the yeast mitochondrial ATP synthetase complex (*20*). It is not yet known whether, like yeast, *Neurospora* also has 4-phosphopantetheine bound to subunit 6 and whether the *cel*$^-$ strains are deficient in 4-phosphopantetheine bound to subunit 6. However, the sensitivity of the *cel*$^-$ strains to fatty acids and to oligomycin may be related to a common defect in the addition of 4-phosphopantetheine to two proteins: the acyl carrier protein and subunit 6. Therefore, the basis for the effects of *cel*$^-$ mutation on circadian rhythmicity may be a defect in the F_0 portion of the ATP synthetase complex, as is the case of the *oli*^{r} mutations.

It still remains to be elucidated how the *oli*^{r} mutations affect the mechanism of circadian timekeeping. One possibility is that the *oli*^{r} mutations in *Neurospora* may affect proton transport in the mitochondria, since subunit 9 purified from yeast *oli*^{r} mutants shows reduced and oligomycin-resistant proton transport (as compared to wild-type subunit 9) when purified and incorporated into liposomes (*21*). Subunit 9 from wild-type and *oli*^{r} strains of *Neurospora* has yet to be tested in such an isolated system. However, the *Neurospora* protein has 50 percent sequence homology with yeast subunit 9 (*5*) and, therefore, might be expected to act similarly in liposome preparations. The analogous subunits of both chloroplast and bacterial ATP synthetase have also been shown to translocate protons in liposome preparations (*22*). Another possible effect of *oli*^{r} mutations is that they may lead to secondary effects on mitochondrial membrane structure, as has been proposed for yeast *oli*^{r} mutations (*23*).

Our finding that a change in mitochondrial function can lead to a periodicity change suggests three possible approaches to the study of the biochemistry of rhythmicity. (i) Mitochondrial functions and sensitivity to inhibitors could be analyzed in the strains carrying period-altering mutations. (ii) Existing strains of *Neurospora* that bear mutations in the mitochondrial genome could be analyzed for their effects on circadian rhythms. This approach has already proved worthwhile in that the *poky* mutation, a maternally inherited mutation, when introduced into the *bd* strain, was 200-fold less sensitive than the normal *bd* strain to the inhibition of the conidiation rhythm caused by exposure to light (*24*). (iii) New mutants selected on the basis of defective mitochondrial function could be obtained and tested for their rhythmic properties. For example, a broad class of mitochondrial mutants could be isolated as nongrowers on glycerol (*25*), and mutants with defects in ATP synthetase could be selected on the basis of resistance to compounds that specifically inhibit this complex, such as aurovertin, rutamycin, and venturicidin (*26*). The three lines of investigation listed above, coupled with biochemical studies of the ATP synthetase complex in wild-type and mutant strains such as *oli*^{r}, *frq*, and *cel*, may lead to the understanding of the role of mitochondria in generating circadian periodicity.

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17 July 1979; revised 22 October 1979

Feedback Control of Juvenile Hormone Synthesis in Cockroaches: Possible Role for Ecdysterone

Abstract. *Inactive female corpora allata implanted into adult males become active and continue to synthesize juvenile hormone at high rates. However, when an ovary is implanted together with the corpora allata, rates of juvenile hormone synthesis decline as the oocytes complete maturation. Injections of ecdysterone mimic the effect of an implanted ovary.*

Ovarian development in the viviparous cockroach *Diploptera punctata*, as is the case in many insects, is dependent on the synthesis and release of juvenile hormone (JH) by the corpora allata (CA) (*1*). In other cockroach species, JH stimulates the fat body to produce vitellogenins (yolk precursors) and also stimulates the oocytes to take up and store these as yolk (*2, 3*). The synthesis of JH correlates precisely with the cycle of egg maturation, as demonstrated by in vitro radiochemical assay of *D. punctata* CA (*4*). Also, in other species, ovaries need not be present for the synthesis of vitellogenin by the fat body (*2, 5*), and therefore it could be hypothesized that the ovaries do not influence the CA. However, we have found evidence that ovaries do influence the CA: after ovariectomy the cycle of JH synthesis is suppressed; and, when two pairs of CA of different ages are implanted into a host, both pairs simultaneously terminate their cycle of JH synthesis in close correlation with the maturation of oocytes (*6, 7*), suggesting that termination of the cycle is related to the presence of a mature ovary or a physiological event associated with the completion of vitellogenesis.

