J. exp. Biol. (1975), 62, 611–627
With 1 plate and 9 figures
Printed in Great Britain

CORPUS ALLATUM ACTIVITY *IN VITRO* DURING OVARIAN MATURATION IN THE DESERT LOCUST, *SCHISTOCERCA GREGARIA*

BY STEPHEN S. TOBE* AND GRAHAME E. PRATT

Agricultural Research Council, Unit of Invertebrate Chemistry and Physiology, University of Sussex, Brighton BN1 9QJ, U.K.

(*Received 22 October* 1974)

SUMMARY

1. Radiochemical *in vitro* assays show that there are large and rapid changes in the spontaneous rate of juvenile hormone synthesis and release during the course of sexual maturation in the locust.

2. Parallel observations on glands incubated with farnesenic acid show that the rate-limiting step is always prior to the stage of esterification of farnesenic acid to the final intermediate, methyl farnesoate.

3. Corresponding changes in oocyte morphometrics do not reveal any clear correlation between endocrine activity of the corpus allatum and either the induction or maintenance of rapid vitellogenesis.

4. Peaks of synthetic activity in the corpus allatum correspond well with the onset of previtellogenic growth in the oocytes.

5. The data do not provide any evidence that 'corpus allatum insufficiency' is responsible for resorption of growing oocytes.

6. It is concluded that short-term *in vitro* radio-assays provide a valid method for estimating quantitatively the physiological activity of the corpus allatum.

INTRODUCTION

There is now ample evidence that the hormone of the corpus allatum (CA) of many insect species, including the desert locust, is necessary for sexual maturation and for maintenance of oocyte development (Engelmann, 1970; Wigglesworth, 1970). However, the precise role of the CA hormone in these processes remains undefined, and although there is little doubt that the hormone affects such related phenomena as 'patency' of the follicular epithelium (Telfer, 1965; Pratt & Davey, 1972), synthesis of female-specific protein by the fat body (Engelmann, 1971) and resorption of developing oocytes (Highnam, Lusis & Hill, 1963*a*, *b*), the mechanism of integrated control of ovarian maturation by CA hormone remains speculative. It has been difficult to relate these physiological events with the activity of the CA because there has been no direct and reliable assay for CA activity; activity could only be inferred from experiments involving gland extirpation plus hormone replacement therapy (Highnam *et al.* 1963*a*; Joly & Meyer, 1969), gland implantations (Joly, Joly, Porte & Girardie, 1968) and bioassay of extracts of haemolymph (De Wilde *et al.* 1968).

* Address as of 15 November 1974: Department of Zoology, University of Toronto, 25 Harbord Street, Toronto, Ontario M5S 1A1, Canada.

Previous studies on the role of CA hormone in sexual maturation have also been hampered by the fact that the identity of this hormone was unknown. Recently, however, the CA of *Schistocerca* spp. have been shown to synthesize the juvenile hormone C_{16}JH (methyl 10,11-epoxy-3,7,11-trimethyl-*trans*,*trans*-2,6-dodecadienoate) *in vitro* (Judy *et al.* 1973; Pratt & Tobe, 1974*a*, *b*). The identification of this hormone has permitted the development of an assay system for CA activity *in vitro* (Pratt & Tobe, 1974*a*; Tobe & Pratt, 1974, 1975) and it is now possible to monitor directly the activity of CA from the desert locust, *S. gregaria*, using simple *in vitro* procedures. In the present study we have employed these techniques to measure the activity of CA synthesizing C_{16}JH spontaneously, and when stimulated with the exogenous precursor, farnesenic acid (Pratt & Tobe, 1974*a*, *b*) during the first two gonotrophic cycles of the adult female desert locust. Using parameters such as oocyte length, patency of follicular epithelium and resorption of developing oocytes, we have attempted to correlate ovarian development with the rate of JH synthesis by the CA. This is the first report of the changes in synthetic activity of CA, measured by direct means, during the sexual maturation in any insect species and our results will demonstrate the complexity of the relation between JH synthetic capacity and sexual maturation.

MATERIALS AND METHODS

Rearing of locusts

Desert locusts, *S. gregaria*, were reared on fresh wheat seedlings. Their diet was supplemented with rolled oats and with an agar-based medium containing antibiotic (see below). The temperature of the rearing rooms cycled between 25 and 38 °C in synchrony with a photoperiod of 12L:12D.

Prior to this investigation it was discovered that our *S. gregaria* colony was infected with the parasitic protozoan *Malamoeba locustae* (Henry, 1968). Infected adult female *S. gregaria* do not undergo the normal colour change associated with maturation (from pink to yellow (Pener, 1967*a*)) and the rate of oocyte maturation is significantly depressed (unpublished observations). We tried to find a 'clean' colony within the U.K. so that we could establish a 'clean' colony in our laboratory but were unsuccessful, so we began treatment of our entire locust colony with the triple sulpha antibiotic Thipyrameth (Burns Biotec, 7711 Oakport, Oakland, California 94621, U.S.A.) as recommended by Henry (1968). The antibiotic (26% active ingredient) was incorporated into an agar-base medium containing salts, amino acids, lipids, sugars and wheat powder, at a final concentration of 0·5% (i.e. 10 ml antibiotic/l of medium), as suggested by Henry (personal communication). This medium was fed to all nymphal locusts daily. Adult insects received fresh wheat seedlings sprayed with a 1% solution of Thipyrameth once a week. Over a period of 6 months the infection was eradicated and this medium is now routinely fed to all locusts in this laboratory, as a prophylactic measure. All locusts used in the present study have been treated with the antibiotic throughout their nymphal life, but the adult insects were not treated. Under our rearing regime, female locusts oviposit their first batch of oocytes 12 days after fledging and their second wave 15–17 days after fledging.

Measurement of JH biosynthesis by CA

Corpora allata from adult female locusts from each day of the first two gonotrophic cycles (up to 16–17 days after fledging) were dissected as previously described (Pratt & Tobe, 1974*a*). Glands were incubated in 0·1 ml of Millipore-filtered TC 199 medium (20 mM Hepes buffered at 7·2) (Flow Laboratories, Edinburgh) containing Ficoll (20 mg/ml) and the appropriate radiolabelled compound as described by Tobe & Pratt (1974). At the end of the incubation period, the glands and medium were separated and each separately extracted, chromatographed by TLC and assayed for radiolabel incorporation into C_{16}JH as previously described (Pratt & Tobe, 1974*a*).

In order to assess the activities of the CA, two different experimental procedures were adopted. In the first series, each pair of CA from each insect was split between two tubes, each of which contained 4 CA from different animals per 0·1 ml medium and incubated for 3 h as previously described (Tobe & Pratt, 1974). The medium contained [*Me*-^{14}C]methionine (Radiochemical Centre, Amersham, U.K.; final specific radioactivity 36·5 mCi/mmol) in the case of one tube and [*Me*-^{14}C]methionine plus [^{3}H]farnesenic acid (25 mCi/mmol; final concentration, 20 μM) in the case of the other tube. Thus, the split pairs of CA were monitored for both spontaneous and stimulated C_{16}JH biosynthesis. In the second series of experiments, CA from four animals were incubated as individual pairs for 2 h in medium containing [*Me*-^{14}C]methionine. At the end of this period the medium was removed from the CA by aspiration and the CA were washed for 15 sec in non-radioactive medium. The wash was then removed by aspiration, and the combined media plus washings extracted and analysed for JH content. In the case of two of the pairs of glands the incubation was then terminated and the contents of the glands analysed as noted above. In the case of the remaining two pairs of glands, a further incubation of 2 h was carried out in medium containing both [*Me*-^{14}C]methionine and [^{3}H]farnesenic acid. At the end of this time, glands and medium were extracted separately as noted above. Four individual pairs of CA were used for each experimental day. Using this method, the capacity of individual CA to release C_{16}JH both spontaneously and when stimulated with [^{3}H]farnesenic acid can be measured on the same CA.

