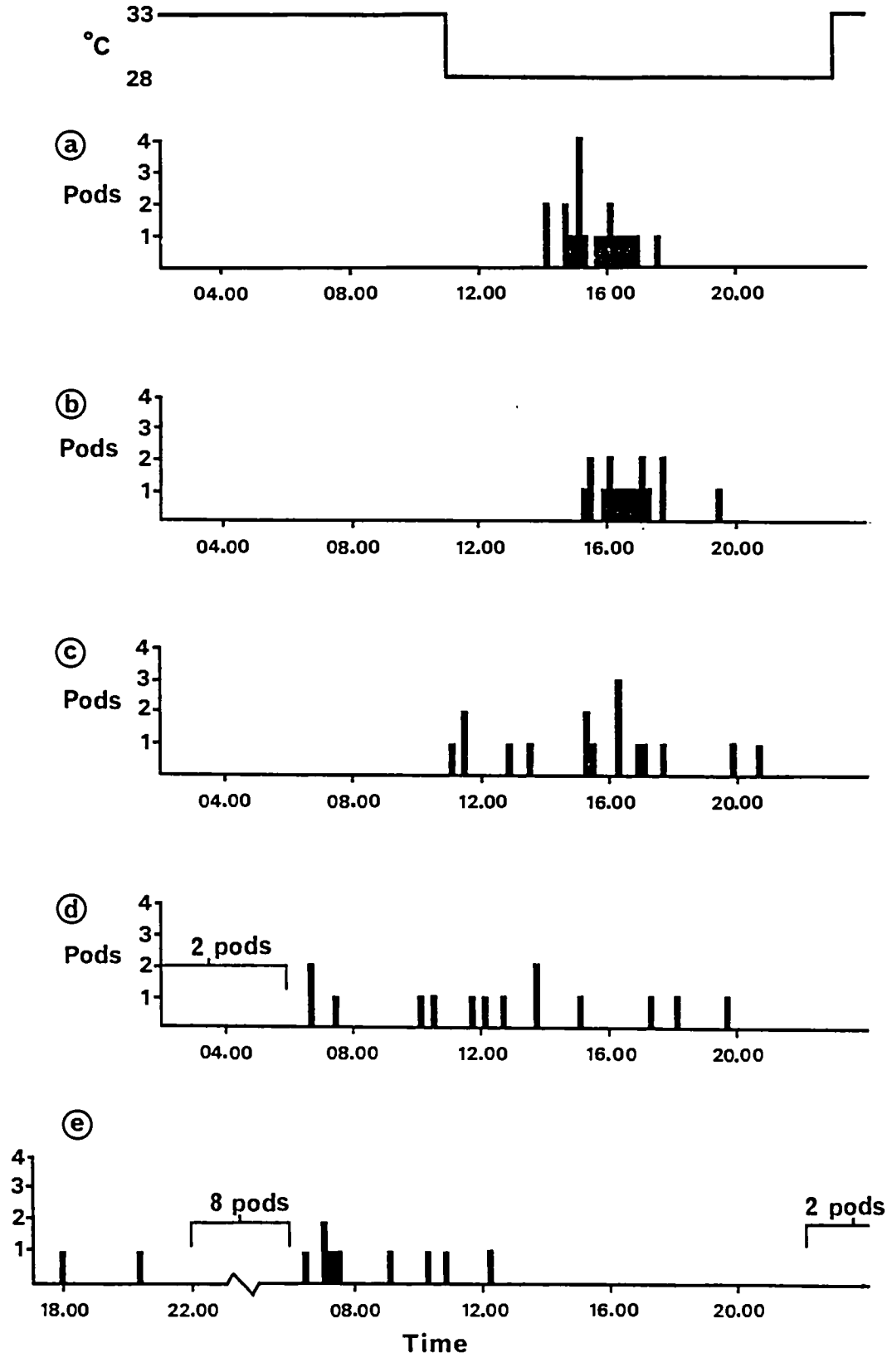


Fig. 15. The distribution of emergence times of two groups of egg pods given either a cycled incubation temperature throughout development (upper figure) or a cycled incubation temperature until the penultimate day, the temperature then being kept constant (lower figure).

Fig. 16. The distribution of emergence times of five groups of egg pods kept at constant temperature for different periods over the last days of incubation.

a) Incubation with completely cycled temperatures.
b) The last day at constant temperature.
c) The last two days at constant temperature.
d) The last three days at constant temperature.
e) The last four days at constant temperature.


33
°C
28
ⓐ
Pods
4
3
2
1
04.00
08.00
12.00
16 00
20.00
ⓑ
Pods
4
3
2
1
04.00
08.00
12.00
16.00
20.00
ⓒ
Pods
4
3
2
1
04.00
08.00
12.00
16.00
20.00
ⓓ
Pods
4
3
2
1
2 pods
04.00
08.00
12.00
16.00
20.00
ⓔ
4
3
2
1
8 pods
2 pods
18.00
22.00
08.00
12.00
16.00
20.00
Time

b) The effect of an early cold stimulus.

Since egg pods given no cold stimulus on the last day emerged at the correct time there is the suggestion that hatching is to an extent independant of the final cold drop. However experiments relating to gated emergence have shown an increased readiness to hatch amongst pods emerging a day later than expected. It is probable therefore that these pods hatching on the second day complete development at some stage between the first and second emergence gates. They then remain quiescent until stimulated to hatch. If a quiescent period exists for these later pods it is probable that the same is true of some of the pods hatching on the first day. Data from Fig 8 have shown that amongst a group of pods given the same incubation temperature some pods will hatch as much as 35 hours in advance of others. Therefore under natural conditions the most advanced pods may well have completed development and entered a phase of quiescence before the first emergence gate. This possibility of fully developed embryos entering a quiescent phase before hatching was investigated by giving the egg pods an early cold stimulus. This was first done under field conditions at the Jeddah research station.

25 egg pods were collected from the adult laying cages at approximately 08.00h. No record of the exact time of laying was made but due to the pattern of oviposition there was probably no more than 12 hours difference in their ages. These pods were then incubated at the ambient air temperature for 12 days i.e., until the penultimate day of development. At 09.00 h. on the morning of this last day the 25 pods were divided up into five groups of five. Groups 1-4 were transferred to an incubator maintained constant at 32.5^{o}C whilst the fifth group was left to incubate at

the ambient air temperature. Each incubator group was then given a cold stimulus of a drop of 4°C at a different time. Group 1 was stimulated at 10.00 h, group 2 at 14.00 h, group 3 at 19.00 h and group 4 at 23.00 h. The emergence time of each of these pods was recorded together with the emergence times of the control group receiving only ambient stimulation. The results are given in Fig 17.

The results show that eggs can be induced to emerge up to almost 16 hours before emergence would normally occur but that this is not universally true for all pods. However at approximately 6 hours before hatching would normally occur the majority of egg pods could be induced to emerge and at 3 hours before expected hatching all the pods could be induced to emerge. Thus even when egg pods are incubated with a fluctuating daily cycle, which would tend to entrain them to emerge at a particular time of the day, this entrainment can be broken by an early cold stimulus. However not all of the pods respond, some hatching closer to the time predicted by entrainment. In an attempt to clarify more fully the relationship between entrainment and the final cold stimulus further experiments were carried out under laboratory conditions.

Egg pods were incubated for 12 days on a 28/33°C cycle. During the final warm period groups of egg pods were periodically removed from the incubator at 33°C and were cooled by either 5°C to 28°C or by 10°C to 23°C. The emergence times from these pods were recorded, the data being given in Figs 18 and 19 and tables 4 and 5.

Included in Figs 18 and 19 are data from Fig 15 giving emergence times from egg pods kept at constant temperature on the last day of incubation after being on a 28/33°C cycle for the previous 12 days.

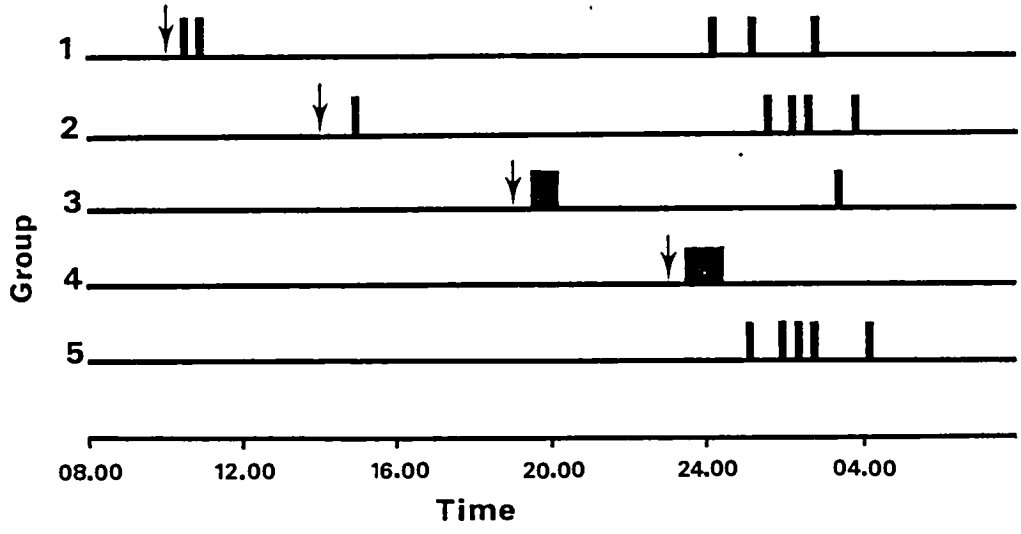


Fig. 17. The distribution of emergence times for egg pods given an early cold stimulation. The arrow ↓ marks the time of stimulation of each group. (Group 5, ambient stimulation).

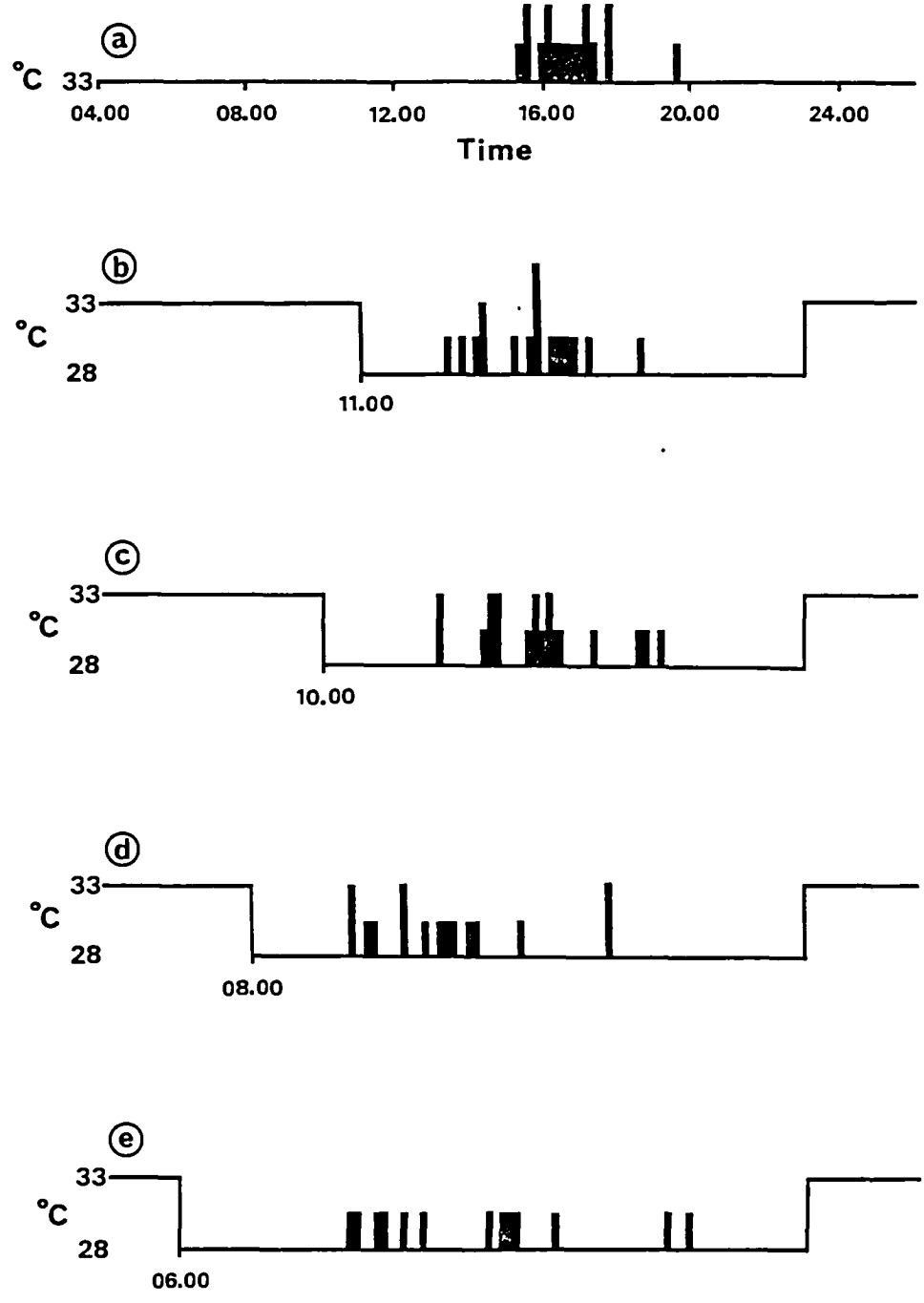


Fig. 18. The distribution of emergence times of egg pods given either no cold drop, a 5°C cold drop at the normal time or a 5°C cold drop 1, 3 and 5 hours earlier than normal.

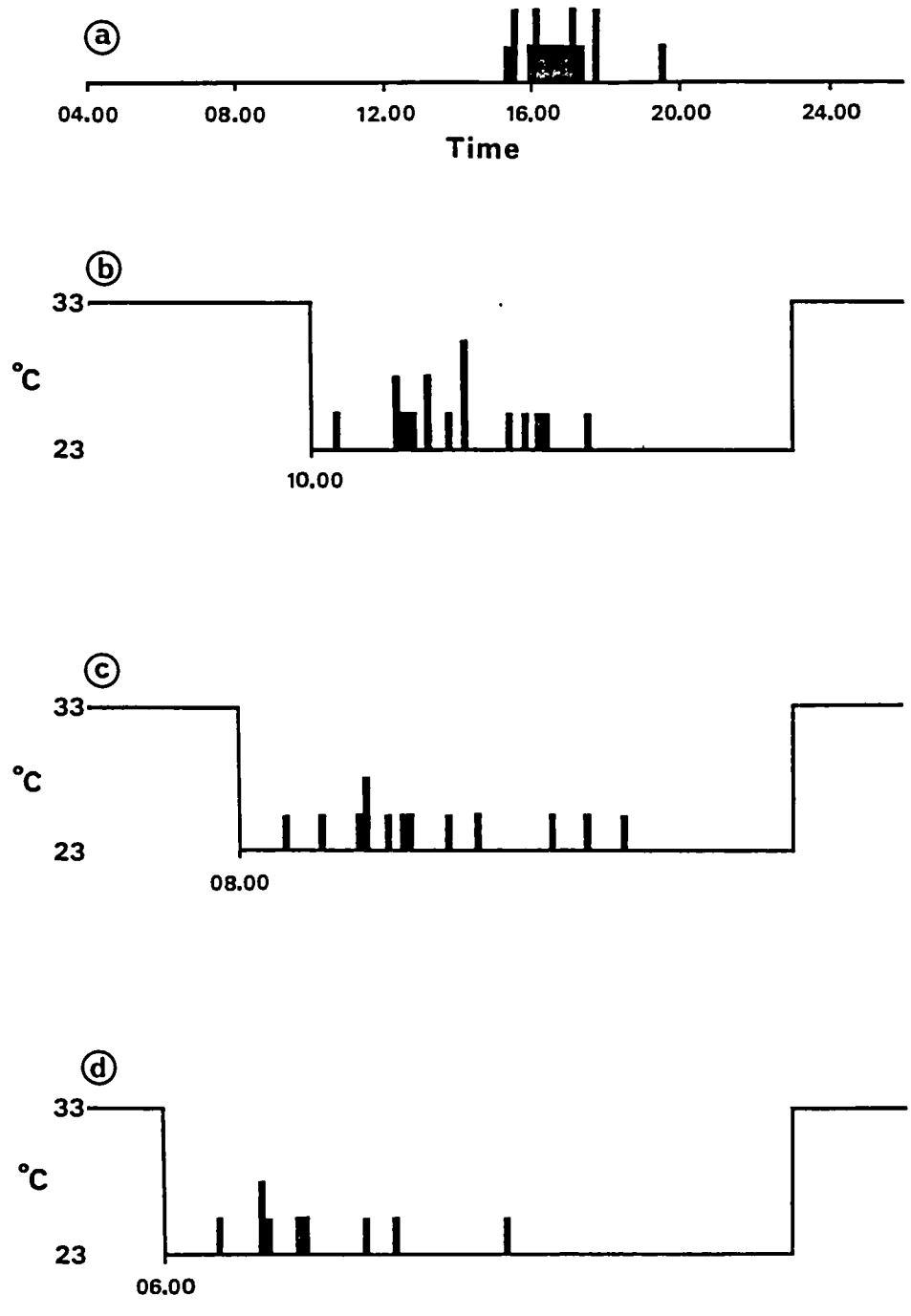


Fig. 19. The distribution of emergence times of egg pods given either no cold drop or a 10° C cold drop 1, 3 and 5 hours earlier than normal.

Time of 5°C cold stimulus.	None	11.00h	10.00h	08.00h	06.00h
Number of pods.	20	18	18	16	18
Number emerging on "correct" day.	18	16	18	15	13
Number emerging on the following day.	2	2	0	1	5
Mean emergence time of the pods emerging on the "correct" day.	16.38h	15.31h	15.44h	13.25h	13.59h
Standard deviation.	1h, 01 min	1h, 23 min	1h, 46 min	2h, 20 min	3h

Table 4. The time of emergence in relation to the time of an early reduction in temperature of 5°C.

Time of 10°C cold stimulus.	None	10.00h	08.00h	06.00h
Number of pods.	20	21	15	19
Number emerging on 'correct' day.	18	16	13	9
Number emerging on the following day.	2	5	2	10
Mean emergence time of pods emerging on the 'correct' day.	16.38h	13.57h	13.13h	10.14h
Standard deviation.	1h, 01 min	1h, 47 min	2h, 50 min	2h, 23 min

Table 5. The time of emergence in relation to the time of an early reduction in temperature of 10°C.

The data are best considered in the light of known influences controlling egg pod emergence. These are the thermoperiod which establishes entrainment and gated emergence, and the cold stimulus which, at the time of completed embryonic development, induces hatching behaviour. In Fig 18a there is no cold stimulus and the mean emergence time of 16.38h on the final day thus represent the component due to entrainment only. In Fig 18b the cold stimulus is given at the normal time and the emergence mean is brought forward slightly to 15.32h on the final day. Additionally the distribution of emergence times, as indicated by the standard deviations, increases from 1h, 01 min to 1h, 23 min. Thus whilst the two influences are reinforcing one another in the broad sense that they are inducing hatching at about the same time of the day, within the limits of the hatching period they are having a divisive influence. In group 18c there is no advance of the emergence mean, and in fact a slight retarding, but as before the standard deviation increases from 1h, 23 min to 1h, 46 min. Thus with the further separation of the entrainment component from the cold stimulus component the standard deviation increases. However, unlike 18b, where the cold stimulus appeared to influence the greater number of pods, in 18c, the entrainment influence appears the greater. In 18d, the emergence mean advances considerably to 13.25 h and once again the standard deviation increases to 2h, 20 min. Finally in 18e the emergence mean is less advanced than before but the standard deviation again increases to 3h. Whilst the mean emergence time is not always advanced by an advance in the cold stimulus the emergence of the first pod is progressively earlier with every advance of the stimulus. Thus with increasing separation of the time when the cold stimulus should produce emergence from when entrainment should

produce emergence there is a general advancement of the emergence time. However, more significantly, this separation of the two factors leads to an ever increasing distribution of the emergence period. By variance ratio analysis (F-test) of the standard deviations in Fig 18 only groups 18a and 18e are significantly different in the statistical sense (5% level). However the correlation between the earlier application of the cold stimulus and the increase in standard deviation for all the data in Fig 18 is strong, ($r = 0.7496$).

In Fig 19 there is broadly the same pattern with the standard deviations increasing in progression from 1h, 01 min in 19a to 1h, 47 min in 19b to 2, 50 min in 19c. The standard deviation of 2, 23 min in 19d is a slight reduction but the number of pods emerging is also less than usual. Unlike Fig 18, in Fig 19 the mean emergence time is brought forward as the cold stimulus is brought forward in every case. This may reflect the greater influence of a cold stimulus of $10^{o}C$ as compared to one of $5^{o}C$.

In both Figs 18 and 19 a number of pods failed to emerge with the main group but hatched either overnight or on the following day. These are recorded with the other data from these two figures in Tables 4 and 5.

The data in Figs 18 and 19 suggest that in general the influence of the cold stimulus and entrainment are approximately equal. The response of an egg pod to one or other of these factors therefore most probably depends on the degree of development of that pod. Those pods most fully advanced are physiologically able to respond to the early cold stimulus and do so, hatching some 3-5 hours later. Those pods slightly less advanced are physio-

logically unable to respond to the early cold stimulus but are sufficiently developed to be able to emerge at the later time that entrainment determines. Finally there is the third group which are the least developed. These are most noticeable where the cold stimulus is either particularly early or of large magnitude, i.e. 10°C. In these pods the cold stimulus has the normal physiological effect of reducing the metabolic rate and retarding development and subsequent emergence until the following day.

Comparing the data for the 5°C drop given in Fig 18 with that of the 10°C drop given in Fig 19 with respect to the first and last emergence times in each group gives the picture presented in Fig 20. The emergence period for each group is represented as a horizontal bar and as with the data given in Fig 14 the time of a first emergence can be seen to be related to the magnitude of the cold drop. However unlike Fig 14 the close adherence to an approximately 4 hour emergence period is not seen.

Data for the total duration of incubation of the egg pods in Figs 18 and 19 is given in Fig 21. Since the time of oviposition was not recorded exactly the mean oviposition time was calculated from the data given in Fig 4. This was 17.00 h. Fig 21a gives the total duration of incubation in degree days up until the time of the cold drop at 11.00 h. on the last day. Figs 21b-h give the total incubation in degree days for each of the groups in Figs 18 and 19 up until the time of stimulation on the last day and at the mean emergence time.

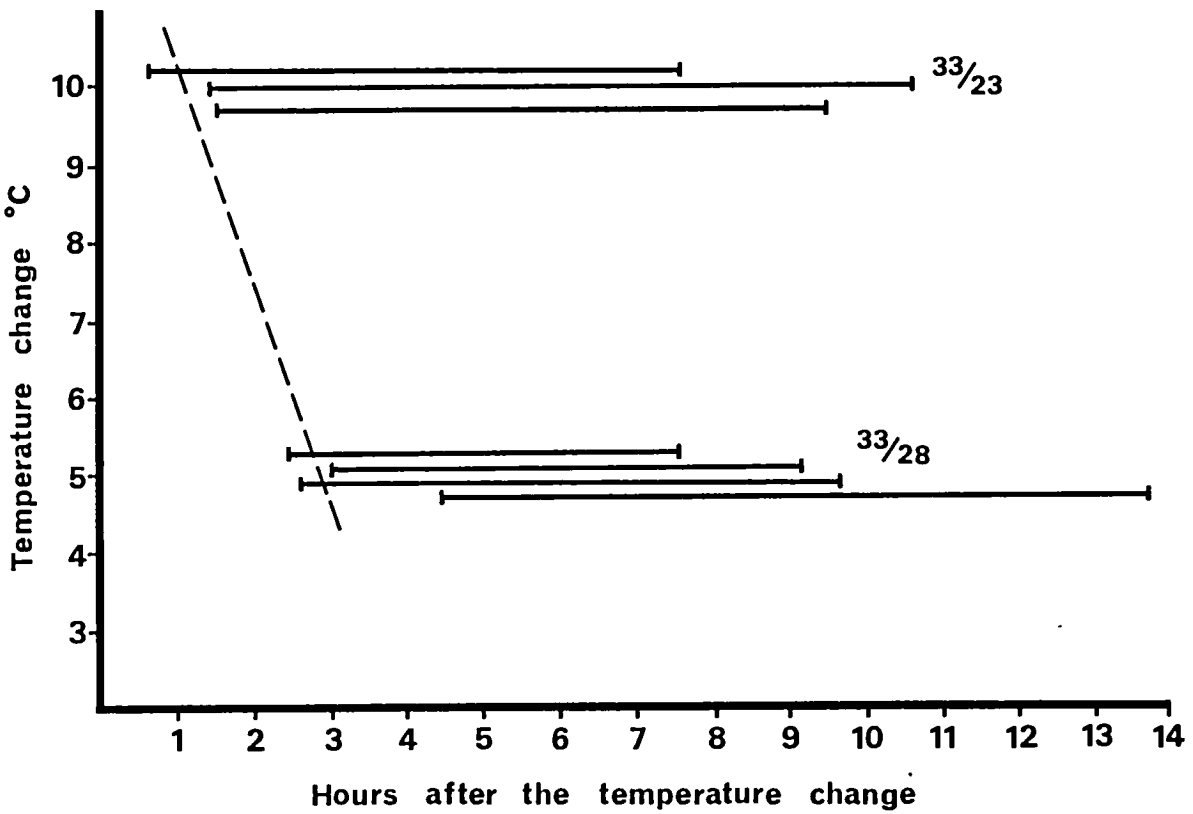


Fig. 20. The relationship between the timing and duration of the emergence period with the magnitude of the stimulating temperature change. (Data from Figs. 18 and 19).

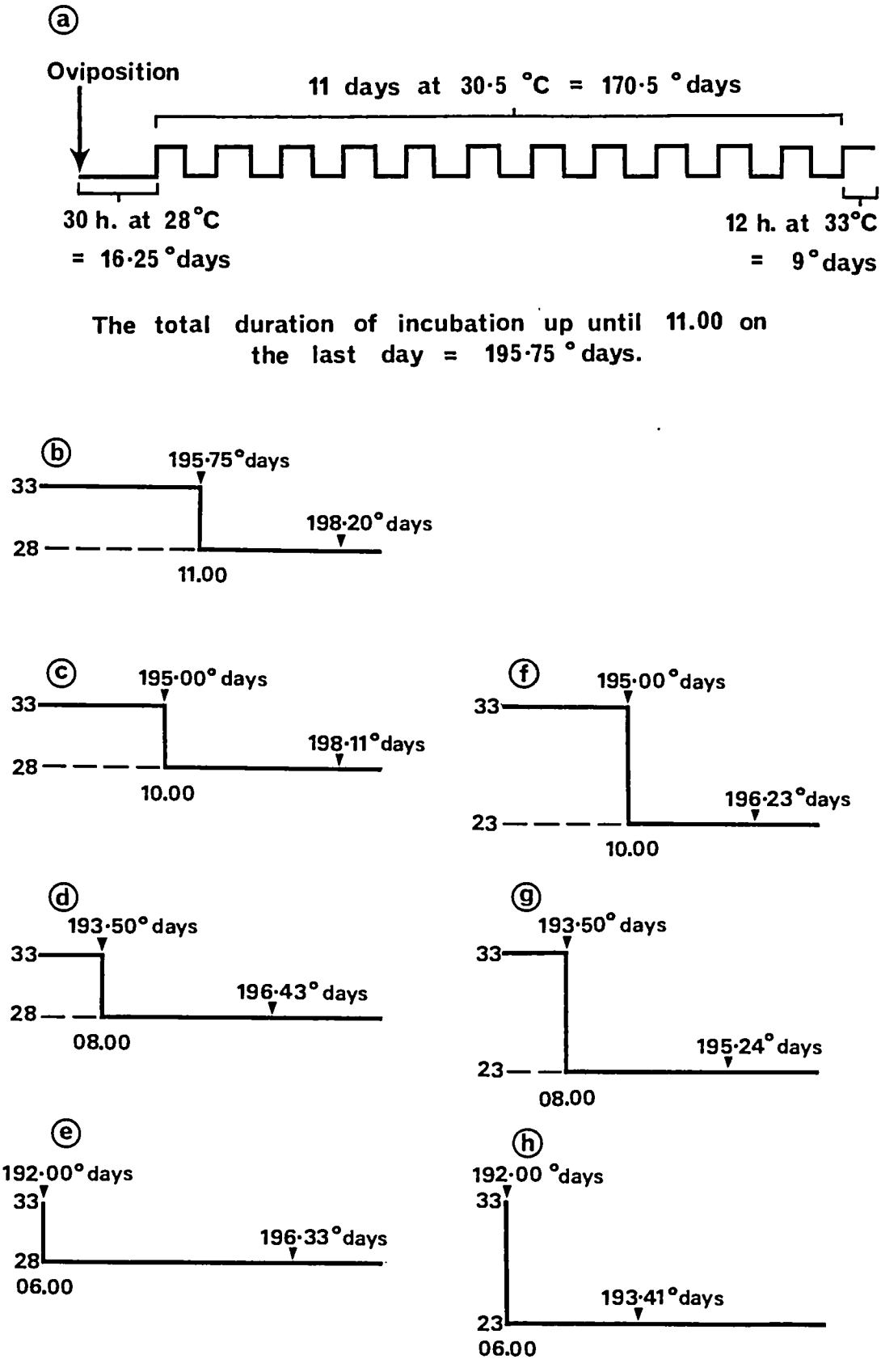


Fig.21. The total duration of incubation at the time of the cold drop and at the mean emergence time for each of the groups shown in Figs 18 and 19.

The results show that the mean duration of incubation is reduced as the time of the stimulating cold drop is advanced. However in group 19c a large proportion of the pods, 10 out of 19, failed to emerge on the first day of hatching. The total duration of incubation at the time when the cold stimulus was given is 192 degree days. Thus the behaviour of this group of egg pods would suggest that 192 degree days is approaching the minimum incubation period, over 50% of the pods being unable to hatch when stimulated at this time. For the pods which did hatch the mean duration of incubation was 193.41 degree.days. Comparing this figure with the data given in Fig 8 it can be seen that 186 degree days was the lowest duration of incubation recorded. Thus at 192 degree days the egg pods are approaching the minimum of incubation and it might be expected that a large percentage of the population would still be insufficiently developed to hatch at this time. However group 18e (21e) are also stimulated at 192 degree days and this group shows a much larger emergence on the first day, only 5 out of 18 pods carrying over to the second day. This pattern of a higher percentage of pods emerging on the first day seems to be a characteristic of all the groups given a 5°C cold drop compared to the groups given the 10°C cold drop.

Comparing Figs 18 and 19 a total of 6 out of 52 pods given an early 5°C drop hatched on the second day whilst 17 out of 54 pods given an early 10°C drop emerged on the second day. Thus whilst results from this and earlier experiments show that the time of emergence on the first day is proportional to the magnitude of the cold drop it appears that a large cold drop which is given early may be inhibitory to the least fully developed section of the population.

iii) The influence of a gradual cold drop on the time of emergence.

It has been demonstrated in previous experiments that both entrainment and a reduction in temperature will induce hatching at a particular time in relation to whichever stimulus is given. However under natural conditions, whilst environmental temperatures are such that entrainment is established, there is never a sudden reduction in temperature similar to those which have been shown to be stimulating in the laboratory. Thus there is no evidence that the gradual cold drop experienced under field conditions on the last day of incubation is in itself stimulating.

Attempting to determine whether or not this is the case has practical difficulties. Ideally egg pods should be maintained at a constant temperature so that entrainment has no influence and then at the end of the incubation period the temperature slowly reduced. However the variation in emergence times at constant temperature is sufficiently great to make the interpretation of results from this type of experiment impractical. Therefore since it is known that an early sharp reduction in temperature is stimulating and advances the mean emergence time the same procedure was adopted for slow reductions in temperature.

A total of 44 egg pods was incubated with a fluctuating daily temperature of 12h 33°C / 12h 28°C for 11 days (plus the part-day on which oviposition occurred) with the change from the warm to the cool period occurring at 13.00h on each day. At 08.00h on the last day of incubation the egg pods were divided into three groups. Group 1, consisting of 17 pods, was placed in a waterbath cooling slowly at the rate of 5°C / 7 hours. Group 2, also consisting of 17 pods, was placed in a second waterbath cooling slightly more

quickly at the rate of 5°C / 3h. Group 3, consisting of 10 pods, remained in the incubator where the temperature was maintained constant at 33°C until the normal changeover time at 13.00h when the temperature dropped quickly to 28°C. The emergence times of the pods of each of the three groups were then recorded and are shown in Fig 22.

In Fig 18 it is shown that an early sharp reduction in temperature on the final day of incubation advanced the mean emergence time. Additionally the standard deviation for the emergence times of any one group increased proportionally to the advancement of the cold drop time. From the data given in Fig 22 it appears that a gradual cold drop is far less stimulating than a rapid one. The mean emergence time is advanced but by less than half an hour in both cases compared to $1\frac{1}{2}$ hours for the 5 hour early 5°C cold drop in Fig 18. There is an increase in the standard deviation in both groups 1 and 2 compared with group 3, the greater standard deviation being in group 2. This perhaps suggests that whilst neither of the two groups show such a marked reaction to the cold drop as the groups in Fig 18 there is a tendency for the emergence distribution to become spread with increasing rate of temperature reduction when the stimulus is given early. This is shown in Fig 23. Comparing the variance ratios, the distributions in the three groups are not significantly different from one another but an increasing distribution of emergence times with increasing speed of temperature reduction is suggested from direct observation of the data.

Under field conditions much the same pattern exists. In the soil the rate of temperature fall is low, approximately 4°C / 14 hours (5°C / 18 hours) and in this case the distribution of

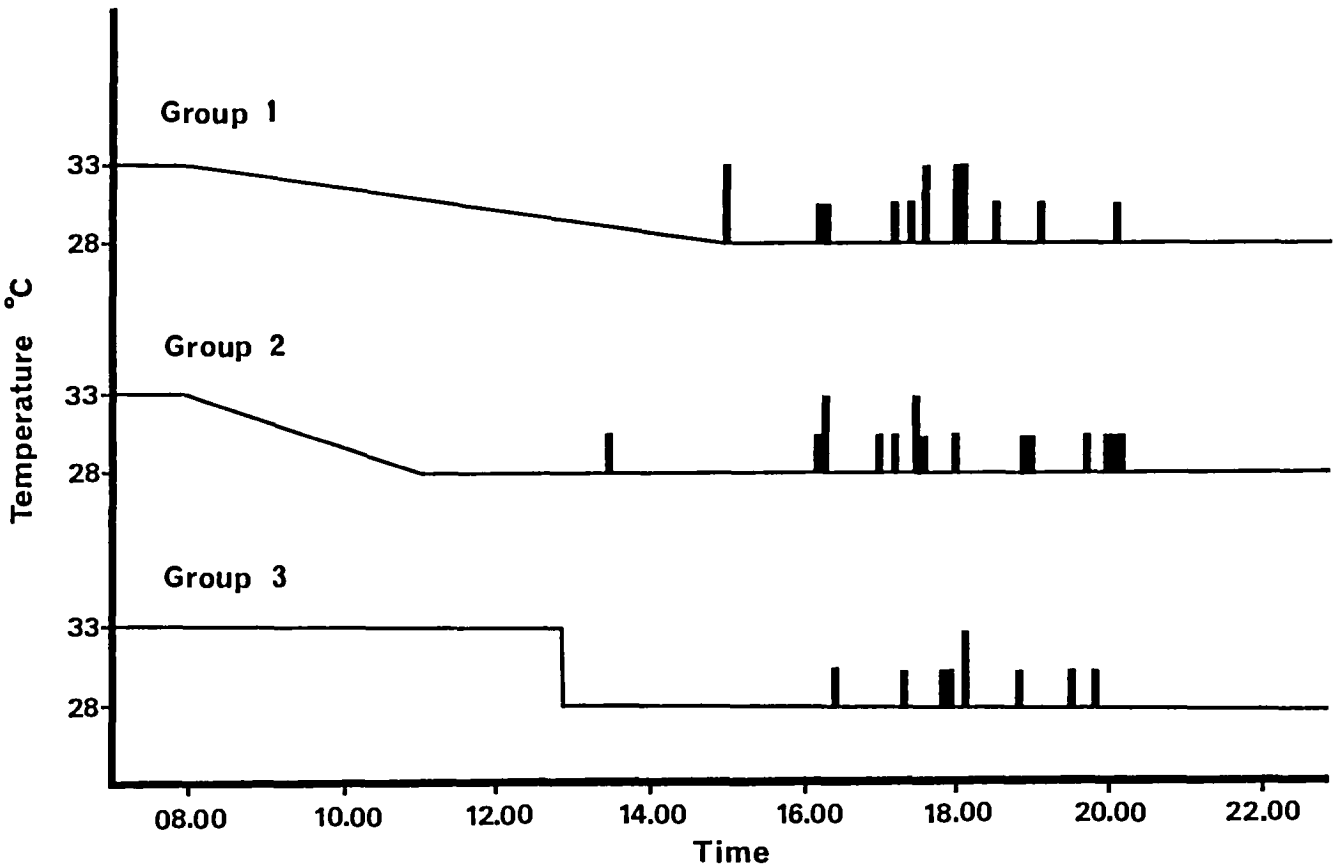


Fig. 22. The distribution of emergence times of egg pods given a gradual decrease in the incubation temperature on the last day. Group 1, 5°C/7 h. Group 2, 5°C/3 h. Group 3, sharp 5°C cold drop.

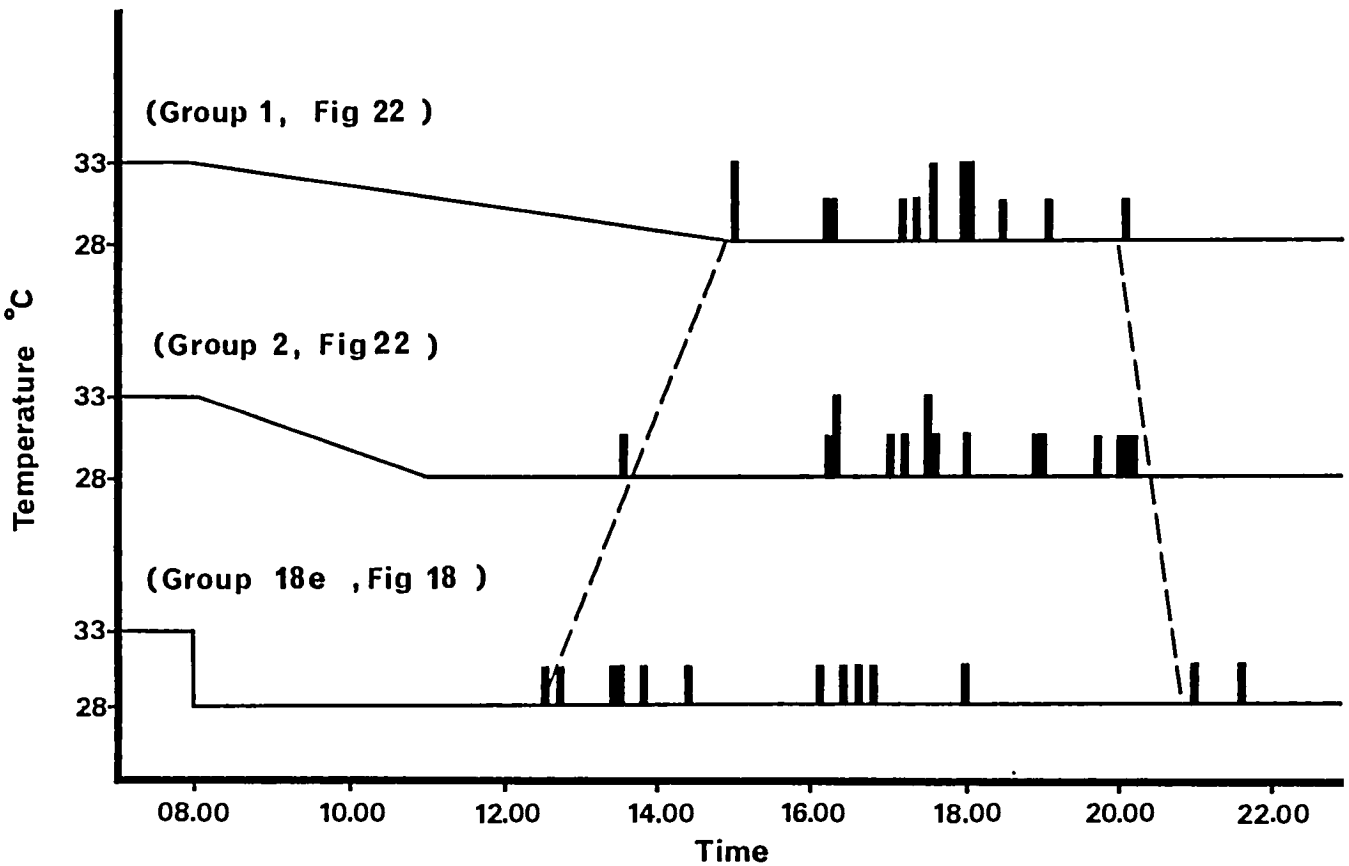


Fig. 23. A comparison of the distribution in emergence times in relation to the rate of fall of temperature. (Temperature drop begins five hours earlier than normal in each case). Group 1, mean latency from the time of the normal cold drop, 4 h. 29 min., s.d. 1 h. 24 min. Group 2, mean latency from the time of the normal cold drop, 4 h. 57 min., s.d. 1 h. 50 min. Group 3, mean latency from the time of the normal cold drop, 2 h. 55 min., s.d. 2 h. 57 min.

emergence is only $1\frac{1}{2}$ hours. At the ambient air temperature the rate of fall is approximately $5^{o}C$ / 4 hours and here the emergence distribution was spread over approximately 6 hours. Thus there is at least limited evidence that the gradual reduction in the ambient temperature during the period immediately prior to hatching tends to have a synchronising influence on the emergence time distribution of a population of egg pods. If this is the case the relationship between entrainment, the reduction in temperature on the final day, and emergence time would be as follows:-

1. The fluctuating temperature during incubation would lead to entrainment and result in hatching at a particular time of the day.
2. The gradual cold drop in the soil temperature under field conditions is of a magnitude sufficient to establish this entrainment but is sufficiently gradual on the last day not to promote any activity response of the type shown by insects to a wide variety of abrupt stimuli such as change in temperature, light or mechanosensation.
3. With increasing rate of fall of the cold stimulus on the last day there is an increase in the response to it. However, as many of the experiments have shown, the population is not uniform in its maturity so that the individual reaction of each egg pod within the population is variable. Those egg pods which are most developed respond to the cold stimulus and emerge shortly afterwards whilst those which are not as developmentally advanced fail to respond but emerge as entrainment predicts. Hence the spread in emergence times. This effect should be at its minimum when no cold drop at all is given and entrainment only is influential. However whilst the spread in emergence

times is small under conditions of constant temperature on the last day the distribution of emergence times is still greater than that shown by pods incubated in the soil under field conditions. Thus the very slow drop may not be completely without influence, perhaps tending to synchronise the population even more.

4. In the laboratory the standard incubation cycle (12h 28°C / 12h 33°C) leads to an emergence time mean approximately $4\frac{1}{2}$ hours after the cold drop with a distribution of about two hours on either side of this mean. Thus even on this cycle when the cold stimulus is given at the "normal" time there is a greater spread of emergence times than under field conditions in the soil. It has been demonstrated that a separation of the cold stimulus from entrainment leads to a greater distribution of emergence times. Thus even with the standard laboratory cycle this may also be the case.

When the temperature is maintained constant on the last day of incubation, after a previously cycled temperature incubation, the emergence mean is at 16.38h (s.d. 1h, 01 min). This is approximately midway through what would have been the cold period had temperatures not been maintained constant on the last day. The emergence gate established by entrainment can thus be represented as approximating to this mean $\pm$ two standard deviations. The entrainment established emergence gate is therefore between 14.36h and 18.40h. However when the final cold drop is given the emergence mean is slightly in advance of the entrainment mean, being at approximately 15.30h. This suggests that the biological clock mediating the entrainment rhythm is not influenced by this final temperature change but assigns the position of the emergence gate on the basis of the shape of the preceding

temperature rhythm as a whole. However the physiological mechanism receptive to the cold drop on the final day is receptive to this final temperature change. In relation to the entrained emergence gate this final cold drop is early even on the "standard" temperature cycle (see methods section p. 46). This would then explain the advance of the emergence time mean of the pods given the final cold drop compared to those kept at constant temperature on the last day. This relationship is shown diagramatically in Fig 24.

By this hypothesis the closer the final cold stimulus is to the entrained hatching gate the smaller the hatching time distribution should be. Since emergence rarely starts before two hours after the cold drop it is possible to give the cold stimulus at least two hours later than normal on the last day. This experiment was carried out.