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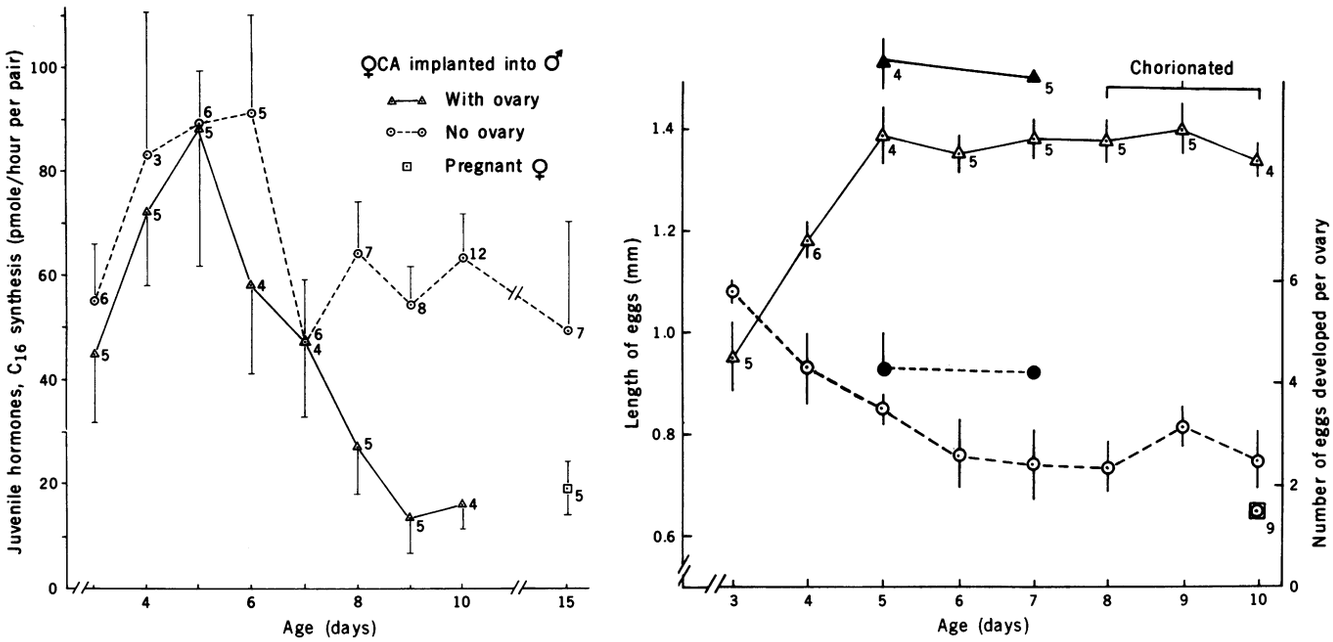


Fig. 1 (left). Rates of synthesis of C_{16}JH by female CA implanted into males with (△---△) or without (○---○) an ovary; implants from 0-day females to 0-day males; the rate of JH synthesis in normal pregnant females on day 15 is shown by (□). Each point is the mean of the number of individual measurements shown adjacent to the point; vertical bars show standard error of means (S.E.M.). On days 8 through 10, the differences between experimental and control values are significant ($.01 > P > .001$; Student's t-test). Fig. 2 (right). Lengths of basal oocytes of ovaries implanted into males on day 0 (△---△) (only oocytes with no visible defect in development are included). Each point is the mean of the number (adjacent to length point) of individual ovaries measured; the number of oocytes which appeared to be developing normally in the ovary is shown (○---○). The mean oocyte length for ovaries implanted into males without female CA is shown for day 10 after implantation (⊡). The mean length (▲—▲) and the mean number of eggs matured (●---●) are shown for ovaries implanted with female CA into ovariectomized females (on day 0) on days 5 and 7 after implantation.