Oocyte length and patency of follicular epithelium

The lengths of the terminal (T), penultimate (T − 1) and antepenultimate (T − 2) oocytes of all experimental animals were measured on both fresh and modified Karnovsky-fixed (Huebner & Anderson, 1972) ovarioles by standard microscopical procedures. At least four ovarioles from each animal were measured. In addition, the number of ovarioles and the number of resorptive oocytes (see Lusis, 1963) were counted, and the number of resorptive oocytes expressed as a percentage of the total ovariole number.

The patency of the follicular epithelium of the T and T − 1 oocytes was assessed by the dye penetration method of Pratt & Davey (1972), using 1% Evans Blue in Ficoll–glucose–Ringer (Pratt & Tobe, 1974*a*). The penetration, if any, of the dye into the follicular spaces was observed on a Leitz inverted microscope and the follicles classified as either patent or non-patent. This distinction is easily made (see Fig. 10, Plate 1).

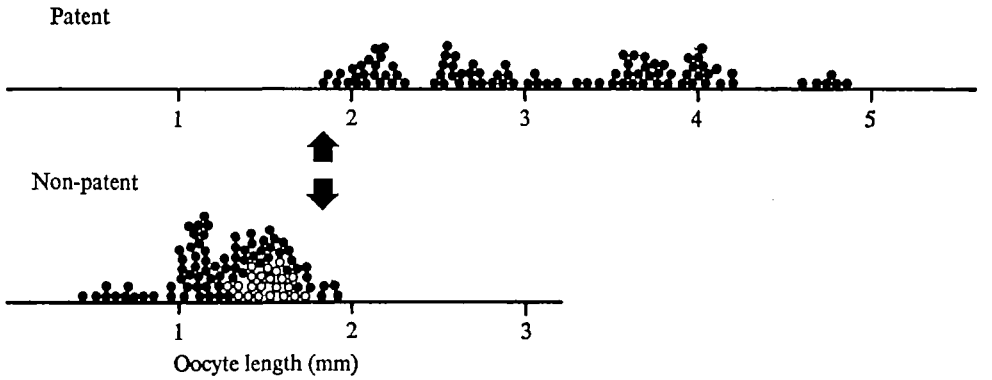


Fig. 1. Oocyte length at which spaces appear in the follicular epithelium. ○, Oocytes containing yellow lipid droplets but no protein yolk spheres. ●, Other classes of oocytes. Arrows indicate the critical size of activation into vitellogenesis: above 1·8 mm the follicles are patent, while below this value the follicles do not have visible intercellular spaces.

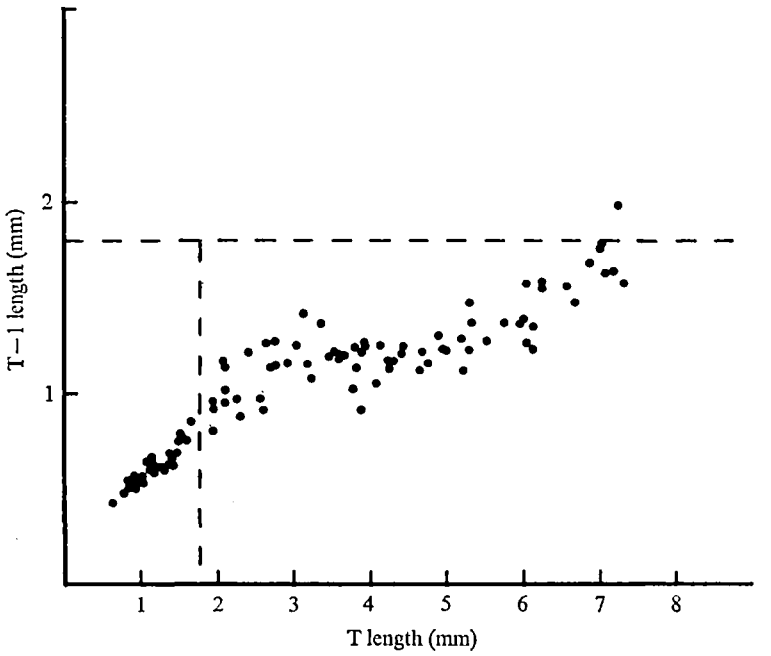


Fig. 2. The length of the T oocyte plotted against that of the T−1 oocyte during the first 17 days of adult life. Each point represents the mean values derived from measurements on not less than four ovarioles from each animal. The broken lines indicate the lengths at which the T and T−1 oocytes enter vitellogenesis (1·8 mm).

RESULTS

Patency and oocyte growth

Spaces between the follicular epithelial cells of the T oocyte, as determined by the dye penetration test, appear when the oocyte has attained a length of 1·8 mm (Fig. 1). We associate this event with the onset of vitellogenesis (see Pratt & Davey, 1972) since only after this time are protein yolk spheres visible in the ooplasm. However, yellow lipid spheres can be observed in the ooplasm after the oocyte attains a length

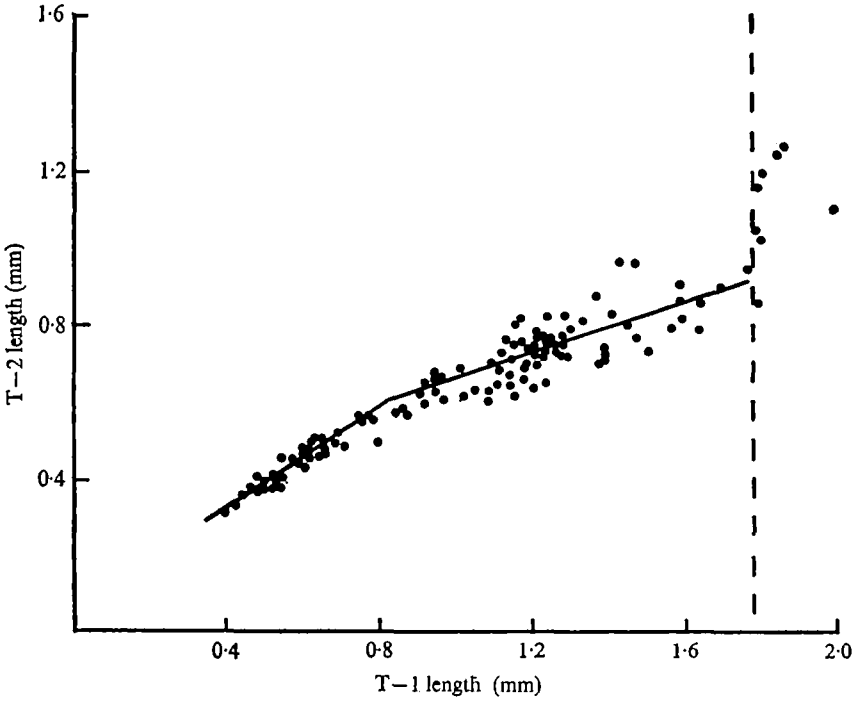


Fig. 3. The length of the T−1 oocyte plotted against that of the T−2 oocyte during days 3 through 17 of adult life. Each point represents the mean values derived from measurements on not less than four ovarioles from each animal. The broken line indicates the length at which the T−1 oocytes enter vitellogenesis (1·8 mm). The solid line was fitted to the data by eye and its point of inflexion indicates the stage (0·8–0·9 mm) at which the T−1 oocytes enter pre-vitellogenesis and start to grow proportionately faster than the T−2 oocytes.

of 1·3 mm (open circles on Fig. 1) but because they cannot be associated with the patency of the follicular epithelium, they are not regarded as vitellogenic yolk. Similar lipid material has been reported to appear in the ooplasm prior to vitellogenesis, at an oocyte length of 1·4 mm (Lusis, 1963) in good agreement with the present study. There can be no doubt that spaces appear between the follicle cells after the oocyte is 1·8 mm in length (see Fig. 10(*b–d*), Plate 1) but these spaces are not apparent in smaller oocytes (Fig. 10(*a*), Plate 1). The spaces appear to increase in relative dimension as the oocytes increase in length (Fig. 10, Plate 1), and the follicle cells become progressively flattened. These spaces allow the surface of the oocyte to come into direct contact with the haemolymph and, it is probably this route which the oocyte 'uses' for the uptake of vitellogenin.