26 egg pods were incubated under standard incubator conditions of 12h 28°C / 12h 33°C, until the final day of development. On the last day the temperature change from 33°C to 28°C was not given at the usual time of 11.00h but was delayed until 13.00h. The emergence times of the pods were recorded and are shown in Fig 25.

The emergence times of those pods given the final temperature reduction two hours later than normal show a mean which is approximately $1\frac{1}{2}$ hours later than the mean of the "standard" group. This tends to support the proposed hypothesis that the reduction in temperature given at the 'normal' time (i.e. at 11.00h) is out of phase with the entrained emergence gate and leads to both an advance of the mean emergence time and an increased spread of times. A comparison of the standard deviations of each of the individual groups shown in the lower histogram of Fig 25 with that of the upper histogram is given in Table 6. It can be seen that the standard

(1) The point of direct cold drop temperature stimulation. This can stimulate the onset of the hatching effort period and results in hatching approximately 1-4 hours later Its influence is independent of the temperature rhythm given during the previous days of incubation.

(2) The Emergence Gate. The cycled temperature rhythm given during incubation results in hatching at a specific time of the day. The stimulation due to this influence is independent of the immediately preceding temperature change but is dependent upon the temperature rhythm given during the previous days of incubation.

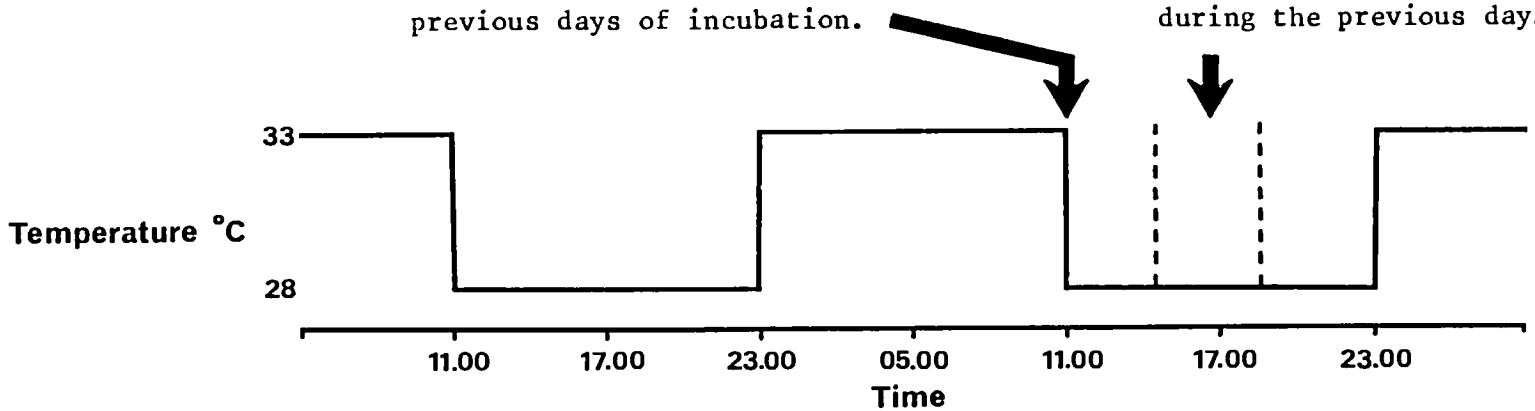


Fig. 24. The times of influence of direct temperature stimulation and indirect temperature stimulation due to entrainment with the standard incubation regime. Individual egg pods are stimulated to hatch by one or other, and in some cases probably both, of the two temperature stimuli. The degree of coincidence of direct and indirect temperature stimulation will therefore influence the degree of synchrony of emergence shown by the population as a whole.

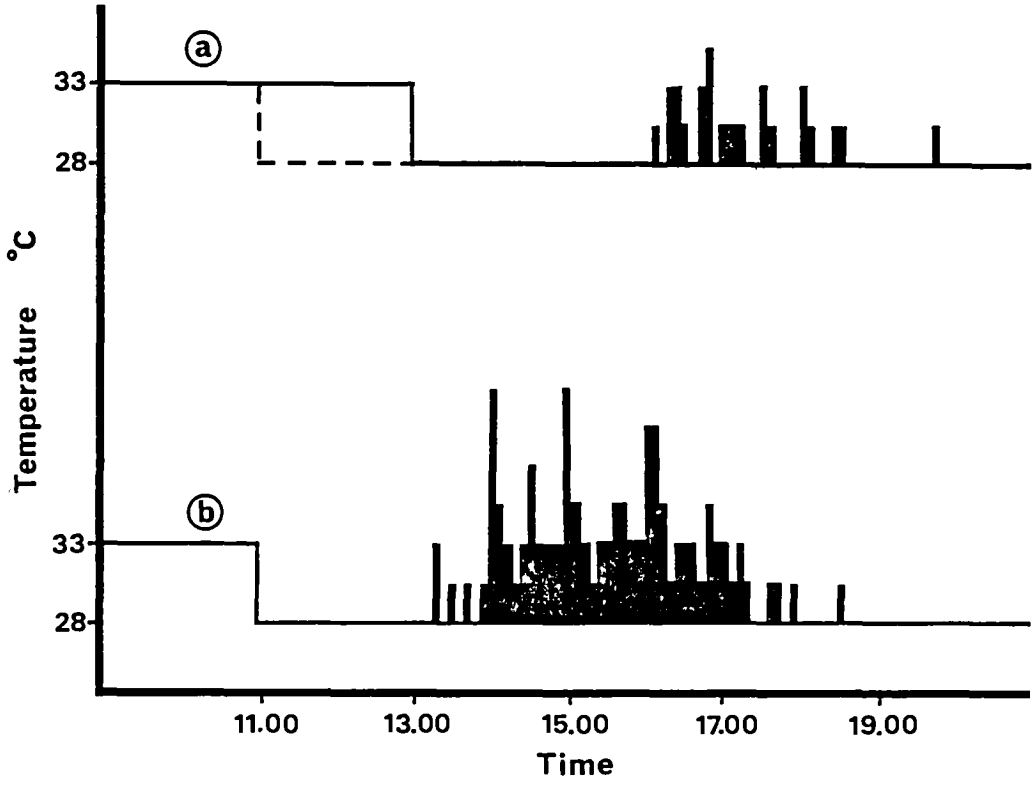


Fig. 25. The distribution of emergence times of egg pods given a reduction in temperature two hours later than on the previous 12 days of incubation (upper figure). For comparison the cumulative emergence time data from Figs. 11, 12a, 12b, 12c,18b and 22/3 which are egg pods experiencing the temperature reduction at the normal time are given in the lower figure.

25a) mean emergence time 17.14 h., s.d. 0 h. 53 min.
25b) mean emergence time 15.29 h., s.d. 1 h. 08 min.
The difference between the means is significant ($p < 0.001$).
The variance is not significantly different.

Egg pod group	Standard deviation of emergence times
Fig 11.	0 h. 55 min.
Fig 12a.	1 h. 19 min.
Fig 12b.	0 h. 58 min.
Fig 12c.	1 h. 04 min.
Fig 18b.	1 h. 23 min.
Fig 22c.	1 h. 05 min.
Fig 25a.	0 h. 53 min.

Table 6. The standard deviations of the emergence times of six groups of egg pods given the final reduction in temperature at the normal time (Figs 11 - 22c) compared with one group given the final reduction in temperature two hours later (Fig 25a).

deviation of the group given the final cold stimulus two hours later than on the previous days of incubation is lower, (though not statistically so) than any of the groups given thecold drop at the normal time. However the distribution of emergence times of the group shown in Fig 25a is still much greater than of pods incubated in the soil under field conditions. This failure to achieve the high degree of synchrony seen in the field may be the result of conditions not being identical between the two. Laboratory incubated egg pods receive a sharp cold drop prior to hatching whilst those in the field receive a very gradual temperature reduction. Also if coincidence of entrainment and immediate temperature stimuli are the cause of a very closely synchronised emergence pattern then even with the two hour late cold drop the two are still 2-4 hours out of phase. Finally the field data are based on a very limited sample. Reports from other field workers suggest that in an extensive natural oviposition site emergence is not quite as sharply defined and may extend over at least three to four hours. This being the case the distribution in Fig 25a is not dissimilar.

D. The relationship between environmental factors other than temperature and the time of emergence.

i) Soil moisture.

Soil samples taken from the oviposition site area described on page 71, were taken at a depth of 8-10 cm at four times during each day for the last five days of the incubation period. These times were 06.00h, 12.00h, 18.00h and 24.00h. The samples were then weighed, dried in an oven at 105°C for 2 days and their percentage moisture calculated. The mean percentage moisture did not vary over a 24 hour cycle but remained almost constant throughout the period of observation (Table 7). There is a very slight tendency for the percentage moisture to increase between 12.00h and 18.00h and again between 18.00h and 24.00h each day. However the change is very slight and since Bernays (1970) has demonstrated that neither wetting nor drying out at the end of the incubation period is a significant hatching stimulus this line of investigation was not pursued further.

ii) Light.

Unlike soil moisture there is an obvious daily photoperiod cycle and dawn is one of the two times during the day when a major reversal of light conditions occurs, i.e. from scotophase to photophase. Künckel d'Herculais (quoted in Uvarov 1928), working with the Moroccan locust, demonstrated that fully developed eggs kept in a dark box did not hatch. However hatching began as soon as the eggs were exposed to light and stopped again as soon as the box was closed. Similarly La Baume (quoted in Uvarov 1928) suggested that the intermediate moult could only be completed in the light. In contradiction, Duck (1944) showed that with Schistocerca obscura (Fabricius) both hatching and the intermediate moult could be completed in darkness. Thus existing evidence as to the influence

Day	06.00h	12.00h	18.00h	24.00h
1	11.3	11.1	11.4	11.1
2	10.9	11.0	11.0	11.3
3	10.6	10.8	11.1	11.5
4	11.5	11.2	11.4	11.4
5	10.7	11.1	11.0	11.2

Table 7. The percentage soil moisture at a depth of 8-10 cm over the last five complete days of incubation of the egg pods described in Table 1.

of light is contradictory and therefore worthy of further examination.

To test the possible influence of light on hatching two groups of adult locusts were allowed to oviposit in prepared soil and the pods marked with matchsticks as in earlier experiments. This time however each pod in one group was covered with a metal tube whilst the other group were left uncovered. It seems unlikely that there is any light penetration to a depth of 6-8 cm through the soil but it is possible that light penetration may occur down the length of the foam plug. The pods were incubated in the soil for 17 days under ambient field conditions and the times of emergence recorded. The results are given in Table 8. They show that there is no significant difference in the emergence times of egg pods whose froth tubes are exposed and those whose froth tubes are covered. Also these observations, together with those from earlier field work, show that emergence does not begin at dawn but starts almost an hour before first light. Thus all these points suggest that the beginning of photophase at dawn is not the stimulus for hatching.

Pod No	Covered/Uncovered	Emergence time
1	Covered	05.20 h
2	"	05.45 h
3	"	06.05 h
4	"	05.35 h
5	"	05.40 h
6	"	06.15 h
7	"	05.15 h
8	"	05.55 h
9	"	06.35 h
10	Uncovered	06.00 h
11	"	05.10 h
12	"	05.50 h
13	"	06.15 h
14	"	05.30 h
15	"	05.25 h
16	"	05.25 h

Table 8. The emergence times of egg pods incubated in the soil at the ambient temperature with either the foam tubes covered (pods 1-9) or uncovered (pods 10-16).

2. THE TIMING OF HATCHING : INTRINSIC FACTORS.

A. Embryonic activity.

i) Introduction.

With the exception of the gross movements of the embryo occurring at blastokinesis, activity during development has received scant attention. Bernays (1970) describes activity during the last three days of Schistocerca embryonic development. At three days before hatching (i.e. at approximately the beginning of the pharate first instar stage.) movements are generally uncoordinated and consist of jerks of appendages or contractions of thoracic or abdominal segments. In slightly older larvae these movements become more coordinated and take the form of irregular waves passing down the body. Finally there is the coordinated activity, termed the hatching effort by Bernays, which leads to the rupture of the egg shell. At the onset of the hatching effort period activity is sporadic and consists of waves passing longitudinally down the body, which Bernays suggests may ensure complete detachment of the body from the serosal cuticle. This is followed by an abrupt increase in activity, the hatching effort proper. In this final phase all the abdominal segments contract simultaneously with great force causing a narrowing of the abdomen and an enlargement of the anterior end. This may be repeated many times, interspersed with waves of dorso-ventral contraction passing down the body until finally the egg shell, under continually increasing pressure from hatching efforts, ruptures. In the present study activity is considered not from the point of view of particular muscular efforts, since this is well documented by Bernays (1970), but in terms of overall activity throughout the last 5-6 days of embryonic development with particular reference to the influence of temperature.

ii) Time lapse cinematography of embryonic development with cycled temperature incubation.

Two single dechorionated eggs were mounted in a sealed glass container as described in the methods (p.46), this then being mounted in front of a time lapse camera. The whole of the apparatus was contained within an environmental cabinet giving a cycled temperature regime of 12h 33°C / 12h 28°C. A two second exposure was taken every two minutes. This was repeated three times to give data on six eggs. The developed film was analysed using a slow speed photoanalyser, the number of changes in the position of each embryo in a sequence of 87 frames (3 hours filming time) being recorded with a hand tally counter. The activity of each of the six embryos is plotted in histogram form in Fig 26. The onset of the hatching effort periods as distinct from general body movements are marked on the histograms with arrows ▼).

Filming started on day 9 of development and at this time the embryos were already showing regular activity. This is much earlier than previous examinations have suggested and demonstrate the advantage of the time lapse camera over direct visual observations for this type of study. The activity itself is seen principally as anterior-posterior jerks of the head, the dorsal surface being tilted backwards and forwards in much the same type of head motion described by Bernays (1970) at the time of hatching. This activity persists at much the same level throughout days 9 to 11. There are small fluctuations but these vary between individuals and cannot be correlated with changes in temperature. During day 12 all of the embryos examined showed a distinct drop in activity to about 50% of that of the previous three days. This sharp drop precedes a further steady decline in activity for the remainder of day 12

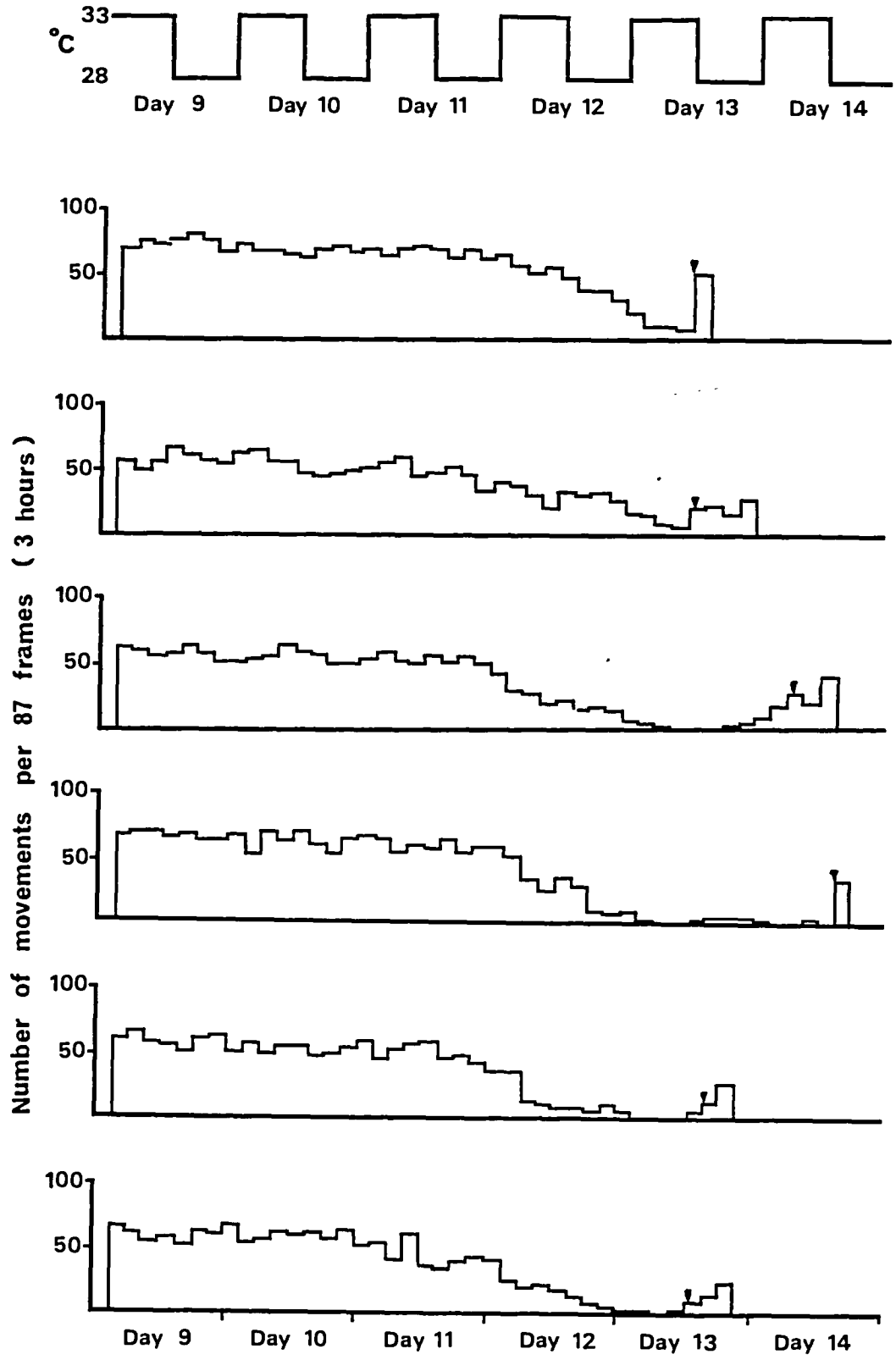


Fig. 26. The number of changes in position per 87 frames (3 hours) for six single embryos over the last five to six days of development with a cycled temperature incubation.

and into day 13 when activity may cease altogether for some hours. In two of the six embryos filmed this period of quiescence persisted throughout the whole of day 13 and into day 14. Finally, on the last day of incubation the quiescent period is broken by the onset of the hatching effort period. Unlike earlier activity which was principally seen as movements of the head the hatching effort is seen typically as dorso-lateral longitudinal indentations of the abdomen. The intensity and duration of the hatching effort period varies considerably from one embryo to another and this aspect is considered subsequently in relation to the behaviour of both isolated and clustered eggs. Throughout the period of observation none of the changes in the magnitude of activity could be correlated with changes in temperature. Only the onset of the hatching effort period could be correlated on a temporal basis with a change in temperature. As seen with many of the previous experiments hatching occurred during the cold period.

Fig 27 gives the mean activity of the above six single embryos (plus two from the following section) for each of the earlier temperature periods when all of the insects were still in phase with one another. There is no temperature related cycle of activity.

iii) Time lapse cinematography of embryonic development with two days at constant temperature at the end of a cycled temperature incubation.

The previous experiment showed that the developing insect displays a distinct pattern of activity in the last 5 days before hatching. Also, with the exception of the time of hatching itself, the activity is independent of temperature. To examine the relationship with temperature further the activity of embryos was filmed

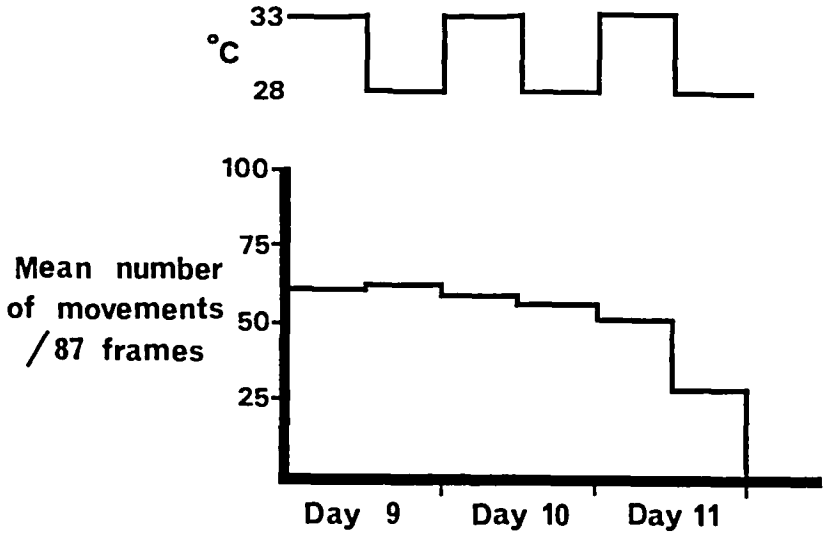


Fig. 27. The mean activity of the eight embryos shown in Figs. 26 and 28 during the period of high activity prior to the quiescent period.

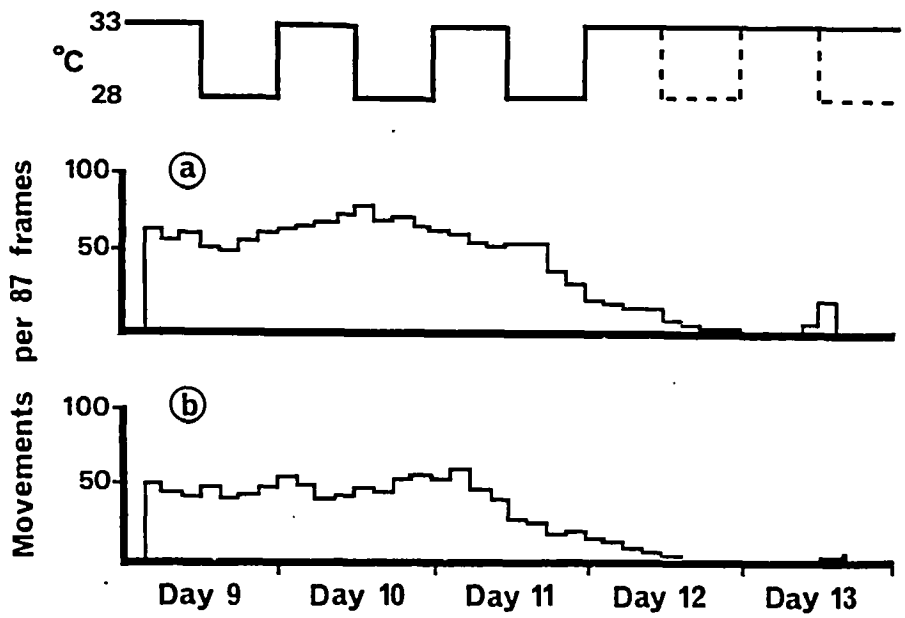


Fig. 28. The number of changes in position per 87 frames (3 hours) for two single embryos over the last five days of incubation, with the last two days at constant temperature.

under an initially cycled temperature which was then maintained constantly at 33°C for the last two days. A 2 second exposure was taken every 2 minutes as in the previous experiment and the number of movements within a sequence of 87 frames (3 hours filming time) is shown in histogram form in Fig 28.

The data given in Fig 28 shows much the same profile as that shown in Fig 26. There is an initial period of sustained activity seen as anterior-posterior jerks of the head and this persists into day 12. There is also evidence of a sudden decline in activity, seen more clearly in Fig 28a, comparable with that seen in the previous experiment. The continuous high temperature over the last two days of incubation seems to make no difference to the activity pattern. As before there is a steady decline leading to the complete cessation of activity which persists until the onset of the hatching effort period. Earlier results have demonstrated that, given a cycled incubation temperature, the insect becomes entrained to this rhythm and hatches at the "correct time" (i.e. during the emergence gate) even when temperatures over the final two days are kept constant. This behaviour is seen in both of these embryos, the larvae hatching in the early part of what would have been the cold period on day 13.

iv) Time lapse cinematography of activity within a whole pod.

Insects in isolated eggs all show the same basic pattern of activity but there is a degree of temporal variation between the eggs in the duration of the different phases. To determine to what extent this variation was a function of the isolation of the eggs and to what extent it occurred when a batch of eggs was in close association a whole pod was filmed. A whole egg pod was prepared

and mounted in front of a time lapse camera as described in the methods (p. 46). The pod was incubated at a cycled temperature of 12h 33°C / 12h 28°C and filmed with a two second exposure every minute. A diagramatic representation of the pod is given in Fig 29 each of the visible embryos being numbered from the top of the pod downwards. The number of movements per 100 frames (1 hour 40 min filming time) of each embryo which was sufficiently visible was then counted with a hand tally counter, the data being given in Figs 30 and 31.

As with the isolated embryos the activity within a whole pod shows three phases (Fig 30). There is an initial period of high activity, the embryos showing the typical head-jerk motion in embryos where the head is visible or a wave of contraction passing down the body where the head is not visible. This is followed by the quiescent period which coincides with the final warm period of incubation after which the hatching effort period begins at about the time of the final drop in temperature. The activity of individual embryos is shown in Fig 31. It should be noted that actual levels of activity are somewhat subjective since some embryos were more easily visible than others, thus tending to bias the results. However this factor does not bias the duration of the phases of activity for each of the embryos which was the aspect principally under consideration. After the quiescent period activity is resumed in two stages. There is an initial increase in activity of the type seen prior to the quiescent period, i.e., head-jerk movements and longitudinal waves of contraction. However after some hours this is replaced by the typical hatching effort activity of strong dorso-ventral contractions of the body. Since the activity does not occur in discrete 100 frame sections as

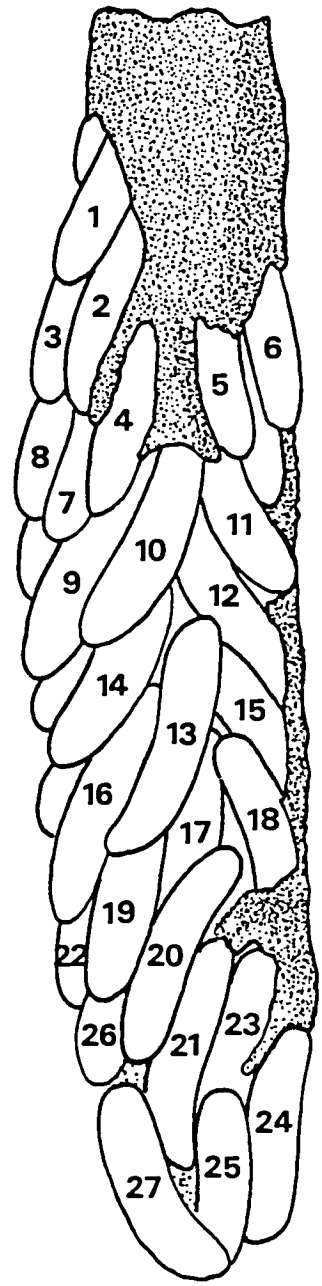


Fig. 29. Diagram of the whole egg pod, aspects of the activity of which are shown in Figs. 30, 31, 32 and 33. (Each of the visible eggs are given an arbitrary number for identification purposes).

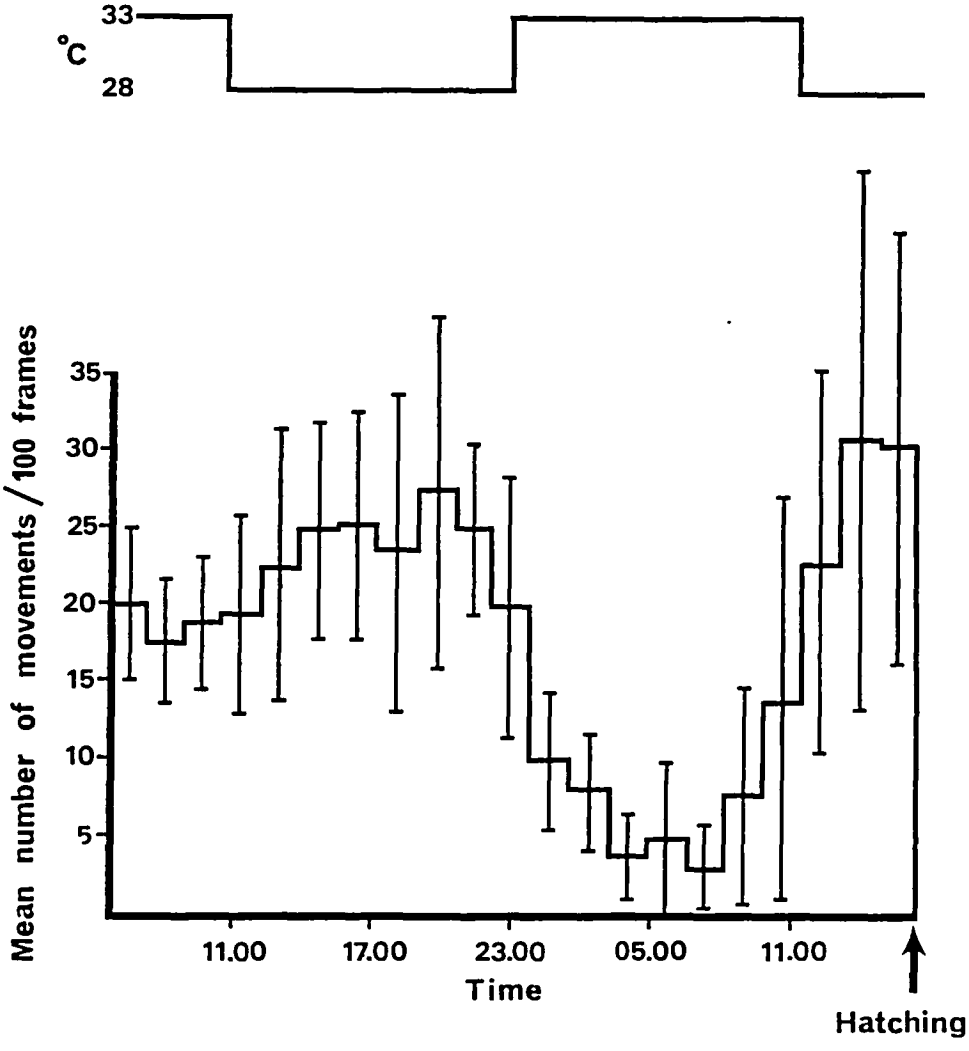
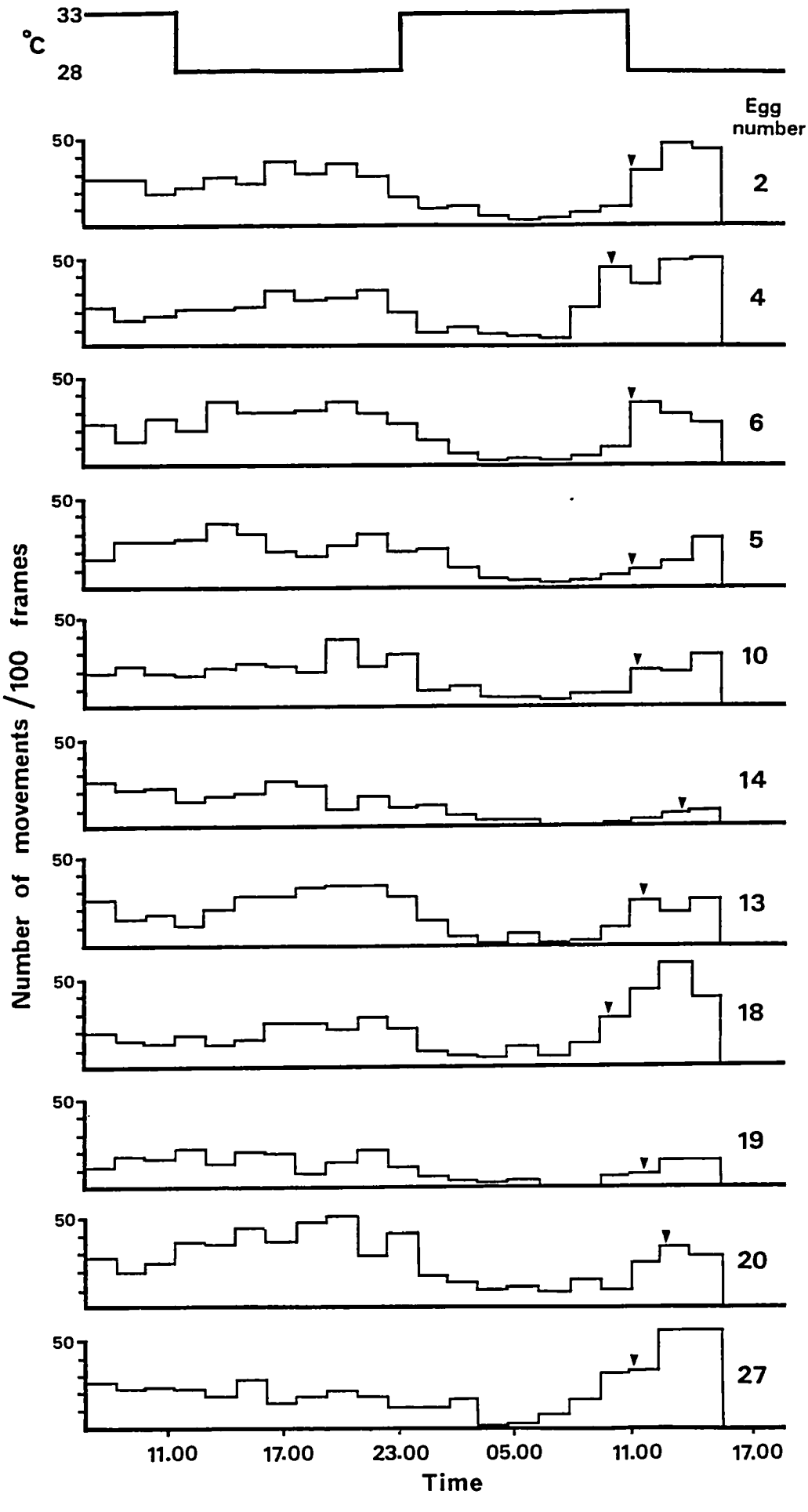


Fig. 30. The mean activity (±1 s.d.) of the eleven visible pharate first instars within the whole pod over the last two days of development.

Fig. 31. The number of movements per 100 frames (100 minutes) for the eleven visible pharate first instars in a whole egg pod over the last two days of development. An arrow ▼ marks the onset of the hatching effort period.


°C
33
28
Egg number
50
2
4
6
5
10
14
13
18
19
20
27
Number of movements /100 frames
11.00
17.00
23.00
05.00
11.00
17.00
Time

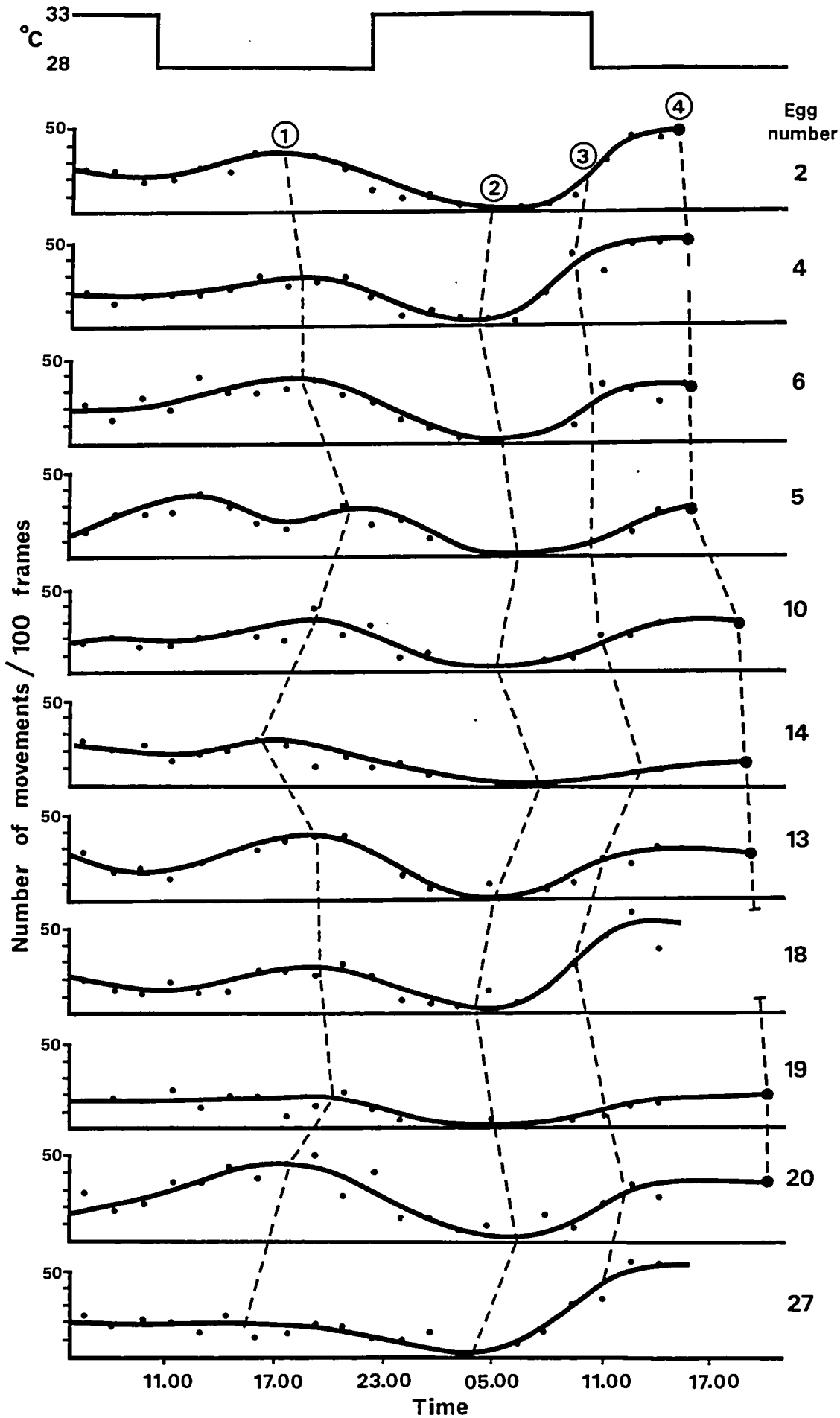
depicted in Fig 31 the data has been redrawn as a continuous phenomenon in Fig 32. The points of maximum activity prior to the quiescent phase, the minimum of the quiescent phase, the onset of the hatching effort period and the time of hatching for each of the visible embryos have been joined to give a comparison in time and duration of the phases. No data are given for any of the eggs after the onset of hatching since unhatched eggs were both obscured and physically moved by hatched vermiform larvae and first instars so making observations impossible. No hatching time is given for eggs 18 and 27 since these did not hatch during the duration of the filming.

Comparing activity with the temperature cycle it can be seen that, with the possible exception of number 14 which was one of the pharate first instars not easily visible, there is a close correlation between the onset of the hatching effort period and the final reduction in temperature. Additionally, with at least two of the pharate first instars, numbers 4 and 18, strong hatching effort activity is seen prior to the cold drop. This would suggest that the function of entrainment is not simply to take over in the absence of the final sharp cold drop but that in some pharate first instars it will initiate the hatching effort behaviour in advance of the final cold stimulus. This will be of even more significance under field conditions where the reduction in temperature overnight is very gradual.

The data in Fig 32 and subsequently in Fig 34 shows that the sequence of hatching within a whole pod is from the top of the pod downwards. The activity of the pharate first instars during the earlier phases shows a less stringent pattern. Looking at individual pharate first instars gives no very clear sequence through the pod. For example a pharate first instar from the lower half of

Fig. 32. The data from Fig. 31 expressed as a continuous phenomenon and with the times of a change in activity of each embryo joined by dotted lines.

(1) The onset of the decline in typical embryonic activity.
(2) The onset of general hatching activity.
(3) The onset of the specific hatching effort period.
(4) Hatching.


°C
33
28
Egg number
2
4
6
5
10
14
13
18
19
20
27
50
Number of movements / 100 frames
11.00
17.00
23.00
05.00
11.00
17.00
Time

the pod, number 18, is one of the earliest to show hatching effort activity and yet this insect failed to hatch during the filming period. Similarly insect number 5 from the top of the pod terminated the quiescent period later than number 19 from the bottom and yet hatched before it. However comparing the mean time for the upper five pharate first instars, numbers 2,4,5,6 and 10 with the mean time for the lower five pharate first instars, numbers 13,18,19,20 and 27 for each of the phases gives the distribution shown in Fig 33. Whilst trends are less distinct for the earlier periods of activity than at hatching an overall pattern throughout the pod can be seen. Going from the top of the pod to the bottom there is a slight overall tendency for the whole of the prehatching period to take longer. The bottom eggs begin the period of quiescence earlier than those at the top but by the time of the minimum of the quiescent period the eggs throughout the pod are exactly in phase with one another. However by the time of the onset of the hatching effort period the top eggs are slightly in advance of those at the bottom and at the time of hatching this advance has been greatly increased. The reduced prehatching period of the upper eggs in the pod compared to those at the bottom is suggestive of a greater physiological fitness amongst the upper embryos. This is a characteristic which has been commented upon by other workers (Papillon 1960, Venter and Potgieter 1967) with reference to other physiological and behavioural parameters. From the data in Fig 31 it is noteworthy that the onset of the hatching effort period is invariably coincident with the time of the final temperature reduction. The period of the hatching effort is thus approximately represented by the latency from the time of the cold drop to the time of hatching. This is of significance in relation to the correlation between the magnitude of the final cold

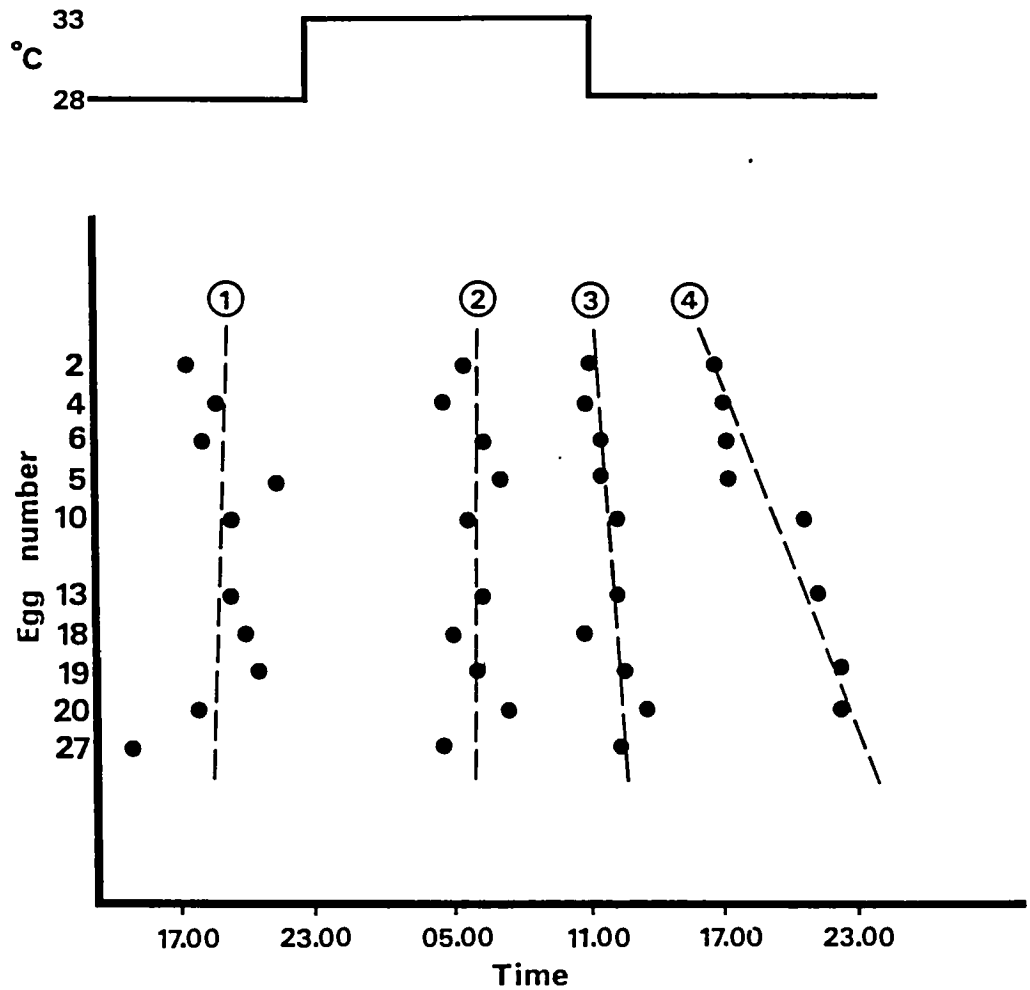


Fig. 33. The relationship of each of the phases of prehatching activity between top and bottom embryos in a whole pod.

(1) The onset of the decline in typical embryonic activity.

(2) The onset of general hatching activity.

(3) The onset of the specific hatching effort period.

(4) Hatching.

stimulus and the latency of hatching seen in section 1C (Fig 14), and is given further consideration in the discussion.