To investigate the effect of the ovary on the CA, we used males as culture media for female CA and ovaries (*8*). Normally no vitellogenin is detected in male blood (*9*), presumably because male CA synthesize insufficient JH (*10*). However, when female CA are implanted into males, vitellogenin appears in sufficient quantity to support deposition of yolk in implanted ovaries (*9*). We now report a comparison of the rates of JH synthesis by female CA implanted into males in the absence and presence of an implanted ovary. There is a striking difference: the ovary effects a final decline in JH synthesis and this effect can be mimicked by injection of the molting hormone ecdysterone.

CA were removed from females at adult ecdysis (day 0) and implanted into males at day 0. On days 3 through 10 and day 15 after the operation the rates of C_{16}JH synthesis (*11*) by the implanted CA were measured over a 3-hour period. The mean rate of JH synthesis by these implanted glands (Fig. 1) increased rapidly from 3 to 4 and reached maximum rates of 90 pmole per pair per hour on days 5 and 6. By day 7 the rate of JH synthesis declined to 47 pmole per pair per hour, but thereafter there was no further decline. Even 15 days after implantation, the rate of JH synthesis remained relatively high (about twice that in a female of this age, pregnant with developing embryos in the brood sac) (Fig. 1). Thus, between days 3 and 7 the pattern of JH synthesis by the female CA in males was similar to that observed for denervated glands in mated females (Fig. 3) (*7*); thereafter it differed in that the denervated glands in otherwise normal females declined to a lower synthetic rate on days 8 through 10.

Because the decrease in rates of JH synthesis between day 6 and 7 occurred independently of the ovary in these experiments, we proposed that a high titer of JH in the hemolymph acts by negative feedback to suppress JH synthesis either directly or indirectly. Indeed, such negative feedback has been demonstrated by topical application of JH analog to normal females (*12*).

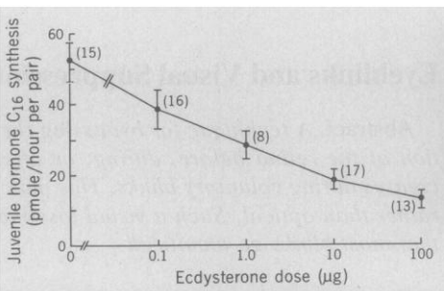
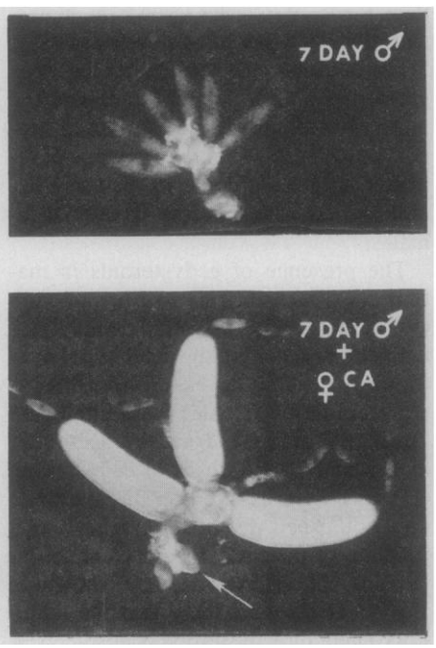


Fig. 3 (left). Rates of C_{16}JH synthesis by female CA implanted into males as a function of total ecdysterone dose. The CA were implanted on day 0 and assayed on day 8; hormone was injected on days 6 and 7. Each point is the mean of individual measurements shown in parentheses. The differences between control animals and those injected with 1, 10, or 100 μg of ecdysterone are significant ($P < .005$, Student's t-test). Fig. 4 (right). An immature ovary implanted into a male shows no growth of basal oocytes after 7 days (top). However, if an ovary and female CA (arrow) are implanted together, several basal oocytes are mature in 7 days (bottom).



If a pair of female CA and a 0 day ovary were implanted into the males, the pattern of JH synthesis (Fig. 1) resembled that of normal females and was identical in timing to that of females with denervated CA (Fig. 3) (*7*). The final decline in the cycle of activity of implanted CA was achieved by implanting an ovary, and this occurred in concert with the completion of oocyte maturation. This inhibition of JH synthesis by the implanted ovary must occur by way of a humoral pathway.

