lized' ATPase activity. At concentrations of 100 μU/ml and higher an enhancement of the ATP hydrolysis could be observed. The highest increase was at 500 μU/ml (172% of the normal activity). 20,000 μU/ml did not affect the enzyme-caused P_i liberation (not shown in the table). The behavior of hypothalamus-derived ATPase was identical. The addition of antiinsulin serum reduced the activity of ATPase to the control level, thus clearly indicating that the effects registered are due to the presence of the hormone.

Discussion. The demonstrated effect of insulin on cerebral NaK-ATPase is in contrast to reports which have shown the failure of insulin to influence ion fluxes in the brain[15,16]. The observed similar behavior of ATPase from hippocampus (a CNS region with an expressed blood-brain barrier) and hypothalamus (lacking a BBB) indicates that the stimulatory influence of insulin is not restricted to the blood vessel-associated part of ATPase activity, which is possibly involved in the maintenance of BBB. There are a few reports dealing with insulinbinding sites at synaptosomes derived from rat CNS[17,18] and axonal terminals[19]. Since nerve endings and the axolemma are densely populated with NaK-ATPase molecules it cannot be excluded that the effect of the hormone was directed to these neuronal particles. Recently, an effect of insulin on the activity of another enzyme in the CNS, ornithine decarboxylase, was suggested[20]. In any case the functional consequences of these findings will remain unclear until new data appear that can help to clarify the significance of insulin for brain metabolism.

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Radiochemical assays of corpus allatum activity in adult female cockroaches following ovariectomy in the last nymphal instar

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Summary. Surgical removal of the ovaries from nymphal *Periplaneta americana* results in lower than normal corpus allatum activity in the adult insect and an apparent absence of the manifestly cyclic pattern of juvenile hormone biosynthesis found in intact mated females. The results suggest that the presence of synchronously developing ovaries is necessary for the attainment of normal synthetic activity in the corpus allatum of this species.

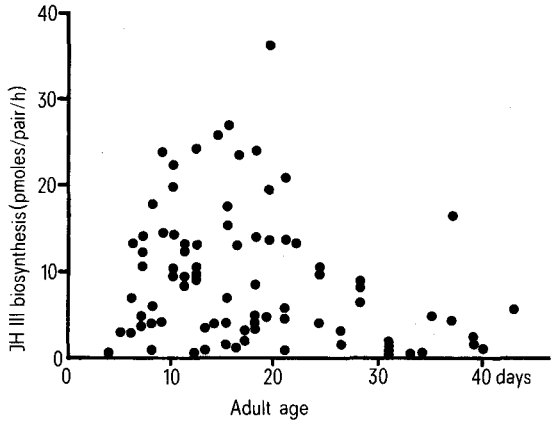
It has been demonstrated that juvenile hormone (JH) is necessary for both the synthesis of vitellogenins and their uptake into the oocytes in *Periplaneta americana,* and radiochemical determinations of JH biosynthesis by isolated corpora allata (CA) indicate that the glands of adult females undergo cycles of activity in vivo[3,4]. The peaks of JH biosynthesis correspond temporally with the later stages of vitellogenesis of terminal oocytes and, at the same time, with the early stages of vitellogenesis of penultimate oocytes[3,4], but the precise physiological basis of the cycles of hormone production is imperfectly understood and the regulatory factors are unknown. Previous studies have proposed feedback loops between the ovaries and the CA in a variety of insect species[5-7] but, with a single exception[7], these have been limited to measurements of glandular size or to histological appearance which are not necessarily indicative of precise glandular activity[8]. In this paper we report the effects of ovariectomy on JH biosynthesis by the CA from *P. americana* which is part of a programme to fully investigate the control of reproduction in this species.

Materials and methods. Last (5th) nymphal instar *P. americana* were anaesthetized with nitrogen, surface sterilized with ethanol, and surgically ovariectomized through 2 small incisions in the dorsal cuticle of abdominal segment VII. The wounds were then sealed with wax. Antibiotics were not used, but survival and successful moulting of operated insects was better than 80%. The operations were performed in the nymphal stage in order to allow time for any artefactual disturbance to dissipate. The stimulatory effects of sham surgery on cockroaches appear to be short-lived[7] and it was therefore considered unnecessary to perform such operations on controls. When moulting to the adult instar had occurred the operated insects were maintained with intact male and female adults under conditions previously described[3,4].

Ovariectomized and control adult females were sacrificed at various times after adult emergence and activities of their CA assessed by the incorporation of radiolabel from methyl ^{14}C-methionine (Radiochemical Centre, Amersham, U.K.) into JH III in vitro using published methods[3,4].