In the whole pod the onset of the hatching effort period, whilst showing a slight tendency to occur first in the upper pharate first instars, invariably occurs within an hour on either side of the time of the cold drop in all the visible embryos. This behaviour is also seen in the isolated pharate first instars. Six out of the eight single pharate first instars filmed show hatching effort behaviour close to the time of the final cold drop (or presumptive cold drop in the case of the two pharate first instars kept at constant temperature). Only in the case of the two pharate first instars hatching a day later than expected did hatching effort behaviour occur considerably in advance of the cold drop.

The sequence of hatching is shown in Fig 34. Immediately before hatching all of the visible pharate first instars were showing the characteristic hatching effort behaviour. The cumulative effect of this was to cause the pod as a whole to expand and contract visibly. This becomes especially apparent around the uppermost eggs which become almost detached from one another, the particles of sand between the eggs falling away and the adhesive froth cracking. The appearance of the pod after hatching of the first 10 larvae is shown in Fig 34b. Not all of the hatched larvae were visible as eggs but from the position in which they first appeared it is probable that they all came from the top third of the pod. The hatched larvae which were visible tended to come from one side of the upper third of the pod. Fig 34c shows the appearance of the pod after hatching of larvae 11-20 and again these appear to come from the upper third of the pod. However the sequence was not strictly from the top downwards, several eggs at the very top of

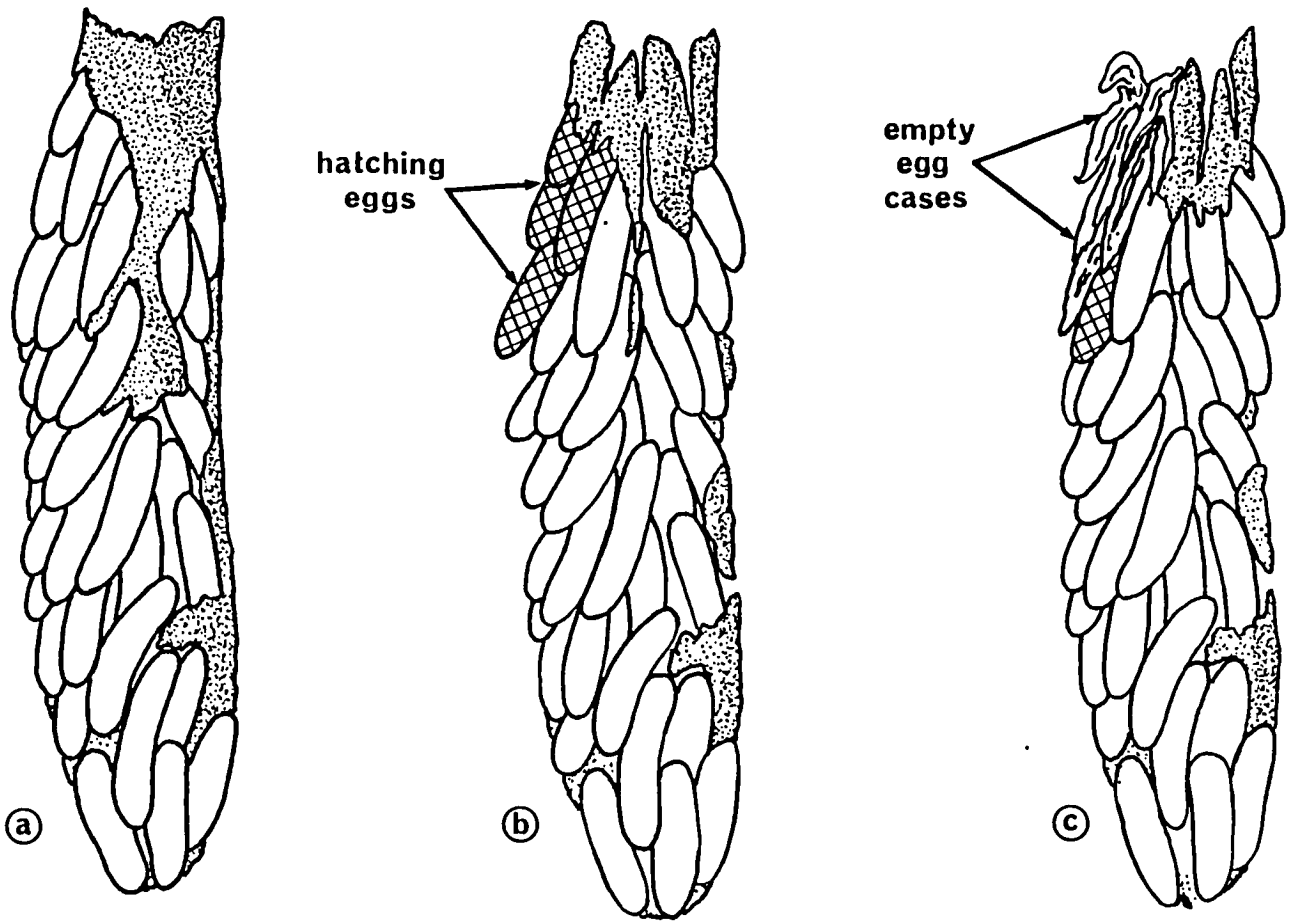


Fig. 34. The sequence of hatching from a whole egg pod. Each drawing represents the appearance of the egg pod after intervals of 10 eggs hatching (not all of which were necessarily originally visible). Visible eggs hatching since the previous drawing are marked with crossed stripes.

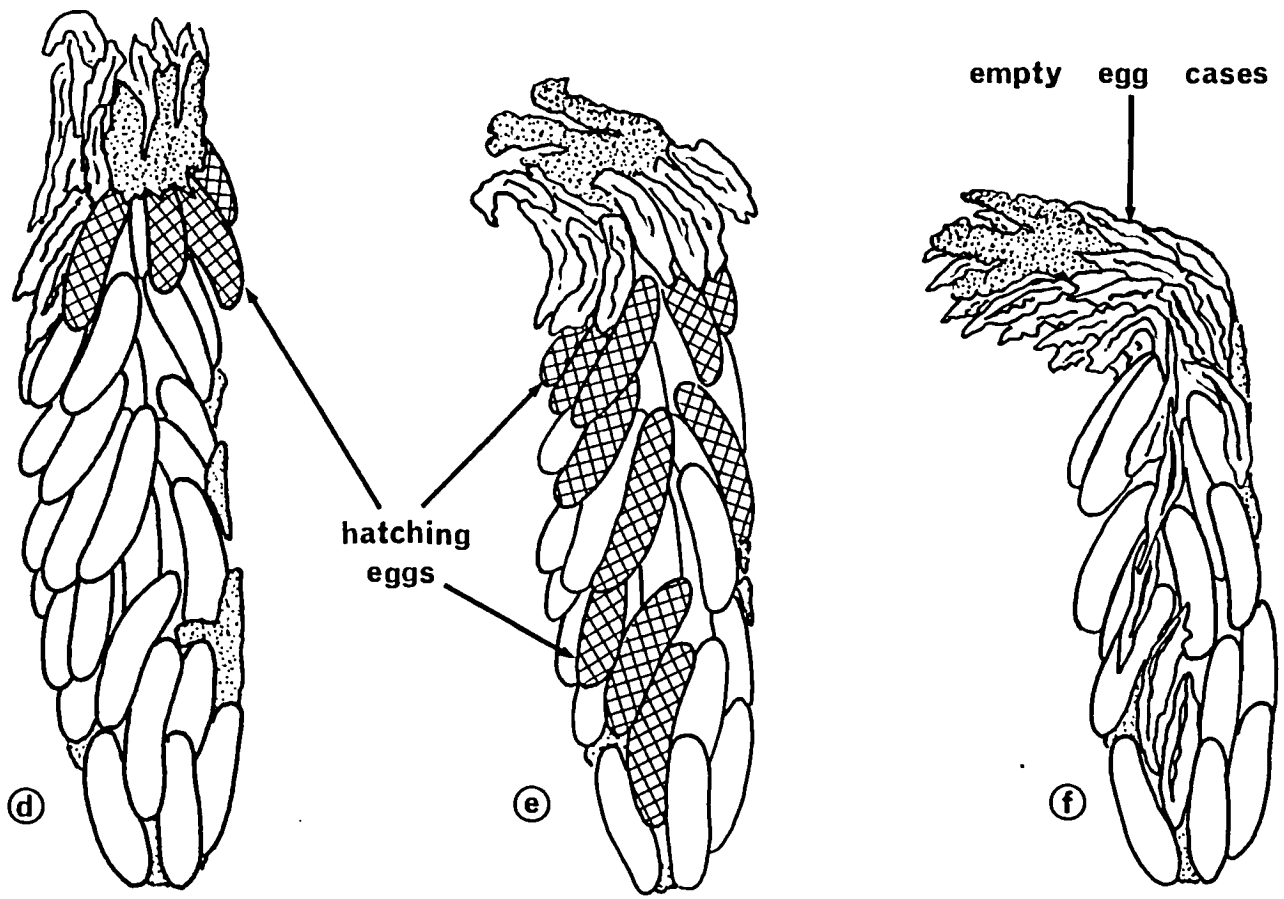


Fig. 34. (continued)

the pod still being unhatched whilst lower eggs had done so. Hatching of larvae 21-30 (Fig 34d) tends to rectify the situation. In this group all the uppermost unhatched eggs hatched so that only embryos in the middle and bottom of the pod remain. By Fig 34e larvae 31-40 have hatched and these include representatives from both the middle and the bottom of the pod. At about this stage the rate of hatching begins to decline. Initially larvae had been hatching at the rate of about 2 per minute but the last ten larvae to hatch took much longer, hatching at a rate of about one every 4-5 mins. This decline was followed by a complete cessation of all further hatching for over two hours and the appearance of the pod at the end of filming was as shown in Fig 34f.

B. The sequence of emergence of the individual pharate first instars of an egg pod.

i) Introduction.

The results of the time lapse cinematography of a whole egg pod have shown that whilst the pattern of hatching is not a completely inflexible sequence there is a general tendency for the top eggs to hatch first, the middle eggs next and the bottom eggs last. Working with the brown locust, Locustana pardalina, Venter and Potgieter (1967) report a similar finding. Using pods divided into top and bottom eggs they demonstrated that frequently the upper eggs of a pod would hatch completely before hatching had even started among the lower eggs of the same pod. In addition to this variation in hatching times there is also evidence of physiological variation between top and bottom eggs. The work carried out by Papillon (1960) established that in Schistocerca heavy, black hoppers with a reduced number of ovarioles are mainly produced by the upper two thirds of the pod and most of the small green forms hatched from the lower third. Papillon (1963, 1969) suggests that these differences are due mainly to stress encountered by the eggs during embryogenesis according to their position in the pod. Venter and Potgieter (1967) similarly found that not only did top eggs hatch first but that they were also heavier, more viable and produced heavier and more viable hoppers. In contrast, Donaldson (1970) working with Locusta found no difference in either emergence times or viability between top and bottom eggs. Thus existing evidence is contradictory. Also since Papillon gives no data with respect to the hatching times of top and bottom eggs of Schistocerca there is no evidence of a physiological basis to the hatching sequence seen in the time lapse film. The emergence times of isolated eggs from the same egg pod were therefore investigated.

ii) The emergence times of isolated eggs.

Eleven egg pods were collected from the adult laying cages at the field research station in Jeddah. These were incubated intact for seven days at the ambient air temperature (max 35°C, min 29°C, mean 32°C). Each pod was then carefully divided up into top, middle and bottom eggs, the individual eggs being reburied in damp sand contained in small glass vials. Nine of the eleven pods then continued their incubation as single eggs at the ambient temperature whilst two pods as single eggs were maintained at a constant temperature of 32°C. The emergence time of each larva was then recorded.

The results, given in Figs 35 and 36, tend to support the work of Venter and Potgieter (1967). When incubated at the fluctuating ambient air temperature the mean emergence time of the top eggs is 01.27h whilst that for the middle is 02.03h and for the bottom is 02.45h. Thus under both whole pod and isolated egg conditions there is a tendency for the top eggs to emerge first. However the sequence of hatching from a whole pod is much faster than that shown by isolated eggs. The close association of the eggs within a pod must therefore be influential in achieving this very close synchrony. Bernays (1970) has shown that mechanical disturbance is an important hatching stimulus and it is probable that this is how cohesion between the individual eggs at hatching is achieved. An egg towards the top of the pod will tend to hatch first due to the natural tendency for the upper eggs to be most advanced. The disturbance caused by the first larva hatching then stimulates others adjacent to it. Under natural conditions it is common for 80-90% of the eggs of a pod to emerge within 15-20 minutes of one another. However under laboratory conditions the spread of emergence times

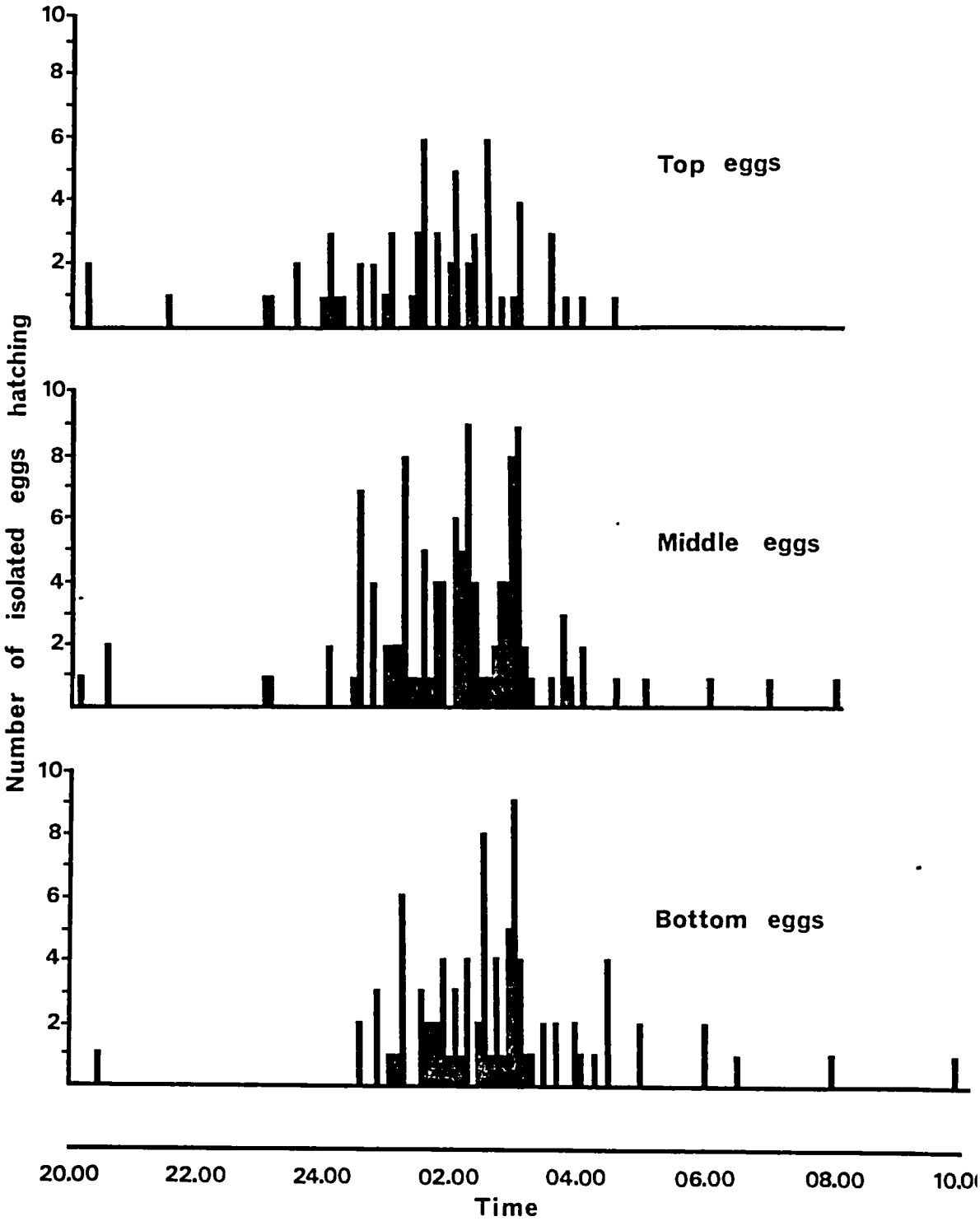


Fig. 35. The distribution of emergence times of isolated eggs from the top, middle and bottom of the egg pod. (Incubation at the ambient air temperature of the Jeddah field station, max 35°C, min 20°C, mean 27°C).

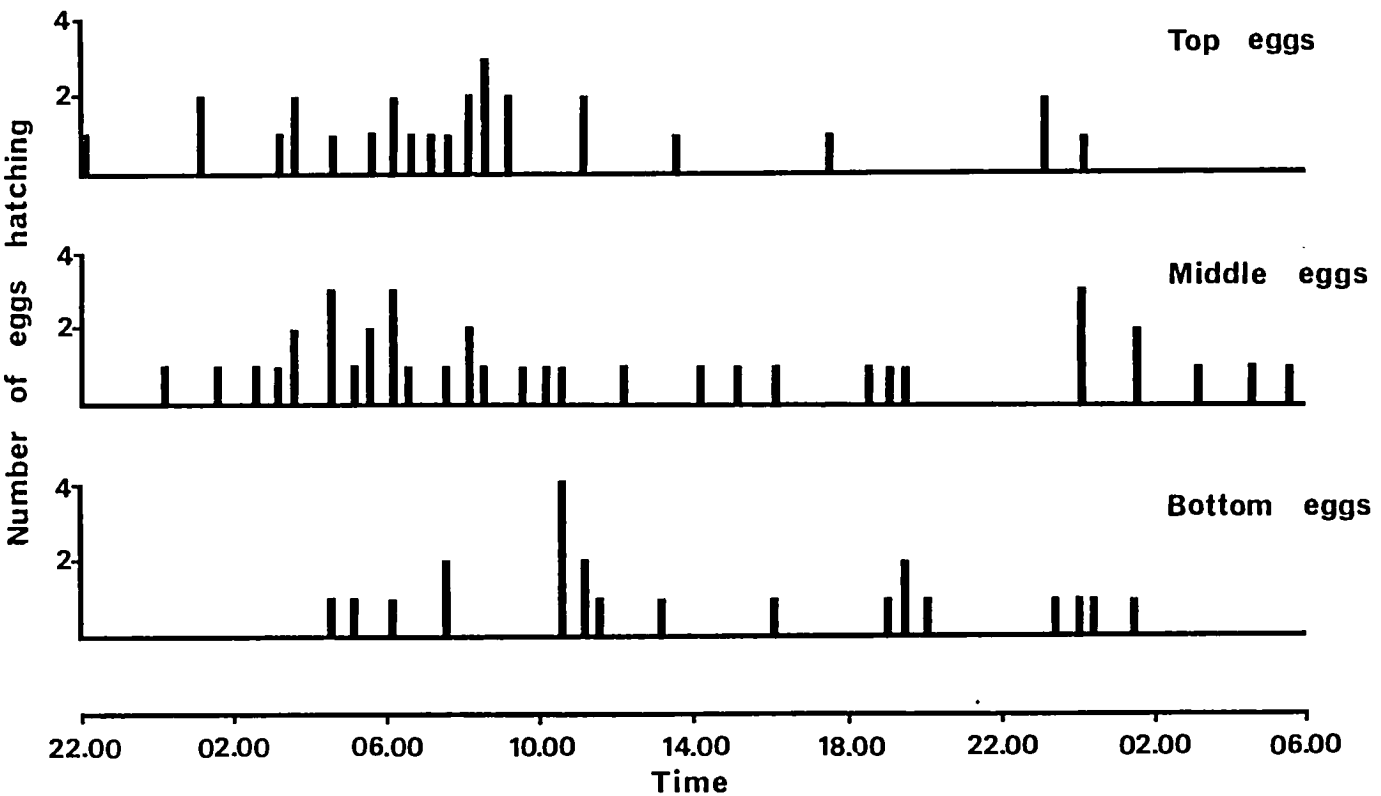


Fig. 36. The distribution of emergence times of isolated eggs from the top, middle and bottom of the egg pod. (Incubation at a constant temperature of 32°C).

from a single pod is often greater than this. In the whole pod hatching film in section 2A (p.136) hatching of the first 40-45 larvae took approximately an hour and this extended hatching time is seen in the results of other workers (Tyrer 1970, Venter and Potgieter 1967).

iii) The distribution of serosal cuticle weights at the time of hatching.

Bernays (1970) has shown that hatching is only possible if the serosal cuticle is digested away to a weight no greater than approximately 50µg. However even below this weight it might be expected that the time taken for a fully developed pharate first instar to break the serosal cuticle would be proportional to the weight of the cuticle. This possibility was examined experimentally.

Three egg pods, each 7 days old, were divided up into single eggs, dechorionated and placed on damp filter paper in petri dishes in an incubator. They were then incubated with a cycled temperature of 12h 33°C / 12h 28°C, until hatching started. In each of the three groups approximately 10 eggs were allowed to hatch and the serosal cuticles of these hatchlings collected. The remaining eggs were then divided into two groups in each of the three dishes. One half of the pharate first instars were removed from their serosal cuticles whilst the other half were left to hatch naturally. Of the larvae removed from their serosal cuticles each was kept isolated and a record made of whether or not it performed the intermediate moult. At this late stage of development it is almost impossible to characterise the exact stage of development of a pharate first instar by its anatomical features. Whether or not it performed the intermediate moult was therefore used as a criterion

of complete or incomplete development. The distribution of serosal cuticle dry weights for each group, those hatching naturally, those being artificially removed but moulting naturally and those artificially removed and failing to moult, from all three of the pods (pooled data) is given in Fig 37.

It might be expected that those eggs which are most fully developed as indicated by their unaided emergence, would show the most fully digested serosal cuticles whilst those least developmentally advanced, as indicated by their failure to moult, would have the highest serosal cuticle weights. Data from Fig 37 suggests that this is the case,with the mean serosal cuticle weight being lowest in group A and highest in group C. However the difference in weights between group B and group C is not significant, only group A being significantly lighter than either of the other two groups ($p = 0.005$).

A comparison of the serosal cuticle dry weights within each pod is given in Fig 38. In pod number 3 the relationship between the cuticle weights in each class is almost linear though this is less true in pod number 1 and is not the case in pod number 2. However pod number 2 has only three individuals in the least developed class which allows the weight of one cuticle to make a significant difference to the distribution.

As described initially, after the hatching of approximately 10 eggs from each of the three pods the remaining eggs were divided into two groups in each pod. The first group was used for serosal cuticle weight determinations whilst the second group was allowed to hatch so as to give the distribution of hatching times for a comparable group of eggs. The distribution of serosal cuticle weights and hatching times for sibling animals in each of the three

Fig. 37. The distribution of serosal cuticle dry wieghts. (Pooled data from three egg pods).

Group A. After normal hatching of the larvae.

Group B. After artificial removal of the cuticle at a stage when the larvae were sufficiently developed to carry out the intermediate moult.

Group C. After artificial removal of the cuticle at a stage when the larvae were not sufficiently developed to carry out the intermediate moult.

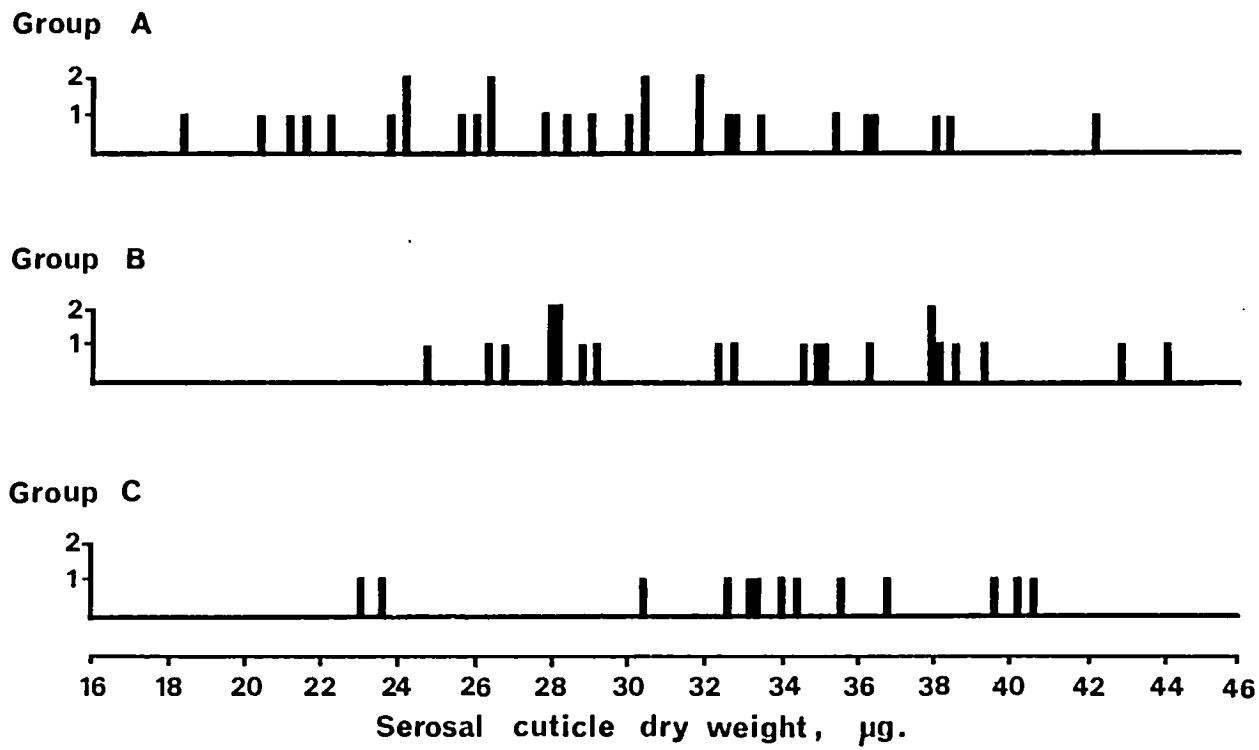
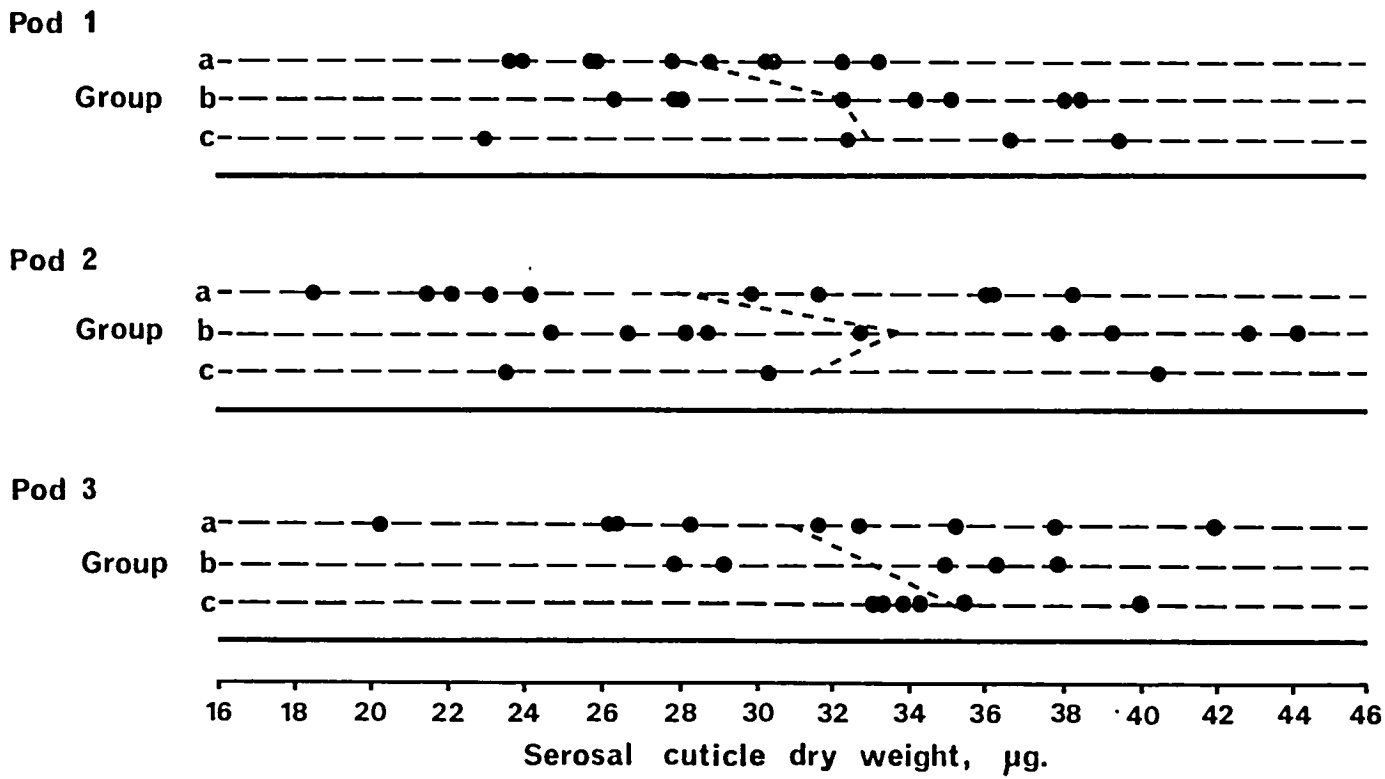

Group A
2
1
Group B
2
1
Group C
2
1
16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46
Serosal cuticle dry weight, µg.

Fig. 38. The distribution of serosal cuticle dry weights for three stages of development within three egg pods. The dotted lines join the means.

Group A. After normal hatching of the larvae.

Group B. After artificial removal of the cuticle at a stage when the larvae were sufficiently developed to carry out the intermediate moult.

Group C. After artificial removal of the cuticle at a stage when the larvae were not sufficiently developed to carry out the intermediate moult.


Pod 1
Group
a
b
c
Pod 2
Group
a
b
c
Pod 3
Group
a
b
c
16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46
Serosal cuticle dry weight, µg.

pods is given in Fig 39. The dotted lines joining the mean cuticle weights and the mean hatching times for each pod show the relationship of the three pods to one another with respect to these two characters. The relationship is in fact very similar, pod 1 hatching earliest and having the lowest serosal cuticle weights whilst pod 3 hatches last and has the highest serosal cuticle weights.

Thus under the laboratory incubation conditions of the experiment serosal cuticle digestion has a distinct influence on the time of hatching. It is also noteworthy that serosal cuticle digestion would appear to be occurring almost up until the time of emergence under laboratory conditions. Under field conditions however the hatching stimulus is less abrupt and occurs at the end of an extended overnight cold period. In view of the very close synchrony of emergence which then occurs it is suggestive that serosal cuticle digestion is completed during the quiescent period so that at the time of hatching its influence as a variable factor throughout the population of egg pods is minimal.

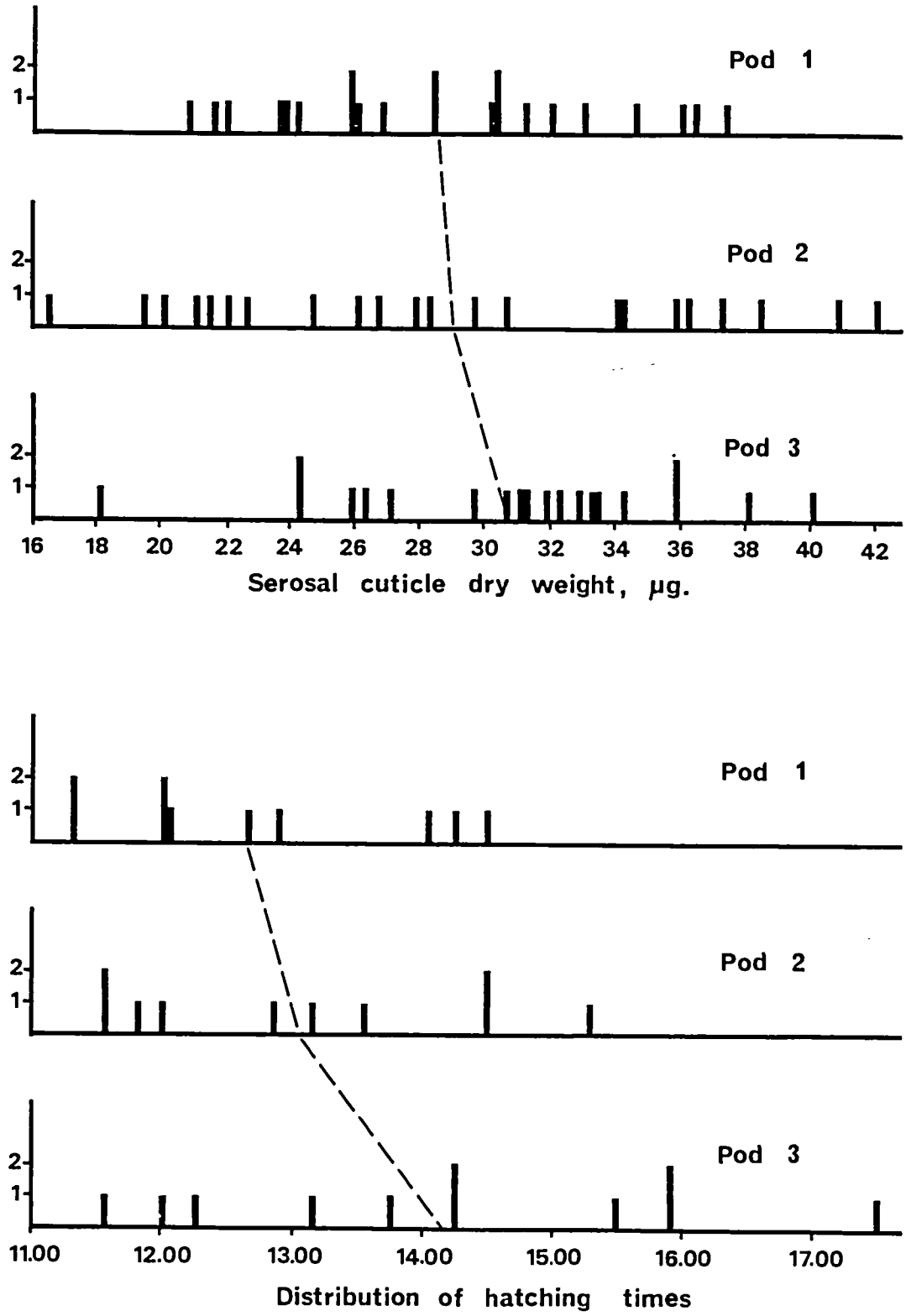


Fig. 39. A comparison of the distribution of serosal cuticle dry weights in three egg pods with the distribution of hatching times of sibling eggs from the same three pods. The dotted lines join the means in each case.

C. Respiratory activity.

i) Introduction.

The relationship between embryonic development, temperature and respiratory activity was first considered by Bodine (1929) working principally with the grasshopper Melanoplus differentialis Uhler. The eggs of Melanoplus are laid in the autumn, undergo a diapause during the winter and hatch in the spring. The respiratory activity of the eggs can be correlated with the environmental temperatures. They show an initial burst of activity soon after they are laid, the respiratory rate then drops throughout the winter and there is then a marked increase in the spring which continues up until hatching. However the respiratory rate is not an exact function of temperature. The eggs show a drop in their respiratory rate more rapidly than the onset of the cold winter temperatures and then in late winter begin to increase the respiratory rate whilst the temperatures are still below the theoretical developmental zero. Bodine examined the relationship between temperature and respiratory rate further with eggs kept at a constant high temperature. In this case the oxygen consumption does not show a steady increase as might be expected but shows an initial peak in the first 6 weeks of development. This is followed by a drop during the period which corresponds with the onset of winter under ambient conditions and the final peak of respiratory activity is resumed some months later. Thus whilst the overt behaviour of the egg suggests only a facultative diapause, since no cold period is necessary, the pattern of respiratory activity suggest an obligate diapause of metabolism independent of temperature. Thus for Melanoplus temperature is not the only factor controlling respiratory rates during development.

The respiratory rate of developing Schistocerca eggs has been examined by Moloo (1968) who gives attention to correlations between respiratory rates and changing developmental processes. During a 15 day developmental period at constant 28°C, oxygen consumption is low for the first 4 days, rises sharply between days 4 and 7 and shows a gradual increase over the next 6 days. During day 14 there is a final peak of respiratory activity whilst on the last day the level declines slightly.

In the present study the respiratory rates of developing Schistocerca embryos are examined in relation to two objectives. Firstly the respiratory rate is examined in detail throughout the last nine days of development to determine to what extent it is linked to the incubation temperature. Secondly, given a period of constant temperature at the end of development, the pattern of respiratory activity was examined for evidence of circadian entrainment.

ii) Respiratory rates in the developing Schistocerca embryo given a cycled incubation temperature.

Single eggs were placed in damp sterile sand in the chambers of four respirometers as described in the methods section (p. 46). The respirometers were immersed in a waterbath, the temperature being cycled at 12h 33°C / 12h 28°C each day for the last 9 days of development. Readings were taken either half hourly or hourly throughout the nine days and the data are presented graphically in Figs 40 and 41. In Fig 40 the daily mean is given whilst in Fig 41 the data are pooled into two hourly periods.

The data in Fig 40 are comparable with that of Moloo (1968). The rates of oxygen consumption are higher in the present study

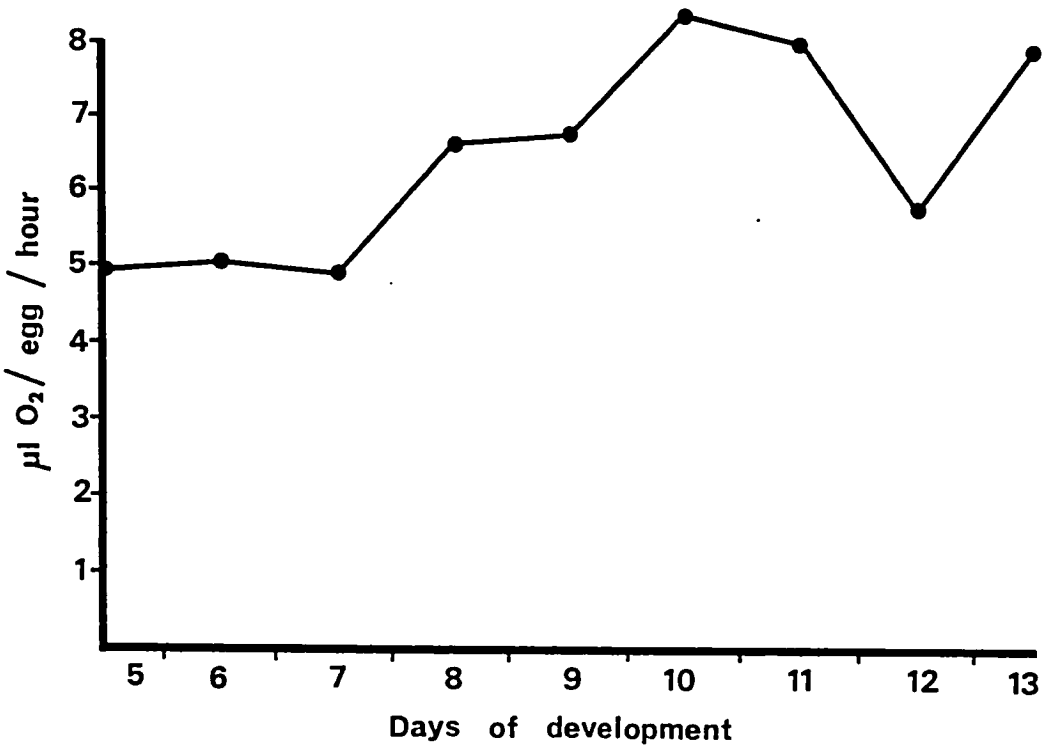


Fig. 40. The mean rate of oxygen consumption per day over the last nine days of embryonic development at a mean temperature of 30.5°C.

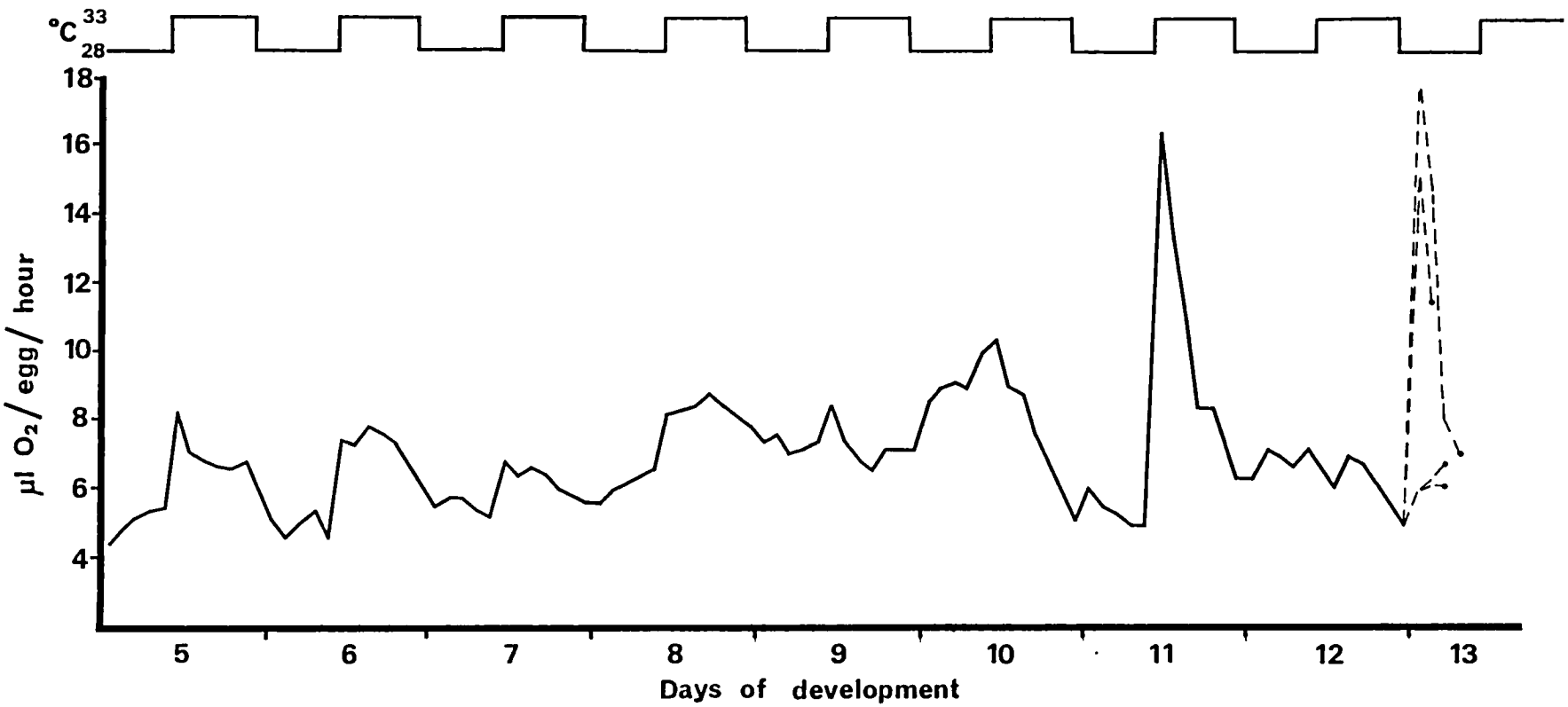


Fig. 41. The mean rate of oxygen consumption of four insects measured at two hourly intervals over the last nine days of embryonic development. The data for each insect are plotted individually during the hatching effort period.

than those of Moloo but the mean temperature is also higher, (30.5°C compared with 28°C). Throughout the middle of the developmental period both studies show a period of constant oxygen uptake being followed by an elevated oxygen consumption towards the end of development. Similarly, on the last full day of development there is a decline in respiratory metabolism in both cases. Finally, on the last day there is a period of elevated oxygen consumption immediately prior to hatching.

In Fig 41 the mean oxygen consumption every two hours is given. Unlike Fig 40 which suggested a steady increase in respiratory metabolism throughout development, Fig 41 shows that oxygen consumption shows marked fluctuations each day. For the first 11 days of incubation these can largely be correlated with temperature, oxygen consumption increasing in the warm period and decreasing in the cool period. In particular there is a very marked increase in the respiratory rate at the beginning of the warm period, this being followed by a decline. In the cold period the pattern is more variable but generally the rate is steady at a rate lower than the ensuing warm period. During days 12 and 13 this largely temperature dependent pattern of oxygen consumption becomes completely reversed. The initial cold period of day 12 is as expected but with the onset of the warm period there is no sudden peak of oxygen consumption as seen on previous days and the level never goes above that of the preceeding cold period. However, in contrast, the final cold period does show a peak of respiratory metabolism though this is very variable in magnitude as seen from Fig 41. During the hatching effort period the individual oxygen consumption curves rather than the mean of the 4 embryos are given and it can be seen that 2 embryos show a very large increase whilst the other two show very little

increase in respiratory rate. It is of significance that the profile of oxygen consumption over the last two days is almost exactly the same as that of activity (see section 2A page131). This relationship is considered subsequently in the discussion.

iii) Respiratory activity of developing Schistocerca embryos given a period of constant temperature at the end of a cycled temperature incubation.