The relationship between the length of the T oocyte and the T−1 oocyte has been investigated with a view to defining the points at which oocyte growth is regulated. Prior to the onset of vitellogenesis (and patency) in the T oocyte and for a short period thereafter, the length of the T−1 oocyte increases proportionately to the T length (Fig. 2). However, when the T oocyte attains a length of 2·5–3·0 mm, the length of the T−1 oocytes remains at *ca.* 1·2 mm until the T oocyte is 6·0 mm in length (Fig. 2). After this length has been reached, both the T and the T−1 increase rapidly in length and when chorionation begins in the T oocyte (at *ca.* 7·0 mm), the length of the T−1

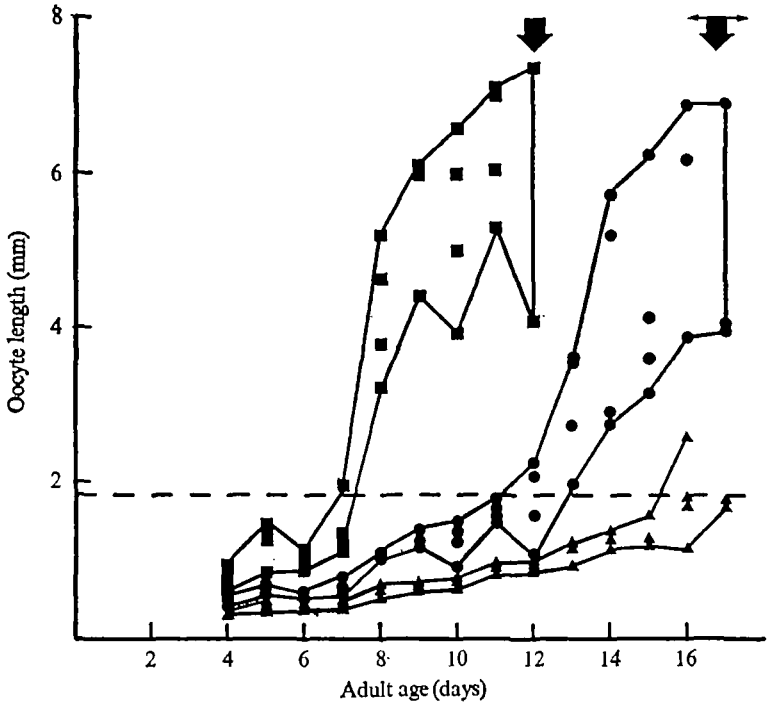


Fig. 4. Lengths of T, T−1 and T−2 oocytes as a function of adult age. ■, First T oocytes; ●, T−1 oocytes; ▲, T−2 oocytes. The horizontal line indicates the length at which oocytes enter vitellogenesis. Arrows indicate the age at which successive ovulations occur. Each point represents the mean value of measurements on at least four ovarioles from each animal in the first experimental series.

oocyte is just below 1·8 mm, the length at which vitellogenesis begins (Fig. 2). Vitellogenesis in the T−1 oocyte does not usually begin until the T oocyte has been ovulated into the oviduct.

The relationship between the lengths of the T−1 and T−2 oocytes has also been investigated. There is a proportional increase in the lengths of the oocytes up to a length of about 0·8 mm (T−1) and 0·6 mm for the T−2 (see Fig. 3). From this point, the length of the T−2 oocyte increases more slowly relative to the T−1 oocyte (Fig. 3). As Fig. 3 indicates, the T−1 oocyte becomes vitellogenic (which corresponds to its becoming the T oocyte) when the T−2 oocyte is about 0·9 mm in length; the T−2 oocyte (now the T−1) rapidly increases in length to about 1·2 mm and remains at this length for much of the gonotrophic cycle.

The lengths of the T, T−1 and T−2 oocytes as a function of age are shown in Figs. 4 and 5. As noted in the methods, two completely separate sets of observations have been obtained and although the CA from each set have been subjected to different experimental procedures, the oocyte data from each set are directly comparable. Thus Fig. 4 shows the observations from one set and Fig. 5, observations from the other set. It should be noted that each set of observations were performed on a single batch of locusts which all ecdysed within 12 h of one another. The importance of this synchrony in experimental animals can be seen from the highly synchronized gonotrophic cycles shown in Figs. 4 and 5.

It can be seen from Fig. 4 that the T oocyte becomes vitellogenic about 7 days after

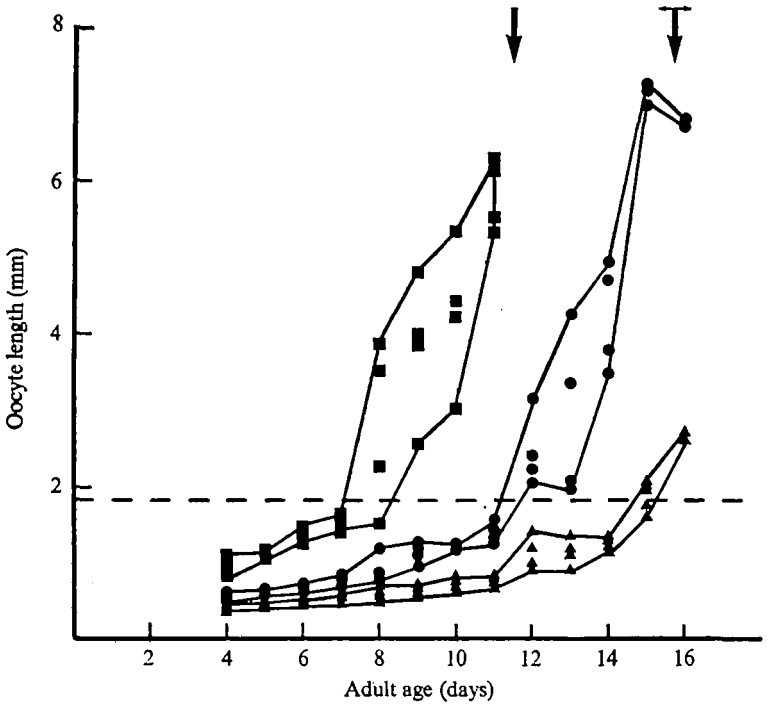


Fig. 5. As for Fig. 4; data from animals in second experimental series.

fledging. Vitellogenesis and chorionation last for a maximum of 4–5 days, with ovulation occurring 11–12 days after fledging. At this, time the T – 1 oocyte enters vitellogenesis and once again, the maturation of this wave of oocytes requires about 4–5 days, with ovulation occurring between days 16 and 17. Thus it appears that each gonotrophic cycle occurs over a 4–5 day period. A similar pattern can also be seen in Fig. 5 which presents data from the second series of experiments, although in this case each gonotrophic cycle lasted for a maximum of 4 days. Thus vitellogenesis begins on days 7–8, 11–12 and 15 for the first, second and third gonotrophic cycles respectively, with ovulation taking place on days 11–12 and 15–16.