Results. A broad range of CA activities was observed during the 2nd and 3rd weeks of adult life in ovariectomized females, but cyclicity of JH biosynthesis was not apparent (figure). During this period the CA of intact females exhibit 5 clearly defined cycles of activity[4]. CA from certain operated females exhibited moderately high activities but the rates of JH biosynthesis by these glands (20–26 pmoles/pair/h) were far lower than values frequently observed for CA from control females (up to 48 pmoles/pair/h)[4]. The highest activity observed in the ovariectomized group was 35 pmoles/pair/h (1 insect=1.2%), whereas 14% of control insects had CA activities above this level. After the 3rd week there is a sharp decline in CA activity of ovariectomized females but glands from control insects still exhibit peaks of high activity. Although the operation appears to diminish overall CA activity, there is no change in storage or release of hormone. Separate extraction of glands and medium in 20% of the incubations indicated that there was essentially no storage of newly synthesized JH in CA from ovariectomized females; regression analysis of JH released against JH synthesized gives $\alpha=0.39$, $\beta=1.02\pm0.01$, r=0.999, which agrees well with the reported analysis for unoperated females[4]. Finally, examination of the spermathecae revealed that ovariectomy had no effect upon sexual receptivity because all females had mated by the 6th day.

Discussion. The observed effects of ovariectomy upon CA activity in *P. americana* can be interpreted in at least 2 ways. The CA of certain operated females may have low JH biosynthetic capacity at all times, whilst glands from others may exhibit a rise to moderate activity followed by a gradual decline. Alternatively, the CA in each individual may exhibit cyclic activity, but there is no synchronisation between different animals, in which case the observations would be random samples from an asynchronous fluctuating population. In that case there would still be a bias towards lower than normal activity, for example the data from the first 3 weeks of adult life would indicate that approximately 40% of the time was spent synthesizing JH at rates of less than 5 pmoles/pair/h, considerably more than that so spent by normal females[4]. The methods employed in the present study did not permit more than a single determination for each individual insect, and consequently we cannot distinguish between these 2 theories. The observed pattern of CA activity is somewhat similar to that reported previously for virgin female *P. americana*[4], but the causal mechanisms may be different because ovariectomized females mate normally. Ovariectomy results in the appearance of large quantities of 'secretory' material in the corpora cardiaca and CA of *P. americana*[9], and also in a doubling of haemolymph total protein concentration and a 20-fold increase in haemolymph vitellogenin levels[2]. It is possible that the build up of material within the retrocerebral complex is a manifestation of a CA inhibitor, and equally possible that high levels of haemolymph proteins are part of a negative feedback signal. Diminished CA activity following ovariectomy in another cockroach species, *Diploptera punctata,* has been indicated by histological observation[10]and confirmed by measurement of JH biosynthesis[7], but 2 studies of the dimensions and histological appearance of the CA from ovariectomized *Leucophaea maderae*[11,12] suggest that the operation results in CA hyperfunction in this latter species. The 'histological' hyperactivity of CA from ovariectomized *L. maderae* has yet to be confirmed biochemically. Therefore, either cyclic CA activity is achieved via different control loops in this species, or the histological appearance of the glands is a particularly misleading indicator of their rate of JH synthesis. We conclude, from our in vitro assays of CA activity in both ovariectomized (reported here) and virgin[4] *P. americana,* that synchronously developing ovaries are necessary for the attainment of full synthetic activity within the CA of this species, and that stimuli of ovarian origin may coerce random fluctuations in JH biosynthesis such that a precisely phased relationship with ovarian maturation ensues. The nature of these stimuli remains to be elucidated. Stay et al.[13] have concluded, from careful experiments carried out in male *D. punctata* implanted with female CA and ovaries, that chorionating follicles might be a source of negative stimuli (perhaps indirect) to the CA. The effect could be mimicked by rather large (10–100 µg) injections of ecdysone. Clearly, such a phenomenon could not be responsible for low levels of JH synthesis in ovariectomized animals. Probably the most interesting question is whether the reduced levels of JH synthesis in ovariectomized animals are the result of an absence of an important primary stimulus from the ovary, or are the result of accumulating feed-back inhibitions resulting from the lack of fulfillment of the gonotrophic stimulus from the CA. High titres of vitellogenin, JH (as yet unmeasured), or other factors might be responsible.



Rates of juvenile hormone biosynthesis by isolated corpora allata from 84 adult female *Periplaneta americana* in relation to age from adult emergence following ovariectomy in the last nymphal instar. Rates for unoperated insects have been omitted because they were essentially the same as those previously published for mated adult females of this species[4]. Individual pairs of CA were incubated for 3 h at 30 °C in 0.1 ml of TC-199 modified by the addition of 20 mg/ml Ficoll and 20 mM Hepes, adjusted to pH 7.3, and containing [methyl-^{14}C] methionine at a final concentration of 0.20–0.24 mM and final sp. act. of 30–35 mCi/mmole (in different incubations). The extraction, separation and quantitation of radiolabeled JH III were as previously described[3,4].

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