The data given in Fig 41 show that the respiratory activity fluctuates each day when the insects are given a cycled incubation temperature. However the fluctuation, with the exception of the last day, appears to be directly temperature dependent. To examine whether or not any circadian fluctuation is established embryos were incubated with the standard cycled incubation temperatures for 10 days and then maintained constant at 33°C for the last three days. The respiratory rate of the individual insects were recorded for the last 5 days of development and the results are given in Fig 42.

The data for the individual insects have not been pooled since whilst they all show a similar pattern of behaviour the timing and magnitude of the changes show some temporal variation. Thus the individual trends become rather obscured when combined. The variation between individuals is also of some interest when considering the overall pattern of hatching behaviour.

For each of the four insects the first two days of recording (days 9 + 10) show the same basic pattern as in Fig 41 with a higher respiratory rate in the warm period than in the cold. With the onset of the continuous warm period on day 11 the pattern of behaviour is somewhat variable. However the general trend is for a

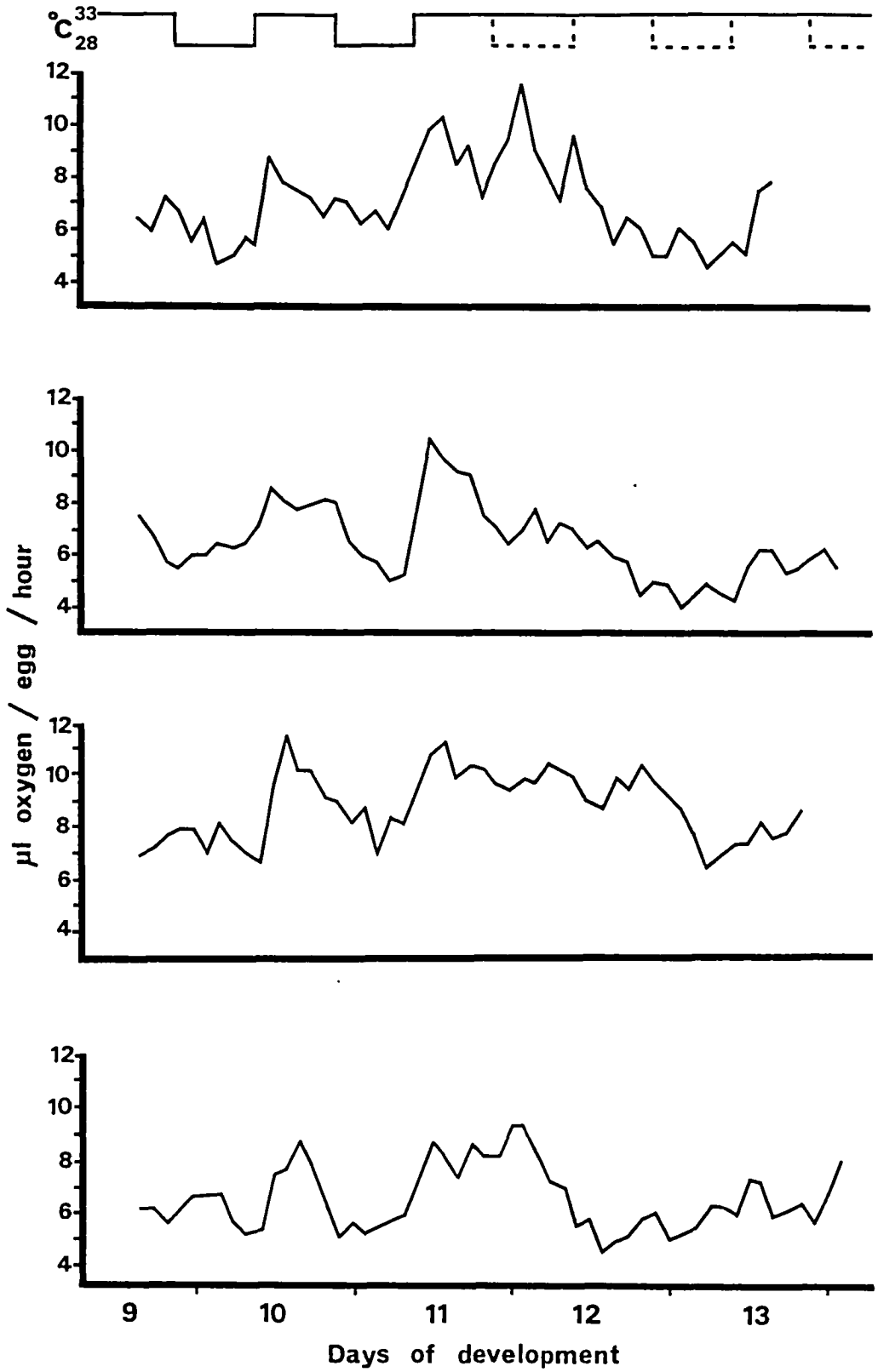


Fig. 42. The rate of oxygen consumption of four individual insects given the last three days of embryonic development at constant temperature.

peak of respiratory activity at the beginning of the warm period to precede a trough throughout day 12. Finally, immediately prior to hatching, there is an elevated period of respiratory activity lasting up to 4 hours. From comparison with the activity films (p.133) it is suggestive that this period of elevated respiration coincides with the hatching effort period.

The evidence from Figs 41 and 42 demonstrates two types of response to temperature, apparently dependent on the age of the embryo. Up until day 11 the rate of oxygen consumption shows a direct correlation with temperature, being high when the temperature is high and vice versa. There is no evidence that a cycled incubation temperature leads to the establishment of any entrained cycles of respiratory activity which are maintained at constant temperature. During days 11 and 12 oxygen consumption declines, now independent of any temperature influence, and then shows a final peak prior to hatching, again independent of temperature. It should be noted however, that whilst the magnitude of respiratory activity in the last two to three days of incubation is largely temperature independent, the duration of the period of low metabolism and the onset of the peak prior to hatching are temperature dependent where a cycled temperature exists.

D. Circadian fluctuations in haemolymph metabolites.

i) Introduction.

The biochemical composition of the insect embryo has been reviewed by Chen (1971) and there is a large body of data relating to metabolic changes occurring throughout embryogenesis. However the functional significance of many of these changes are less well understood and with respect to the present study there is almost no record of circadian fluctuations in insect embryo metabolites. In the adult house cricket there is both a circadian cycle of locomotor activity and a circadian fluctuation in haemolymph sugar levels though these are not coincident. It is suggested that the peak in haemolymph sugar is not a function of the locomotor and feeding peak since it occurs also in last instar nymphs which show no marked daily pattern of locomotion and feeding, (Nowosielski and Patton 1964). In adult Periplaneta Hillard and Butz (1969) demonstrated a rhythmic drop in uric acid concentration which was correlated with the period of peak locomotion. A less distinct rhythm of trehalose concentration is reported by Nayar (1969) in fourth instar larvae of the mosquito Aedes taeniorhynchus (Wiedemann). Brady (1967, 1968), prompted by the work of Ellis and Hoyle (1954) on ion concentrations and activity in the locust, examined changes in the haemolymph sodium and potassium ion concentration in Periplaneta in relation to circadian activity patterns. However whilst a fall in haemolymph potassium ions did occur coincident with the onset of locomotion in some insects, overall the results were inconclusive.

In the Schistocerca embryo the pattern of hatching behaviour shows the characteristics of circadian entrainment. In view of this

behavioural rhythm the metabolism of the developing embryo was therefore examined for possible evidence of physiologically coincident rhythms which might bear some functional relationship to the hatching rhythm.

ii) Haemolymph sugar levels (Anthrone positive material).

Seven-day old Schistocerca egg pods were removed from their original laying tubes and divided up into single eggs in a large sand filled incubation box. This was then placed in an incubator on a temperature cycle of 12hr 33°C / 12hr 28°C. On the tenth day determinations of haemolymph sugar concentrations were started. Each determination was made on a 10 µl haemolymph sample. At ten days old this required four or five embryos for sufficient haemolymph whilst at 13 days old two pharate first instars were sufficient. At each sample enough eggs for the determination were removed from the incubator, the rest remaining undisturbed. For the quantitative estimation an Anthrone method was used as described in the methods section (p. 49). No attempt was made to distinguish between reducing sugars such as glucose and non-reducing sugars such as trehalose. Since the last four days of development were to be examined in their entirety, for practical purposes, the period was divided up into overlapping half days. In the first group estimations were made approximately hourly from 08.00h to 21.00h (incubator time) each day whilst in the second group estimations were from 20.00h to 09.00h (incubator time) each day. In addition to these overlapping half days, estimations were also made continuously throughout days 10 and 11 and likewise for days 11 and 12. The data were then pooled and is given in Fig 43. On day 13 most of the pharate first instars emerged between 14.00h and 20.00h. The readings on day 13 therefore most probably represent

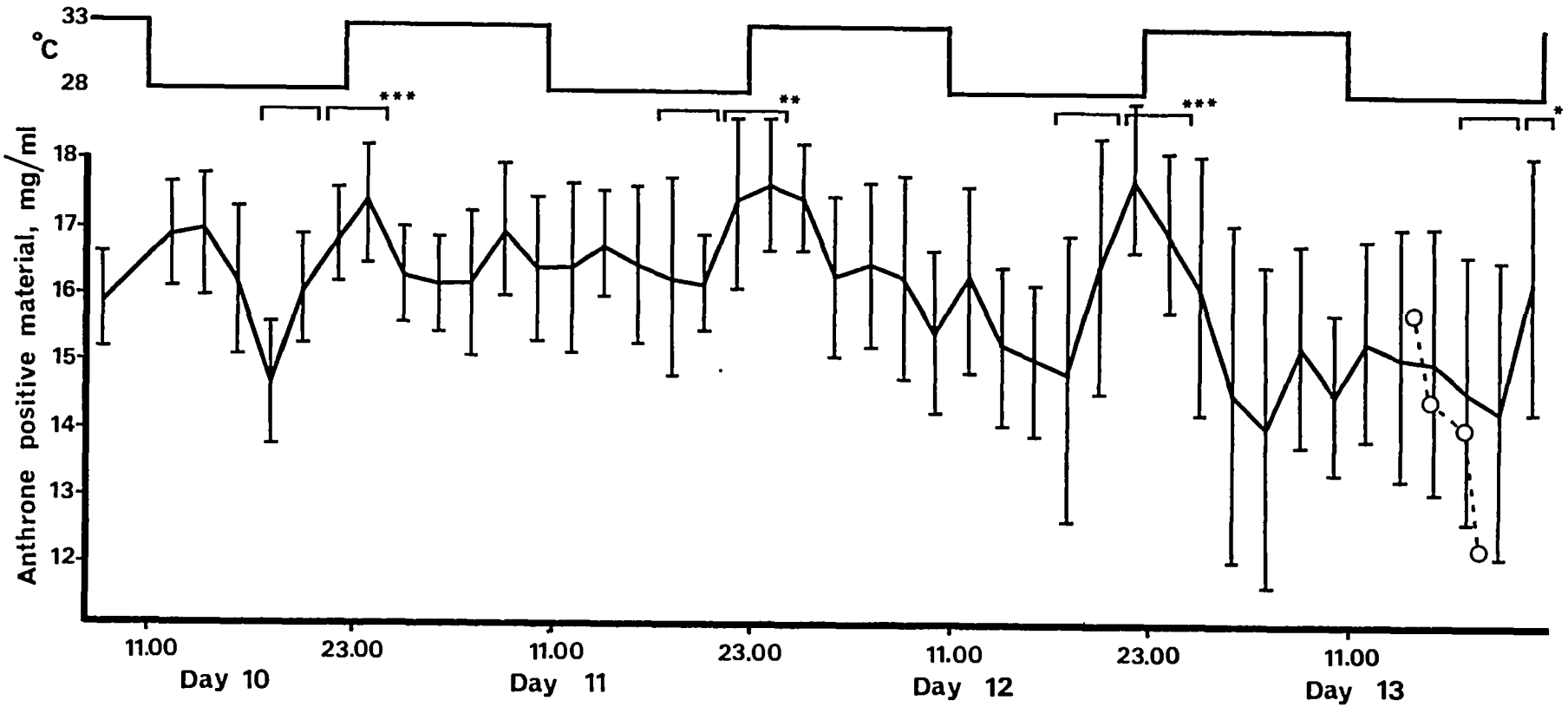


Fig. 43. The concentration of haemolymph sugar (Anthrone positive material) (±1 s.d.) over the last four days of embryonic development. *$p < 0.05$. **$p < 0.005$. ***$p < 0.001$. The dotted line on day 13 represents data from newly hatched first instars.

pharate first instars which were not going to hatch until day 14. The dotted line on day 13 represents data from first instar hoppers 0, 1, 3 and 4 hours after ecdysis from the embryonic cuticle. The line is arbitrarily started at 16.00h, this being approximately the mean emergence time.

Comparing the fluctuations in the level of haemolymph sugar with the temperature cycle it can be seen that a fluctuating daily trend does exist. Towards the end of the cold period each day the level of haemolymph sugar shows an increase reaching a peak either at the end of the cold period or at the beginning of the warm period. The level then shows a gradual decline throughout the warm period and into the beginning of the cold. Comparing the levels between 18.00h to 22.00h and 22.00h to 02.00h each day there is always a significant difference. (See Fig 43 for the probabilities of the null hypothesis being valid for each day).

In the data given in results section 1 (Fig 15) it was shown that pharate first instars entrained to a temperature cycle emerged at the 'correct' time on the last day even if the temperature is maintained constant. In the above data on sugar concentrations there is a daily rhythm which suggests a similar entrainment to the environment. In order to compare these behavioural and metabolic rhythms further sugar levels were estimated in pharate first instars given a cycled temperature up until the penultimate day of development and then kept at a constant 33°C on the last day. The results are given in Fig 44.

The fluctuations in haemolymph sugar concentration show the same pattern for pharate first instars kept at constant temperature on the last day as for those given a complete cycled temperature

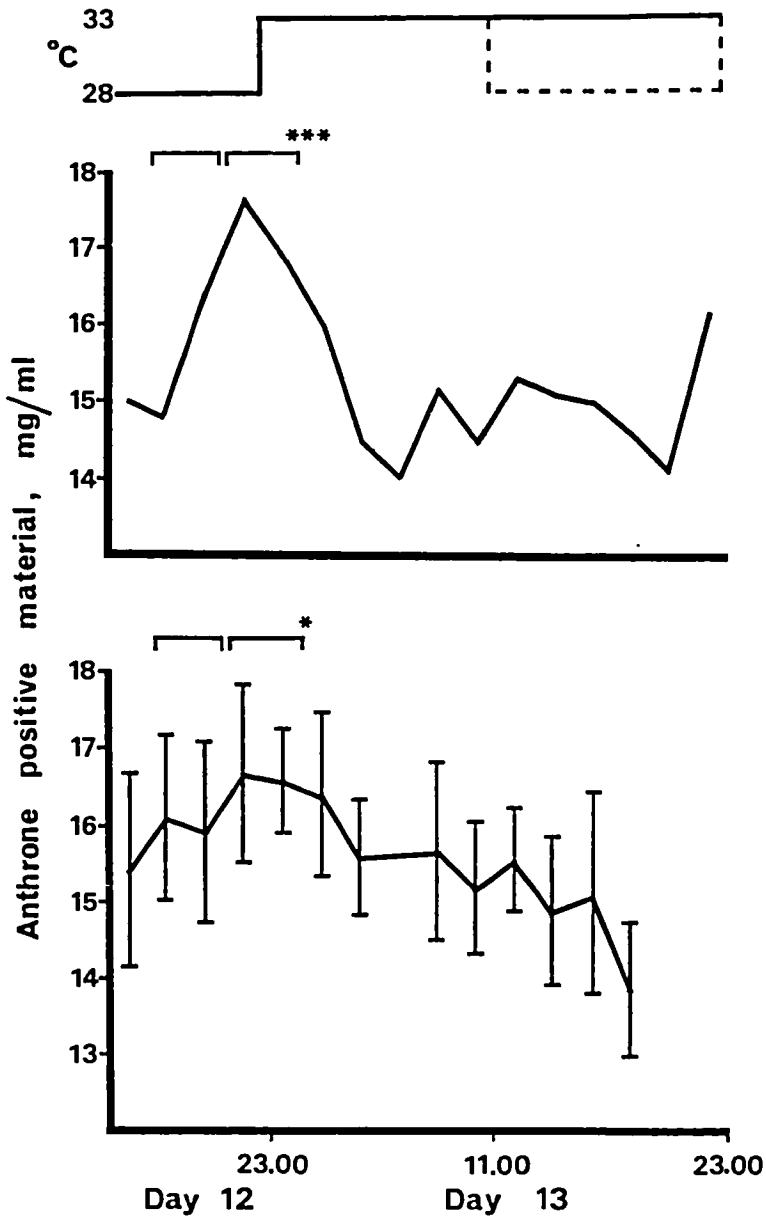


Fig. 44. The concentration of haemolymph sugar (Anthrone positive material) (±1 s.d.) for pharate first instars kept at constant temperature over the last day of development (lower graph). For comparison the concentration over the same period with a cycled temperature (Fig. 43.) is shown above. *$p<0.05$. ***$p<0.001$.

regime. The difference between the level at 18.00h to 22.00h and 22.00h to 02.00h is significant (probability of the null hypothesis being valid is less than 0.05). The rise is however less marked than for the comparative time in the cycled temperature group, ($p < 0.001$).

The haemolymph sugar levels over the last four days of development show a trend towards a regular daily cycle, the amplitude of which tends to increase each day. Since this fluctuation persists in the absence of a cycled temperature on the last day it is suggested that there is good evidence for a circadian basis to the fluctuations. A comparison of this rhythm with those of oxygen consumption and activity are considered in the discussion.

iii) Haemolymph amino acid levels.

Haemolymph amino acid determinations were carried out in the same way as for haemolymph sugars but for only the last three days of development. The method of quantitative estimation is given in the methods section (p. 49). As before most of the pharate first instars had hatched by 20.00h on the last day and so readings after this time represent larvae probably due to emerge on the following day. The results are given in Fig 45.

In comparison with the haemolymph sugar fluctuations the amino acid levels show no apparent daily cycle but remain almost constant throughout the last three days. Since no rhythmic fluctuations seemed to exist no further experiments were carried out with respect to amino acid levels.

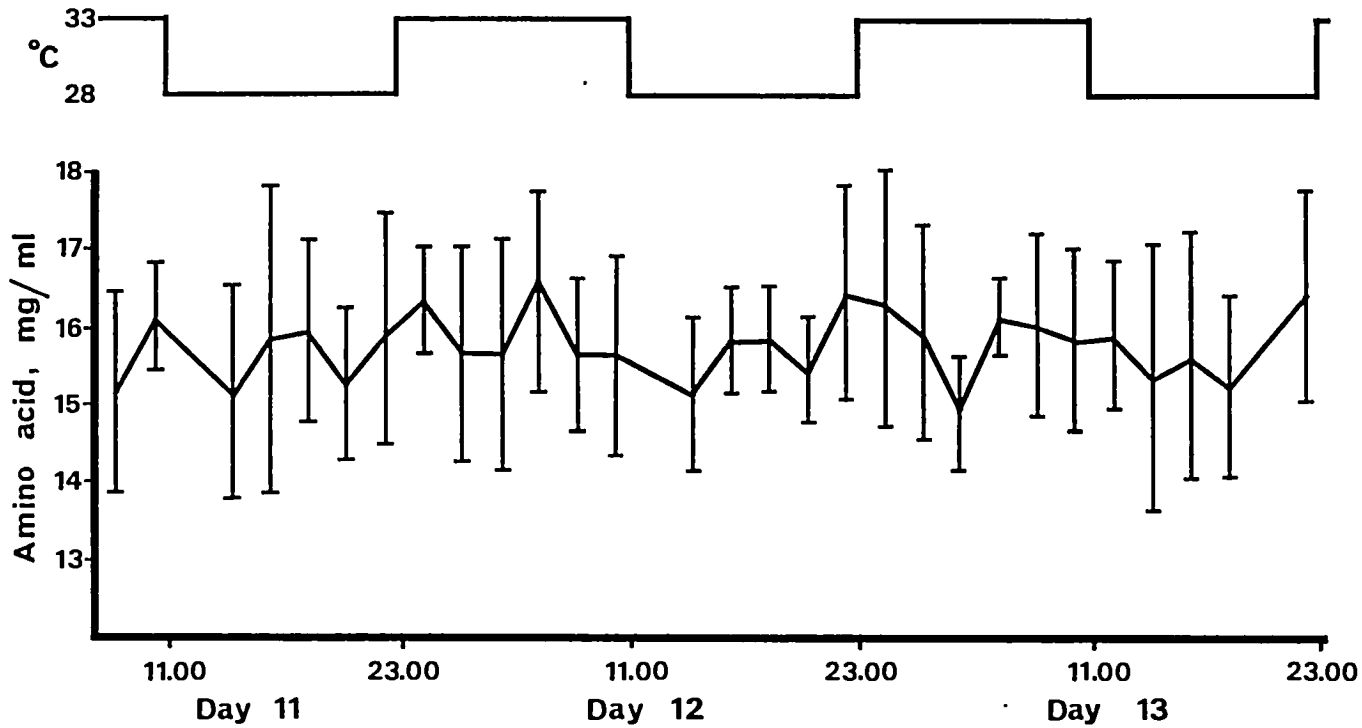


Fig. 45. The concentration of haemolymph amino acid (±1 s.d.) over the last three days of embryonic development. (Cycled temperature incubation).

E. The influence of humoral and pharmacologically active factors on the induction of hatching.

i) Introduction.

The results from the experiments concerning the role of the ambient temperature cycle on the time of hatching have shown that the establishment of gated eclosion is one of its principal influences. Such gating of emergence have been demonstrated in the hatching of other insects (see Minis and Pittendrigh 1968, Kalmas 1935, Meng, Chang, Du and Ge 1973, Edwards 1964), however in none of these instances is there any evidence for the underlying physiological mechanism linking circadian entrainment with hatching. If, however, one regards hatching, not as a specific activity, but rather as simply another form of general activity then possible neuro-hormonal links between circadian rhythmicity and overt activity are evident from published data.

Truman and Riddiford (1970) working with Saturniid moths demonstrated that the eclosion of the adult from the cocoon is synchronised with the environmental photoperiod. Further, by experiment with implanted detached brains, it was demonstrated that there is a clock controlled release of a specific eclosion hormone which initiates the behaviour pattern leading to adult emergence (Truman and Sokolove 1972). Subsequently Truman, Fallon and Wyatt (1976) have shown that the effect of the silkmoth eclosion hormone is duplicated by injection of cyclic-AMP and they conclude that an early step leading to the release of the eclosion behaviour is an endogenous increase in cyclic-AMP in the hormone target neurones.

In addition to eclosion, other steps in the moulting process may also show a sensitivity to environmental entrainment. For example Rensing (1971) demonstrated that in Drosophila melanogaster (Meig) release of prothoracotropic hormone from the brain shows a gated release pattern in phase with the environmental photoperiod. Similarly, Truman (1972) has shown that the times of the larval moults of Manduca are circadian entrained and that this arises from a circadian entrainment of the endocrine events which initiate the moulting process.

Evidence for a neuro-hormonal link between circadian entrainment and general locomotor activity has come from work with the cricket Acheta domesticus, (L.). Cymborowski (1970b) has demonstrated that the circadian locomotor activity is controlled by the neurosecretory cells of the pars intercerebralis. These show a circadian based synthetic and secretory rhythm (Cymborowski 1970c, Cymborowski and Dutkowski 1970), release of the neurosecretion having an inhibitory influence on locomotion. It is further suggested that the stimulation of activity is brought about by an increase in endogenous levels of 5-hydroxytryptamine. Cymborowski (1970a) and Cymborowski and Muszynska (1974) have shown that injected 5-hydroxtryptamine causes an increase in locomotor activity whilst injected reserpine, which inhibits the normal action of 5-hydroxytryptamine, inhibits activity. Circadian rhythms of 5-hydroxytryptamine in the brain of larval, pupal and adult Drosophila have been shown by Fowler, Goodnight and LaBrie (1972) and these cycles parallel the respiratory and locomotory rhythms as reported by Rensing (1964).

Rhythmic fluctuations in the level of acetylcholine esterase in the cricket brain have also been demonstrated (Cymborowski,

Skangiel-Kramska and Dutkowski 1970). However the relationship with the circadian activity pattern is anomolous in so far as high locomotor activity coincides with a sharp drop in acetylcholine esterase levels.

Direct evidence for neuropharmacological control of circadian activity rhythms is provided by Rao and Gropalakrishnareddy (1967). Working with the scorpion Heterometrus fulvipes they found that perfusion of isolated nerve cords with haemolymph or cephalothoracic ganglion extracts produced both increases and decreases in spontaneous neuronal activity depending upon the time of day at which the blood or ganglia sample was taken. The induction of the highest spontaneous output coincides with blood or ganglia samples taken at the time of peak circadian locomotor activity.

Emergence of the Schistocerca vermiform larva from the chorion almost immediately precedes the intermediate moult and by analogy with later instars one might expect a high titre of 20-hydroxyecdysone in the period immediately prior to hatching (Morgan and Poole 1976). However 20-hydroxyecdysone appears to be implicated more with the stimulation of new cuticle formation rather than with apolysis (Ellis, Morgan and Woodbridge 1972) and its appearance at the end of the instar supports this hypothesis. Whether or not this hormone or any other ecdysteroid influencesthe final stage of moulting, the actual ecdysis, remains unclear but the data of Haskell and Moorhouse (1963) suggest that it might. They have shown that treatment of the central nervous system of Schistocerca with either the blood of a moulting locust or an extract of ecdysone and 20-hydroxyecdysone produces enhanced activity in the ventral cord and depressed activity in the motor system. This depressed motor activity is clearly seen in

the late instar nymph as it approaches the moult but whether or not this is the case with the intermediate moult is open to speculation. Immediately preceding the intermediate moult is a period of high activity during the hatching effort period and on this basis this and later ecdyses would appear dissimilar. However preceding the hatching effort period is a quiescent phase of half a day or more (see Fig 26 and 30) and the depressed motor activity seen at this time could be argued to be analogous with the pre-ecdysial quiescence of nymphal moults. Evidence in favour of a neuro-hormonal or ecdysteroid involvement in hatching comes from the work of Polivanova (1968). Working with Eurygaster it was demonstrated that there is a build up of neurosecretion in the pars intercerebralis and an inhibition of hormone release by the prothoracic glands when embryos are inhibited from hatching or nymphs inhibited from moulting by subthreshold temperatures. On this basis it is suggested that there is an analogy between the hormonal control of moulting and hatching.

Whilst ecdysone (α ecdysone) is only detectable at relatively low levels in the nymphal stages of the locust it appears to be the principal ecdysteroid in the egg (Laqueux, Hirn and Hoffman 1977). There is thus the possibility that this may also be influential at the time of hatching.

The results of experiments carried out to examine the influence of some of the afore mentioned hormones and pharmacologically active compounds in relation to the possible existence of a specific hatching hormone in Schistocerca pharate first instars are given below.

ii) Haemolymph transfer experiments.

1. The pharate first instar locust bioassay.

Schistocerca egg pods were incubated in two incubators each on a temperature cycle of 12 hr 33°C / 12 hr 28°C. The only difference between the two incubators was the time of temperature change over, one incubator being four hours in advance of the other. Four days before the experiment both sets of egg pods were removed from their original sand incubation tubes and the eggs placed individually on moist filter paper in sealed boxes so as to reduce mechanical disturbance during the subsequent experiment. On the day of hatching those larvae in the incubator which gave the earlier temperature drop started to emerge the sooner and these were used as haemolymph donor larvae. As each larva emerged from the egg shell in the vermiform state a 2µl blood sample was taken from it. This was then reinjected into one of the fully developed pharate first instars from the second incubator which were estimated to be due to hatch in approximately four hours. The recipient larva was then returned to its incubator and the latency between injection and hatching recorded. For each fully developed pharate first instar injected in this way a control pharate first instar was injected with 2µl of haemolymph from an adult locust. The latency between injection and hatching was similarly recorded.

The results given in Fig 46 suggest that there is no evidence for a hatching hormone. The mean hatching time of the group injected with vermiform larvae haemolymph is in advance of the group injected with adult haemolymph but the difference is not significant.

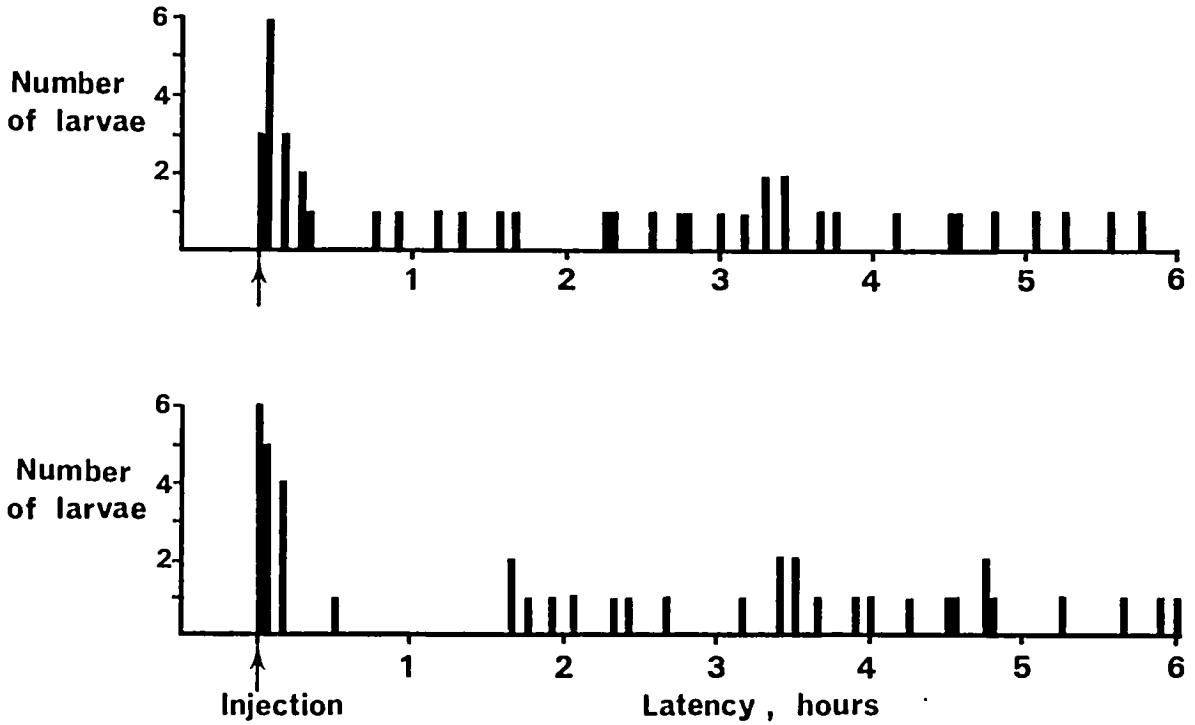


Fig. 46. The latency between the time of injection and hatching for fully developed pharate first instars injected with either vermiform larva haemolymph (upper figure) or adult locust haemolymph (lower figure). The differencesbetween the means and variances are not significant.

2. The pharate adult blowfly bioassay.

Since pharate first instar locusts proved to be a difficult recipient insect with which to test blood samples an alternative recipient insect was sought. As described in the methods section (p. 50) the insect selected was the pharate adult stage of the blowfly Calliphora.

Calliphora larvae were reared from the last instar and the newly formed pupae collected twice daily. At 20°C the pupae take 16 days to metamorphose and when emergence was occurring regularly in a group, pupae from the group half a day younger were used as recipients. The experiment was carried out as before with recipient Calliphora pupae being injected with either 2µl of vermiform larvae blood or 2µl of adult locust blood. The injections were made through a small window cut in the puparial wall which was sealed immediately afterwards. The emergence times of the adults from the puparia were recorded and the results are given in Fig 47. Adults emerging in excess of 8 hours after injection were not recorded.

The results given in Fig 47 suggest that the Calliphora pupae were an improved recipient insect in so far as almost none responded to mechanical disturbance at the time of injection. However there is no significant difference between the eclosion times of the two groups and no evidence of a hatching hormone in vermiform locust larva haemolymph.

3. Pharate first instar locusts as both recipients and donors of haemolymph.

Results given section 2A (p 131) concerning the filming of activity of embryos have shown that the hatching effort, whilst

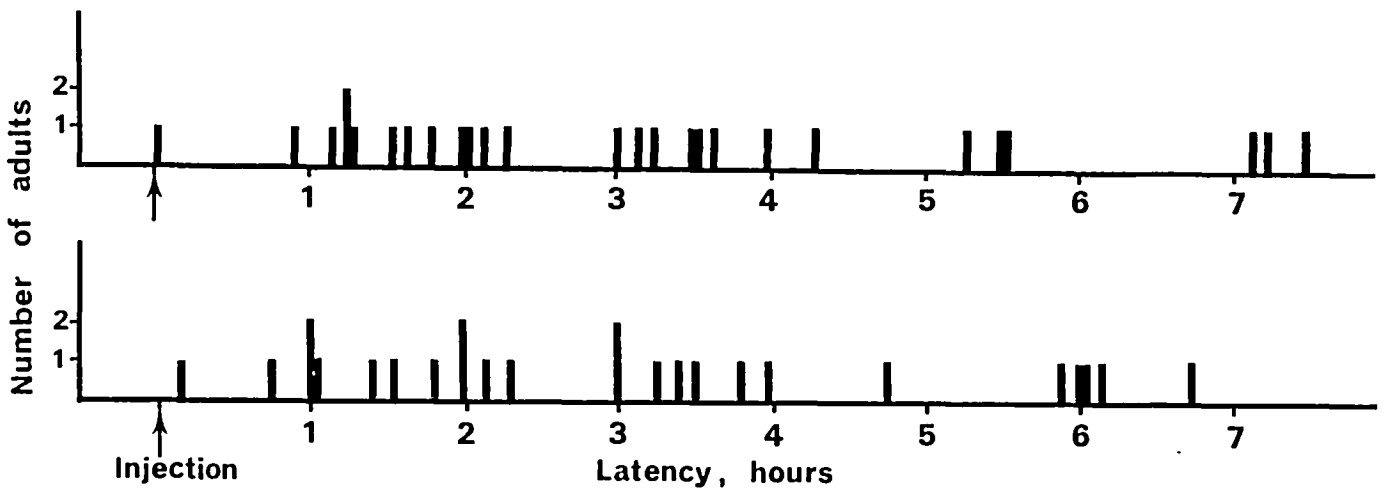


Fig. 47. The latency between the time of injection and emergence for fully developed pharate Calliphora adults injected with either vermiform locust larva haemolymph (upper figure) or adult locust haemolymph (lower figure). The differencesbetween the means and variances are not significant.

variable in duration, may start as much as 5 hours before hatching itself occurs. It is thus probable that if a hormone was initiating hatching behaviour its release into the haemolymph would occur some hours before hatching and it might therefore have been metabolised before the vermiform larvae appeared. This could then account for the failure to detect any hatching hormone in vermiform larvae haemolymph. In view of this possibility the first experiment was repeated using pharate first instars showing hatching effort activity as haemolymph donors and slightly younger larvae showing no hatching effort behaviour as recipients. The results are given in Fig 48.

The mean hatching time of those quiescent pharate first instars injected with haemolymph from pharate first instars showing hatching effort activity is earlier than for the control group injected with adult haemolymph. However the difference is not significant, ($0.1 > P > 0.05$). Unlike the vermiform larvae in Fig 46 there is no hatching response shown by a percentage of the pharate first instars to the mechanical disturbance of the injection. This is probably due to the larvae in Fig 48 being younger than those in Fig 46 and as such being unable to respond to the mechanical disturbance.

iii) The role of the neuroendocrine system.

1. The injection of corpora cardiaca homogenate.

The injection of haemolymph from pharate first instars showing hatching effort activity into slightly younger pharate first instars gave no evidence for a specific hatching hormone. However the functional endocrine physiology of the locust embryo is largely unknown and as such the previous experiments may have given negative results because of some shortcoming in the experimental method. For example the time of release of the proposed hormone was estimated

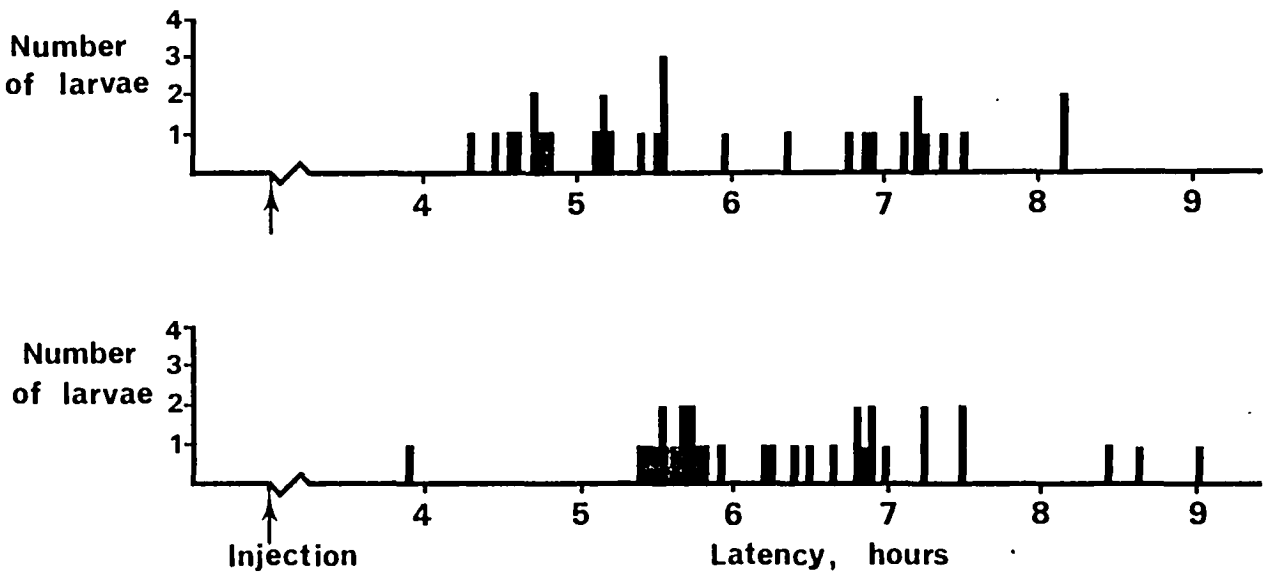


Fig. 48. The latency between the time of injection and hatching for fully developed pharate first instars injected with either haemolymph from pharate first instars showing hatching effort activity (upper figure) or haemolymph from adult locusts (lower figure). The differences between the means and variances are not significant.

and may have been wrong. For this reason the initial results are not taken as conclusive evidence for the absence of a hormone and further possibilities were investigated.

The corpora cardiaca are a potent source of insect hormones, acting as both storage and release sites of neurohormones from the brain as well as possessing their own intrinsic endocrine cells. (For reviews see Highnam and Hill 1969, Mordue and Goldsworthy 1969). Homogenates of corpora cardiaca were therefore tested for evidence of hatching inducing activity.

The extract was prepared as described in the methods (p 52), 1µl aliquots being injected into 35 recipient fully developed pharate first instars. Alternately with every extract injection a control pharate first instar was injected with 1µl of Ringer. The injected insects were incubated as described in the methods section and the latency between injection and hatching recorded. The results are given in Fig 49.

Of the group of pharate first instars injected with corpora cardiaca extract 33 out of 35 hatched within four hours of injection, the mean latency being 1h, 09 min, (s.d. 34 min). In the control group 30 out of 35 pharate first instars hatched within four hours of injection, the mean latency being 1h, 16 min, (s.d. 41 min). The difference is not significant.

2. Histology of the brain-corpora cardiaca neurosecretory complex.

The brain and corpora cardiaca were examined histologically using paraldehyde fuchsin stain at six stages of development:-

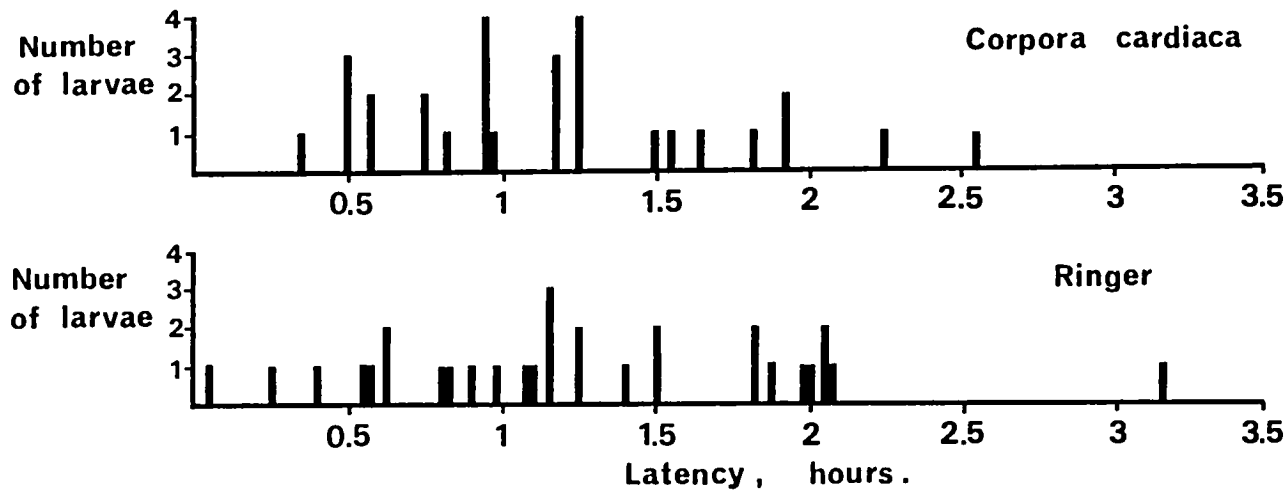


Fig. 49. The latency between the time of injection and hatching for fully developed pharate first instars injected with either corpora cardiaca extract (upper figure) or Ringer (lower figure). The differences between the means and variances are not significant.

a) Pharate first instars showing red tips to the mandibles. (12-18 h prehatching).

b) Pharate first instars showing brown tips to the mandibles. (6-12 h prehatching).

c) Pharate first instars showing black tips to the mandibles. (3-6 h prehatching).

d) Vermiform larvae.

e) Larvae immediately after the ecdysis from the embryonic cuticle.

f) Larvae one hour after ecdysis.

Stained sections of brain of ten larvae from each stage were examined for changes in the amount of stainable material present. At the earliest stage examined (12 - 18 hours prehatching) the corpora cardiaca showed large amounts of PF staining neurosecretory material. However, in the neurosecretory cells of the pars intercerebralis almost no PF staining neurosecretory material was present and the neurosecretory axon tracks through the brain were totally unstained. In subsequent stages both the high level of stainable material in the corpora cardiaca and the minimal level in the neurosecretory cells of the pars intercerebralis persisted. There is thus no evidence of any change in concentration of brain neurosecretion at the time of hatching.

It is possible that if a neurosecretory hatching hormone were present its release from the brain or corpora cardiaca might make no significant difference to the amount of stainable material remaining. Alternatively it may not stain with paraldehyde fuchsin in the first place. Thus the histological evidence does not preclude the existence of a hatching hormone but neither does it provide any evidence for it.

iv) Injection of dibutyryl cyclic-AMP.

Fully developed pharate first instars were injected with either 1µl of Ringer, 1µl of theophylene (5µg/µl) or 1µl of theophylene followed by 1µl of dibutyryl cyclic-AMP (0.1 mg/µl). The theophylene was injected with the dibutyryl cyclic-AMP to inhibit the cyclic nucleotide phosphodiesterase activity in the haemolymph and injected alone as an additional control. The injected pharate first instars were incubated in the usual way and the hatching times recorded. The results are given in Table 9.

The most significant factor from this set of results is the induction of premature cuticular melanization in 17 out of 21 of the pharate first instars injected with cyclic-AMP. VandenBerg and Mills (1974) demonstrated that the injection of cyclic-AMP mimics the effect of bursicon release in Periplaneta and this also appears to have occurred with these Schistocerca pharate first instars. A side effect of this premature induction of cuticular tanning is to inhibit the escape from the chorion after approximately an hour from the time of injection. Thus the mean latency of those pharate first instars which do hatch is much less than either of the two control groups but this is an artefact due to the early onset of tanning.

The results therefore provide no evidence for a hatching hormone acting in the same way as the silkmoth eclosion hormone. However the possible role of cyclic-AMP as an intermediate cannot be entirely ruled out since physiological responses will depend on specific concentrations at the target organs and these cannot be reliably mimicked by injection into the haemocoele as a whole.