In view of the data presented in Figs. 2–5 it would appear that oocyte maturation can be roughly divided into four periods: (1) an early growth period during which the oocytes reach a length of 0·8–0·9 mm; (2) a previtellogenic period when the oocytes increase in length from 0·8–0·9 mm to 1·8 mm; (3) the vitellogenic period when oocyte length increases from 1·8 mm to about 7·5 mm; and (4) chorionation, when oocytes have reached their maximum length. As has been noted above, a previtellogenic oocyte (i.e. T – 1) cannot enter vitellogenesis until the vitellogenic oocyte (i.e. T) has completed chorionation. Similarly, it would appear that an oocyte in the early growth period cannot enter the previtellogenic stage until the proximal oocyte has become vitellogenic. Thus, two events appear to take place simultaneously when the penultimate (T – 1) oocyte reaches a length of 0·8–0·9 mm: (*a*) the T oocyte enters vitellogenesis (see Fig. 3) and (*b*) the T – 1 oocyte enters previtellogenesis (as shown by the inflexion of the line in Fig. 3).

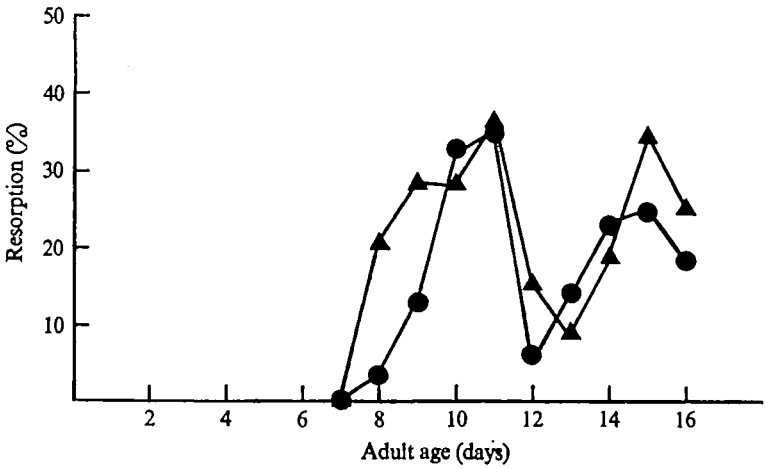


Fig. 6. Percentage of ovarioles containing resorptive oocytes, as a function of adult age during the first two gonotrophic cycles. ▲, Data from first experimental series; ●, data from second experimental series. Each point represents the mean determination on four animals for each day.

Oocyte resorption

In order to assess the effect of C_{16}JH synthesis on oocyte resorption during the first two gonotrophic cycles, the percentage of resorptive oocytes in each ovary has been determined and plotted as a function of age. Such a plot might also provide some insight into the age at which resorption is initially determined. It appears that the number of resorptive oocytes increases progressively during each gonotrophic cycle with the maximum number of resorptive oocytes (about 35 %) being observed shortly before ovulation (Fig. 6). This progressive increase in the number of resorptive oocytes can be seen in both sets of experiments. However, this progressive increase should not be construed as an indication that the percentage of resorptive oocytes increases with the growth (i.e. length) of the oocytes, as other workers have suggested (see Highnam *et al.* 1963*b*). In fact, when oocyte length is plotted against percentage of resorptive oocytes (data from first and second gonotrophic cycles from both sets of experiments combined) as in Fig. 7, it can be seen that the percentage does not increase significantly once the oocytes reach a length of about 5 mm. Thus, further resorption is not initiated after the oocytes attain a length of about 5 mm.

Synthesis of C_{16} JH by isolated CA

The ability of isolated CA to synthesize C_{16}JH on each day of the first two gonotrophic cycles has been measured on both pooled (first series) and individual (second series) pairs of CA. Both spontaneous synthesis and synthesis stimulated by the addition of farnesenic acid to the incubation medium have been monitored. Spontaneous synthesis has been measured through the incorporation of the [^{14}C]methyl moiety of [*Me*-^{14}C]methionine into C_{16}JH and the efficacy of this method has been previously discussed (Pratt & Tobe, 1974*a*; Tobe & Pratt, 1974; Pratt *et al.* 1975); similarly, stimulated synthesis has been followed using the incorporation of both [^{3}H]farnesenic acid and [*Me*-^{14}C]methionine into C_{16}JH. Spontaneous and stimulated synthesis of C_{16}JH have been observed over a period of 2 h (second series) or 3 h

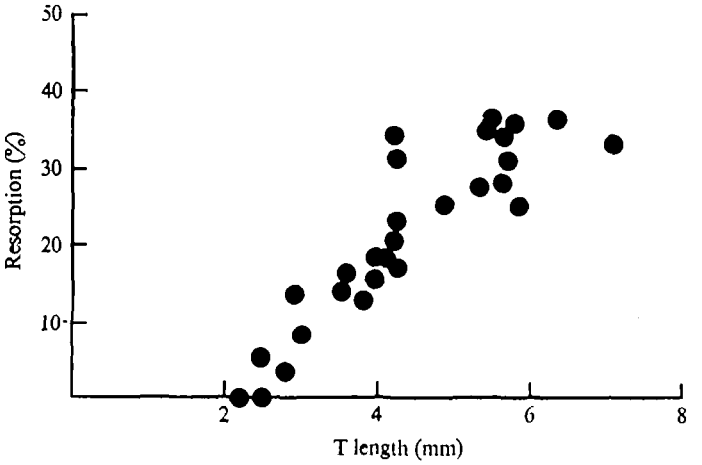


Fig. 7. Relationship between percentage resorption of T oocytes, and length of remaining unresorbed oocytes observed at different times throughout the first two gonotrophic cycles. Points represent the means of values for four separate animals on each experimental day of each series of observations. Resorption is observed only after oocytes have entered vitallogenesis (exceeded 1·8 mm in length) and does not take place after oocytes reach a length of 4·5–5 mm.

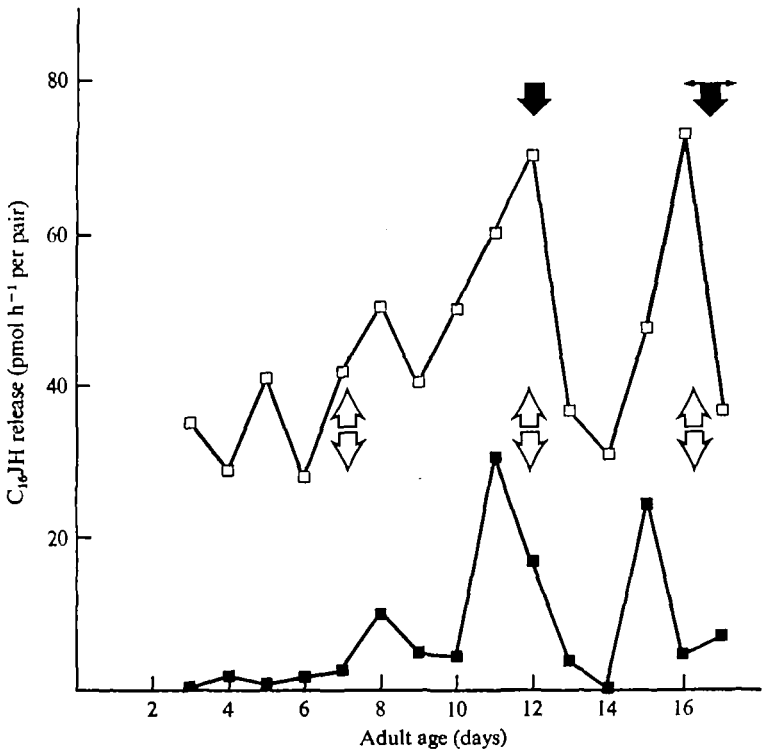


Fig. 8. Rates of release of JH by corpora allata under conditions of spontaneous (■) and farnesenic acid stimulated (□) synthesis, throughout the first two gonotrophic cycles. Open arrows show the times of activation of vitellogenesis in the first three generations of T oocytes. Solid arrows indicate the times of ovulation of the first two generations of T oocytes. Data from first experimental series.