Figure 4 shows ovaries implanted into males with and without CA. Ovaries implanted into males in the absence of female CA showed no growth. Even after 10 days the basal oocytes remained in the previtellogenic stage as at implantation (0.65 ± 0.001 mm in length) (Fig. 2). Hence the production of JH by male CA was insufficient for oocyte development. However, in the presence of female CA the oocytes increased in length to 1.40 ± 0.06 mm by day 5 and complete chorion deposition occurred between days 7 and 8. These observations indicate that the event leading to the decline in rates of JH synthesis after day 7 is the completion of vitellogenesis as signaled by chorion formation.

In *D. punctata*, an ovary usually contains six ovarioles (*13*). In ovaries implanted into males, only a fraction produced mature oocytes (Fig. 2). To compare the relative suitability of male and female environments for ovarian growth, oocyte lengths were measured in ovaries implanted into females ovariectomized and implanted on day 0 with one ovary and a pair of CA from day 0 females. On days 5 and 7 after the operation, the mean lengths of the oocytes were greater than those of ovaries grown in males; and more oocytes have developed (Fig. 2). In addition, the vitellin content was greater by a factor of 10 (*9*). Hence the male environment, although able to support oocyte growth in the presence of female CA, did not do so to the same extent as that which occurs in normal females.

The presence of ecdysteroids in mature cockroach ovaries and hemolymph (*14*) suggests that ecdysteroids may participate in regulating the rates of JH synthesis by the CA. To test this hypothesis, CA from day 0 females were implanted into day 0 males and males injected on days 6 and 7 with doses of ecdysterone (*15*). CA were assayed for JH synthesis on day 8. The rates of JH synthesis as a function of the total dose (two equal injections) of ecdysterone are shown in Fig. 3. These data show that the inhibitory effect of ecdysterone is dose-dependent. At 100 μg and 10 μg the rates of JH synthesis are low, 13.15 ± 2.04 and 18.45 ± 2.59 pmole per pair per hour, respectively. These low rates are similar to those measured at the end of the gonadotrophic cycle in normal females (*4*) and in males implanted with an ovary and female CA (13.40 ± 5.91 and 15.69 ± 4.92 pmole per pair per hour on days 9 and 10, respectively) (Fig. 1). The rates are significantly lower than those of the controls (Fig. 3).

Whether ecdysterone from the ovary limits the activity of the CA at the end of a normal gonadotrophic cycle either directly or indirectly remains to be determined. However, our results show that the ovary is acting humorally to limit JH synthesis, supporting the hypothesis that it is doing so by production of ecdysterone. Engelmann's observation, in another cockroach species, that either implanted prothoracic glands or injection of the hormone ecdysone inhibit both egg maturation and increase in volume of CA (*16*), is consistent with this hypothesis. Ecdysone from mosquito ovary has been shown to control production of vitellogenin (*17*); the fly ovary is thought to produce an oostatic hormone (*18*). The demonstration of an endocrine function for the cockroach ovary makes it likely that the endocrine functions of insect ovaries are more widespread than previously thought.

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1 October 1979

Eyeblinks and Visual Suppression

Abstract. *A technique for bypassing the eyelids permits equivalent visual stimulation of the retina before, during, or after a blink. Sensitivity to these stimuli decreases during voluntary blinks. This indicates that the source of the deficit is neural rather than optical. Such a visual loss may help to explain the common experience that most blinks go unnoticed.*

Under normal conditions of vision, eyeblinks occur every few seconds (*1-3*), each blink producing an almost total interruption of light and pattern reaching the eye. It is a surprising feature of visual perception that the interruption of vision typically goes unnoticed, and the subjective visual world remains continuous and stable. The effect is even more surprising when one considers the magnitude and duration of this interruption. Consider the voluntary blink diagramed in Fig. 1A. As the upper lid drops over the pupil, it severely attenuates the light reaching the eye (*4*) and wipes out nearly all visual information in the form of contour and contrast. The time during which the pupil is completely obscured is relatively long: 110 msec in the example shown (*5, 6*). Yet the blink is scarcely noticed. By contrast, in the absence of a blink, a much briefer interruption of room lights has a pronounced visual effect (*3, 7*).

The observation that the perceptual effect of a blink is small with respect to the

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