Injection	Number hatching	Mean latency	Number not hatching	Number melanizing
Ringer	24	2h, 08 min	6	0
Theophylene	26	2h, 04 min	4	0
Theophylene + cyclic AMP	7	40 min	21	17

Table 9. The effect of injecting theophylene and dibutyryl cyclic-AMP into fully developed pharate first instars compared with Ringer and theophylene controls.

v) Injection of ecdysone and 20-hydroxyecdysone.

Solutions of ecdysone and 20-hydroxyecdysone were prepared at concentrations of 1ng/µl, 5ng/µl, 10ng/µl, and 100ng/µl made up in Ringers solution as described in the methods section (p. 53). Fully developed pharate first instars were then anaesthetised with carbon dioxide and injected with either 1µl of ecdysteroid or 1µl of Ringer. The injected insects were then returned to the incubator and their hatching times recorded. The latency between the time of injection and the time of hatching for each of the groups is given in Figs 50 and 51. Insects not having hatched within 4 hours of injection were scored as unhatched.

None of the groups injected with either ecdysone or 20-hydroxyecdysone showed any significant reduction in the latency between injection and hatching compared with the control group injected with Ringer. It thus seems unlikely that ecdysteroids play any significant role in the initiation of hatching.

vi) Injection of 5-hydroxytryptamine (serotonin).

Pharate first instar larvae were anaesthetised with carbon dioxide and then injected with 1µl of the creatine sulphate salt of 5-hydroxytryptamine at a concentration of 80µg/ml. Injections were made into the heads of the larvae as described in the methods section (p.53). A control group of pharate first instars were injected with 1µl of Ringer, also into the head. The injected insects were then incubated in the usual way, the latency between the injection and hatching being recorded. The experiment was repeated on five occasions, the results being given in Fig 52.

For each of the five individual experiments the difference in latency between the Ringer and 5-hydroxytryptamine groups are not

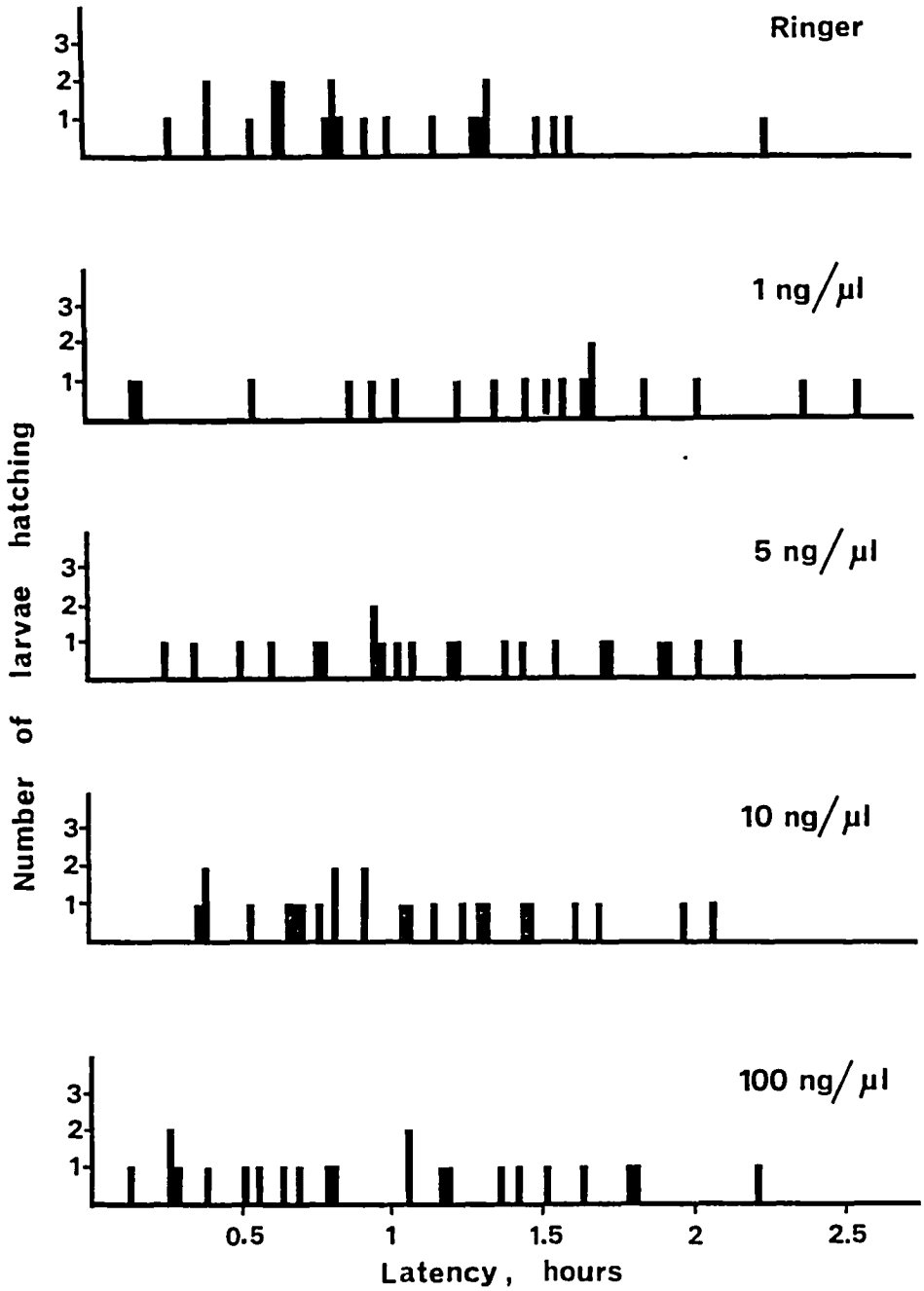


Fig. 50. The latency between the time of injection and hatching for fully developed pharate first instars injected with 20-hydroxyecdysone. The differences between the means of any of the groups are not significant.

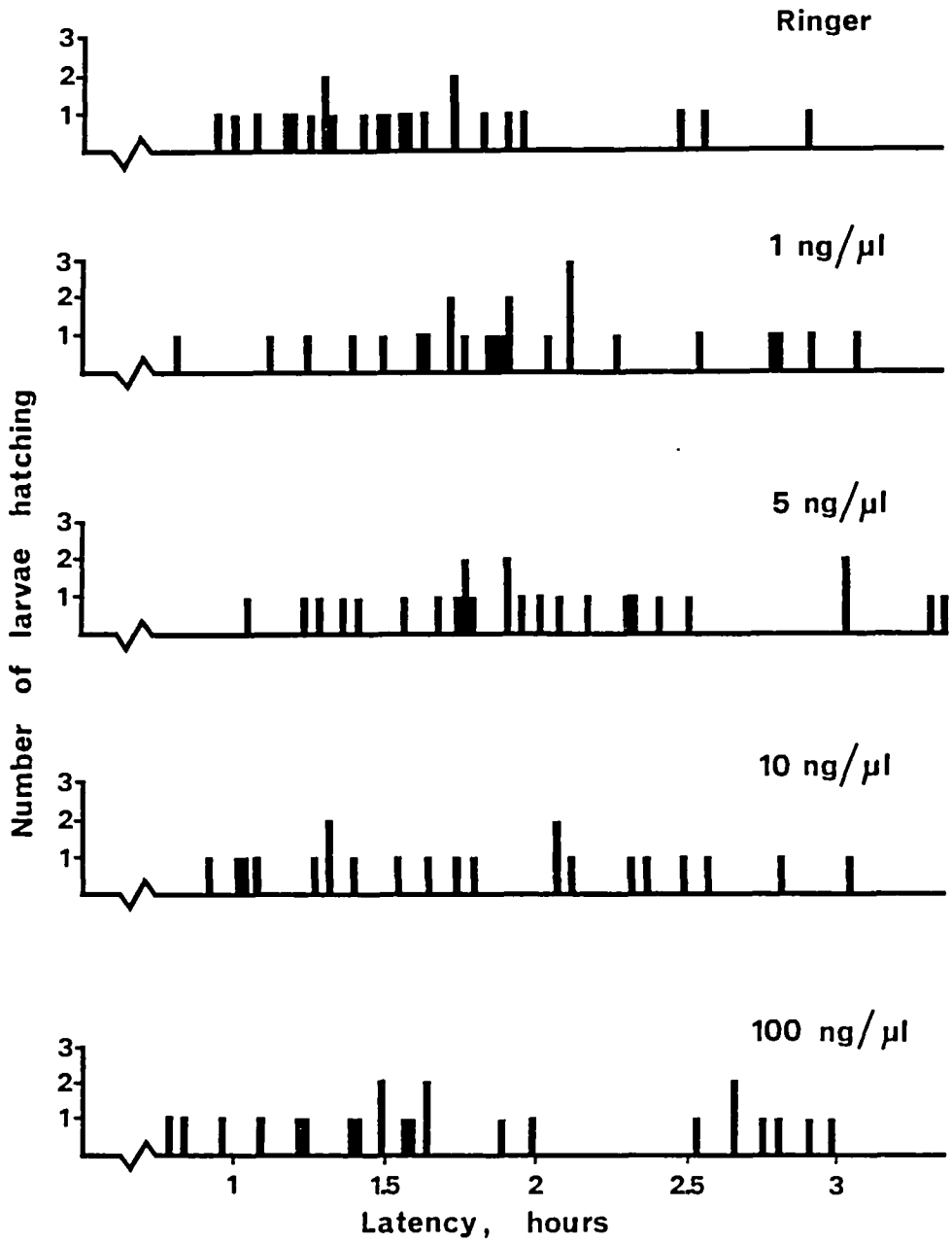
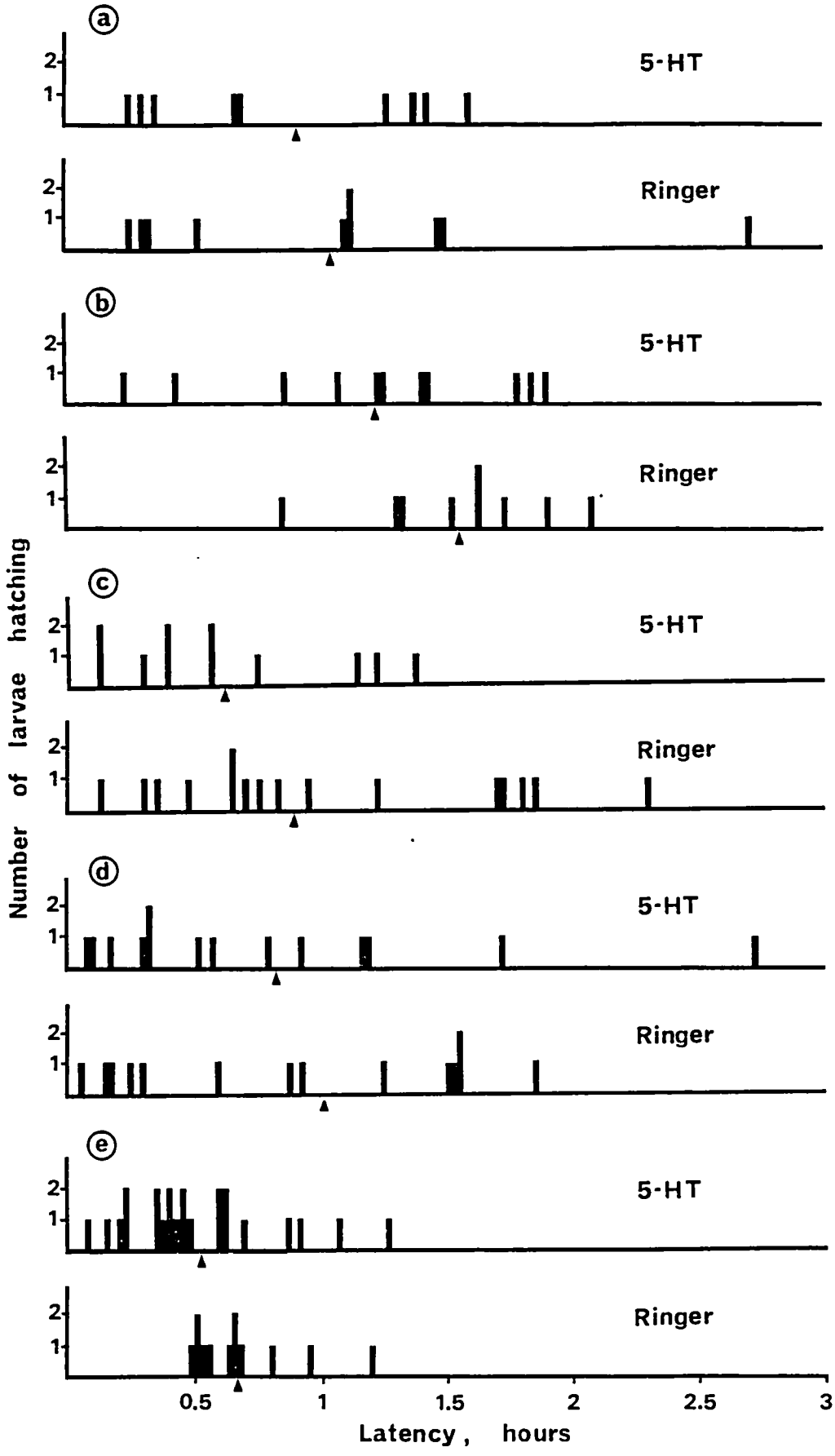


Fig. 51. The latency between the time of injection and hatching for fully developed pharate first instars injected with ecdysone. The differencesbetween the means of any of the groups are not significant.

Fig. 52. The latency between the time of injection and hatching for five groups of fully developed pharate first instars injected with either 5-hydroxytryptamine or Ringer. The groups injected with 5-hydroxytryptamine show a reduced latency compared to their respective controls and the difference is significant ($p < 0.05$) when the data are pooled. (Means are marked with an arrow ▼).


a
5-HT
Ringer
b
5-HT
Ringer
c
5-HT
Ringer
d
5-HT
Ringer
e
5-HT
Ringer
2
1
Number of larvae hatching
0.5
1
1.5
2
2.5
3
Latency, hours

significant with either t - test or analysis of variance. This is similarly true when the data for each of the five experiments are pooled. However it is noteworthy that in each of the five experiments the mean latency for the 5-hydroxytryptamine group is less than that for the respective Ringer injected group. Also, since the number of 5-hydroxytryptamine and Ringer injected insects are not identical with one another in each group a weighted analysis of variance can be used with the pooled data (Snedecor and Cochran 1967). Such analysis shows that there is a difference between the treatments (5-hydroxytryptamine or Ringer) which is significant at the 5% level.

vii) Injection of reserpine.

Reserpine is known to inhibit the activity of 5-hydroxytryptamine in the mammalian nervous system and in the mollusc Mercenaria it has been shown to block the 5-hydroxytryptamine induction of cardio-excitation (Cottrell and Laverack 1968). In the ant Formica rufa L. ingestion of reserpine with the food causes an inhibition of spontaneous locomotor activity (Kostowski, Beck and Meszaros 1966) and in the cricket it will reverse 5-hydroxytryptamine induced activity. Reserpine also causes a shift in the time of the peak of the circadian locomotor rhythm in the cricket (Cymborowski 1970a). The emergence of Schistocerca hatchlings show circadian entrainment and there is also a suggestion from the previous results that 5-hydroxytryptamine may induce hatching activity. Therefore the possible inhibitory effect of injected reserpine on both the level and the time of hatching activity was considered.

Two Schistocerca egg pods approximately seven days old were divided up into single eggs and placed in an incubator giving

12h 33°C / 12h 28°C. As discussed in results section 1C (Fig 15) incubation under cycled temperature conditions leads to entrainment of the pharate first instars with emergence then occurring approximately 4-5 hours after the onset of the final cold period. On the penultimate day of development, approximately 24 hours prior to the time when hatching should occur, 30 pharate first instars were injected with 1µl of 80µg/ml reserpine (approximately 5µg/g body weight) and 30 pharate first instars with 1µl of Ringer as a control (see methods section p 54). The insects were then returned to the incubator and their emergence times recorded. The results are given in Fig 53.

Despite the apparent suggestion of stimulated hatching activity in the group of insects injected with 5-hydroxytryptamine there was no inhibition of activity due to the injection of reserpine and no indication of any change in the characteristic emergence time during the day.

viii) Acetylcholine esterase levels in the brain during embryonic and early post-embryonic development.

The levels of brain acetylcholine esterase (ACHE) were determined as described in the methods (p 55). Determinations were made at 10 stages throughout the last five days of embryonic development and in the newly emerged first instar. The results are shown in Figs 54 and 55. Fig 54 shows the activity per unit wet weight of brain. The level of ACHE increases in an approximately linear progression over the four days prior to the penultimate day. On the final day of development the activity per unit wet weight increases more sharply to reach a plateau at the time of hatching.

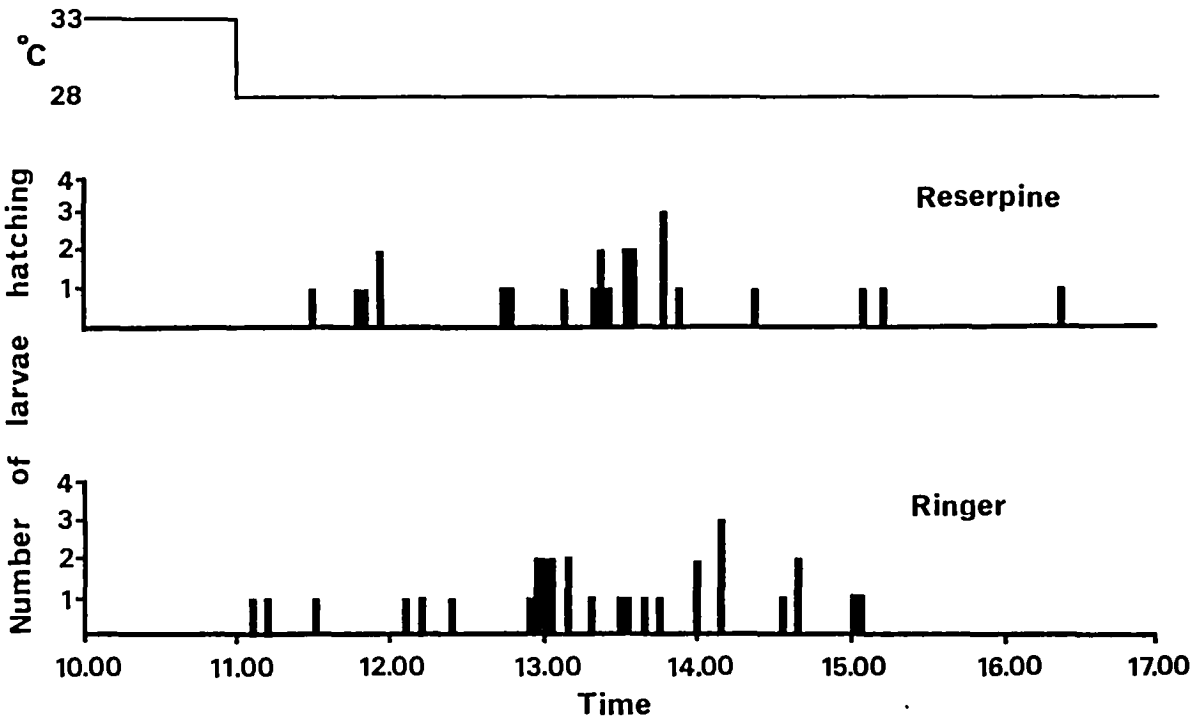


Fig. 53. The emergence times during the day of larvae injected 24 hours previously with either reserpine (upper figure) or Ringer (lower figure). The differencesbetween the means and variances are not significant.

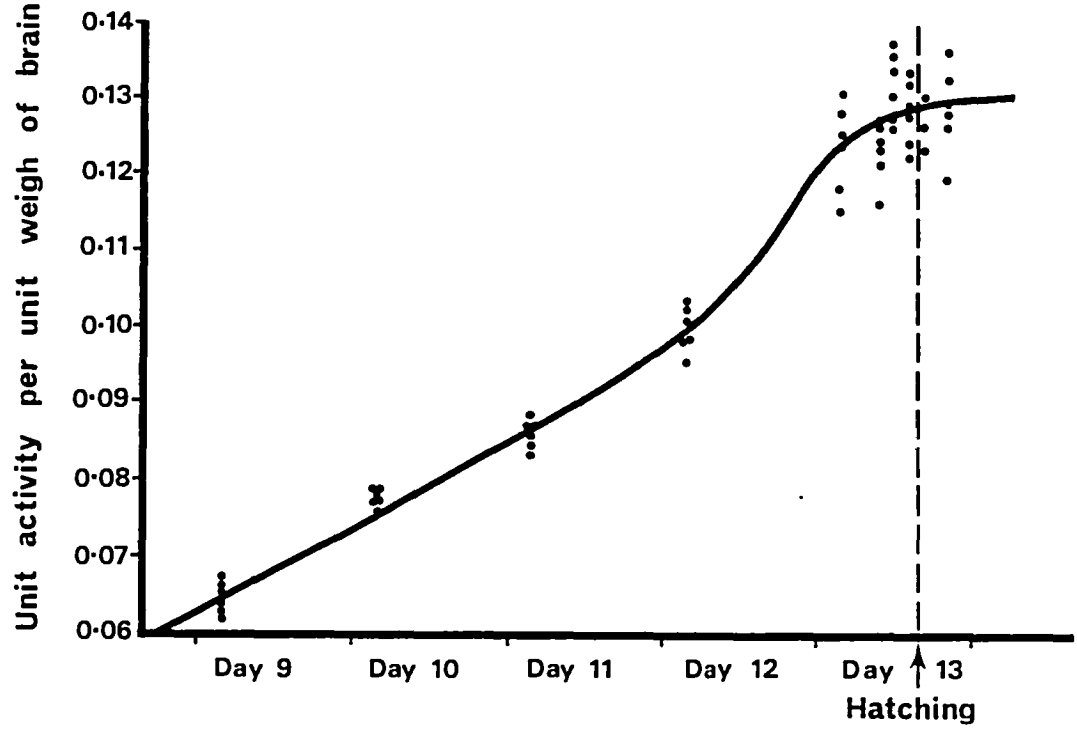


Fig. 54. The acetylcholine esterase activity per unit wet weight of brain over the last five days of development.

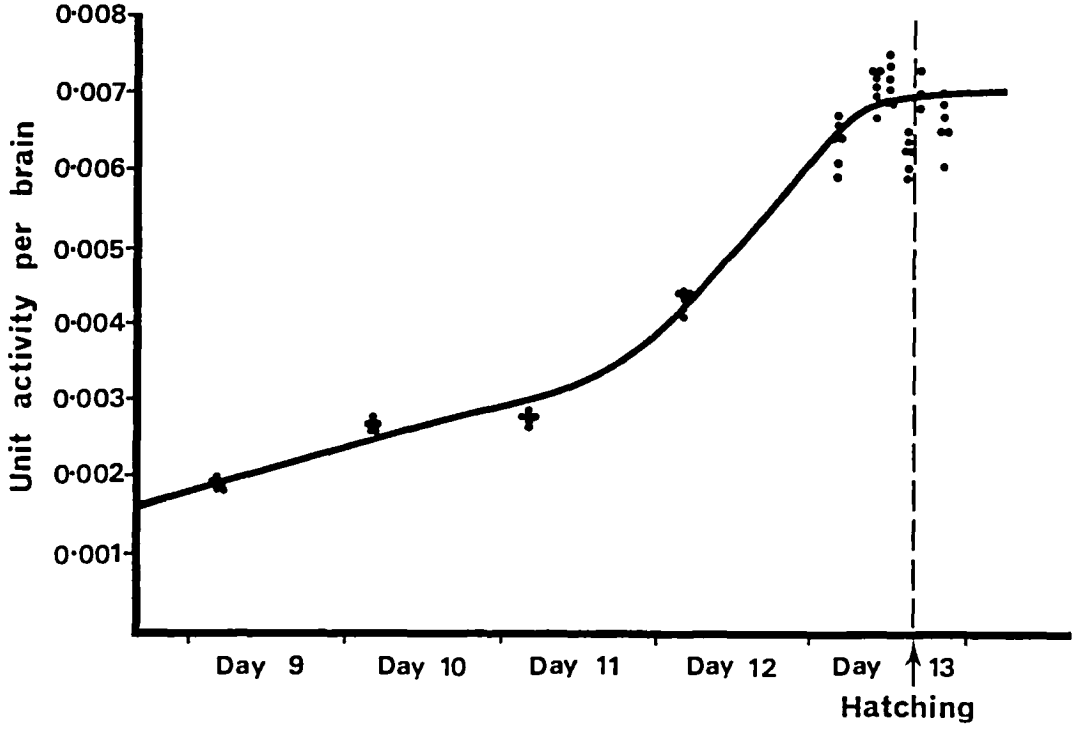


Fig. 55. The acetylcholine esterase activity per whole brain over the last five days of embryonic development.

In addition to the levels of ACHE showing an increase per unit wet weight of brain during the developmental period the total mass of the brain also increases. Thus the total content of ACHE per brain rises more rapidly than the increase per unit wet weight. This is shown in Fig 55. As with Fig 54 there is an initial linear increase but at approximately two days prior to hatching there is the onset of both an increase in ACHE activity per unit wet weight and an overall increase in brain weight. This results in a rather sharper rise in overall ACHE content which again reaches a plateau at the time of hatching. The slight drop immediately prior to hatching is the result of a low brain weight recorded for this group. Since brain weight is unlikely to decrease this low result for total activity is considered to be spurious.

If the level of brain ACHE is indicative of the relative level of brain functional organisation the results suggest that initially the rate of brain development is comparatively slow. At about minus $1\frac{1}{2}$ days, however, the rate of brain development shows a marked increase, the rate declining again as the pharate first instar nears the completion of development. Whilst the results give no indication as to the relationship of the brain to emergence it is significant that the onset of the more rapid phase of development is at about minus 2 days. This approximately coincides with the onset of the establishment of entrainment as shown by hatching experiments in which the temperature was maintained constant over the final days of development. (See results section 1C, Fig. 16.)

3. THE CONTROL OF THE INITIATION OF POST INTERMEDIATE MOULT CUTICULAR TANNING.

A. The control of melanization.

i) Introduction.

The hormonal control of cuticular hardening and darkening has been investigated in a number of insects. Fraenkel and Hsiao (1962, 1963, 1965) were the first to report the presence of a neurosecretion controlling tanning in the blowfly and named the hormone bursicon. In the fly, bursicon is to be found in the neurosecretory cells of the pars intercerebralis and in a higher concentration in the combined ganglia of the thorax. The initiation of tanning and melanization results from the release of the hormone from the thoracic ganglia. This in turn is dependent upon these secretory cells receiving an activating neuronal stimulus from sensory receptors in the cuticle conducted via the brain. The hormone is thought to be released within 3 minutes of the emergent fly reaching an unconfined space.

In the cockroach Periplaneta, Mills, (1965) has shown that ligatures placed around the body always confine the cuticular darkening to the posterior portion and they suggest that release of the hormone is from the terminal abdominal ganglion. Release is thought to take place within 20 minutes of ecdysis, though some results suggest that it may be sooner than this (Mills, 1966).

Vincent (1971) has shown in adult Locusta that although the largest amounts of bursicon are to be found in the brain and corpora cardiaca, ligatures placed around the neck of emerging adults result in the heads remaining small and shrivelled while the rest of the body tans normally. Again it is suggested that bursicon is released

from the terminal abdominal ganglion, in this instance within 6 to 10 minutes of the start of ecdysis.

In the tobacco hornworm, Manduca sexta, abdominal perivisceral organs (PVOs) appear to be the main site of bursicon release, the hormone appearing in the haemolymph almost immediately after the larval-pupal ecdysis and reaching a peak within 15 minutes, (Truman, 1973).

The present study describes the neural and humoral control of melanization in first instar larvae of Schistocerca.

ii) Haemolymph melanizing activity.

Two-microlitre samples of haemolymph were taken from donor larvae at fixed times from 0 hr (vermiform larvae) to 4 hr after hatching. Each sample was injected into a recipient vermiform larvae and after incubation the larvae were scored on a 0 to 5 scale of darkening. The results are shown graphically in Fig 56.

It can be seen that there is a rapid increase in the melanizing activity of the blood in the first few minutes after hatching. This is followed by a plateau of maximum activity for about 40 minutes, after which activity declines. In order to quantify the initial hour rather more precisely, a second trial was carried out using the concentration to which the haemolymph could be diluted, and still remain active, as a measure of its initial activity. As before, haemolymph was taken from donor larvae at time periods from 0 to 4 hr. This haemolymph was then diluted with Ringer solution before injection: a dilution of 1:4 was used for one trial and of 1:9 for the other. The recipient larvae were again incubated and scored for melanization. The pooled results are shown graphically

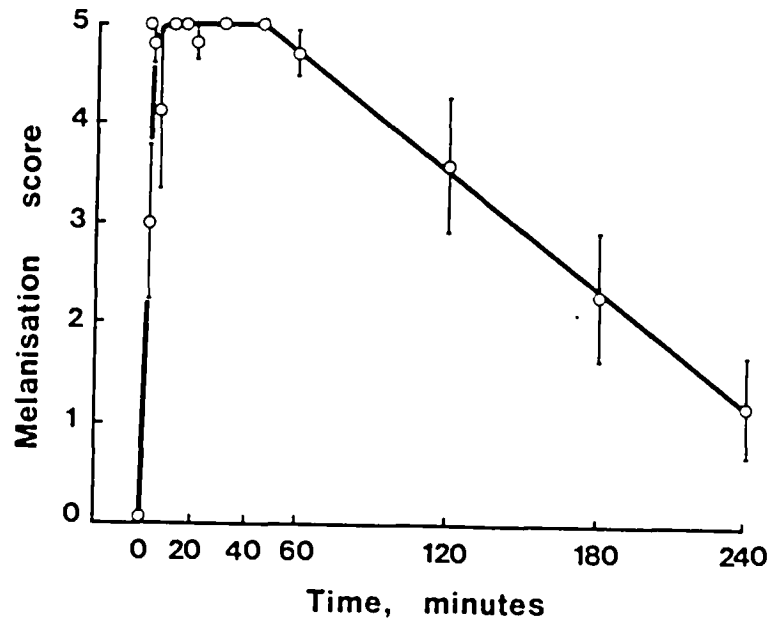


Fig. 56. The time course of melanizing activity in undiluted blood of newly ecdysed first instar larvae. Mean and standard error with a sample size of 6 to 8.

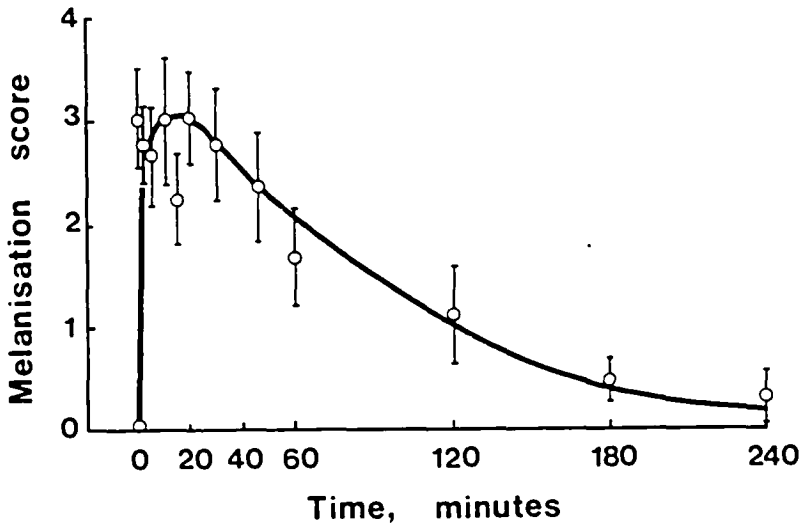


Fig. 57. The time course of melanizing activity of diluted blood of newly ecdysed first instar larvae. Mean and standard error with a sample size of 10 to 21.

in Fig 57, in which scores obtained using the 1:9 dilution have been doubled so as to be comparable with those of the 1:4 dilution.

A maximum level of activity is reached within 2 to 3 minutes and this level is maintained until 10 to 15 minutes after hatching. After this, the level of activity declines steadily.

iii) The release site of the melanization inducing factor.

The release site of the melanizing factor was located by ligatures applied in five different positions and at four different times as described in the methods section (p 58). These five positions and four times gave 20 combinations. After 3 hr incubation the larvae of each group were scored for melanization or non-melanization: the results are given in Table 10. The characteristic responses for each of the 20 groups are shown diagramatically in Fig 58. For ease of reference each group is designated by a letter indicating the position of the ligature and a number indicating the time of application.

It is evident that the release site of the melanizing factor is not in the head or the terminal abdominal ganglion since the rest of the body will melanize when either positions A or E are ligatured. Conversely positions C and D do influence which part of the body will melanize. In those larvae ligatured at D, melanization is often limited to the anterior part of the body (Figs 59, 60 and 61) whilst in those ligatured at C, melanization is often limited to the posterior part of the body (Fig 62). The release site thus seems to lie between these two points.

In addition to the position of the ligature, the time at which it is applied can influence the result. Thus at time 1 melanization

Group	Anterior body	Posterior body	Number
A1	Green	Green	24
A2	Green	Black	15
	Green	Green	6
	Black	Black	7
	Black	Green	1
A3	Green	Black	24
	Black	Black	6
A4	Black	Black	21
B1	Green	Green	22
B2	Green	Green	27
	Black	Green	9
	Green	Black	4
	Black	Black	5
B3	Green	Black	32
	Green	Green	3
	Black	Black	9
B4	Black	Black	13
C1	Green	Green	11
C2	Green	Green	21
	Green	Black	5
	Black	Black	2
C3	Green	Black	20
	Black	Black	5
C4	Black	Black	9
D1	Green	Green	8
D2	Black	Green	12
	Green	Green	1
D3	Black	Green	21
	Green	Green	2
	Black	Black	6
D4	Black	Black	12
E1	Green	Green	6
E2	Black	Green	7
E3	Black	Green	8
	Black	Black	2
E4	Black	Black	5

Table 10. Development or inhibition of melanization on either side of ligatures placed at positions A-E at times 1 to 4. (See Fig. 58 for diagrammatic summary).

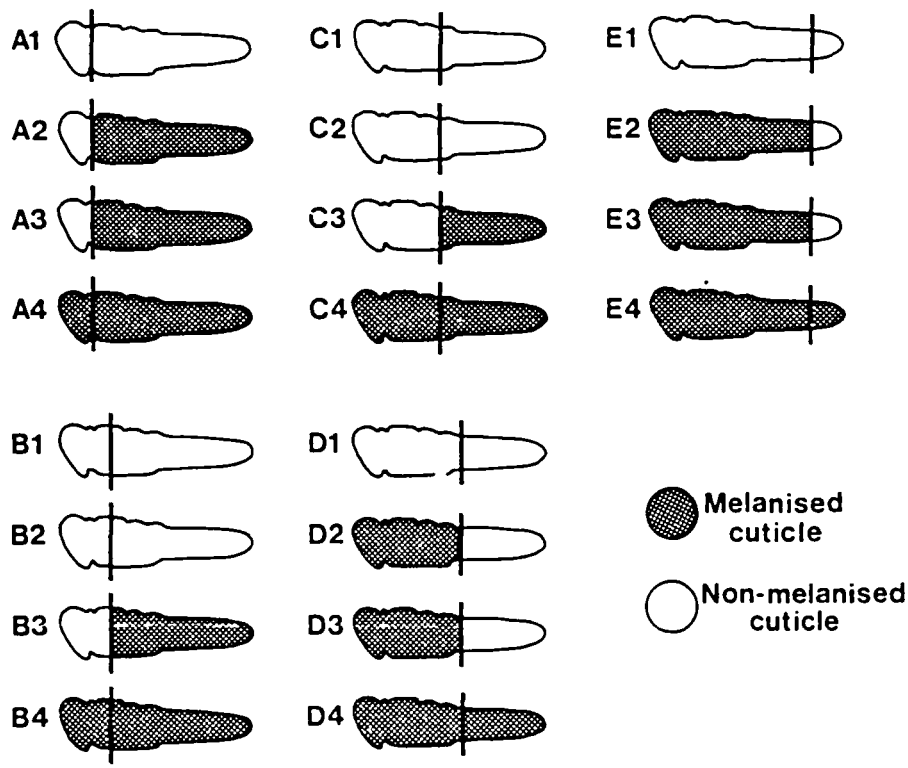


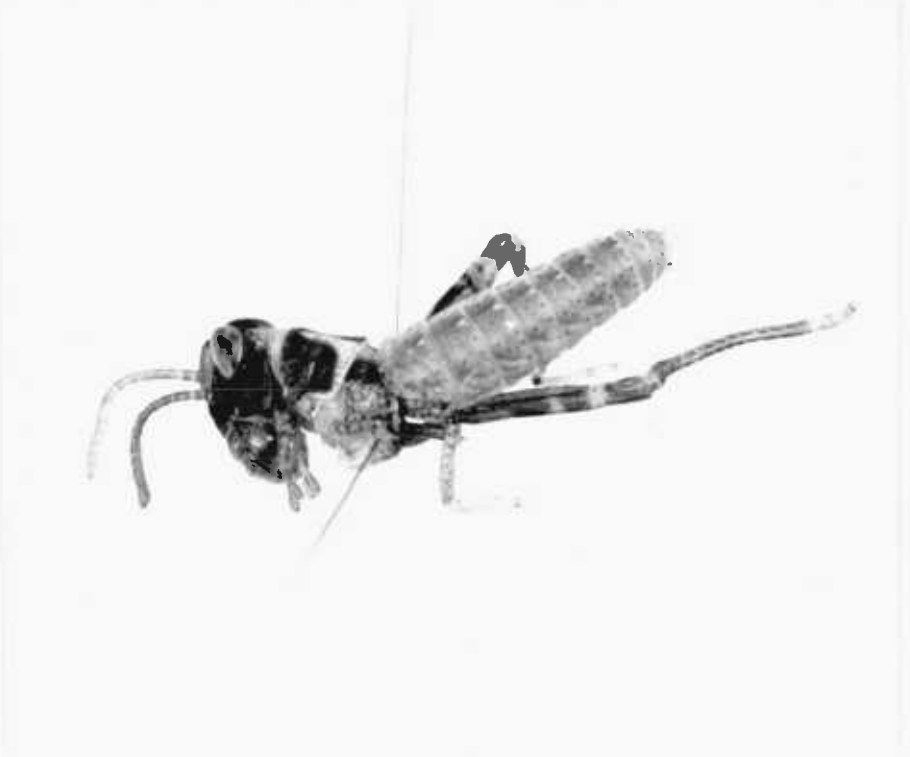
Fig.58. Diagram of the principal result for each of the 20 ligature groups given in Table 10.

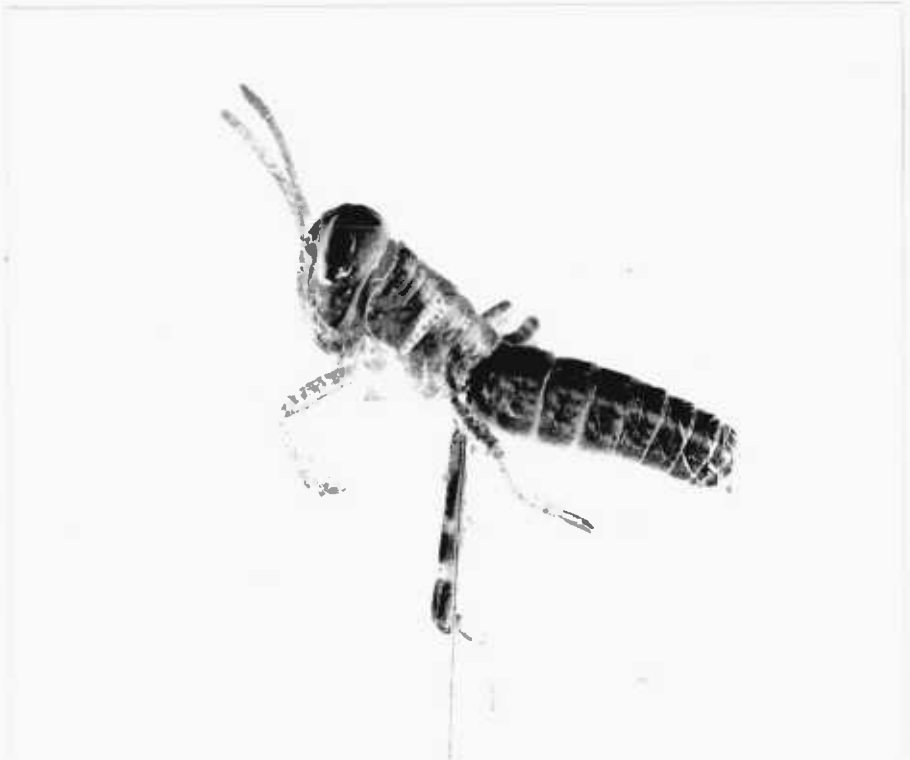
Fig. 59. The localisation of melanization by a ligature around the body. The larva is ligatured behind the metathoracic legs so that the release site of the melanization hormone is anterior to the ligature.

Fig. 60. The localisation of melanization by a ligature around the body. The larva is ligatured as in Fig. 59 and again shows melanization anterior to the ligature but not in the posterior part of the body.

Fig. 61. The localisation of melanization by a ligature around the body. The larva is ligatured diagonally between the metathoracic legs. This is the most anterior ligature position which gives melanization in the anterior part of the body. The release site of the melanization hormone is therefore immediately anterior to this ligature.

Fig. 62. The localisation of melanization by a ligature around the body. The larva is ligatured immediately in front of the metathoracic legs. This is the most posterior ligature position which gives posterior melanization. The release site of the melanization hormone is therefore immediately posterior to this ligature.





is always totally inhibited whilst at time 4 it always proceeds to completion throughout the body. At time 2 both neck and abdominal ligatures (A, D and E) result in melanization being restricted to that part of the body containing the release site. However, ligatures at B and C totally inhibit the process. At time 3 this inhibition is lost and melanization develops in the half of the body containing the release site, no matter where the ligature is placed.

Two sets of control experiments were carried out. In order to confirm that the melanizing factor was present in a region which darkened and was absent from an undarkened region, blood samples were taken from the two regions and injected into vermiform larvae for bioassay. In 31 out of 32 cases in which blood was taken from a darkening segment it induced darkening in the vermiform larvae, while no darkening was induced by any of the 23 samples of blood from undarkened segments. To confirm further that a failure to darken was not due to a lack of competence of the epidermis to respond, perhaps as a result of damage by the ligature, blood from newly hatched larvae was injected into undarkened segments of ligatured larvae and after 3 h incubation the larvae were scored for melanization. In the majority of cases melanization had taken place (Table 11).

iv) Severance of the ventral cord.

Ecdysing vermiform larvae were clamped with micro-arterial clamps transversely across the ventral region of the neck, along the ventral surface of the thorax and at points along the ventral surface of the abdomen. After 3 h incubation at room temperature these larvae were scored for melanization (Table 12).

Number of larvae	Unmelanized region injected	Number of larvae melanized after 3 h	Percentage positive
14	Head and prothorax	9	64
15	Head and pro/ mesothorax	13	87
15	Meso/metathorax and abdomen	12	80
12	Abdomen	9	75

Table 11. Control experiment in which unmelanized ligatured body sections were injected with actively melanizing blood from newly ecdysed first instars.

Number of larvae	Position of clamp	Number of larvae melanized after 3 h
14	Ventral neck	14
23	Between pro- and mesothoracic ganglia	4
16	Between meso- and metathoracic ganglia	3
15	Between first and second abdominal ganglia	14
8	Between third and fifth abdominal ganglia	8
7	Dorsal thorax	7
7	Dorsal abdomen	7

Table 12. The effect of severing the ventral nerve cord, at the time melanization is triggered, on subsequent melanization.

Melanization occurred in nearly every case, except when the clamp was applied to the ventral region of the thorax.

v) The origin of the initial neuronal trigger.

Since appearance of the melanizing factor in the blood coincides with the first split in the embryonic cuticle along the mid dorsal line of the thorax, it was thought that a change in the orientation of trichoid mechanoreceptors in this region might be responsible for the initial stimulation. Larvae can be prevented from actually splitting the embryonic cuticle by the application of warm wax to the dorsal surface of the thorax; whilst such larvae remain vermiform, they do harden and darken. Additionally, vermiform larvae in which the embryonic cuticle is artificially removed do not melanize but die within a few hours. It would thus seem that it is not the actual shedding of the cuticle which initiates the process of melanization but some other aspect of the normal behaviour pattern occurring at this time.

vi) Electrocoagulation of areas of the nervous system.

Electrocoagulatory lesions were made at points along the ventral surface of vermiform larvae adjacent to the prothoracic, mesothoracic, metathoracic and some abdominal ganglia and also between the metathoracic and first abdominal ganglia. Those larvae which subsequently ecdysed were kept at room temperature for 8 h and then scored for melanization (Table 13).