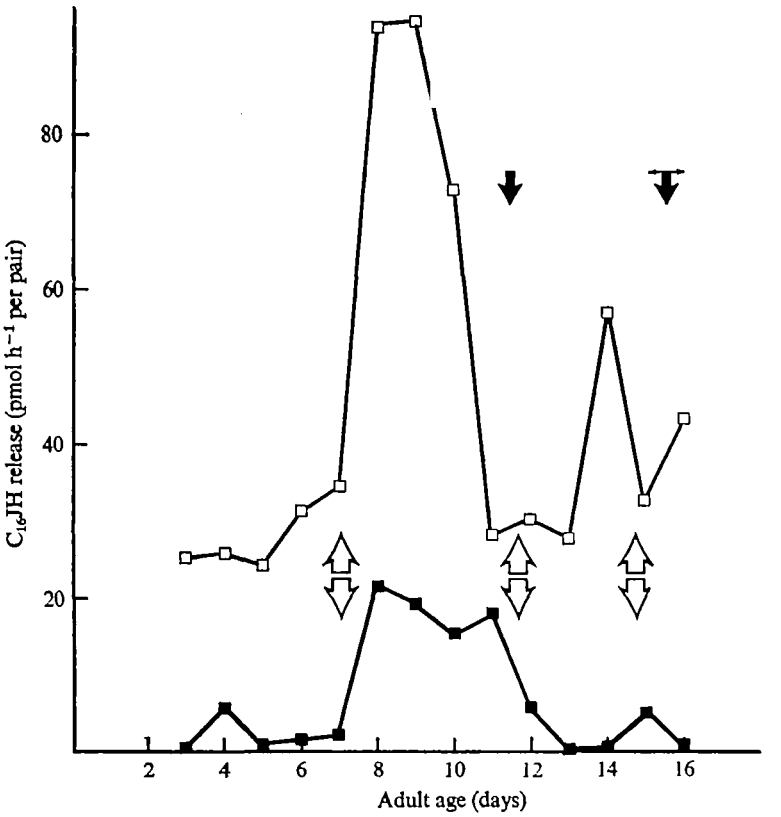


Fig. 9. As for Fig. 8 using data from second experimental series.

(first series) since it has been previously demonstrated that the rate of synthesis and release of the hormone is linear for a period of 3 h in both glands synthesizing hormone spontaneously (Pratt *et al.* 1975) and in stimulated glands (Tobe & Pratt, 1974). Our direct comparisons between the rates of synthesis and release of JH under conditions of both spontaneous and stimulated JH synthesis confirm a previous report (Tobe & Pratt, 1975) that release is strictly proportional to synthesis under all conditions.

Figs. 8 and 9 show the rate of release of C_{16}JH by CA, under both spontaneous and stimulated conditions, during the first two gonotrophic cycles of *S. gregaria*. Fig. 8 shows the results of experiments using pooled CA while Fig. 9 shows the results of experiments on individual pairs of CA. The general similarity in the pattern of C_{16}JH release rate in both sets of experiments is striking. Thus, a small peak in JH release rate at 4 days and larger peaks at days 8, 11 and 15 are apparent during spontaneous release. In the case of stimulated glands it is obvious that the release rate of C_{16}JH is appreciably higher than the spontaneous rate at all times during the first two gonotrophic cycles. High stimulated release rates can be observed on days 11–12 and 15–16 in the case of the first set of observations (Fig. 8), while in the second set high stimulated release rates are observed slightly earlier, at days 8–10 and day 14 (Fig. 9), than in the previous set. It should be remembered that the rate of oocyte development,

'as measured by oocyte length and time of ovulation, is about 1 day faster in the second series than in the first (compare Figs. 4 and 5). This may be related to the slightly earlier peaks in release rate under stimulated conditions observed in the second series.

From the observations presented in Figs. 8 and 9 it would appear that the major periods of spontaneous JH synthesis and release are (i) a short period about 4 days after adult emergence, (ii) a period lasting throughout the first gonotrophic cycle (days 8–11 or 12), and (iii) a short period near the end of the second gonotrophic cycle. The high release rates observed under stimulated conditions cannot be easily correlated with these periods of spontaneous JH release, except during the first gonotrophic cycle.

It is of interest that the onset of the previtellogenic period, as defined above in terms of oocyte lengths, coincides with peaks in spontaneous JH synthesis by the CA. Thus, on day 4 the T oocyte becomes previtellogenic, while on day 7–8 the T − 1 oocyte becomes previtellogenic. Similarly, on days 11–12 the new T − 1 oocyte (formerly the T − 2) enters previtellogenesis. It might therefore appear that the inflexion point in Fig. 4, where the T − 1 oocytes begin to increase in length rapidly relative to the T − 2 oocytes, correlates well with the synthesis of JH.

DISCUSSION

Effects of Malamoeba

The importance of eliminating infections by *M. locustae* in experimental cultures of locusts cannot be overemphasized. As has been noted in the Methods, the parasite appears to be capable of altering the metabolism of the host so as to delay both the development of the ovaries and the colour change associated with maturation. It is not known how the parasite influences these phenomena, but the possibility that the synthesis of JH by the CA is altered cannot be excluded. Certainly both ovarian development and colour change are known to be dependent on the presence of 'active' CA in *S. gregaria* (Highnam *et al.* 1963*a*; Pener, 1967*a*). It is significant that our attempts to obtain uninfected *S. gregaria* from other laboratories proved unsuccessful: this might explain the tremendous variations in the values reported in the literature for the time required for ovarian development and for colour change. There can be no doubt that before physiological experiments are performed on either locusts or grasshoppers, the animals must be shown to be free of parasitic infections. We are confident that the locusts used in the present study were free from *M. locustae* and we believe that the highly synchronous and rapid maturation reported in this paper can be attributed, at least in part, to this fact. In addition, we believe that the rearing conditions, particularly the use of adequate supplies of fresh wheat seedlings, also contributed to the synchrony of the colony and the rapidity of maturation.

Ovarian development

The results presented in Figs. 4 and 5 give an indication of the high rate of ovarian development observed in the present study. Each of the first two gonotrophic cycles lasts for a maximum period of 4–5 days and it would appear that the period of somatic growth (the interval between fledging and the onset of vitellogenesis; see Hill, Luntz & Steele, 1968) is about 7 days. This rate of development is considerably faster than

that reported by other workers (see Highnam & Lusis, 1962; Hill *et al.* 1968) and, as noted above, we believe that part of this difference can be attributed to the diet. It is significant that Hill *et al.* (1968) experienced difficulty in synchronizing batches of locusts and thus found it necessary to express their results on the basis of oocyte length rather than age; such difficulties have not been encountered in the present study.

In the present work, oviposition sites (tubes of damp sand) were provided from day 10 after fledging onward. This allowed females with mature oocytes to oviposit immediately and few females were observed to retain mature oocytes in their oviducts. As indicated in Figs. 4 and 5, ovulation occurred between days 11 and 12. All females in the present study had oviposited by the end of day 12. Similarly, for the second gonotrophic cycle, all females had oviposited by the end of day 18 in the first set (Fig. 4) and day 16 in the second set (Fig. 5).

Oocyte development has been divided into four phases in the present study and these periods are similar to those suggested by Lusis (1963). However, this author considered oocytes between 0·5 and 1·4 mm to be early growth phase (stage 1) and oocytes 1·4–1·6 mm in length as previtellogenic (stage 2). Lusis (1963) used the histological appearance of the oocyte and follicular epithelium as the criteria for his classification, while in the present work we have used the relation between the length of the T – 1 and T – 2 oocyte (i.e. the point at which the rate of growth of the T – 1 oocyte, relative to the T – 2, increases). Thus, if we rely upon morphometric criteria, there is a well-defined stage in the growth of the follicle (stage 2) which immediately precedes vitellogenesis and whose initiation is co-ordinated with the initiation of vitellogenesis (stage 3) in the neighbouring T oocyte. This initiation of the previtellogenic growth phase (stage 2) takes place at an oocyte length of 0·8–0·9 mm (see (Fig. 3).

There is considerable confusion in the literature regarding the control of resorption in *S. gregaria*. Certainly there can be no doubt that surgical allatectomy of females containing vitellogenic oocytes results in the resorption of these oocytes (Highnam *et al.* 1963*a*) and that partial ovariectomy results in a decreased percentage of resorptive oocytes (Highnam *et al.* 1963*b*). However, the reasons for these effects remain obscure and this is due in part to the fact that the precise temporal sequence for resorption in maturing locusts remains undefined. Thus, Highnam & Lusis (1962), Lusis (1963) and Highnam *et al.* (1963*b*) suggested that resorption can occur at any time during the growth of the oocytes (even before vitellogenesis has begun) and that the percentage of resorptive oocytes increases progressively during the maturation process. On the other hand, Hill *et al.* (1968) maintained that oocyte resorption occurred only during the 'final half' of oocyte development, when the oocytes 'demand for protein' was maximal, and that the high percentage of resorptive oocytes observed in their study might be a result of increased rate of maturation.

In our experiments, resorption only occurred in oocytes which had already entered vitellogenesis, and was never induced in oocytes which had exceeded 5 mm in length. We cannot agree with the suggestion of Hill *et al.* (1968) that a high percentage of resorptive oocytes can be attributed to a rapid rate of maturation since in our study the rate of maturation was significantly higher than theirs and yet the percentage resorption was considerably less. This is not to say, however, that their high levels of

resorption were not due to a 'protein deficiency', perhaps imposed by their rearing conditions.

It appears that the extent and pattern of oocyte resorption varies between animals reared in different laboratories, which makes it difficult to interpret the phenomenon in terms of possible control mechanisms. Highnam *et al.* (1963*b*) concluded that CA deficiency could compound 'protein deficiency' in producing high levels of resorption. Unfortunately, their apparently successful experiments involving hormone therapy with the JH analogue farnesol are difficult to interpret, owing to the fact that the required dose of farnesol was not examined quantitatively. Therefore, we cannot be certain that the relatively massive quantities of farnesol employed by Highnam *et al.* (1963*b*) were acting as a specific mimic of naturally released JH.

Our observations permit us to correlate precisely the synthetic and release activity of the CA with the appearance of resorptive oocytes in the ovary. A detailed quantitative comparison of these two parameters, in both series of experiments, has not revealed any obviously meaningful relationship between glandular activity and manifest levels of resorption. The matter is further complicated by the fact that there is no precise information on the contemporaneity or otherwise of the determinative event(s) which initiate the resorptive process, and the overt manifestation of resorption. In conclusion, whilst we do not dismiss the possibility that high levels of oocyte resorption can be induced by sub-optimal levels of CA activity (Highnam *et al.* 1963*b*), we do believe that this theory should be the subject of serious question until such time as the results of more definitive experiments are available.

CA activity and ovarian development

The present study represents the first time that it has been possible to relate the synthetic capabilities of the CA to the ovarian development of an insect. However, the role of JH in oocyte development remains unclear. Of course, it is not sufficient to measure the synthetic capabilities of the CA, it is also necessary to know the haemolymph titre of JH as determined by precise chemical means, the concentration and affinity of JH-specific binding protein (see Kramer *et al.* 1974), and the rate of turnover of JH in the animals. The present study provides a first step in the elucidation of the role of JH in ovarian development and work is continuing in this laboratory on these other aspects. The present work thus provides not only a specific assay for the rate of biosynthesis of locust JH but also an indication of when high titres of JH might be found *in vivo*. It also suggests that, provided the turnover of JH *in vivo* is proportionately rapid, the levels of JH in the animal must change dramatically during the course of the first two gonotrophic cycles of *S. gregaria*. This follows from the large changes observed in the spontaneous synthetic capabilities of the CA during this period and there is no doubt that the rate of spontaneous JH release by the CA can change by a factor of at least 10 within certain 24 h periods in maturing locusts. Thus the assay described in this paper provides a unique opportunity for determining the factor(s) involved in these large changes in JH synthesis and release. We do know that there is no appreciable storage of JH at any time during the first two gonotrophic cycles of *S. gregaria* (Tobe & Pratt, 1975) and that these rapid changes in the release of JH by the CA are a consequence of changes in the rate of JH synthesis.

It has been shown previously (Pratt & Tobe, 1974*a*, *b*) that when optimum con-

centrations of farnesenic acid are added to the incubation medium the rate of production of JH reveals the competence of the glands to effect just the last two stages in hormone biosynthesis (namely esterification of the olefinic acid and epoxidation of the olefinic ester), independently of their spontaneous rate of hormone biosynthesis. We show here (Figs. 8, 9) that at no time during the first gonotrophic cycles does the rate of spontaneous hormone release reach that of stimulated release. It would therefore appear that the rate-limiting step in the synthesis of JH must occur prior to the esterification of farnesenic acid to methyl farnesoate, at all times during reproductive development of the female locust. Furthermore, the changes in the ability of the CA to synthesize JH from optimum concentrations of farnesenic acid are small (about 2- to 3-fold) in comparison to the changes occurring in the spontaneous synthetic rate (over 50-fold). However, the periods of maximum farnesenic acid stimulated activity do correspond fairly well with peaks in spontaneous synthetic rate; this relationship will be discussed further in a separate paper (Tobe & Pratt, in preparation).

Validity of the assay

The question arises as to how we may compare the measured rates of JH synthesis of CA *in vitro* with their presumed activity *in vivo*. In particular we need to know whether or not CA which have been deprived of all nervous connexion and isolated in chemically defined culture medium (as is their condition during our *in vitro* assay) are capable of expressing their predetermined level of physiological activity. A concensus of the evidence available for *Schistocerca* spp. and *Locusta migratoria* makes it seem likely that nervous connexion to the brain or the presence of circulating neurosecretion are necessary for either the induction or maintenance of high levels of endocrine activity in the CA *in vitro*. Thus Strong (1965*a*) observed the effects on oocyte development of implanting CA into allatectomized female *Schistocerca*; he concluded that CA from immature females did not become 'active' after transplantation, and that CA from mature females 'rapidly lost activity'. Strong (1965*b*) further showed that severance of the NCA I, but not NCA II, in immature female *S. paranensis* prevented subsequent oocyte growth and maturation. Similarly, Pener (1967*b*) observed in *S. gregaria* that although severance of the NCA I in immature females did not prevent the initiation of vitellogenesis in successive generations of oocytes, it did result in the complete resorption of the developing oocytes. Total denervation of the CA did not produce any further effect on ovarian development, and he concluded that the NCA I normally has a stimulating effect upon the CA and that severance of this nerve results in 'subnormal' functioning of the gland. These observations could be given an alternative interpretation: that severance of the NCA I interferes with the release of brain neurosecretion which is necessary either to stimulate the CA or to permit the ovaries to respond to JH. The recent report (Moulins, Girardie & Girardie, 1974) that electrical stimulation of the neurosecretory cells of the pars intercerebralis in immature females of *Locusta migratoria* restores their ability to mature and oviposit a batch of oocytes after section of the NCA I is germane to this discussion. If we can exclude the possibility that the neurosecretion released after electrical stimulation is acting pharmacologically rather than physiologically, these findings indicate that at least in *L. migratoria*, an intact NCA I is not normally essential for the continued functioning of the CA. Similarly, Johnson & Hill (1973) have shown by bioassay that high titres of

JH activity can be observed in the haemolymph of male *L. migratoria* several days after denervation of the CA.