The majority of ecdysing larvae melanized in the normal way but a small number of those with lesions in areas of the metathoracic ganglion and immediately posterior to this ganglion either remained green or showed reduced melanization 8 h after ecdysis.

Number of larvae	Position of electrocoagulation	Percentage with less than normal melanization	Percentage mortality
10	Prothoracic ganglion	0	60
8	Mesothoracic ganglion	0	37
16	Metathoracic ganglion	33	44
23	Between the metathoracic and first abdominal ganglion	50	44
9	First abdominal ganglion	0	55
6	Third abdominal ganglion	0	33
8	Fifth abdominal ganglion	0	25

Table 13. The effect on subsequent melanization of electrocoagulation of regions of the central nervous system.

vii) High potassium Ringer extraction of the melanization factor.

High potassium Ringer (Maddrell and Gee 1974) was used as a neurohormone releasing agent to give extracts of anterior, middle and posterior body segments as described in the methods section, (p 61). The extract solution was then injected directly into vermiform larvae and after four hours incubation the larvae were scored for darkening, (Table 14).

There is a significant increase in the degree of melanization induced by the extract of the middle region compared with the effect of either the anterior or posterior region extracts. The difference between the effects of anterior and posterior extracts is not significant.

viii) Acetaldehyde fuchsin and paraldehyde fuchsin staining.

Both acetaldehyde fuchsin (AF) and paraldehyde fuchsin (PF) were used to stain wax-embedded sections of the ventral cord of vermiform larvae as described in the methods section, (p 61).

The results, (Table 15) suggest that there is no stainable material in the region of the ventral cord implicated as the release site by previous experiments.

ix) Electron microscopy of the release site.

Since both AF and PF staining gave no evidence of the presence of neurosecretory material in the release site region recognized by ligatures and cautery, this area was examined under the electron microscope for the presence of electron-dense granules. (See Maddrell (1966) and Brady and Maddrell (1967) for correlations between electron dense granules in electromicrographs and neurosecretory activity of the tissue). The results are shown in Figs

Number of larvae	Region of extract	Melanization score		
		Range	Mean	S.D.
28	Head and prothorax	0-2	0.21	0.49
34	Meso/metathorax plus first and second abdominal segments	0-3	0.91	0.86
31	Posterior abdomen	0-1	0.16	0.37

Significance: Anterior-middle $t = 3.78$, P 0.001, Posterior-middle $t = 4.46$, P 0.001, Anterior-posterior $t = 0.46$, N.S.

Table 14. Activity of extracts from the anterior, middle and posterior regions of vermiform larvae in inducing melanization when injected into other vermiform larvae.

Region of C.N.S.	PF reaction	AF reaction
Median neurosecretory cells of the brain	+	+
Lateral neurosecretory cells of the brain	-	+
Corpora cardiaca	+	+
Corpora allata	-	+
All the ventral ganglia, the ventral connectives and the segmental nerves	-	-

Table 15. The paraldehyde fuchsin and acetaldehyde fuchsin staining reactions of the vermiform larva nervous system.

63 to 74.

The nervous elements present immediately posterior the metathoracic ganglion consist of the two large ventral connectives and seven smaller nerves containing approximately 200 to 400 axons each. The two ventral connectives are devoid of electron-dense granules but all seven of the smaller nerves contain axons with electron-dense spherical inclusions. Close to the metathoracic ganglion the axons containing electron-dense granules tend to lie centrally within the small nerves, but posteriorly these axons come to lie on the periphery of the nerves directly adjacent to the neural lamella or even running outside the main neural lamella.

The electron-dense material is confined to the length of the small nerves delimited by the ligatures C and D. Within this area the distribution of granules is diffuse. Nowhere are there large accumulations of granules comparable with the corpora cardiaca but throughout a length of almost a millimetre there are many hundreds of small accumulations of electron-dense granules. The individual granules are approximately 100 to 150 nm in diameter.

Both vermiform larvae and newly ecdysed first instar larvae were examined and, while there was no gross change in the number of granules present, there did seem to be a suggestion of a decrease in granule concentration after ecdysis. Figs 63-67 show transverse sections of some of the small nerves posterior to the metathoracic ganglion in vermiform and first instar larvae. Around the periphery of each of these nerves can be seen axons containing electron dense granules and each is directly adjacent to the neural lamella. Figs 68 to 74 show the individual axons containing electron-dense granules.

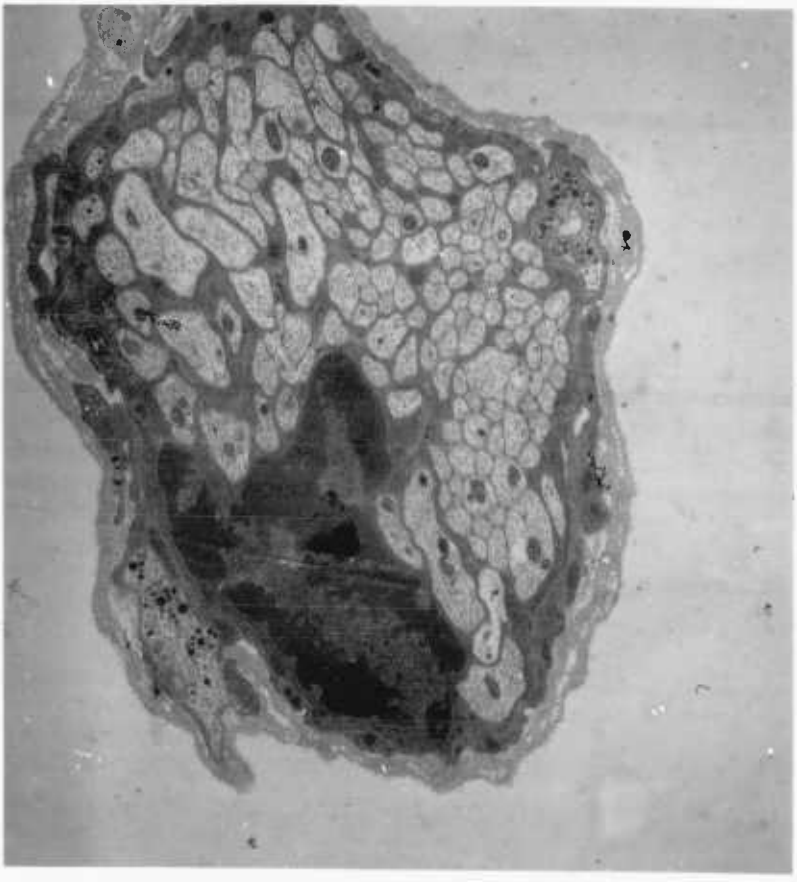


Fig. 63. A transverse section of one of the small nerves posterior to the metathoracic ganglion in a vermiform larva (x 13,000). Note the peripheral axons in the upper right and lower left hand corners containing accumulations of electron dense neurosecretory granules.

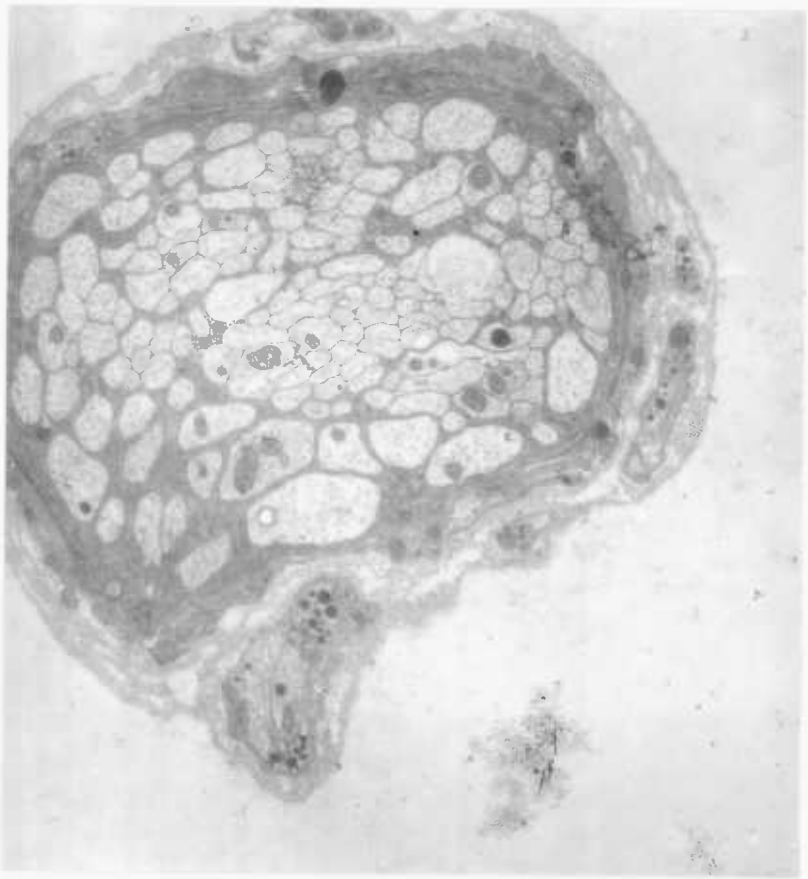


Fig. 64. A transverse section of one of the small nerves posterior to the metathoracic ganglion in a vermiform larva (x 13,000). The axons containing electron dense neurosecretory vesicles can be seen to be lying completely detached from the main nerve and bounded only by the neural lamella.

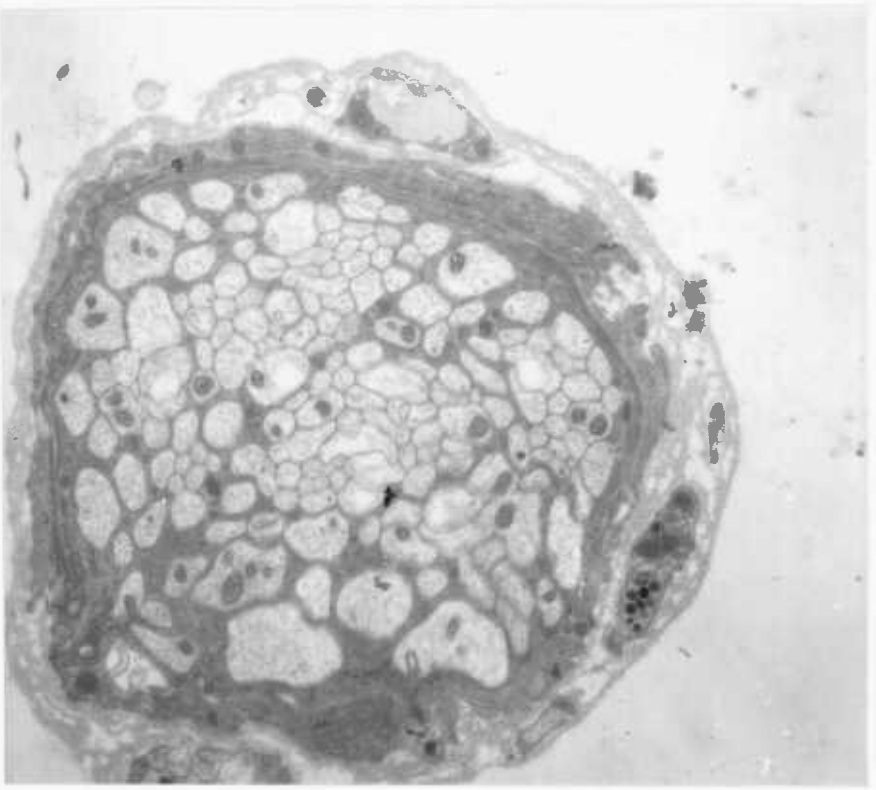


Fig. 65. A transverse section of a small nerve posterior to the metathoracic ganglion in a vermiform larva (x 13,000). The small accumulation of electron dense neurosecretory vesicles seen in the axon in the lower right hand corner is typical of the diffuse nature of the release site.

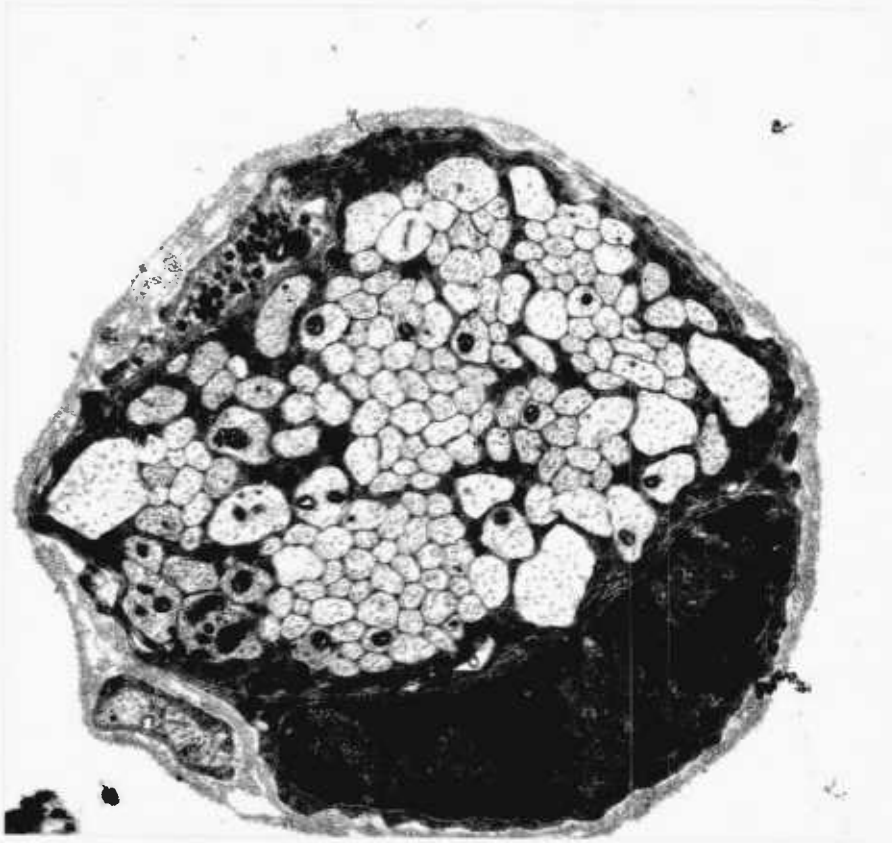


Fig. 66. A transverse section of one of the small nerves posterior to the metathoracic ganglion in a vermiform larva (x 13,000). The axon containing the electron dense neurosecretory vesicles in the upper left hand corner is lying peripherally but is not completely surrounded by the neural lamella. This is typical of nerve sections lying slightly nearer to the metathoracic ganglion than those showing completely detached axons as in Fig. 65.

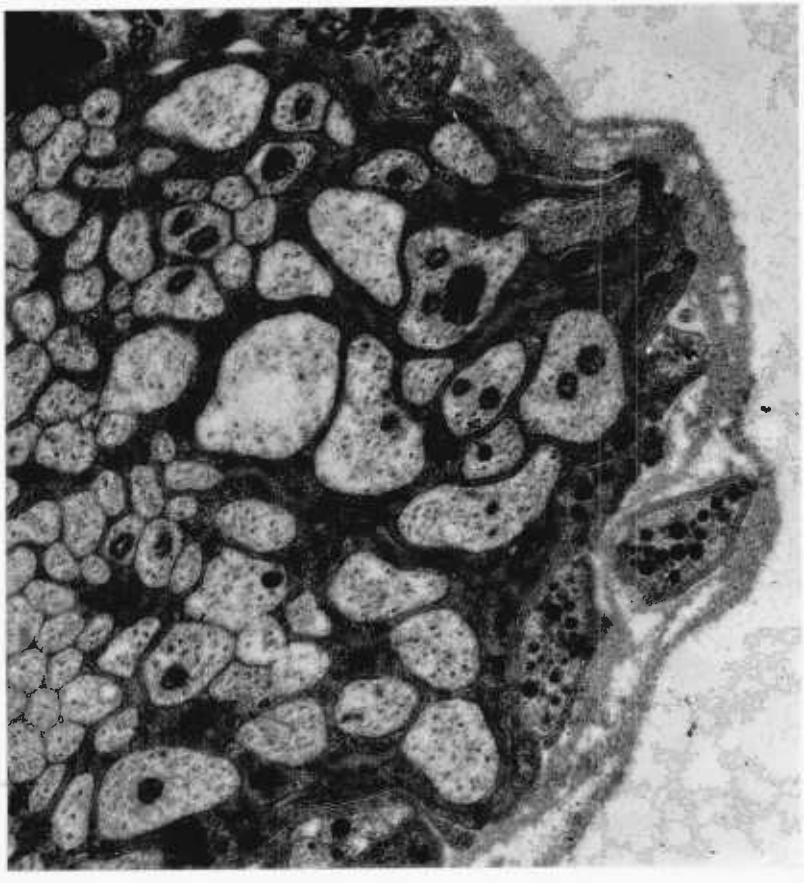


Fig. 67. A transverse section of part of one of the small nerves posterior to the metathoracic ganglion in a vermiform larva (x 20,000). Electron dense neurosecretory vesicles can be seen in three of the peripheral axons.

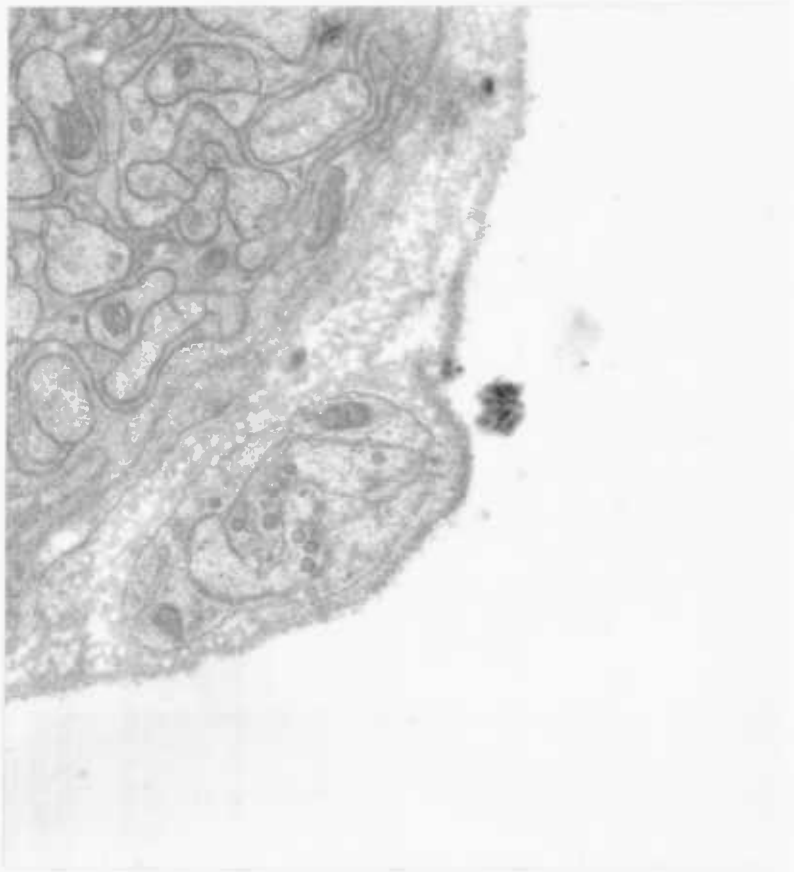


Fig. 68. A transverse section of peripheral axons in one of the small nerves posterior to the metathoracic ganglion in a first instar larva (x 26,000). A small group of axons containing electron dense neurosecretory vesicles lie in the neural lamella, detached from the main nerve.

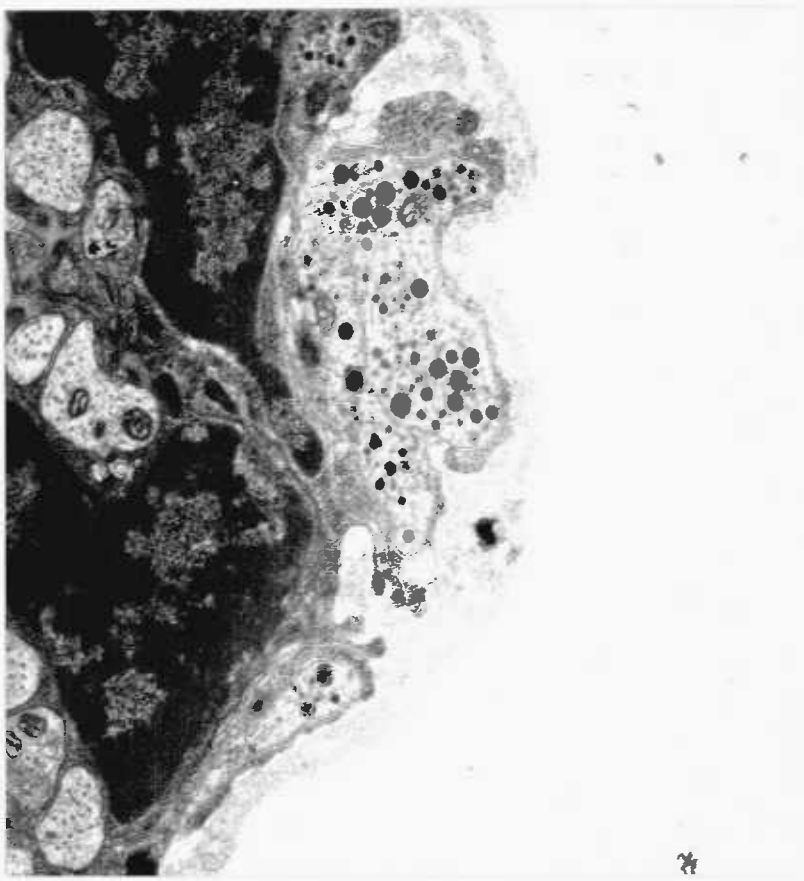


Fig. 69. A transverse section of the periphery of one of the small nerves posterior to the metathoracic ganglion in a vermiform larva (x 26,000).

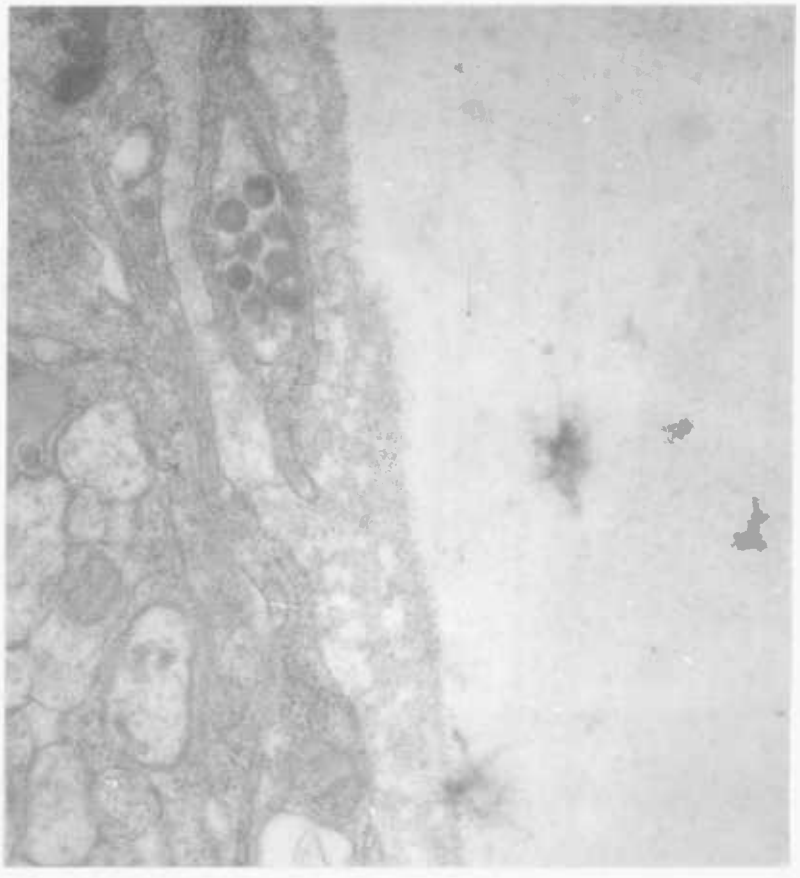


Fig. 70. A transverse section of a detached, vesicle containing axon, lying within the neural lamella of one of the small nerves posterior to the metathoracic ganglion in a first instar larva (x 50,000).

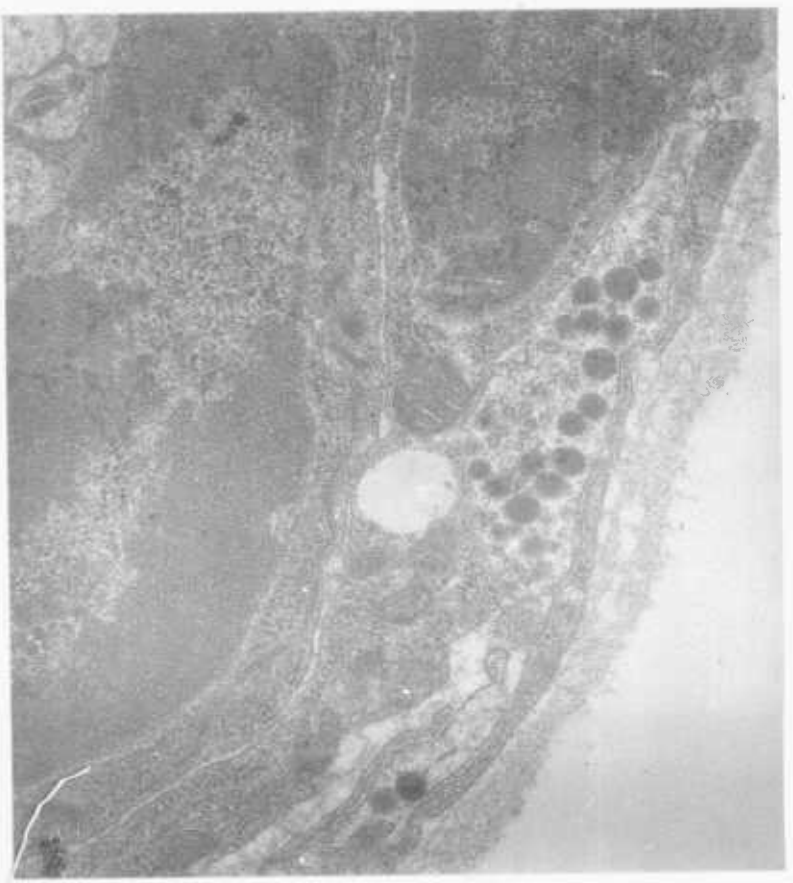


Fig. 71. A transverse section of an accumulation of electron dense neurosecretory vesicles within a peripheral axon of a small nerve posterior to the metathoracic ganglion in a vermiform larva (x 41,000).

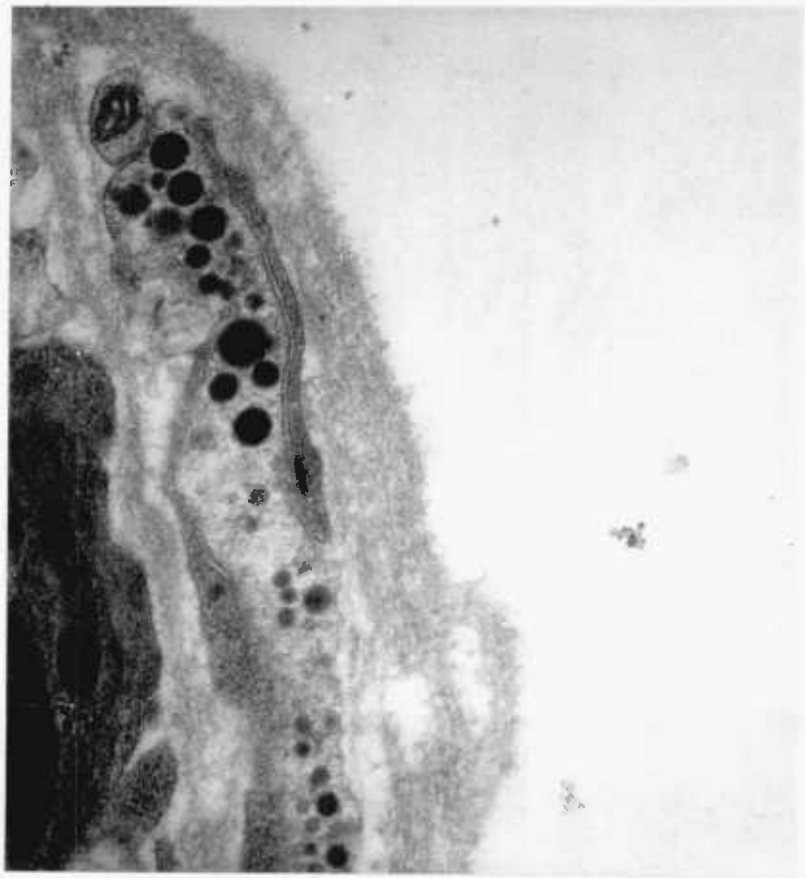


Fig. 72. A transverse section of an accumulation of electron dense vesicles, some of which can be seen to be bounded by a double membrane. The axon is at the periphery of a small nerve posterior to the metathoracic ganglion in a vermiform larva (x 66,000).

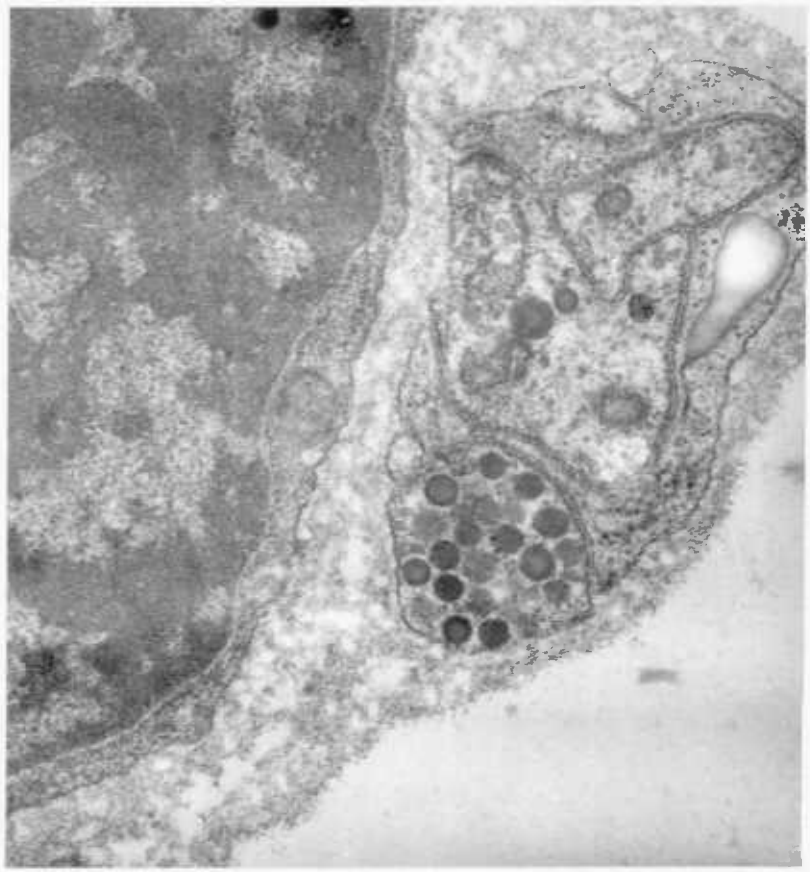


Fig. 73. A transverse section through a small group of axons containing electron dense vesicles. These axons are lying at the periphery of one of the small nerves posterior to the metathoracic ganglion in a first instar larva (x 50,000). As in other figures the neurosecretory vesicles are in close proximity to the haemolymph.

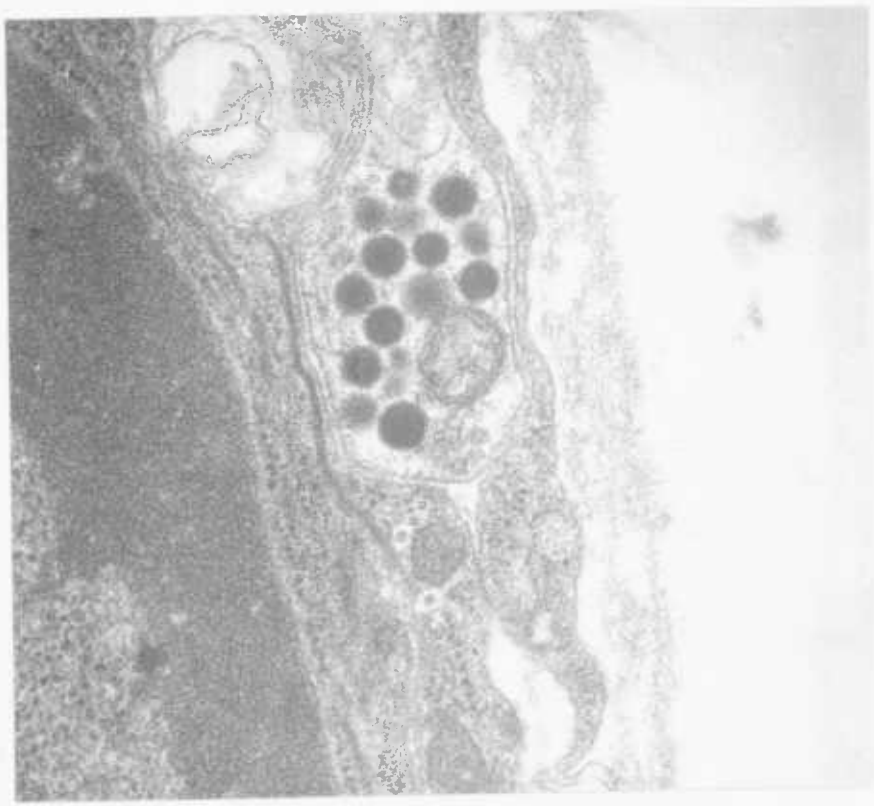


Fig. 74. A transverse section through an accumulation of electron dense vesicles in an axon at the periphery of one of the small nerves posterior to the metathoracic ganglion in a vermiform larva (x 66,000). As with Fig. 72 these vesicles can be seen to be bounded by a double membrane.

B. The relationship between the hormonal control of melanization and sclerotization.

i) Introduction.

In results section 3A (p 203) evidence was given for a blood-borne factor controlling the initiation of cuticular melanization. However since the experimental insects were examined only for melanization the analogy of this blood-borne factor with bursicon, the tanning hormone of other insects, was not demonstrated. Fränkel and Hsiao (1962, 1963, 1965) who originally described bursicon used the blowfly Sarcophaga bullata as their assay insect whilst Mills, Mathur and Guerra (1965) used neck ligatured Musca domestica L. to assay the blood of Periplaneta and Vincent (1972) used neck ligatured Phormia terraenovae Rob.-Desv. to assay Locusta and Schistocerca haemolymph. All of these flies proved to be successful recipient assay insects for bursicon and in the present study another blowfly, Calliphora erythrocephala was used as the assay recipient insect.

Whilst melanization and sclerotization are biochemically distinct (Dennell 1958), they are considered as having the same hormonal control by the hormone bursicon (Fogel and Fraenkel 1969). This relationship between sclerotization and melanization has been investigated by a comparison between the sclerotizing and melanizing potential of blood from newly ecdysed melanizing, non-melanizing green, and albino first instar larvae of Schistocerca.

ii) The Blowfly melanization bioassay for bursicon.

Both neck-ligatured blowflies and vermiform larvae of Schistocerca were used as bioassay recipients. Into these were injected haemolymph from either newly hatched first instar larvae of Schistocerca or from 20 minute postemergence adult Calliphora.

The injection of locust haemolymph into vermiform larvae and of blowfly haemolymph into ligatured blowflies served as controls. After 3 h incubation the neck-ligatured blowfly adult and vermiform larvae recipients were scored for melanization on their respective scales. The results are given in Table 16.

There is no significant difference in the degree of melanization produced by either blowfly or locust haemolymph when injected into either bioassay insect.

iii) Cuticle digestion.

The digestibility of larval cuticle was used as a measure of its degree of sclerotization, the assay procedure being as described in the methods section, (p.63). Larvae at six stages on either side of hatching were assayed. The quantity of protein going into solution was measured spectrophotometrically. Mean optical density readings for each of the six stages are given in Fig 75.

It is evident that there is a slight decrease in the amount of extractable protein per unit weight during the 14 hr before hatching. After hatching the quantity of extractable protein decreases markedly over a period of about an hour, after which the decline is less pronounced. In terms of cuticular sclerotization these results suggest a slow increase during the terminal stages of development but a very sharp increase immediately after hatching.

iv) Cuticular histology.

It is noteworthy that both the onset of rapid sclerotization and the appearance of melanizing activity in the haemolymph are coincident with ecdysis. The association between melanization and sclerotization can be demonstrated further in ligatured larvae

Bioassay recipient	Bioassay donor	Melanization score in recipient			
		2	2.5	3	4
Neck-ligatured blowflies	First instar locusts	10	12	3	-
Neck-ligatured blowflies	Adult blowflies	2	13	6	-
Vermiform locusts	First instar locusts	3	-	12	19
Vermiform locusts	Adult blowflies	-	-	8	13

Table 16. Melanization induced in neck-ligatured blowfly adults and vermiform Schistocerca larvae when injected with haemolymph from newly ecdysed first instar Schistocerca larvae or from 20 min post-emergence adult Calliphora. The Table gives the number of insects giving each particular score.

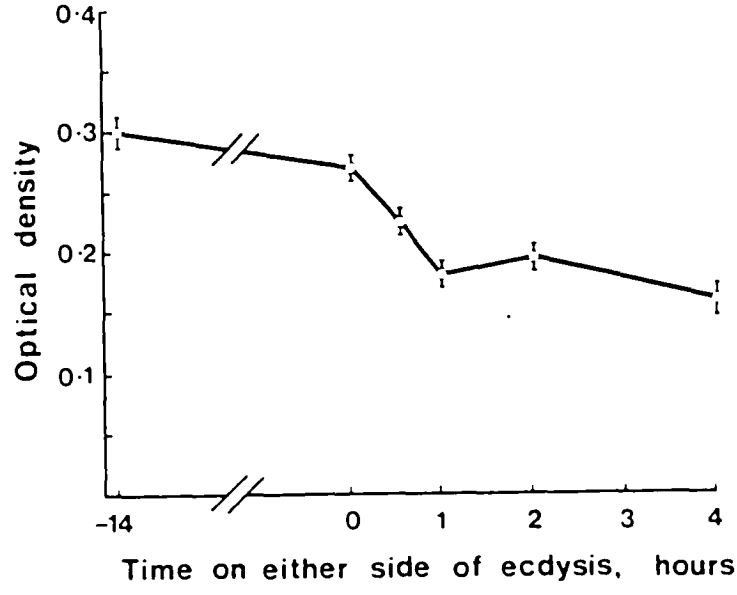


Fig. 75. Changes in the amount of cuticular protein soluble in weak alkali in the period from 14 hours before eclosion to 4 hours after eclosion. Vertical bars represent one standard deviation on either side of the mean.

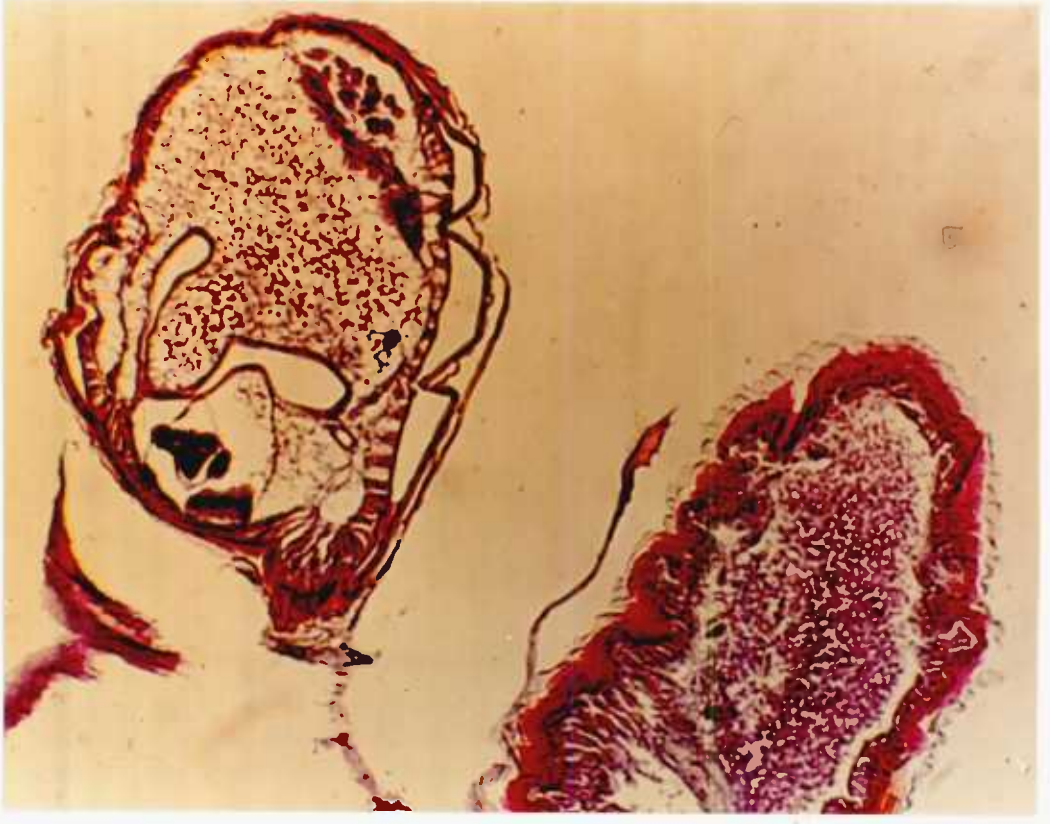
which were scored for melanization and then stained histologically for changes in sclerotization. Such a ligatured larva is shown in Fig 76, the ligature being between the prothoracic and mesothoracic legs. As described previously (p.207), the release site of the melanization hormone is immediately posterior to the metathoracic ganglion and thus the ligatured larva shows melanization posterior to the ligature but none anterior. The cuticular histology of this same larva is shown in Fig 77. The section cuts the prothoracic and mesothoracic femora. The prothoracic femur which shows no melanization, also exhibits the typically thick bright red staining cuticle of unsclerotized vermiform larvae. In contrast, the melanized mesothoracic femur is both more refractory to the acid fuchsin staining and shows the typically thin extended cuticle described by Bernays (1972b) for newly sclerotized 4 hr post-eclosion Schistocerca larvae. Similarly, when a larva is ligatured posterior to the release site, melanization and sclerotization are confined to the anterior end of the body.

v) Interchanges of haemolymph between melanizing, non-melanizing green, and non-melanizing albino larvae of Schistocerca.

Haemolymph from newly ecdysed larvae of each of the three locust forms was injected into vermiform larvae of a different form as described in the methods section, (p 64). Thus, haemolymph from green first instar larvae was injected into potentially melanizing vermiform larvae and vice-versa. Haemolymph from albino larvae was similarly interchanged. Melanization was recorded by observations of darkening, and sclerotization by the change in the histological appearance of sections to Mallory's triple stain from strongly acid fuchsin positive cuticle to thin non-staining cuticle. The results

Fig. 76. A first instar larva ligatured between the prothoracic and mesothoracic legs at the onset of the intermediate moult. Melanization is limited to that part of the body posterior to the ligature.

Fig. 77. A transverse section of the prothoracic femur (right hand side) and the mesothoracic femur (left hand side) of the ligatured larva shown in Fig. 76. The prothoracic femur shows the thick, strongly acid fuchsin positive cuticle characteristic of an unsclerotized vermiform larva. Conversely the mesothoracic femur shows the thinner, more extended cuticle, which is refractory to acid fuchsin staining, characteristic of a sclerotized first instar larva.



of these reciprocal blood transfers, together with those for two control groups, are given in Table 17.

Haemolymph from newly ecdysed melanizing, non-melanizing green and non-melanizing albino larvae is capable of inducing sclerotization and melanization in vermiform gregarious larvae even though the latter two forms do not themselves melanize. Conversely haemolymph from a newly ecdysed gregarious donor, which induces melanization in the donor, does not do so in either the green non-melanizing or the albino vermiform recipients, although it does induce sclerotization in both.

Number of larvae	Donor	Recipient	Melanization in recipient	Sclerotization in recipient
16	Non-melanizing first instar, just ecdysed	Potentially melanizing vermiform larva	16	16
9	Melanizing first instar, just ecdysed	Potentially non-melanizing vermiform larva	0	9
28	Albino first instar, just ecdysed	Potentially melanizing vermiform larva	27	27
25	Melanizing first instar, just ecdysed	Albino vermiform larva	0	25
10	Potentially melanizing vermiform larva	Albino vermiform larva	0	0
10	Albino first instar 5 h after ecdysis	Potentially melanizing vermiform larva	0	0

Table 17. The melanizing and sclerotizing potential of haemolymph from newly ecdysed melanizing, non-melanizing green and non-melanizing albino locust donors when injected into reciprocal recipients of these forms.