Although the results of these *in vivo* experiments suggest that it may not be possible to measure the physiological activity of the CA by *in vitro* procedures, recent research in this laboratory has shown that this need not be the case. We have previously reported that, in the case of glands stimulated with farnesenic acid, newly synthesized JH was detectable in the medium within minutes of adding the radiolabel to the freshly isolated glands (Tobe & Pratt, 1974). Furthermore, the rate of synthesis of JH by a given set of CA was constant for at least 4 h. Similar results have now been obtained with glands synthesizing hormone spontaneously (Pratt *et al.* 1975). Therefore, in as much as (*a*) the measured rate of synthesis and release of JH is constant for at least 3 h *in vitro*, and (*b*) the method reveals large changes in the spontaneous activity of the glands during development, we are confident that the *in vitro* procedure we have employed here faithfully reveals the consequences of regulatory changes in the synthetic capability of the glands which have occurred in the animal prior to the isolation of the CA. This conclusion is not in conflict with those experimental findings referred to above in which the effect of various surgical operations of 'CA activity' was investigated in the whole animal, because in those experiments the 'activity' of the CA was assessed over a period of days rather than hours. It is entirely possible that a CA deprived of its nervous connexions and suspended in a medium containing no humoral trophic factors may lose its predetermined level of JH synthetic activity after a period of several hours.

What is the relation between CA activity and ovarian development?

It is tempting to speculate on the observed relationship between CA activity and events occurring within the ovary. It is apparent from the results presented in this paper that the synthesis and release of JH cannot be exclusively associated with the onset of vitellogenesis. In fact, in the case of the first gonotrophic cycle, the glands exhibit a burst of activity well before vitellogenesis starts, but then the T oocytes enter vitellogenesis just before the CA exhibit their second peak of JH synthetic activity. At the beginning of the second gonotrophic cycle, however, maximum JH synthesis by the CA precedes the onset of vitellogenesis. Thus, there appear to be qualitative differences between the first and subsequent gonotrophic cycles, with respect to the related activity of the CA. We emphasize that these changes in CA activity take place very rapidly, and would probably not be observable in experimental animals that were any less well synchronized, with respect to their sexual maturation, than those employed in the present study. In addition there appear to be quantitative differences between first and subsequent gonotrophic cycles: in general, the rates of JH synthesis observed during the course of the first cycle are higher than those observed during the second cycle. These quantitative differences could perhaps be a consequence of the qualitative differences described above. Certainly, our findings do not provide encouraging prima facie evidence for a simple involvement of the CA in initiating vitellogenesis.

Strong (1965*a*) has previously shown that intact CA are necessary for previtellogenic growth of oocytes in *S. paranensis*, and our results do indeed indicate that peaks of JH synthesis occur at the beginning of the 'pre-vitellogenic' period when the

T oocytes in the first cycle and T – 1 oocytes in the second and third cycles reach a length of 0·8–0·9 mm, namely on days 4, 7–8 and 11–12. JH could thus be an important trigger in the initiation of previtellogenic growth, and this situation may be similar to the activation of mosquito oocytes previously described by Gwadz & Spielman (1973). None the less, until such time as the precise site and mode of action of JH in the ovaries is elucidated, the exact relationship between JH synthesis and ovarian development will remain unclear.

We are grateful to our colleagues Dr A. F. White and Miss M. M. Blight for kindly providing [^{3}H]farnesenic acid and synthetic reference compounds; to Dr J. E. Henry for advice and information on the use of Thipyrameth as a prophylactic agent against *Malamoeba* infection, and to the late Joe Da Costa for rearing the locusts. S. S. Tobe acknowledges receipt of a National Research Council of Canada post-doctoral fellowship.

REFERENCES

ENGELMANN, F. (1970). *The Physiology of Insect Reproduction.* Oxford: Pergamon Press.

ENGELMANN, F. (1971). Juvenile hormone controlled synthesis of female specific protein in the cockroach *Leucophaea maderae*. *Archs Biochem. Biophys.* **145**, 439–47.

GWADZ, R. W. & SPIELMAN, A. (1973). Corpus allatum control of ovarian development in *Aedes aegypii*. *J. Insect Physiol.* **19**, 1441–8.

HENRY, J. E. (1968). *Malamoeba locustae* and its antibiotic control in grasshopper cultures. *J. Invert. Pathol.* **11**, 224–33.

HIGHNAM, K. C. (1962). Neurosecretory control of ovarian development in *Schistocerca gregaria*. *Q. Jl Microsc. Sci.* **103**, 57–72.

HIGHNAM, K. C. & LUSIS, O. (1962). The influence of mature males on the neurosecretory control of ovarian development in the desert locust. *Q. Jl Microsc. Sci.* **103**, 73–83.

HIGHNAM, K. C., LUSIS, O. & HILL, L. (1963*a*). The role of the corpora allata during oocyte growth in the desert locust, *Schistocerca gregaria* Forsk. *J. Insect Physiol.* **9**, 587–96.

HIGHNAM, K. C., LUSIS, O. & HILL, L. (1963*b*). Factors affecting oocyte resorption in the desert locust *Schistocerca gregaria* (Forskal). *J. Insect Physiol.* **9**, 827–37.

HILL, L., LUNTZ, A. J. & STEELE, P. A. (1968). The relationship between somatic growth, ovarian growth and feeding activity in the adult desert locust. *J. Insect Physiol.* **14**, 1–20.

HUEBNER, E. & ANDERSON, E. (1972). A cytological study of the ovary of *Rhodnius prolixus*. I. The ontogeny of the follicular epithelium. *J. Morph.* **136**, 459–94.

JOHNSON, R. A. & HILL, L. (1973). Quantitative studies on the activity of the corpora allata in adult male *Locusta* and *Schistocerca*. *J. Insect Physiol.* **19**, 2459–67.

JOLY, P. & MEYER, A. S. (1970). Action de l'hormone juvenile sur *Locusta migratoria* (Orthoptère) en phase grégaire. *Archs Zool. exp. gén.* **111**, 51–63.

JOLY, L., JOLY, P., PORTE, A. & GIRARDIE, A. (1968). Etude physiologique et ultrastructurale des corpora allata de *Locusta migratoria* L. (Orthoptère) en phase grégaire. *Archs Zool. exp. gén.* **109**, 703–27.

JUDY, K. J., SCHOOLEY, D. A., HALL, M. S., BERGOT, B. J. & SIDDALL, J. B. (1973). Chemical structure and absolute configuration of a juvenile hormone from grasshopper corpora allata *in vitro*. *Life Sci.* **13**, 1511–16.

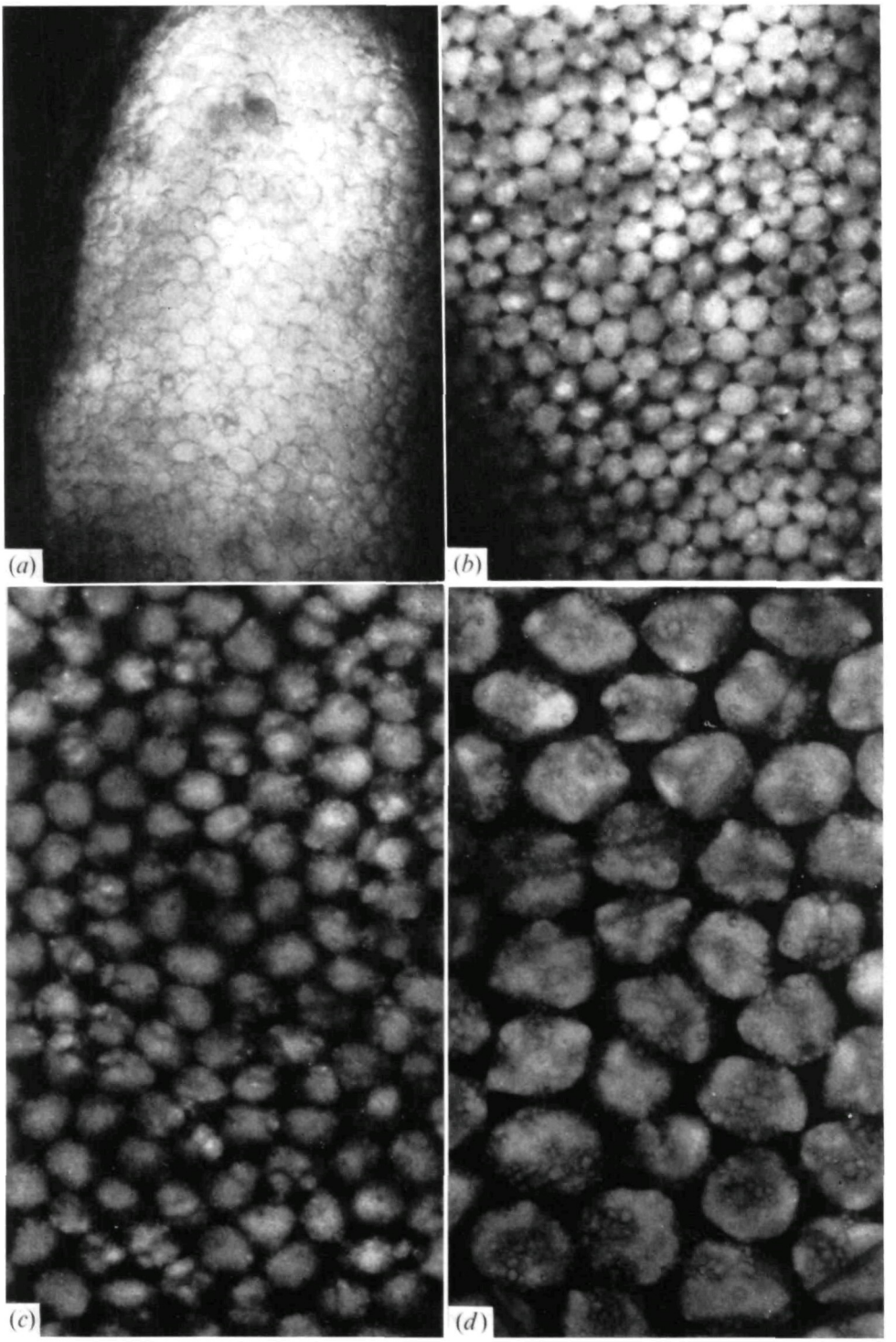
KRAMER, K. J., SANBURG, L. L., KEZDY, F. J. & LAW, J. H. (1974). The juvenile hormone binding protein in the hemolymph of *Manduca sexta* Johannson. *Proc. natn. Acad. Sci. U.S.A.* **71**, 493–7.

LUSIS, O. (1963). The histology and histochemistry of development and resorption in the terminal oocytes of the desert locust, *Schistocerca gregaria*. *Q. Jl Microsc. Sci.* **104**, 57–68.

MOULINS, M., GIRARDIE, A. & GIRARDIE, J. (1974). Manipulation of sexual physiology by brain stimulation in insects. *Nature, Lond.* **250**, 339–40.

PENER, M. P. (1967*a*). Comparative studies on reciprocal interchange of the corpora allata between males and females of adult *Schistocerca gregaria* (Forskal) (Orthoptera: Acrididae). *Proc. R. ent. Soc. Lond.* A **42**, 139–48.

PENER, M. P. (1967*b*). Effects of allatectomy and sectioning of the nerves of the corpora allata on oocyte growth, male sexual behaviour and colour change in adults of *Schistocerca gregaria*. *J. Insect Physiol.* **13**, 665–84.


(a)
(b)
(c)
(d)

PRATT, G. E. & DAVEY, K. G. (1972). The corpus allatum and oogenesis in *Rhodnius prolixus* (Stal.). I. The effects of allatectomy. *J. exp. Biol.* **56**, 201–14.

PRATT, G. E. & TOBE, S. S. (1974*a*). Juvenile radiobiosynthesised by corpora allata of adult female locusts *in vitro*. *Life Sci.* **14**, 575–86.

PRATT, G. E. & TOBE, S. S. (1947*b*). *In vitro* double-label biosynthesis of juvenile hormones by locust corpora allata. *Gen. comp. Endocr.* **22**, 402–3.

PRATT, G. E., TOBE, S. S., WEAVER, R. J. & FINNEY, J. R. (1975). Spontaneous synthesis and release of juvenile hormone by isolated corpora allata of the locust *Schistocerca gregaria* and cockroach *Periplaneta americana*. *Gen. comp. Endocr.* (in the Press).

STRONG, L. (1965*a*). The relationships between the brain, corpora allata, and oocyte growth in the central American locust, *Schistocerca* sp. I. The cerebral neurosecretory system, the corpora allata, and oocyte growth. *J. Insect Physiol.* **11**, 135–46.

STRONG, L. (1965*b*). The relationships between the brain, corpora allata, and oocyte growth in the central American locust, *Schistocerca* sp. II. The innervation of the corpora allata, the lateral neurosecretory complex, and oocyte growth. *J. Insect Physiol.* **11**, 271–80.

TELFER, W. H. (1965). The mechanism and control of yolk formation. *A. Rev. Ent.* **10**, 161–84.

TOBE, S. S. & PRATT, G. E. (1974). The influence of substrate concentrations on the rate of insect juvenile hormone biosynthesis by corpora allata of the desert locust *in vitro*. *Biochem. J.* **144**, 107–13.

TOBE, S. S. & PRATT, G. E. (1975). Juvenile hormone release from corpus allatum: dependence upon intra-glandular content. *Nature, Lond.* (in the Press).

WIGGLESWORTH, V. B. (1970). *Insect Hormones*. Edinburgh: Oliver and Boyd.

DE WILDE, J., STAAL, G. B., DE KORT, C. A. D. & BAARD, G. (1968). Juvenile hormone titre in the haemolymph as a function of photoperiodic treatment in the adult Colorado beetle (*Leptinotarsa decemlineata* Say.). *Proc. K. ned. Akad. Wet* C **71**, 321–6.

EXPLANATION OF PLATE

Fig. 10. Bright-field photomicrographs of typical freshly dissected ovarioles subjected to the Evan's Blue dye penetration test. All at times 222 magnification.

(*a*) Oocyte of length 1·9 mm, just prior to the development of intercellular spaces in the follicle epithelium.

(*b*)–(*d*) Oocytes in various stages of active vitellogenesis, showing the rapid penetration of dye across the follicular epithelium via the intercellular spaces. Oocyte lengths as follows: (*b*) 3·0 mm, (*c*) 4·3 mm, (*d*) 6·9 mm.