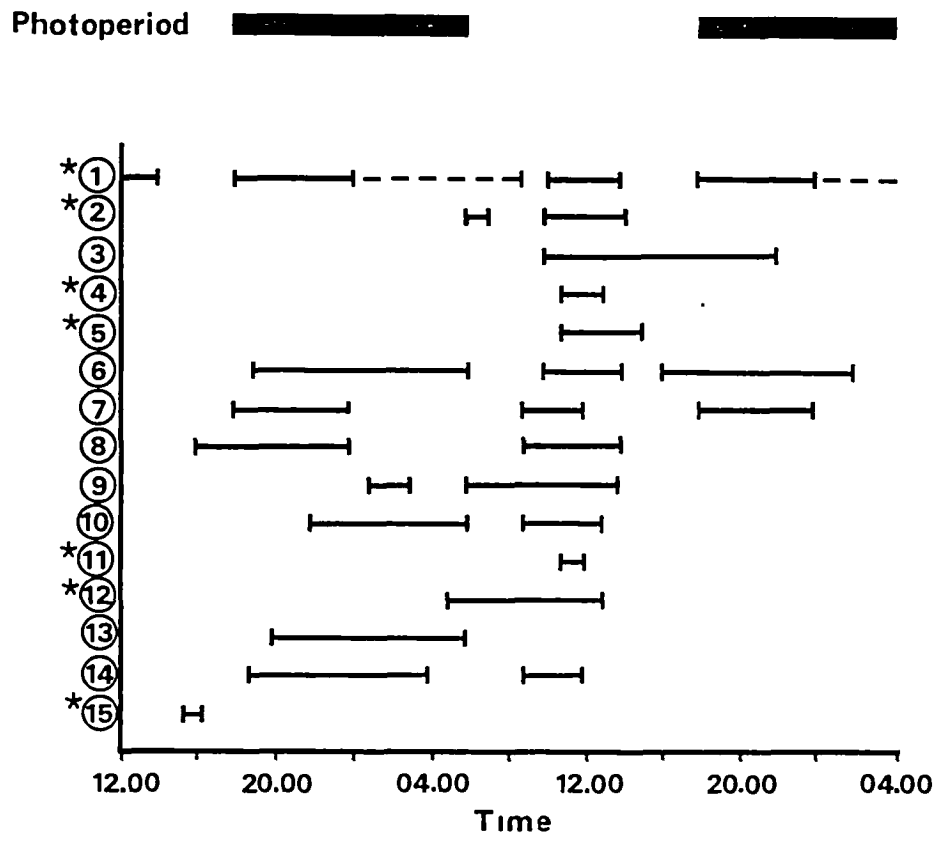
DISCUSSION

The behaviour and physiology of the pharate first instar and hatching locust.

It is a common feature of field reports concerning the behaviour of Acridids that the hatching of egg pods is synchronous throughout an oviposition site such that emergence is limited to a particular time of day. Uvarov (1966) suggests that for Acridids in general temperature must be important in stimulating hatching since below a given threshold the muscular activity associated with hatching is not possible. Rubtzov (1935) showed that on a hot sunny day emergence of the eggs of the Siberian grasshopper occurred during the morning whilst on a cold day emergence was protracted but occurred mainly in the afternoon. This would imply a direct relationship between temperature and hatching activity of the type envisaged by Uvarov (1966). A similar situation is reported by Pickford, (1966, 1976). The eggs of the two grasshoppers Camnula pellucida (Scudd) and Melanoplus bivittatus emerge at different times of the day but in both cases the onset of emergence is initiated by a rise in the soil temperature to the hatching threshold and emergence is inhibited if the soil temperature fails to reach this threshold on a particular day. Field reports of the emergence times of Schistocerca egg pods however suggest a rather different temperature relationship. In general Schistocerca egg pods are reported to emerge in the early morning, often before dawn, (Ellis and Ashall 1957, Ashall and Ellis 1962, Uvarov 1977, Hunter-Jones 1966a, Wardhaugh, Ashour, Ibrahim, Khan, and Bassonbol 1969, Roffey personal communication). At this time of day temperatures are close to the minimum and this suggests that any stimulation due to temperature must be in response to a fall in temperature rather than to an increase.

The observations of emergence times under semi-natural conditions at the Jeddah Research Station are in agreement with these earlier field reports. Emergence started at 05.05h and finished at approximately 06.30h with dawn at approximately 06.10h. Since emergence from Schistocerca egg pods shows no relationship to an increase in temperature and since the ambient temperatures throughout the invasion area almost never fall below the hatching threshold it seemed possible that hatching might be independent of temperature. The synchronous emergence might then be explained on the basis of synchronised oviposition by the adult females and an almost constant incubation period. Under natural conditions on the Arabian Tihama, under semi-natural conditions at the Jeddah Research Station and under artificial conditions at the laboratory at COPR there is a tendency for oviposition to occur in the evenings and to a lesser extent in the mornings (personal observations). To a large extent these observations are confirmed by those of other field workers, a summary of field data being given in Fig 78. Where observations have been continuous throughout 24 hours or more oviposition is frequently seen to commence at about sunset and to continue overnight. There is then a less extended oviposition period in the late morning. Since the egg pods collected in the laboratory at COPR were intended only to provide pods of exactly known age no attempt was made to examine oviposition times continuously. No oviposition tubes were therefore provided between 23.30h and 09.00h. Thus the extended overnight oviposition given in field data are not at variance with the present laboratory data in this respect. Both the present data and the earlier field data do however suggest that oviposition times show a much greater variability than reported emergence times and as such suggest that synchronous emergence is not a function of synchronised oviposition.

Fig. 78. The daily distribution of oviposition times as recorded by laboratory observations (upper line) or field observations (all other data). (* less than 24 hour observations).



*(1) Laboratory data.
*(2) Somalia.....Popov (1958)
(3) Somalia "
*(4) Kenya "
*(5) Kenya "
(6) Kenya "
(7) Kenya "
(8) Kenya "
(9) Kenya "
(10) Kenya "
*(11) Iran.....Popov (1954)
*(12) Iran "
(13) Ethiopia.....Ashall and Ellis (1962)
(14) Eritrea.....Stower, Popov and Greathead (1958)
*(15) Egypt.....Ballard, Mistikawa and Zoheiry (1932)

With respect to the duration of incubation in terms of degree days there is good evidence of a linear relationship between the rate of development and the incubation temperature. Hunter-Jones (1966a) reports that for Schistocerca the incubation period, when temperatures are between the limits of 15° and 35°C, is almost constant at 224 ± 12 degree days and calculation of the coefficient of linear correlation for this data gives r = 0.9196 (very strong). This relationship is reported to be valid with either constant, daily, cycled or mixed temperature incubation. Confirmation of this linear relationship is provided by the data of Wardhaugh, Ashour, Ibrahim, Khan and Bassonbol (1969) working with Schistocerca under semi-natural conditions, (Jeddah Research Station). Calculation from their data of the coefficient of linear correlation between development rates and incubation temperature gives r = 0.9590 (very strong), with a mean incubation of 226 degree days. Earlier data of Hamilton (1936) give a much longer mean incubation period of 265 degree days. However these data fail to show a good linear relationship over a range of three incubation temperatures and is therefore of doubtful value. Subsequent data (Hamilton 1950) give a closer linear relationship and the mean of 223 degree days is almost identical to that of Hunter-Jones (1966a). Shulov and Penner (1963) give data for incubation periods at only two temperatures, 27 and 20°C. However in this instance the incubation periods were 207 degree days at 37°C and 252 degree days at 20°C. Thus a greater total quantity of heat would appear to be required at 20°C than at 27°C. It would seem plausible that this extended incubation at 20°C might be due to the closeness of the incubation temperature to the hatching threshold of 19-20°C reported by Hunter-Jones. However Hunter-Jones himself shows that rates of

development remain linear with temperature down to 19.7°C and thus contradicts this explanation of the extended development period. The mean incubation period of 207 degree days at 27°C is rather lower than previous reports but the data of Tyrer (1970) tends to confirm that incubation periods may deviate considerably from the 224 degree days mean of Hunter-Jones. In the data of Tyrer (1970) incubation periods extend from 179 degree days to 222 degree days (all at 28°C) and have a mean of 198 degree days. Information concerning the methods of recording times of oviposition and hatching vary considerably in the above reports. The data of Tyrer (1970) would seem to be the most accurately timed, the oviposition time being accurate to within three hours and the emergence times being exact. In contrast the data of Wardhaugh et al (1969) is timed to within 24 hours at oviposition and to within 12 hours at emergence. In other cases no information is given. In the present study an effort was made to reduce errors in timing to a minimum. With the exception of three emergences times which were estimated to within an error of no greater than ± 3 hours all times are accurate to the nearest half hour. In view of the extended and variable oviposition time of two hours or more any increase in the accuracy of this time is of doubtful value. Similarly the emergence of even the majority of the eggs in a pod may take 15 minutes or more and so again any increase in accuracy is unlikely to be meaningful. The range of incubation periods are 186 to 219.6 degree days for pods incubated with a cycled temperature of 12 hours 29°C / 12 hours 33°C and 192.5 to 221 degree days for pods incubated with a constant temperature of 31.5°C. The respective means are 201 and 207 degree days. Thus whilst the linear relationship between the rate of development and incubation temperature is valid a review of the

present and earlier data suggests that the mean of 224 degree days may not be wholely representative of incubation periods in general. Thus with respect to both oviposition times and incubation periods there would seem to be sufficient natural variation in each to negate any hypothesis of synchronised emergence being the result of synchronised oviposition and constant incubation periods. It should also be noted that whilst the number of degree days of incubation may remain relatively constant the duration of incubation will vary with temperature. Thus deviations from a constant temperature - incubation time relationship must occur to accommodate the discontinuous daily pattern of emergence.

Whilst the preceding results demonstrate a lack of any quantitative relationship between oviposition time and emergence time they do suggest a relationship with temperature similar to that seen in the field. In both cases the eggs emerge with a high degree of synchrony during the cold period of the day. Further it is shown that under both field and laboratory conditions the time of emergence is not related to absolute temperature but rather to the magnitude of the change in temperature experienced by the pharate first instars immediately prior to emergence. Thus a drop in temperature of 10°C from 35°C to 25°C resulted in emergence approximately three hours after the cold drop whilst a drop of only 2°C from 32 to 30°C resulted in emergence approximately six hours after the cold drop. In contrast when a drop in temperature of 5°C is given at three different absolute temperatures, 38 to 33°C, 33 to 28°C, and 28 to 23°C, the latencies between the temperature drop and emergence are 4 h, 0 min, 4 h, 30 min and 4 h, 54 min respectively. These three distributions are not significantly different from one another.

However the coefficient of linear correlation between the magnitude of the temperature change and the latency to emergence, as shown in Fig 14 is strong ($r = 0.8190$). Under field conditions a similar relationship is seen. Those egg pods incubated in the soil and experiencing a relatively small change in temperature (approximately 4 - 5°C) emerge at approximately 06.00h on the final day. However those pods incubated at the ambient air temperature experience a greater change in temperature, (approximately 12 - 13°C) on the final night of incubation, and emerge at approximately 02.30h. This relationship is difficult to quantify because of the necessity to apply an arbitary time for the onset of the cold stimulus. However qualitatively the field data are consistant with the temperature change/emergence latency relationship demonstrated in the laboratory.

The reason for the above relationship is not immediately obvious but data from the filming of developing pharate first instars suggest an explanation. It has been shown that the onset of the hatching effort period coincides very closely with the onset of the cold period. Thus the latency between the temperature drop and hatching can be assumed to represent approximately the hatching effort period. Unfortunately all but one of the filmed hatchings were after a temperature drop of 5°C, the other being with no temperature drop. Thus the only comparison which can be made of activity after temperature drops of different magnitudes is with 5°C and 0°C. Comparing the activity of those pharate first instars given the 5°C cold drop with those pharate first instars kept at constant temperature gives filmed hatching efforts at a mean of approximately 5/87 frames (3 hours) for the group given no cold drop and 25/87 frames for the group given the 5°C cold drop. Thus

the cold drop would appear to be a stimulus to activity and by projection a greater cold stimulus might be expected to produce a stronger series of hatching efforts resulting in earlier hatching. This hypothesis is not inconsistent with the behaviour of hopper and adult locusts in which a transient increase in activity in response to a reduction in temperature has been demonstrated in both (Kennedy 1939, Chapman 1955, 1959). The film evidence contradicts this hypothesis to an extent in so far as the mean hatching time of the pharate first instars given two days at constant temperature is earlier than those given the 5°C cycled incubation. However as demonstrated in Fig 16 periods of more than a day at constant high temperature tend to produce both an increase in the variability of the emergence time and a general advancement of the mean emergence time. Thus the earlier hatching time of the group given no temperature drop does not invalidate the correlation between the cold stimulus and the increased hatching effort activity.

Of the various cycled temperature regimes used to establish a rhythm of hatching activity the smallest temperature change given was 2°C. Whilst the latency of response to this temperature change was greater than that of larger temperature changes it was shown to be sufficient to establish synchronised emergence. Thus the pharate first instar has a physiological mechanism capable of detecting changes in temperature at least as small as 2°C. This is not however in the least unique amongst insects. The tarsal cold receptors of Periplaneta have been demonstrated to be sensitive to a 1°C temperature reduction (Kerkut and Taylor 1957), Cimex is sensitive to a 1°C increase in temperature (Sioli 1937) and bees are similarly sensitive (Heran 1952).

The relationship between the magnitude of the stimulating temperature drop and the activity of the pharate first instar at the time of hatching is interesting in so far as it explains the behaviour of the pharate first instars subjected to a rapid temperature change but cannot in itself be the only influential factor. Under both field conditions and conditions of constant temperature on the last day of incubation in the laboratory no rapid temperature change occurs. However despite this, emergence from a group of egg pods is both synchronous with one another and at approximately the same time of day as with egg pods given the final cold stimulus. This suggests that the pharate first instars become entrained to the environmental temperature rhythm during incubation and will then initiate the pattern of behaviour leading to hatching in the absence of the final environmental cues.

The establishment of a circadian rhythm of behaviour is a characteristic common to a vast number of insects, (see Brady 1974, Saunders 1976 appendix B), but a rhythm of hatching has only previously been demonstrated in Dixippus morosus (Brunner) (Kalmas 1938), in the moths Halisidota argentata Pack. and Nepytia phantasmaria Stkr. (Edwards 1964) and in the pink bollworm Pectinophora gossypiella (Saunders) (Minis and Pittendrigh 1968). With both Nepytia and Pectinophora the peak of hatching coincides with the onset of the environmental photoperiod. With Halisidota however the emergence peak is between 20.00h and 24.00h under ambient environmental conditions and with Dixippus is between 24.00h and 04.00h.

With the eggs of Schistocerca the data from experiments in which the developing pharate first instars were given 1 - 4 days

at constant temperature at the end of incubation, after a previously cycled temperature incubation, suggest that entrainment may be established at some early stage of embryonic development but that this is then gradually lost with increasingly longer periods at constant temperature. Alternatively rhythmicity may only be established towards the end of incubation, the long periods of constant temperature then preventing the entrainment of hatching. In fact, as Minis and Pittendrigh (1968) suggest, the truth may be a combination of both of these factors. It is known that many simple acellular life-forms are capable of sustained circadian oscillation and on this basis it is suggested that a lack of functional development within the brain cannot be regarded as evidence of an inability to sustain circadian oscillations. Thus circadian rhythmicity as a general phenomenon may well be established at an early stage of embryonic development. However Minis and Pittendrigh go on to suggest that the neuronal element within the brain which controls hatching may not become functional until the later stages of development and thus the specific entrainment of hatching is not phase set until this time. There is some support for this suggestion within the present data. A circadian rhythm of metabolite concentration in the haemolymph is evident at four days before hatching and may extend back before this. However the functional organisation of the brain, as indicated by the activity of acetylcholine esterase, shows its most rapid development over the last two days of incubation. (See Smallman and Mansingh, 1969 for review of correlations between the activity of acetylcholine esterase and nervous system development in insect embryos). Thus whilst the embryo may be receptive to entrainment from a very early stage, those functions which then become entrained will depend

on the state of development of the embryo. It is probable that the neuronal elements controlling such a specific behaviour pattern as this do not become a functional part of the brain until the period of rapid development within the last two days and are thus unreceptive to entrainment until this time.

Such an argument does, however, assume that the brain is the site of the circadian oscillator controlling hatching behaviour. This has not been demonstrated in the present study but work with both the cockroach (Roberts 1966, Brady 1967) and the cricket (Tyshchenko 1973) have shown that circadian activity rhythms are inhibited by either decapitation, cutting of the connectives between the brain and the thoracic ganglia or electrocoagulation of neural areas of the pars intercerebralis. There is thus indirect evidence to implicate the brain in the control of circadian hatching activity in Schistocerca pharate first instars. It should be noted that the results of Harker (1956, 1960) are at variance with other workers in so far as the suboesophageal ganglion rather than the brain is implicated as the site of circadian oscillation control. However this data remains to be confirmed.

The synchronous early morning emergence from a natural Schistocerca egg pod field, which is frequently reported in ecological references, would thus appear to be the result of a number of influences. Due to a variety of factors such as parental physiology and local variations in the microclimate of any given pod within the egg field the time of attainment of completed development will vary from one egg pod to another (Van Horn 1966). Thus for synchronous emergence to occur the most advanced egg pods must experience a delay in the expression of their innate emergence behaviour. The

quiescent period of variable duration provides the basis for this temporal manipulation of the behaviour of the population. Given this variable quiescent period the synchronisation of emergence is then achieved by an entrainment of the individual pharate first instars to the daily ambient temperature cycle. Hatching behaviour is then only initiated at the time of specific emergence gates in the early morning when temperatures are lowest. Superimposed upon this gated emergence there would also appear to be an innate behavioural tendency to become more active in response to a lowering of the ambient temperature. This behaviour is not seen other than on the final day of development of the pharate first instar but is seen throughout subsequent stages of the life cycle. The absence of this behaviour in the embryo is perhaps related to its relatively complex neuronal basis which might then be expected to be undeveloped until morphogenesis is virtually complete. The relative influence under field conditions of cold stimulation is difficult to ascertain. It would appear from experiments in which the temperature was maintained constant on the last day that the final cold drop is completely unnecessary and as such any response to it is unnecessary. However the fact that fully developed pharate first instars do respond positively to both sharp cold stimulation and to a gradual reduction in temperature makes it seem unlikely that this does not play a role in the synchronisation of the population at the time of hatching.

The eclosion of adult Drosophila from the pupa shows circadian entrainment to the ambient photoperiod cycle (Brett 1955) and Pittendrigh (1954) suggests that, as with proposals in the present study, development may be completed at any time overnight, the pharate adult then remaining quiescent until the dawn emergence gate.

However Harker (1965) disputes the existence of a quiescent period at the end of development and suggests that the whole of pupal development is entrained to the photoperiod and that emergence at dawn is the result of a summation effect of circadian rhythms of development at earlier stages. The length of the pupal period is then determined by the time of day, in a 12 : 12 LD rhythm, at which pupation occurred. Thus a larva pupating at 06.00 h might take 95 hours to complete development and the adult would then emerge at dawn. However a larva pupating at 21.00 h takes approximately 15 hours less to develop and therefore also emerges at dawn on the same day. With the present data the proposed quiescent period is demonstrated by the activity filming, respiration measurements and the ability to induce early hatching in some egg pods. Thus an alternative hypothesis of graded rates of development from an earlier fixed point of reference similar to that proposed by Harker for Drosophila seems improbable.

Amongst Acridids inhabiting cooler climates, evidence suggests that direct environmental influences rather than circadian rhythms play a major role in determining the time of day of emergence. Pickford (1967, 1977) has shown that emergence of Melanoplus reaches a peak in the early afternoon while for Camnula the peak is in the late morning and in both cases it is evident that hatching is directly inhibited by low temperatures at other times of the day. Superficially therefore the behaviour of these cool climate Acridids appears to differ from that shown by Schistocerca. However it is known that fully developed pharate first instar Schistocerca subjected to similar environmental conditions as temporate climate Acridids, i.e. sub-hatching threshold temperatures overnight followed by warm days, then behave accordingly and emerge as the temperature

increases (Bernays 1970). It is thus possible that given tropical environmental conditions the eggs of temperate climate grasshoppers may well then display the same circadian hatching behaviour as do Schistocerca eggs. Unfortunately no data are available with respect to this supposition.

In addition to the role of environmental influences on the time of hatching, physiological processes and in particular the digestion of the serosal cuticle, may also influence hatching time. Results presented in the present study demonstrate that there is a relationship between the state of digestion of the serosal cuticle and the latency to the time of hatching. Thus in at least some of the egg pods the onset of hatching may be delayed by this process, this being particularly so of egg pods given an early cold stimulus. Cuticle digestion could then account for some of the variation in hatching times within a group of egg pods hatching within any one day. If the digestion of the serosal cuticle was not subject to the temperature inhibition shown by activity on the final day but started once a specific developmental stage was reached and then continued uninterrupted, it might be expected that at the time of the onset of the hatching effort period serosal cuticle digestion would be variable throughout the population. Thus, given a uniform onset to the hatching effort period, variations in the state of serosal cuticle digestion would result in a variability of the actual time of hatching due to the extra time taken to complete digestion and/or rupture of the thicker cuticles. The data of Bernays (1972c) give serosal cuticle weights over the last three days prior to hatching for eggs from three egg pods. With two of these egg pods there is a steady decrease in serosal cuticle dry weight with no evidence of a quiescent period. In the third pod however there is a distinct

cessation of digestion over the first two thirds of the last day which would thus coincide with the activity quiescent period. The relationship of serosal cuticle digestion to the activity quiescent period thus remains unresolved.

Synchronous emergence is not only a characteristic of a population of egg pods but is also shown by the majority of pharate first instars within a single egg pod. The exact control of this simultaneous emergence has not been conclusively demonstrated but two aspects of pharate first instar behaviour provide an indication as to the mechanism. Bernays (1970) has shown that mechanical disturbance is a hatching stimulus. From the film of hatching from a whole egg pod it is evident that there is considerable activity of each individual embryo immediately prior to hatching. The film does not provide evidence of any apparent chain reaction of one embryo stimulating its neighbour into activity but the effect of activity throughout the pod does appear to be cumulative. At the time when the first vermiform larva hatches there is sufficient activity throughout the whole pod to cause the outer eggs, and in particular those at the top of the pod, to be shaken back and forth with some vigour. In view of the results of Bernays (1970) it is difficult to believe that this is not causing a general stimulation throughout the pod tending to lead to synchronised hatching. The second characteristic aspect of pharate first instar behaviour relating to hatching is not directly linked to the synchrony of hatching but more to the sequence. Results given in the present study have shown that, in agreement with Papillon (1960) and Venter and Potgieter (1967), there is a distinct tendency for the uppermost eggs to hatch first even when the eggs of a pod are isolated from one another. The significance of this in relation to hatching synchrony

is unclear. It might be expected that synchronous emergence would best be achieved if the uppermost eggs were to act as a plug, delaying the escape of the lower eggs until the majority of the eggs were hatched. However this appears not to be the case, simultaneous emergence of the majority of the eggs in a pod being achieved despite this innate tendency for the uppermost eggs to hatch first. This being the case, mechanical stimulation alone is perhaps sufficient to achieve synchronised hatching and the advanced development of the uppermost eggs may ensure the ease of escape for the lower eggs so benefiting the pod as a whole.

Whilst the hatching of Acridid egg pods has been mainly associated with specific environmental temperature conditions it has also been suggested that light and soil moisture may also stimulate hatching. Kunckel D'Herculais (in Uvarov 1928) suggests that light is necessary for the hatching of Dociastaurus moroccanus (Thunberg). There is also evidence that rain may stimulate the hatching of Chortoicetes terminifera (Walker) (Clark, Ashall, Waloff and Chinnick 1969) although whether or not the hatching stimulus provided by rain is a direct effect or acts via a simultaneous cooling is not known. However neither light nor wetting appear to stimulate the hatching of Schistocerca larvae. Field experiments demonstrated that covered egg pods with no access to the possible stimulation of dawn light emerged at the same time as uncovered pods and in both groups at least some of the pods hatched before dawn. With respect to soil moisture the data have shown that there is no significant change on the day prior to hatching and Bernays (1970) has demonstrated that the moistening of eggs on the final day of incubation is not a stimulus to hatching.

The lack of correlation between soil moisture and hatching might be expected on an ecological basis since in the outbreak area of the desert locust rainfall is always infrequent and may be limited to a single day even in a good year for rain.

The existence of the entrainment of overt behaviour to the environmental temperature rhythm is demonstrated by the synchronous emergence of egg pods during either the cold period of the day or the presumptive cold period when temperatures are maintained constant on the day of hatching. However hatching is a 'once only' occurrence and therefore cannot be examined as a daily phenomenon within an embryo population of uniform age. Thus in an attempt to find evidence of rhythmicity in the developing embryo and pharate first instar which might be associated with the hatching rhythm some physiological parameters were investigated.

From the filming of the developing embryos it was shown that by the eighth day of a 13 day incubation persistent activity is well established. However despite the apparent strong activity stimulation caused by a reduction in temperature at the time of hatching there is no evidence of this in the preceding days of development. Conversely there is no evidence of a more normal response to increased temperature in the form of increased activity. Thus despite a strong synchronisation of activity at the time of hatching this does not ensue from a pattern of activity laid down during the later stages of embryonic development. However the specific hatching effort activity shown immediately prior to emergence is distinct from the somewhat random and uncoordinated activity seen prior to the quiescent period. It might therefore be expected that these two behavioural patterns do not show a temporal relationship.

It is also probable that the establishment of an activity rhythm showing higher activity during the cold period than during the warm period might pose physiological problems in the developing embryo. At the time of hatching the muscular system of a pharate first instar is innervated and therefore the pharate first instar possesses a neuromuscular system capable of increased activity in response to a cold drop acting via either cold receptor neurones (Kerkut and Taylor 1958) or possibly direct stimulation of neurosecretory or other cells in the brain. However at three to four days before hatching, whilst embryonic activity is well established, available evidence suggests that the muscular system is uninervated (Tyrer 1968, Provine 1976) and as such incapable of displaying increased activity in response to cold stimulation.

Unlike activity, respiratory rates do show a distinct rhythmic fluctuation when the embryos are incubated with a fluctuating daily temperature rhythm. Thus up until the penultimate day metabolic activity would appear to be directly related to the incubation temperature. This is consistent with the linear relationship between development rates and temperature described by Shulov and Penner (1963) and Hunter-Jones (1966a). However the daily cycle of oxygen consumption is not circadian and thus differs from the rhythmicity demonstrated throughout the life cycle of Drosophila (Rensing 1966, Belcher and Brett 1973). During the penultimate and final days the profile of oxygen consumption shows the same quiescent phase as does activity. Also, as with activity, this quiescent period is characteristically present under both cycled and constant temperature conditions. Following the quiescent period there is then a return to a higher level of respiratory activity which is coincident with

the onset of the hatching effort period. Additionally amongst the eight embryos whose respiratory rates were recorded the variability during the hatching effort period is consistent with the variability in activity seen during the hatching effort period. Thus, whilst during early embryology respiratory rates most probably reflect the rate of developmental metabolism of the embryo as a whole, during the quiescent period and the hatching effort period respiratory rates consistently reflect the muscular activity of the pharate first instar. In Drosophila, Lints and Lints (1967) demonstrated that the rate of oxygen uptake rises more rapidly prior to hatching. Also, as with Schistocerca pharate first instars, the steeper increase coincides with the time of the first muscular contractions associated with hatching.

Whilst there is no circadian rhythm of oxygen consumption in developing embryos, metabolically there is evidence for just such a phenomenon. Concentrations of sugars (Anthrone positive material) in the haemolymph show a distinct fluctuation with a peak in concentration towards the beginning of each warm period. Unlike activity and respiration this rhythm persists throughout the penultimate and final days and also persists in the absence of a fluctuating temperature on the final day. It thus has the characteristics of a true circadian rhythm. It is also noteworthy that whilst the rhythm of oxygen consumption is not circadian, under cycled temperature conditions the peaks of both this and haemolymph sugar concentration are coincident up until the penultimate day. At this time the peak of oxygen consumption undergoes a 12 hour phase change whilst the rhythm of sugar concentration remains constant. In both the cricket Gryllus domesticus (Nowosielski and Patton 1964, 1964) and the cockroach Periplaneta (Hillard and Butz 1969) circadian

fluctuations of haemolymph trehalose have been demonstrated but in neither case could these fluctuations be correlated with either feeding or locomotor activity. In the present study there is also no apparent correlation with any behavioural rhythm and no immediately obvious connection with the time of hatching. Because of the small size of the embryos it was not possible to make reliable determinations of the blood volume and thus a circadian rhythm of blood volume could account for the apparent rhythm of sugar concentration and dilution. However if this were the case it would then imply a coincident fluctuation of amino acid concentration necessary to give the measured constant value. Thus in reality either haemolymph sugars or amino acids or both may be showing rhythmic changes depending upon the magnitude and phase relationship of either with changes in blood volume. However whilst the absolute relationship of changes in haemolymph metabolites have not been resolved the data are sufficient to imply a metabolic circadian rhythm of one or other of these factors. This in itself is significant.

The total concentration of amino acids in the haemolymph over the last three days of development has a mean of 15.8 µg/µl. The only comparable data are that of McGregor and Laughton (1976) for the embryo of Locusta. They report total amino acid concentrations of 60 - 66 µg/egg during the final stages of development. Taking the volume of an egg to be 15 µl then gives a concentration of approximately 4.2 µg/µl. This is a lower figure than in the present study but may reflect the difference between concentrations of free amino acid in the haemolymph and in the egg as a whole. Sugar levels also appear to be within the same order of magnitude

as reported for other Acridid embryos. Quickenden (1970) reports trehalose concentrations with a mean of approximately 1000 mg/100g in the eggs of the grasshopper Aulocara ellioti (Thomas). This compares with a mean of 1600 mg/100 ml of haemolymph in the present study. Again the higher figure given here may reflect differences in concentration between haemolymph and whole egg.

Within recent years an ever increasing number of physiological and behavioural functions within insects have been demonstrated to be under hormonal control (for reviews see Highnam and Hill 1969, Mordue and Goldsworthy 1969). Of particular relevance to the present study are the findings of Truman and Riddiford (1970) who have demonstrated a specific eclosion hormone in the silkmoth and those of Roberts (1966), Cymborowski (1970a, 1970b). Cymborowski and Dutkowski (1970) and Dutowski, Cymborowski and Przelecka (1971) who have demonstrated a relationship between neurosecretory cell activity in the brain and circadian activity rhythms in the cricket. In the silkmoth, eclosion behaviour is triggered by the clock controlled release of a specific eclosion hormone which then initiates the behaviour pattern leading to emergence from the pupa. In the cricket the rhythmic locomotor activity is suggested to be controlled via cyclic release of neurosecretion from the brain which leads to rhythmic inhibition of serotoninergic brain centres and subsequent inhibition of activity.

In the present study no evidence of a blood borne hormonal factor inducing hatching behaviour was found and thus a control system similar to that described by Truman and Riddiford (1970), Truman, Fallon and Wyatt (1976) for adult eclosion in silkmoths seems unlikely. Of the pharmacologically active compounds examined for

their effect on hatching behaviour, only serotonin was found to have any significant influence, resulting in a 20% reduction in the latency between injection and hatching when compared with Ringer injected controls. This is consistent with the results of Fowler and Goodnight (1966) who demonstrated coincident peaks of activity and 5-hydroxytryptamine concentration in the nervous system of the Arachnid Leiobunum longipes. Similarly Hinks (1967) found that administration of 5-hydroxytryptamine caused an increase in both the duration and amplitude of the nocturnal activity of Noctua pronuba L. and Agrotis ipsilon (Hufnagel). There is thus the possibility of a situation analogous to that described by Cymborowski (1970b) for the neurosecretion and serotonin controlled activity pattern seen in the cricket. Since neurosecretion from the brain is suggested to act directly on other brain centres there is no release of an activity stimulating hormone into the haemolymph. This is consistent with the present data.

It is also noteworthy that Van der Kloot (1955) working with Hyalophora cecropia (L.) pupae showed a simultaneous resumption of acetlycholine esterase activity and endocrine activity in the brain after diapause. Whilst no endocrine activity has been demonstrated in Schistocerca pharate first instars, both an acetylcholine esterase increase and serotonin stimulation, which have been correlated with neurosecretory activity in other insects, have been shown to occur. However the initiation of activity in response to the injection of a pharmacologically active compound is not in itself conclusive evidence that this behaviour pattern is initiated in this way endogenously. Thus the possible role of serotonin and neurosecretion in the initiation of hatching activity in Schistocerca must remain speculative. This is especially true

in view of the results of Brady (1967) and Tyshchenko (1973) who suggest that neurosecretion may not play any role in circadian activity control in the cricket.

The increase in levels of acetylcholine esterase activity during the latter part of embryonic development is of some significance independant of any possible correlation with endocrine activity. Clements (1963) has shown that Aedes embryos are stimulated to hatch by a low oxygen tension in the water. This low oxygen tension is perceived by a sensory centre in the head or thorax and maximal sensitivity coincides with a period of maximal activity in the central nervous system as indicated by the concentration of acetylcholine. Since acetylcholine and acetylcholine esterase are functionally interdependant in the brain the plateau in the concentration of acetylcholine esterase at the time of hatching demonstrated in the present study suggests a similar high sensitivity to environmental stimuli at this time.

Chino (1957) working with Bombyx mori L., David (1959) working with Pieris brassicae L. and Mehrotra (1960) working with Musca domestica and Oncopeltus fasciatus Dallas, have all demonstrated that the activity of acetylcholine esterase in embryos shows the same pattern of increase, relative to the rate of development, as in the present study. Additionally where acetylcholine esterase activity has been inhibited by eserine or organophosphate insecticides anatomical embryogenesis is normal up until the completion of development but the insects then fail to hatch. This is suggested to be due to the dual effect of toxic concentrations of acetylcholine and the inability to coordinate the muscular activity required to rupture the chorion. Thus a subthreshold level of

acetylcholine esterase might be at least one of the phenomena responsible for the failure of the lower eggs in a pod to hatch on the same day as middle and upper eggs. It would be interesting to compare the activity of acetylcholine esterase in hatched and unhatched larvae from the same pod. It would also be interesting to examine the activity of Schistocerca embryos poisoned with organophosphate insecticide. At the present time the nature of muscular activity in embryos has not been conclusively demonstrated. Tyrer (1968) has suggested that from the 67% stage of development (minus 2 - 3 days) the muscles are functionally inervated. However activity is well established at minus four to five days and it is probable that this is uninervated muscular activity. By inhibiting acetylcholine esterase activity one might then gain an indication as to whether or not this was the case and perhaps also of the stage at which neurogenic activity begins.

The ecological significance of emergence at, or just before, dawn is open to speculation but a number of adaptive advantages would appear to ensue from such behaviour. Emergence at dawn is a characteristic of many tropical insects, Drosophila, for example, emerge from their pupae shortly before sunrise (Bunning 1964). This behaviour pattern would appear to protect newly emerged insects in which the cuticle is only partially tanned and protected with wax (Wigglesworth 1945) from the lethal effects of desiccation since this is not only the coolest time of day but also the time of highest humidity. Additionally, Bernays (1972b) has shown that femur lengths of first instar Schistocerca are greater in larvae emerging at 25°C than at 31°C. The higher temperatures increase the rate of cuticular tanning resulting in hardening of the cuticle before expansion is complete. A similar effect is seen with the wing

size of emerging Aedes aegypti (L) adults, (Van den Heuvel 1963). Thus emergence into a cool climate will enhance the anatomical fitness of the insect.

The significance of synchronised emergence may be largely related to the adaptive advantage of a dawn emergence for if this is an advantage for one individual it will be an advantage to the population as a whole. However there may be inherent advantages to a synchronised emergence independent of the time of day. The avoidance of predation by the population as a whole is one such possibility. In the preliminary studies of emergence times carried out at the Jeddah Research Station almost all of the emerging larvae from the first day were captured by ants and it seems probable that it was only the large number of larvae present that enabled at least some of the hoppers to escape. However one could argue that synchronised emergence could also increase vulnerability to predation from reptiles capable of eating many larvae. It may be therefore that dawn is a time of relatively low predator activity, for which there is some evidence (See reviews by Cloudsley-Thompson 1961a, 1961b). An adaptive advantage of synchronised emergence of more specific significance to Schistocerca is perhaps related to its essentially gregarious behaviour throughout its life cycle. The adaptive value of gregarious behaviour is very much open to speculation but on the assumption that it does have an adaptive value it would then be advantageous to be gregarious from as early a stage as possible. The mass emergence of first instars within a few hours would provide for this immediate gregarisation of the population.

The control of cuticular melanization and sclerotization in the newly hatched first instar.

The results presented in the present study confirm those of Bernays (1972b) which indicated that melanization in first instar Schistocerca larvae is induced by a blood borne factor. Release of this factor occurs within two to three seconds of the embryonic cuticle first splitting, although it seems that the splitting itself is not actually necessary for the process to be initiated. This is much faster than the six to ten minutes after the start of ecdysis reported by Vincent (1971) for adult Locusta or the 10 to 20 minutes for Periplaneta (Mills 1966) and thus gives a more precise indication of the external triggering stimuli involved. As in the blowfly there is a total inhibition of darkening while the insects are digging up through the soil. Once at the surface, however, sensory input in both insects leads to the initiation of melanization. In the adult blowfly and vermiform larvae of Schistocerca there is an initial period of air swallowing although in neither case does this seem to be the trigger for the release of the melanizing factor. Cottrell (1962) has suggested that since the injection of active blood at the time of air swallowing interferes with expansion, presumably because tanning then starts prematurely, this is not the stimulus for release. Observations on Schistocerca larvae have shown that occasionally larvae will emerge, swallow air, and then do no more. In these instances there is no melanization.

Direct observations suggest that the trigger for release of the humoral factor is the horizontal displacement of the embryonic cuticle. This is effected by waves of abdominal contractions in the

two to three seconds just before and just after the cuticle splits (Bernays 1972c).

Not only does the release of the humoral melanizing factor occur very rapidly after triggering but, as shown in Fig 57, almost maximum activity is achieved within the first 30 seconds, the activity increasing only marginally in the next five minutes. There is then a progressive decline over the next three to four hours. Whilst the attainment of maximum melanizing activity is much more rapid than in adult Locusta (Vincent, 1971) or adult Manduca sexta (Truman, 1973), the general shape of the activity curve is very much the same, with maximum activity being attained very early and being followed by a progressive decline. In contrast, the activity curves for Periplaneta tanning hormone (Mills 1966) and for the blowfly (Fraenkel and Hsiao, 1965) are markedly different. In Periplaneta no activity is seen until ten minutes after ecdysis and maximum activity is attained after 1.5 hours, to be followed by a decline over another 1.5 hours. The blowfly shows some activity much sooner, after only two minutes, but again there is a delay, in this case of about 30 minutes, before maximal activity is attained. These data suggest that there is a difference between the control of the release of the tanning hormone in adult Locusta and Manduca compared to that in Periplaneta and the blowfly. The control of the release of the darkening factor of first instar larvae of Schistocerca belongs to the former category. Of all the insects examined for the nature of the hormonal control of tanning, the system in the cockroach Leucophaea maderae described by Srivastava and Hopkins (1975) is the most similar to that found in Schistocerca first instars. In both cases the hormone is found at a high level in the haemolymph before the process of ecdysis is completed and in

both cases the release of the hormone is from the thorax at a time exactly coincident with the first ecdysial splitting.

The application of ligatures to Schistocerca larvae has shown that the humoral factor controlling melanization is released from a point approximately midway between the metathoracic legs. A ligature in front of the metathoracic legs results in melanization in the posterior half of the body only, whereas a ligature diagonally between the metathoracic legs results in anterior melanization. The area delimited by these two ligatures as the site of release of the darkening factor does not contain any of the ventral ganglia but lies immediately behind the metathoracic ganglion. Although all previous descriptions of the release site of the tanning/melanization hormone have, with the exception of Manduca, related to ganglia, the possibility of its release from axon terminals just outside the main ganglion is not unreasonable. The diuretic hormone of Rhodnius prolixus, Stål. is released from a series of swollen axon terminals lying at the surface of abdominal nerves which fan out from the back of the ganglionic mass in the thorax (Maddrell 1966). It seems probable that this is also the case in Schistocerca first instar larvae. In Locusta the maximum titre of bursicon in the haemolymph occurs at ten minutes after ecdysis when eight gland-equivalents are present. However assay of all the ganglia and the corpora cardiaca before ecdysis give a total of only four gland-equivalents (Vincent 1972). This discrepancy could be due to a loss of material in the paired and segmental nerves attached to the ganglia which were specifically excluded from the assay. In Manduca (Truman 1973) bursicon is released from abdominal perivisceral organs but in Locusta Vincent (1972) specifically excludes these organs as possible release sites of bursicon and in first instar larvae of Schistocerca they often appear to be absent.

It is evident that the release of a hormone at a specific time in response to specific environmental or proprioceptive stimuli requires a neural control. The results suggest that in vermiform larvae of Schistocerca displacement of the cuticle at the start of ecdysis is the trigger. Further evidence for an initial neural pathway leading to the release of the melanization factor is given by ligature experiments, (see Fig 58) which cause severance of the ventral nervous system.

Considering the results on a temporal basis, at time (1), i.e. before ecdysis and with no subsequent ecdysis, the larvae remain unmelanized on both sides of the ligature no matter where that ligature is placed. Thus, unless ecdysis begins, melanization is not triggered. Conversely at time (4), that is some four to five seconds after the onset of the ecdysial split, melanization proceeds to completion on both sides of the ligature, again no matter where that ligature is placed. Intermediate to these two extremes, at times (2) and (3) for each of the five ligature positions, the pattern is more variable.

Considering the neck ligatured larvae, A2 and A3, isolation of the brain and suboesophageal ganglion in no way inhibits melanization in the body posterior to the ligature. The few instances in which darkening of the anterior parts occurred were probably due to the relatively late application of the ligature and this explanation probably accounts for many of the larvae which unexpectedly melanized on both sides of the ligature. As with the head, isolation of the terminal abdomen, E2 and E3, also fails to inhibit melanization in the rest of the body.

With the ligature in positions B, C and D melanization of the body region containing the release site occurred if the ligature was applied at time (3). For the same three ligatures at time (2), only D2 resulted in melanization of the body in the half containing the release site. In both B2 and C2 melanization was invariably totally inhibited. This result suggested that neither anterior to the neck nor posterior to the second abdominal body segment (ligature D) did disruption of the ventral nerve cord inhibit melanization in the body half containing the release site. Between these points, however, a disruption of the nerve cord at time (2) did inhibit melanization in the body half containing the release site. There is thus the suggestion of a neural signal coming by way of the prothoracic ganglion and passing back along the thoracic nerve cord to initiate release posterior to the metathoracic ganglion. The microclamps which were used to sever the ventral cord in various places provide additional evidence in support of this suggestion. When clamps were applied to the neck, dorsal thorax or the dorsal or ventral abdomen melanization was normal. When, however, clamps were applied to any part of the ventral nerve cord between the prothoracic ganglion and the area just posterior to the metathoracic ganglion, inhibition of melanization often occurred. Since these clamps always allowed continuous blood circulation, the results seem to confirm the role of the prothoracic ganglion and the ventral nerve cord posterior to this in stimulation of the release site.

Whilst a neuronal signal is present in the thoracic nerve cord, the origin of this signal is unknown. Since it is possible to ligature off the head once ecdysial movements have started, the brain cannot provide the final stimulus for release of the melanizing

factor. This is in agreement with Srivastava and Hopkins (1975) who demonstrated that the brain does not control the release of bursicon in Leucophaea either. Also with both Schistocerca and Leucophaea early artificial removal of the cuticle does not stimulate hormone release. It thus seems probable that the brain triggers the normal process of ecdysis whilst the ensuing behaviour then triggers the release of the melanizing factor. The brain is thus not required after ecdysis behaviour has started, but artificial removal of the cuticle without brain stimulation of the correct behaviour pattern is inadequate to induce release. Truman (1973) has shown that artificial removal of the pupal cuticle of Manduca will not cause bursicon release but that this occurs later at the time of normal circadian entrained eclosion.

Electrocoagulation of the area implicated by ligatures as the release site tended to confirm this location. There was a high percentage of both mortality and normal melanization in all groups but this probably reflects the narrow limits between excessive damage producing the former and insufficient tissue coagulation resulting in the latter. However, melanization was inhibited only in larvae with lesions in the area of the metathoracic ganglion and just posterior to it. That melanization was not inhibited by electrocoagulation of parts of the prothoracic and mesothoracic ganglia suggests that such electrocoagulation did not reach the relevant axons and interfere with neural conduction. Were it to have done so melanization should have been inhibited since an intact thoracic nerve cord has been shown to be necessary for the melanization inducing factor to be released. Confirmation that conduction can occur through a partially electrocoagulated

ganglion was obtained electrophysiologically by audio recordings from the ventral connectives of first instar larvae. Whole nerve recordings show a similar pattern of discharge to pro-tibia stimulation both before and after lesions to the mesothoracic ganglion.

There is evidence from Periplaneta (Gosbee, Milligan and Smallman 1968), Calliphora (Normann 1970) and Rhodnius and Glossia austeni Newstead, (Maddrell and Gee 1974) that elevated potassium ion Ringer can induce neurohormone release. Since it was found that melanization could be induced by a wide range of suspended homogenates of non-specific tissue, the high potassium Ringer of Maddrell and Gee (1974) was used. The injection of 2µl of the high potassium Ringer into intact vermiform larvae is not by itself sufficient to induce the release of the melanizing hormone. The extracts of anterior, middle and posterior body sections were therefore injected directly into vermiform larvae for bioassay. Both the anterior and posterior extracts gave a reaction which was less than 4% of the total normal melanization score. The middle body extract, however, gave a score of 18% of the normal. Whereas the middle extract gave a relatively low score, it should be noted that Maddrell and Gee (1974) report only a 10% extraction of diuretic hormone after 100 minutes and also that the middle extract gave a significantly higher score than either of the other two extracts.

Staining with acetaldehyde fuchsin and paraldehyde fuchsin gave no positive reaction in any part of the nervous system other than in the brain, corpora cardiaca and corpora allata. This is in agreement with the findings of Vincent (1972) and Farley and Evans (1972) who were unable to make any correlations between paraldehyde fuchsin or acetaldehyde fuchsin staining and bursicon content in or

adjacent to the terminal abdominal ganglia of Locusta and Periplaneta respectively.

Ultrastructural examination of the release site area has suggested that at least some and possibly all of the small nerves which emerge from the posterior end of the metathoracic ganglion have potential release site structures. Particularly characteristic are the axons which contain electron-dense vesicles and which lie adjacent to or outside the main neural lamella. These are very similar, both in overall appearance and granule size, to the neurohaemal organs containing electron dense granules described in the medial nerve of adult Schistocerca by Brady and Maddrell (1967). However in first instar larvae of Schistocerca the majority of granules were electron-dense and there were no large accumulations of the electron transparent granules described by Brady and Maddrell (1967) for adult Schistocerca. It is interesting to note that the ultrastructure of the melanization hormone release site in first instar larvae of Schistocerca closely resembles the diuretic hormone release site in Rhodnius (Maddrell 1966). Both systems have a release site which involves many hundreds of axon terminals lying adjacent to the neural lamella and arising from the fine nerves which radiate from the posterior end of the terminal thoracic ganglion. Additionally both of these hormones can be detected in a single sample of haemolymph and, as Maddrell (1966) points out, a large number of release sites increases the rate at which an effective concentration of the hormone in the haemolymph can be built up. In view of the known rapidity with which the hormone appears and reaches maximum activity in the blood, such a system provides optimum conditions for release.

That the blood borne factor causing cuticular melanization in Schistocerca larvae is analogous with bursicon has been demonstrated in two ways. Firstly in the reciprocal transfers of active haemolymph between vermiform larvae of Schistocerca and ligatured adult Calliphora there was no significant difference in the capacity of undiluted blood from either insect to induce darkening in the bio-assay. Secondly, that the Schistocerca melanization hormone is also a tanning hormone is shown by the ligatured larvae which both melanize and sclerotize in the body half containing the release site but do neither in the other half. There is also evidence for the coincident onset of both sclerotization and melanization. Melanization is initiated at the time of ecdysis. Similarly, as shown in Fig 75 the rapid onset of cuticular sclerotization begins at ecdysis. This is in agreement with the observations of Bernays (1972b) who also reports a decrease in the more soluble fraction of Schistocerca cuticle in the first hour after ecdysis. Thus whilst sclerotization and melanization are biochemically distinct (Dennell 1958) their control at the hormonal level in Schistocerca is mediated by the same hormone, bursicon. This is in agreement with the findings of Fogal and Fraenkel (1969) working with the blowfly Sarcophaga.

The reactions of the melanizing, non-melanizing green and albino larvae to the various reciprocal injections of active blood suggest that the hormone bursicon is acting as a trigger and that the determination of the type and degree of sclerotization and melanization is an epidermal property. Within a gregarious first instar larva there is both dark brown and relatively colourless sclerotization as well as melanized and non-melanized areas of cuticle. Andersen (1974) suggests that these types of sclerotization and the presence or absence of melanization are dependent upon

specific epidermal enzymes. Malek (1957) also attributes the presence or absence of melanin in the cuticle to a differential distribution of phenolase enzymes in the outer layers of the exocuticle. The results given in the present study confirm these suggestions in so far as they show that blood capable of inducing melanization in a potentially melanizing larva does not have this capacity in a green or albino larva. Conversely, blood which has the capacity to induce only sclerotization in a green or albino larva will trigger both sclerotization and melanization in a potentially melanizing larva. Thus, the difference between melanizing, non-melanizing green and albino larvae resides in the epidermis rather than in the hormone.

In gregarious adult Locusta migratoria migratorioides the development of the yellow mature male colouration is controlled by the corpora allata, (Pener Girardie and Joly 1972). Implants of corpora allata into newly ecdysed gregarious males will speed up this yellow colour development. However corpora allata implanted into solitarious Locusta males, which show yellowing on their hind wings, does not induce any further yellow colouration (Pener 1976). Thus, in the development of both the black colouration in first instar larvae of Schistocerca and the yellow colouration in adult male Locusta the hormonal factor which initiates the process in the gregarious phase does not do so in the solitarious phase. In both instances the cuticular or epidermal colouration depends on the potentiality of the epidermal cell and not upon the hormone, which only triggers (first instar Schistocerca) or triggers and maintains (adult male Locusta) this epidermal potentiality if it is present.

SUMMARY

THE TIME OF EMERGENCE : EXTRINSIC FACTORS.

1) Under semi-natural field conditions it has been demonstrated that the hatching of Schistocerca egg pods invariably occurs in the period of the day a few hours before and after dawn (see Fig 3 and Table 1).

2) The synchronisation of hatching is not a function of synchronised oviposition. There is a tendency for oviposition to occur at specific times of the day with peaks in the morning and evening (see Figs 4 and 78), but there is not the constancy of behaviour shown at hatching and field reports suggest that oviposition can occur at any time of the day.

3) The onset of hatching is initiated by a fall in the incubation temperature. However it appears not to be mediated via a directly metabolic process since the absolute temperature (within physiological limits) and the 'quantity of cooling' in terms of degree hours above the developmental zero are not related to the time of the onset of hatching (see Figs 10 and 13).

4) The time of hatching does, however, show a strong correlation with the magnitude of the temperature drop experienced on the last day of incubation (see Figs 12 and 14). A drop in temperature of 10°C results in a reduced latency between the time of the drop and hatching compared to a drop of only 2°C. It is suggested that hatching activity is at least partially mediated via cold receptor neurones which induce hatching effort activity in proportion to the magnitude of the stimulating temperature drop (p 87).

5) Whilst a reduction in temperature on the final day of incubation is a stimulus to hatching and results in a synchronisation of the hatching times of a population of egg pods, synchronous hatching will also occur with a constant temperature on the final day (see Fig 15). There is thus evidence that the cycled incubation temperature sets a biological rhythm within the pharate first instars so that they become entrained to hatch at the 'correct' time on the final day even in the absence of the final environmental cues.

6) With increasingly longer periods at constant temperature at the end of a cycled temperature incubation the synchronisation of hatching becomes progressively reduced (see Fig 16). With three to four days at constant temperature at the end of incubation hatching is random throughout the day. Thus either a rhythm is established and then lost during the period of constant temperature or it is not established until the final two to three days of development.

7) There are thus at least two environmental influences determining the time of hatching. A cycled incubation temperature results in entrainment being established with an ensuing gated hatching time. This is reinforced by the reduction in temperature during the final cold period of development which is itself a hatching stimulus.

8) Under field conditions these two influences are coincident and the duration of the emergence gate is relatively short. Under laboratory conditions, however, these two influences can be separated by giving the final reduction in temperature earlier than on previous days. This then results in an

increase in the duration of the emergence gate (see Figs 18 and 19). It is suggested that this spread of emergence times is brought about by the variability in the physiological age of individual pods within a population. Those pods which are most developmentally advanced respond to the earlier cold stimulus whilst the physiologically younger egg pods are unable to respond to this and hatch some time later at the time of the entrainment influence.

9) In addition to the directly stimulating influence of the reduction in temperature prior to hatching there is also limited evidence that the gradual reduction in temperature experienced by egg pods under natural environmental conditions may have a synchronising influence on the population as a whole (see Figs 9 and 23). However it is likely that this effect is due to the cold stimulus being perceived later, i.e., closer to the time of the entrainment influence, in egg pods experiencing the slow rate of temperature reduction. Thus at the time of stimulation a greater number of individuals within a population of egg pods have attained a state of readiness to hatch and as such emergence is more synchronised.

10) In the present study the mean duration of incubation was approximately 204 degree days. This is within the range of development periods reported by other workers. The present data also broadly agree with the linear relationship between development rate and incubation temperature proposed by Shulov and Penner (1963) and Hunter-Jones (1966a). It is, however, suggested that the phenomenon of gated emergence brings an element of discontinuity to the overall linear relationship.

11) There is no evidence that either light or soil moisture have any significant influence on the direct stimulation of hatching on the final day of development.

THE TIME OF HATCHING : INTRINSIC FACTORS.

12) Time lapse cinematography of developing pharate first instars incubated with a cycled temperature did not demonstrate any pattern of entrained circadian activity during development. However, the filming did demonstrate that the pharate first instars go through a phase of quiescence during the penultimate day. This quiescent phase is then broken with the onset of the hatching effort period which invariably starts at approximately the time of the final cold drop (see Fig 26). The demonstration of this quiescent period confirms behavioural work which suggested its existence in relation to the control of gated hatching.

13) Within a batch of eggs forming a whole egg pod the same pattern of activity behaviour is seen as with single embryos. There is, however, some evidence of a slower rate of development amongst the lower eggs of the pod and this is particularly evident at the time of hatching. The sequence of emergence as shown in Fig 34 is generally from the top of the pod downwards and many of the lower eggs fail to hatch at the time of this initial emergence.

14) These filmed observations are supported by the hatching times of top, middle and bottom eggs incubated in isolation. The hatching times of eggs from 9 egg pods are given in Fig 35. The advance in the hatching times of the top eggs compared to those in the middle and of the middle eggs compared to those at the bottom are highly significant in both cases.

15) Digestion of the serosal cuticle is a significant physiological factor controlling the time of hatching. Evidence given in Fig 39 demonstrates that the weight (thickness) of the serosal cuticles of the eggs in a pod is proportional to the latency to hatching of a similar group of eggs from the same pod.

16) Up until the penultimate and final day of development the rate of oxygen consumption of embryos shows a rhythm which is proportional to the incubation temperature (see Fig 41). However this rhythm is not circadian. On the penultimate day the rhythm becomes reversed, oxygen consumption being low during the warm period and high during the final cold period. To this extent oxygen consumption parallels the activity of the pharate first instar.

17) When given a cycled incubation temperature the haemolymph sugar concentration shows a rhythmic fluctuation (see Fig 43). If the incubation temperature is maintained constant over the last two days of development this rhythmic fluctuation of sugar concentration persists and is thus suggested to be circadian in nature (see Fig 44).

18) Under the same conditions haemolymph amino acid concentrations do not show any rhythmic fluctuation (see Fig 45).

19) No evidence was found to suggest that there is a hatching hormone similar in nature to the pupal eclosion hormone of silkmoths described by Truman, Fallon and Wyatt (1976). Neither the injection of corpora cardiaca homogenate, ecdysone, 20-hydroxyecdysone nor dibutyryl cyclic-AMP induced any significant reduction in the latency to the time of hatching.

20) Of the pharmacologically active compounds injected only 5-hydroxytryptamine (serotonin) induced a significant reduction in the latency to the time of hatching (see Fig 52). It is suggested that since 5-hydroxytryptamine has been shown to induce increased circadian activity levels in other insects this may also be of significance in the initiation of hatching activity in Schistocerca.

21) Reserpine, an antagonist of 5-hydroxytryptamine, was shown to have no apparent influence on the normal pattern of hatching behaviour.

22) The activity of acetylcholine esterase per unit weight of brain was shown to increase over the last five days of development (see Fig 54). Initially this increase is slow but rises more sharply in the two days prior to hatching. Coincidentally there is also an increase in the weight of the brain so that the total activity of acetylcholine esterase increases approximately $3\frac{1}{2}$ times over the last five days (see Fig 55). It is suggested that this may be of significance in relation to the sensitivity of the brain to environmental stimuli, the circadian control of hatching and the overall capacity of the pharate first instar to hatch.

THE CONTROL OF THE INITIATION OF POST INTERMEDIATE MOULT CUTICULAR TANNING.

23) There is a blood borne factor which is released within seconds of the onset of the intermediate moult which induces cuticular melanization. The activity of this factor in the haemolymph reaches a maximum within minutes and then shows a progressive decline over the next three to four hours (see Fig 57).

24) By the application of ligatures to vermiform larvae in the process of moulting the release site of this melanization inducing factor was narrowed down to an area at the junction of the thorax and abdomen (see Figs 58, 59, 60, 61 and 62).

25) By applying ligatures (which sever the ventral nerve cord) at specific times after the onset of moulting it was demonstrated that there is a neural control of the release of the melanizing hormone. This neural control is suggested to be initiated by the horizontal displacement of the embryonic cuticle at the onset of moulting. The sensory information is then conducted via the prothoracic ganglion and the ventral cord to the release site.

26) Both electrocoagulation and high potassium Ringer extraction of the ventral nerve cord suggested a neural origin for the hormone (see Tables 13 and 14). From the position of ligatures the exact point of the release site was shown to be immediately posterior to the metathoracic ganglion.

27) Electron microscopy of the nervous system posterior to the metathoracic ganglion has shown characteristic neurohormonal release sites on seven small nerves. Typically axons containing electron dense neurosecretory vesicles can be seen at the periphery of these small nerves (see Figs 63 - 69). Frequently these axons become completely detached from the other axons of the nerve and are bounded only by the neural lamella (see Fig 70). The distribution of many hundreds of neurohormonal release sites in almost immediate association with the haemolymph provides optimum conditions for the rapid release of the hormone which is as described (see Fig 57).

28) By use of the standard blowfly bioassay the melanization inducing factor in the haemolymph of newly moulted first instar Schistocerca is shown to be analogous with the hormone bursicon (see Table 16).

29) The analogy with bursicon is further demonstrated by showing that cuticular sclerotization is coincident with cuticular melanization on both a temporal and spatial basis (see Figs 75, 76 and 77).

30) The relationship between hormone and epidermis in the control of sclerotization and melanization was examined using two non-melanizing forms of Schistocerca. In both green (solitarious-like) and albino Schistocerca the hormone bursicon is released into the haemolymph at the onset of moulting but in both these cases only sclerotization is initiated. However when haemolymph from either of these two forms is injected into gregarious Schistocerca vermiform larvae both sclerotization and melanization are initiated. Thus whether or not melanization occurs is a property of the epidermis, the hormone bursicon only triggering this potentiality if it is present (see Table 17).

REFERENCES

ALBRECHT, F.O. (1962) Some physiological and ecological aspects of locust phases. Trans. R. ent. Soc. Lond. 114, 335-375.

ANDERSEN, S.O. (1974) Evidence for two mechanisms of sclerotization in insect cuticle. Nature Lond. 251, 507-508.

ASHALL, C. and ELLIS, P.E. (1962) Studies on the numbers and mortality in field populations of the Desert Locust (Schistocerca gregaria Forskål) Anti-Locust Bull. 38. pp59.

BALLARD, E., MISTIKAWI, A.M. and El ZOHEIRY, M.S. (1932) The desert locust, Schistocerca gregaria Forsk, in Egypt. Bulletin 110, Technical and Scientific Service, (Plant Protection Section) Ministry of Agriculture, Egypt.

BELCHER, K.S. and BRETT, W.J. (1973) Relationship between a metabolic rhythm and emergence rhythm in Drosophila melanogaster. J. Insect Physiol. 19, 277-286.

BERNAYS, E.A. (1970) Hatching in Schistocerca gregaria. (Forskål): Processes occurring between initial deposition and final hardening of the first instar cuticle. Unpublished PhD thesis London University.

BERNAYS, E.A. (1971) The vermiform larva of Schistocerca gregaria (Forskål): Form and Activity (Insecta, Orthoptera). Z. Morph. Tiere 70, 183-200.

BERNAYS, E.A. (1972a) The Intermediate Moult (First Ecdysis) of Schistocerca gregaria. (Forskål) (Insecta, Orthoptera). Z. Morph. Tiere 71, 160-179.

BERNAYS, E.A. (1972b) Changes in the first instar cuticle of Schistocerca gregaria before and associated with hatching. J. Insect Physiol. 18, 897-912.

BERNAYS, E.A. (1972c) Hatching in Schistocerca gregaria (Forskål) (Orthoptera, Acrididae). Acrida 1, 41-60.

BODINE, J.H. (1929) Factors influencing the rate of respiratory metabolism of a developing egg. (Orthoptera). Physiol. Zool. 2, 459-482.

BRADY, J. (1967) Control of the circadian rhythm of the cockroach. I. The role of the corpora cardiaca, brain and stress. J. exp. Biol. 47, 153-163.

BRADY, J. (1968) Control of the circadian rhythm of activity in the cockroach. III. A possible role of the blood electrolytes. J. exp. Biol. 49, 39-47.

BRADY, J. (1974) The physiology of insect circadian rhythms. Adv. Insect Physiol. 10, 1-116.

BRADY, J. and MADDRELL, S.H.P. (1967) Neurohaemal organs in the medial nervous system of insects. Z. Zellforsch. mikrosk. Anat. 76, 389-404.

BRETT, W.J. (1955) Persistent diurnal rhythmicity in Drosophila emergence. Ann. ent. Soc. Amer. 48, 119-131.

BUNNING, E. (1964) The Physiological Clock. Springer-Verlag Berlin.

CHAPMAN, R.F. (1955) A laboratory study of roosting behaviour in hoppers of the African migratory locust (Locusta migratoria migratorioides R. and F.) Anti-Locust Bull. 19, pp40.

CHAPMAN, R.F. (1959) Observations on the flight activity of the red locust, Nomadacris septemfasciata (Serville). Behaviour 14, 300-334.

CHAPMAN, R.F. (1971) The Insects : Structure and function. The English Universities Press.

CHEN, P.S. (1971) Monographs in developmental biology. Vol. 3 Biochemical aspects of insect development. S. Karger London + New York.

CHINO, H. (1957) Enzymatic synthesis and hydrolysis of acetylcholine in the eggs of the silkworm Bombyx mori. Annotationes Zool. Japon 30, 106-113.

CLARK, D.P., ASHALL, C., WALOFF, Z. and CHINNICK, L. (1969) Field studies on the Australian Plague Locust (Chortoicetes terminifera Walk). Anti-Locust Bull. 44, pp101.

CLEMENTS, A.N. (1963) The physiology of mosquitos. Pergamon Press, Oxford.

CLOUDSLEY-THOMPSON, J.L. (1961a) Adaptive function of circadian rhythms. Cold Spring Harb. Symp. quant. Biol. 25, 345-355.

CLOUDSLEY-THOMPSON, J.L. (1961b) Rhythmic activity in animal physiology and behaviour. Academic Press New York and London.

COTTRELL, C.B. (1962) The imaginal ecdysis of blowflies. The control of cuticular hardening and darkening. J. exp. Biol. 39, 395-411.

COTTRELL, C.B. (1964) Insect ecdysis with particular emphasis on cuticular hardening and darkening. Adv. Insect Physiol. 2, 175-218.

COTTREL, G.A. and LAVERACK, M.S. (1968) Invertebrate Pharmacology. A. Rev. Pharmac. 8, 273-298.

CYMBOROWSKI, B. (1970a) The assumed participation of 5-hydroxytryptamine in regulation of the circadian rhythm of locomotor activity in Acheta domesticus L. Comp. gen. Pharmac. 1, 316-322.

CYMBOROWSKI, B. (1970b) Investigations on the neurohormonal factors controlling circadian rhythm of locomotor activity in the house cricket Acheta domesticus L. 11. Daily histochemical changes in the neurosecretory cells of the pars intercerebralis and suboesophageal ganglion. Zoologica. Pol. 20, 127-149.

CYMBOROWSKI, B. (1970c) Investigations on the neurohormonal factors controlling circadian rhythm of locomotor activity in the house cricket Acheta domesticus L. 1. The role of the brain and suboesophageal ganglion. Zoologica. Pol. 20, 104-125.

CYMBOROWSKI, B. and DUTKOWSKI, A. (1970) Circadian changes in protein synthesis in the neurosecretory cells of the central nervous system of Acheta domesticus. J. Insect Physiol. 16, 341-348.

CYMBOROWSKI, B. and MUSZYNSKA, M. (1974) The effect of some psychotropic drugs on the circadian rhythm of locomotor activity of Acheta domesticus L. J. interdiscipl. cycle Res. 5, 362-370.

CYMBOROWSKI, B., SKANGIEL-KRAMSKA, K., and DUTKOWSKI, A. (1970) Circadian changes of acetylcholine esterase activity in the brain of house crickets (Acheta domesticus L.) Comp. Biochem. Physiol. 32, 267-370.

DAVID, W.A.L. (1959) The systemic insecticidal action of paraoxon on the eggs of Pieris brassicae (L.). J. Insect Physiol. 3, 14-27.

DENNELL, R. (1958) The hardening of insect cuticles. Biol. Rev. 33, 178-196.

DONALDSON, J.M.I. (1970) Differences between top and bottom eggs and between the resulting hopper and adult populations of two strains of Locusta migratoria migratorioides (R. and F.) Phytophylactica 2, 199-202.

DUCK, L.G. (1944) The bionomics of Schistocerca obscura (Fabr) J. Kansas Ent Soc. 17, 105-119.

DUTKOWSKI, A.B., CYMBOROWSKI, B. and PREZLECKA, A. (1971) Circadian changes in the ultrastructure of neurosecretory cells of the pars intercerebralis of the house cricket. J. Insect Physiol. 17, 1763-1772.

EDWARDS, D.K. (1964) Activity rhythms of Lepidopterous defoliators. II. Halisidota argentata Pack. (Arctiidae) and Nepytia phantasmaria Stkr. (Geometridae) Can. J. Zool. 42, 939-958.

ELLIS, P.E. and ASHALL, C. (1957) Field studies on diurnal behaviour, movement and aggregation in the Desert Locust (Schistocerca gregaria Forskål) Anti-Locust Bull. 25, pp94.

ELLIS, P.E. and HOYLE, G. (1954) A physiological interpretation of the marching of hoppers of the African migratory locust (Locusta migratoria migratorioides R and F). J. exp. Biol. 31, 271-279.

ELLIS, P.E., MORGAN, E.D. and WOODBRIDGE, A.P. (1972) Moult-inducing hormones of the prothoracic glands of insects. Nature 238, No. 5362, 274-276.

ELLMAN, G.L., COURTNEY, K.D., ANDRES, V., and FEATHERSTONE, R.M. (1961) A new and rapid colorimetric determination of acetylcholine esterase activity. Biochem. Pharmacol. 7, 88-95.

EWEN, A.B. (1962) An improved aldehyde fuchsin staining technique for staining neurosecretory products in insects. Trans. Am. microsc. Soc. 81, 94-96.

EWER, D.W. (1977) Two functions of the foam plug of Acridid egg pods. Acrida 6, 1-18.

FARLEY, R.D. and EVANS, S.J. (1972) Neurosecretion in the terminal ganglion of the cockroach Periplaneta americana. J. Insect Physiol. 18, 289-303.

FOGAL, W. and FRAENKEL, G. (1969) Melanin in the puparium and adult integument of the fleshfly, Sarcophaga bullata. J. Insect Physiol 15, 1437-1447.

FOWLER, D.J. and GOODNIGHT, C.J. (1966) The cyclic production of 5-hydroxytryptamine in the Opilionid. Am. Zool. 6, 187-193.

FOWLER, D.J., GOODNIGHT, C.J. and LaBRIE, M.M. (1972) Circadian rhythms of 5-hydroxytryptamine (serotonin) production in larvae, pupae and adults of Drosophila melanogaster (Diptera : Drosophilidae). Ann. ent. Soc. Am. 65, 138-141.

FRAENKEL, G. and HSIAO, C. (1962) Hormonal and nervous control of tanning in the fly. Science, Wash. 138, 27-29.

FRAENKEL, G. and HSIAO, C. (1963) Tanning in the adult fly: A new function of neurosecretion in the brain. Science, Wash. 141, 1057-1058.

FRAENKEL, G. and HSIAO, C. (1965) Bursicon, a hormone which mediates tanning of the cuticle in the adult fly and other insects. J. Insect Physiol. 11, 513-536.

GOODWIN, T.W. (1952) The biochemistry of locust pigmentation. Biol. Rev. 27, 439-460.

GOSBEE, J.L., MILLIGAN, J.V., and SMALLMAN, B.N. (1968) Neural properties of the protocerebral neurosecretory cells of the adult cockroach, Periplameta americana. J. Insect Physiol. 14, 1785-1792.

HACKMAN, R.H. (1967) Melanin in an insect Lucilia cuprina (Wied). Nature, Lond. 216, 163.

HACKMAN, R.H. and GOLDBERG, M. (1971) Studies on the hardening and darkening of insect cuticles. J. Insect Physiol. 17, 335-347.

HAMILTON, A.G. (1936) The relation of humidity and temperature to the development of three species of African locusts - Locusta migratoria migratorioides (R. and F.), Schistocerca gregaria (Forsk), Nomadacris septemfasciata (Serv). Trans. R. ent. Soc. Lond. 85, 1-60.

HAMILTON, A.G. (1950) Further studies on the relation of humidity and temperature to the development of two species of African locusts - Locusta migratoria migratorioides (R.and F.) and Schistocerca gregaria (Forsk). Trans. R. ent. Soc. Lond. 101, 1-58.

HARKER, J.E. (1956) Factors controlling the diurnal rhythm of activity of Periplaneta americana. J. exp. Biol. 33, 224-234.

HARKER, J.E. (1960) Internal factors controlling the suboesophageal ganglion neurosecretory cycle in Periplaneta americana. J. exp. Biol. 37, 164-170.

HARKER, J.E. (1965) The effect of a biological clock on the development rate of Drosophila pupae. J. exp. Biol. 42, 323-337.

HASKELL, P.T. and MOORHOUSE, J.E. (1963) A blood-borne factor influencing the activity of the central nervous system of the desert locust. Nature 197, 56-58.

HAZEF, M. and IBRAHIM, M.M. (1958) Studies on the egg and nymphal stages of Acrida pellucida Klug, in Egypt. Bull. Soc. ent. Egypte 42, 183-198.

HERAN, H. (1952) Untersuchungen uber den temperatursinn der honiybiene (Apis mellifera) unter besonderer berücksichtigung der wahrnehmung strahlender wärme. Z. vergl. Physiol. 34, 179-206.

HIGHNAM, K.C. (1962) Neurosecretory control of ovarian development in Schistocerca gregaria. Quart. J. micr. Sci. 103, 57-72.

HIGHNAM, K.C. and HILL, L. (1969) The comparative endocrinology of the invertebrates. E. Arnold London.

HILLARD, S.D. and BUTZ, A. (1969) Daily fluctuations in the concentration of total sugar and uric acid in the haemolymph of Periplaneta americana. Ann. ent. Soc. Am. 62, 71-74.

HINKS, C.F. (1967) Relationship between serotonin and circadian rhythm in some nocturnal moths. Nature, Lond. 214, 386-387.

HUNTER-JONES, P. (1957) An albino strain of the desert locust Nature, Lond. 180, 236-237.

HUNTER-JONES, P. (1966a) Studies on the genus Schistocerca with special reference to development. Unpublished Ph.D. thesis London University.

HUNTER-JONES, P. (1966b) Rearing and breeding locusts in the laboratory. Anti-Locust Research Centre. London.

JONES, B.M. (1956a) Endocrine activity during insect embryogenesis. Function of the ventral head glands in locust embryos (Locustana pardalina and Locusta migratoria, Orthoptera). J. exp. Biol. 33, 1, 174-185.

JONES, B.M. (1956b) Endocrine activity during insect embryogenesis. Control of events in development following the embryonic moult (Locusta migratoria and Locustana pardalina, Orthoptera). J. exp. Biol. 33, 4, 685-696.

JONES, B.M. and SINCLAIR, W. (1958) Induction of melanin patterns and of hardening as separate processes in the cuticle of albino locusts with internally absorbed phenol substrates. Nature, Lond. 181, 926-927.

KALMAS, H. (1935) Periodizitat und Autochronie als zeitregelnde Eigenschaftern der Organismen. Biol. gen. 11, 93-114.

KARLSON, P., KALLIOPE, E.S., und MARMARAS, V.I. (1969) Die Aminosäurezusammensetzung verchiedener Proteinfraktionen aus

der Cuticula von Calliphora erythrocephala in verschiedenen Entwicklungsstadien. J. Insect Physiol. 15, 319-323.

KASIM, A.A.I. (1973) The effect of neurosecretory nerve section upon the endocrine system of Locusta migratoria together with observations on the function of the lateral neurosecretory cells. Ph.D. Thesis University of Sheffield.

KENNEDY, J.S. (1939) The behaviour of the desert locust (Schistocerca gregaria (Forsk)) (Orthopt) in an outbreak centre. Trans R. ent. Soc. Lond. 89, 385-542.

KERKUT, G.A. and TAYLOR, B.J.R. (1958) The effect of temperature changes on the activity of poikilotherms. Behaviour 13, 259-279.

KOSTOWSKI, W., BECK, J. and MESZAROZ, J. (1966) Studies on the effect of certain neurohormones and psychotropic drugs on bioelectric activity of the central nervous system and behaviour in ants (Formic rufa L.) Acta. physiol. pol. 17, 98-110.

LAGUEUX, M., HIRN, M. and HOFFMAN, J.A. (1977) Ecdysone during ovarian development in Locusta migratoria. J. Insect Physiol. 23, 109-119.

LINTS, F.A. and LINTS, C.V. (1967) Consumption of oxygen by the egg of Drosophila melanogoster. Nature, Lond. 214, 785-787.

LOWERY, O.H., ROSENBOROUGH, N.J., FARR, A.L. and RANDALL, R.J. (1951) Protein measurement with the folin phenol reagent. J. Biol. Chem. 193, 265-275.

MADDRELL, S.H.P. (1966) The site of release of the diuretic hormone in Rhodnius. A new neurohaemal system in insects. J. exp. Biol. 45, 499-508.

MADDRELL, S.H.P. and GEE, J.D. (1974) Potassium - induced release of the diuretic hormones of Rhodnius prolixus and Glossina austeni: Ca dependence, time course and localisation of neurohaemal areas. J. exp. Biol. 61, 155-172.

MALEK, S.R.A. (1957) Sclerotization and melanization: Two independent processes in the cuticle of the desert locust. Nature, Lond. 180, 237.

McGREGOR, D.A. and LOUGHTON, B.G. (1976) Free amino acid content during development of the African migratory locust embryo. Insect Biochem. 6, 153-157.

MEHROTRA, K.N. (1960) Development of the cholinergic system in insect eggs. J. Insect Physiol. 5, 129-142.

MENG, H.L., CHANG, G.S., DU, S.L. and GE, J.Y. (1973) On the activity rhythms and interspecific dominance of three species of spotted bollworms (Lepi. Noctuidae). Acta. Entomol. Sin. 16, 32-37.

MILLS, R.R. (1966) Hormonal control of tanning in the American cockroach III. Hormone stability and post ecdysis changes in hormone titre. J. Insect Physiol. 12, 275-280.

MILLS, R.R., LAKE, C.R., and ALWORTH, W.L. (1967) Biosynthesis of N-acetyl dopamine by the American cockroach. J. Insect Physiol. 13, 1539-1546.

MILLS, R.R., MATHUR, R.B., and GUERRA, A.A. (1965) Studies on the hormonal control of tanning in the American cockroach. 1. Release of an activation factor from the terminal abdominal ganglion. J. Insect Physiol. 11, 1047-1053.

MILLS, R.R. and WHITEHEAD, D.L. (1970) Hormonal control of tanning in the American cockroach: Changes in blood cell permeability during ecdysis. J. Insect Physiol. 16, 331-340.

MINIS, D.H. and PITTENDRIGH (1968) Circadian oscillation controlling hatching: Its ontogeny during embryogenesis of a moth. Science 159, 534-536.

MOLOO, S.K. (1968) Experimental studies on the embryonic development of Schistocerca gregaria. Unpublished Ph.D. Thesis University of London.

MORDUE, W. and GOLDSWORTHY, G.J. (1969) The physiological effects of corpus cordiacum extracts in locusts. Gen. comp. Endocrinol. 12, 360-369.

MORGAN, E.D. and POOLE, C.F. (1976) The pattern of ecdysone levels during development in the desert locust Schistocerca gregaria. J. Insect Physiol. 22, 885-889.

NAYAR, J.K. (1969) The pupation rhythm in Aedes taeniorhynchus V. Physiology of growth and endogenous diurnal rhythm of pupation. Ann. ent. Soc. Am. 62, 1079-1087.

NORMANN, T.C. (1970) The mechanism of hormone release from neurosecretory axon endings in the insect Calliphora erythrocephala. In Aspects of Neuroendocrinology (Ed by Bargmann W. and Scharrer B.) pp 30-42 Springer, Berlin.

NORRIS, M.J. (1952) Reproduction in the Desert Locust (Schistocerca gregaria Forsk.) in relation to density and phase. Anti-Locust Bulletin 13, 49pp.

NOWOSIELSKI, J.W. and PATTON, R.L. (1964) Daily fluctuations in blood sugar concentration in the house cricket, Gryllus domesticus L. Science 144, 180-181.

NOWOSIELSKI, J.W. and PATTON, R.L. (1965) Variation in the haemolymph protein, amino acid and lipid levels in adult house crickets, Acheta domesticus L. of different ages. J. Insect Physiol. 11, 263-270.

PAPILLON, M. (1960) Etude preliminaire de la repercussion du groupement des parents sur les larves nouveaunees de Schistocerca gregaria Forsk. Bull. Biol. 93, 203-263.

PAPILLON, M. (1963) Influence de la temperature d'incubation sur le polymorphisme larvaire de Schistocerca gregaria Forsk. C.R. Acad. Sci. 256, 4098-4100.

PAPILLON, M. (1969) Donnees recentes sur l'origine du polymorphisme larvaire a l'eclosion chez le Criquet pelerin, Schistocerca gregaria Forskål. C.R. Acad. Sci. Paris 268, 1640-1642.

PENER, M.P. (1976) The differential effect of the corpora allata on yellow colouration in crowded and isolated Locusta migratoria migratorioides (R.and F.) males. Acrida 5, 269-285.

PENER, M.P., GIRARDIE, A. and JOLY, P. (1972) Neurosecretory and corpus allatum controlled effects on mating behaviour and colour change in adult Locusta migratoria migratorioides Males. Gen. comp. Endocr. 19, 484-508.

PICKFORD, R. (1966) The influence of date of oviposition and climatic conditions on hatching of Camnula pellucida (Scudder) (Orthoptera: Acrididae) Can. Ent. 98, 1145-1159.

PICKFORD, R. (1976) Embryonic growth and hatchability of eggs of the two striped grasshopper, Melanoplus bivittatus (Orthoptera: Acrididae), in relation to date of oviposition and weather Can. Ent. 108, 621-626.

PITTENDRIGH, C.S. (1954) On the temperature independence of the clock system controlling emergence time in Drosophila. Proc. nat. Acad. Sci. Wash. 40, 1018-1029.

PITTENDRIGH, C.S. (1966) The circadian oscillation in Drosophila pseudoobscura pupae: A model for the photoperiodic clock. Z. Pflanzenphysiol 54, 275-307.

POLIVANOVA, E.N. (1968) On mechanisms of hatching inhibition in insects at low temperatures. XIIIth International Congress of Entomology. Moscow.

POPOV, G.B. (1954) Notes on the behaviour of swarms of the desert locust (Schistocerca gregaria Forskål) during oviposition in Iran. Trans. Roy. Ent. Soc. Lond. 105, part 4 65-77.

POPOV, G.B. (1958) Ecological studies on oviposition by swarms of the desert locust (Schistocerca gregaria Forskål) in Eastern Africa. Anti-Locust Bulletin 31, pp 70.

POST, L.C. (1972) Bursicon: its effect on tyrosine permeation into haemocytes. Biochim. biophys. Acta. 290, 424-428.

POST, L.C. and DE JONG, B.J. (1973) Bursicon and the metabolism of tyrosine in the moulting cycle of Pieris larvae. J. Insect Physiol. 19, 1541-1546.

PREDTECHENSKII, S.A. (1935) Studies on the desert locust (Schistocerca gregaria Forsk) in central Asia and Transcaucasus in 1929-1930. Bull. Pl. Prot. Leningr. (Ent) II pp 92.

PROVINE, R.R. (1976) Development of function in nerve nets. (in) Simpler Networks and Behaviour. ed J.C. Fentress. Sinauer Associates, Massachusettes.

QUICKENDEN, K.L. (1970) Carbohydrates in eggs of the grasshopper Aulocara elliotti during development. J. Insect Physiol. 16, 171-183.

RAO, K.P. and GROPALAKRISHNAREDDY, T. (1967) Blood borne factors in circadian rhythms of activity. Nature, Lond. 213, 1047-1048.

RENSING, L. (1964) Daily rhythmicity of corpus allatum and neurosecretory cells in Drosophila melanogaster (Meig). Science N.Y. 144, 1586-1587.

RENSING, L. (1966) Zur circadianen Rhythmik des Saverstoffuerbranches von Drosophila. Z. vergl. Physiol. 53, 62-83.

RENSING, L. (1971) Hormonal control of circadian rhythms in Drosophila (in) Biochronometry. ed M. Menaker pp 527-540. National Academy of Sciences Press, Washington.

ROBERTS, S.K. de F. (1966) Circadian activity rhythms in cockroaches III. The role of endocrine and neural factors. J. cell. Physiol. 67, 473-486.

ROE, J.H. (1955) The determination of sugar in blood and spinal fluid with Anthrone reagent. J. Biol. Chem. 212, 335-343.

ROSEN, H. (1957) A modified ninhydrin colorimetric analysis for amino acids. Arch. Biochem. Biophys. 67, 10-15.

RUBTZOV, I.A. (1935) Regularities in the development and behaviour of Siberian Acrididae in connection with climatic factors. Bull. Acad. Sci. U.S.S.R. 1935, 789-824.

SALZEN, E.A. (1960) The growth of the locust embryo. J. Embryol. exp. Morph. 8, part 2, 139-162.

SAUNDERS, D.S. (1976) Insect Clocks. Oxford. Pergamon.

SELIGMAN, M., FRIEDMAN, S. and FRAENKEL, G. (1969) Hormonal control of turnover of tyrosine and tyrosine phosphate during tanning of the adult cuticle in the fly Scarophaga bullata. J. Insect Physiol. 15, 1085-1101.

SHULOV, A. and PENER, M.P. (1963) Studies on the development of eggs of the desert locust (Schistocerca gregaria Forskål) and its interruption under particular conditions of humidity. Anti-Locust Bulletin 41, pp 59.

SIOLI, H. (1952) The perception of warmth by the bed-bug Cimex. Zool. Jahrb. Physiol. 58, 284-296.

SLIFER, E.H. (1937) The origin and fate of the membranes surrounding the grasshopper egg; together with some experiments on the source of the hatching enzyme. Quart. J. Micr. Sci. 79, 3, 493-506.

SLIFER, E.H. (1938) A cytological study of the pleuropodia of Melanoplus differentialis (Orthoptera, Acrididae) which furnishes new evidence that they produce the hatching enzyme. J. Morph. 63, 1, 181-205.

SMALLMAN, B.N. and MANSINGH, A. (1969) The cholinergic system in insect development. Ann. Rev. Entomol. 14, 387-408.

SNEDECOR, G.W. and COCHRAN, W.G. (1967) Statistical methods. Iowa State University Press, U.S.A.

STOWER, W.J., POPOV, G.B. and GREATHEAD, D.J. (1958) Oviposition behaviour and egg mortality of the desert locust (Schistocerca gregaria Forskål) on the coast of Eritrea. Anti-locust Bulletin 30, pp33.

SRIVASTAVA, B.B.L. and HOPKINS, T.L. (1975) Bursicon release and activity in haemolymph during metamorphosis of the cockroach Leucophaea maderae. J. Insect Physiol. 21, 1985-1993.

SYMMONS, P.M., GREEN, S.M., ROBERTSON, R.A., and WARDHAUGH, K.G. (1974) The production of distribution maps of the incubation and hopper development periods of the desert locust Schistocerca gregaria (Forsk) (Orthoptera Acrididae) Bull. ent. Res. 64, 443-451.

TREHERNE, J.E. (1974) Insect Neurobiology. North-Holland Publishing Company, Amsterdam and Oxford.

TRUMAN, J.W. (1971) Physiology of insect ecdysis. 1. The eclosion behaviour of Saturniid moths and its hormonal release. J. exp. Biol. 54, 805-814.

TRUMAN, J.W. (1972) Physiology of insect rhythms. I. Circadian organisation of the endocrine events underlying the moulting cycle of larval tobacco hornworms. J. exp. Biol. 57, 805-820.

TRUMAN, J.W. (1973) Physiology of insect ecdysis. III Relationship between the hormonal control of eclosion and of tanning in the tobacco hornworm, Manduca sexta. J. exp. Biol. 58, 821-829.

TRUMAN, J.W., FALLON, A.M. and WYATT, G.R. (1976) Hormonal release of programmed behaviour in Silkmoths: Probable mediation by cyclic-AMP. Science 194, 1432-1434.

TRUMAN, J.W. and RIDDIFORD, L.M. (1970) Neuroendocrine control of ecdysis in Silkmoths. Science 167, 1624-1626.

TRUMAN, J.W. and SOKOLOVE, P.G. (1972) Silkmoth eclosion: Hormonal triggering of a centrally programmed pattern of behaviour. Science 175, 1491-1493.

TYRER, N.M. (1968) Some aspects of the functional developments in the locust embryo. Unpubl. Ph.D. thesis University of Cambridge.

TYRER, N.M. (1970) Quantitative estimation of the stage of embryonic development in the locust, Schistocerca gregaria. J. Embryol. exp. Morph. 23, 3, 705-718.

TYSHCHENKO, V.P. (1973) The role of nervous cells in circadian rhythm regulation in insects. In Neurobiology of Invertebrates Ed by Salanki J. Hungarian Academy of Science Budapest.

UVAROV, B.P. (1928) Locust and Grasshoppers. William Clower and Sons. London.

UVAROV, B.P. (1966) Grasshoppers and Locusts Vol. 1 C.U.P.

UVAROV, B.P. (1977) Grasshoppers and Locusts Vol. 2 C.U.P.

VANDENBERG, R.D. and MILLS, R.R. (1974) Hormonal control of tanning by the American cockroach: Cyclic-AMP as a probable intermediate. J. Insect Physiol. 20, 626-627.

VAN DEN HEUVEL, M.J. (1963) The effect of rearing temperatures on the wing length, thorax length and ovariole number of the adult mosquito Aedes aegypti (L). Trans R. ent. Soc. Lond. 115, 197-216.

VAN DER KLOOT (1955) The control of neurosecretion and diapause by physiological changes in the brain of the Cecropia Silkworm. Biol. Bull. Woods Hole 109, 276-294.

VAN HORN S.N. (1966) Studies on the embryogenesis of Aulocara elliotti (Thomas) (Orthoptera) 11. Developmental variability and the effects of maternal age and environment. J. Morph. 120, 115-134.

VENTER, I.G. and POTGIETER, W.E. (1967) Systematic discontinuities between eggs and hoppers from individual egg pods of the Brown Locust. S. Afri. J. Agri. Sci. 10, 293-298.

VINCENT, J.F.V. (1971) Effects of bursicon on cuticular properties in Locusta migratoria J. Insect Physiol. 17, 625-636.

VINCENT, J.F.V. (1972) The dynamics of release and possible identity of bursicon in Locusta migratoria. J. Insect Physiol. 18, 757-780.

WALL, C. (1974) The effect of temperature on embryonic development in Chesias legatella (Lepidoptera : Geometridae) J. Zool. Lond. 172, 147-168.

WARDHAUGH, K.G., ASHOUR, Y., IBRAHIM, A.O., KHAN, A.M. and BASSONBAL, M. (1969) Experiments on the incubation and hopper development periods of the desert locust (Schistocerca gregaria Forskål) in Saudi Arabia. Anti-locust Bulletin 45.

WIGGLESWORTH, V.B. (1945) Transpiration through the cuticle of insects. J. exp. Biol. 21, 97-114.