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CORRESPONDENCE

On the role of Juvenile Hormone in vitellogenesis in cockroaches

A reply to Holbrook *et al.*, *Physiological Entomology* (2000) 25, 27–34. Juvenile Hormone is essential to induce vitellogenesis in the German cockroach, also in Barcelona.

A recent article of C. Schal and associates (Holbrook *et al*., 2000) on the effects of ovariectomy, the activity of the corpora allata (CA) and Juvenile Hormone (JH) production in *Blattella germanica*, stresses a number of discrepancies with respect to results published by us. However, most of the claimed discrepancies seem more apparent than real, which prompts us to clarify the following points. In the discussion (p. 30), Holbrook *et al*. (2000) say: 'Our results prompt us to make conclusions that differ radically from those made by Maestro *et al*. (1994), who asserted that the corpora allata of unmated, ovariectomised *B. germanica* produced JH at a low, almost constant rate in the first 9 days of adulthood. By contrast, we contend that the activity of the corpora allata markedly increases and then decreases, that is, cycles in ovariectomised virgins. It is worth noting, however, that the data of Maestro *et al*. (1994) do, in fact, support our contention, for their results show a muted cycle of JH synthesis in ovariectomised females, which went unrecognised by the investigators'. Yes, our data showed that activity of the CA increased and decreased, but the differences were not statistically significant. In any case, the text reproduced above argues against the radical difference announced at its onset.

Another claimed discrepancy that does not exist is between two papers coming from Schal laboratory. Holbrook *et al*. (2000) (p. 31), say 'We have now ascertained that mating stimulates the production of JH in females lacking ovaries. This result is at odds with our previous investigation: Gadot *et al*. (1991) reported that the corpora allata of unmated and mated produced similar amounts of JH'. However, Gadot *et al*. (1991) described that 'the CA of ovariectomized females that did not mate (have not become sexually receptive) in daily exposure to males exhibited low and relatively invariable rates of JH synthesis *in vitro* (Fig. 1)'. Compare also with the text reproduced in the previous paragraph.

Rates of production of JH described by Schal are generally higher than those reported by us, but this could be explained because Schal uses the rapid isooctane partition assay (which may measure derivatives of JH in addition to JH), and because he adds some five-fold more $CaCl_2$ than usual to the medium (which greatly enhances JH production). In our experiments, the CA from ovariectomized virgins increase rates of production of JH *in vitro* with time (Maestro *et al*., 1994), whereas the production is linear in intact females (Bellés *et al*., 1987). The extra $CaCl_2$ used by Schal and his associates may even explain why in their experiments the CA from virgin ovariectomized females do not increase rates of production of JH *in vitro* with time (Holbrook *et al*., 2000).

Finally, Holbrook *et al*. (2000) state (p. 32) that 'Greatly at odds with these results [the previous paragraph gives data on the vitellogenic role of JH], Martín *et al*. (1995a) reported that the fat body of ovariectomised virgins of *B. germanica* produced abundant vitellogenin, even though the corpora allata in these females did not synthesize a substantial quantity of JH (Maestro *et al*., 1994). They suggested therefore that JH was not essential, but that other factors probably were, to induce vitellogenesis in the German cockroach (Martín *et al*., 1995a,b, 1996)'. Although the reference is wrong, we certainly reported what is stated in the first sentence (in Martín *et al*., 1996; not 1995a). However, and this is the main stimulus for this reply, we never inferred what is stated in the second. In intact females of *B. germanica*, rates of production of JH as low as ≈ 0.2 pmol/h measured up to day 3 of adult life (Bellés *et al*., 1987; Maestro *et al*., 1994), fire the vitellogenic cycle (Martín *et al*., 1995b). Therefore, low concentrations of JH could be enough to sustain vitellogenesis, which is not contradictory with the fact that JH determines vitellogenesis in this cockroach, as showed at the molecular level by Comas *et al*. (1999). What is obvious is that rates of synthesis of JH and those of vitellogenin production are uncoupled in the second half of the cycle, because when vitellogenin production begins to decrease (Martín *et al*., 1995a,b) synthesis of JH is still increasing (Bellés *et al*., 1987; Maestro *et al*., 1994). This suggests that the JH cycle does not modulate that of vitellogenesis, in the sense that the decrease of vitellogenin production is not determined by a decrease in the rate of synthesis of JH. A plausible hypothesis is that a supplementary factor, possibly coming from the ovary, may be involved in terminating the synthesis of vitellogenin (Martín *et al*., 1995a). What, then, could be the sense of the rising rates of synthesis of JH when vitellogenin production declines? They possibly modulate vitellogenin incorporation into the oocyte, as suggested by Martín *et al*. (1995a), given that the degree of enlargement of the intercellular spaces of the follicular epithelium correlates with the rates of synthesis of JH and with the dynamics of growth of the basal oocyte (Pascual *et al*., 1992).

The discussion of Holbrook *et al*. (2000) finishes with possible explanations for the high rates of production of vitellogenin in ovariectomized females, and with comments on the regulation of vitellogenesis in general, using data published by Schal and associates and by us. Our team has measured JH

(Bellés *et al*., 1987; Maestro *et al*., 1994), vitellogenin (Martín *et al*., 1995a,b, 1996) and ecdysteroids (Pascual *et al*., 1992; Romañá *et al*., 1995) (which are also invoked by Schal as possible regulatory factors), but, unfortunately, no measurements of vitellogenin and ecdysteroids are available in published contributions from Schal's laboratory, which makes the inference of parallelisms less clear.

In science, the true challenges consist in finding regularities, and the vitellogenic role of JH in most insects, including *B. germanica*, is an old regularity not challenged by us. We believe firmly that research effort should be directed towards finding regularities and agreements, not discrepancies, especially if the latter are more apparent that real.

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Reply – Consentience on the necessity of Juvenile Hormone for vitellogenesis in the German cockroach

The role of the ovary in regulating the synthesis of Juvenile Hormone (JH) during reproduction has been a subject of considerable investigation, especially in cockroaches. From this research has emerged the opinion that in adult female cockroaches the ovary must be present for the corpora allata (CA) to reach a high level of activity. Indeed, the CA in ovariectomised females of two well-studied blaberids, *Diploptera punctata* and *Nauphoeta cinerea*, and the blattid *Periplaneta americana*, were shown to produce little JH, even after females had mated (Stay & Tobe, 1978; Lanzrein *et al*., 1981; Weaver, 1981). In sharp contrast, we later showed that the CA of ovariectomised, mated *Blattella germanica* (L.), a blattellid, synthesized as much JH as those of females with ovaries (Gadot *et al*., 1991). Moreover, we found that the CA of ovariectomised females, after an extended period of high JH production, declined only partially and transiently in activity, but not in females whose CA had been denervated from the brain. On the basis of these results, we concluded that the ovary contributes less to activation of the CA in *B. germanica* than in other studied cockroaches.

Maestro *et al*. (1994) later found differently and reported that the CA of *B. germanica* were greatly depressed in activity in females, this time virgins, whose ovaries had been excised. Unexpectedly, however, the fat body in these females produced substantial amounts of vitellogenin (Martín *et al*., 1996), whose synthesis is widely thought regulated by JH (Wyatt & Davies, 1996). This finding led Bellés and co-workers to hypothesize a limited role for JH in regulating vitellogenesis in the German cockroach. Martín *et al*. (1995a) stated as much: 'Maestro *et al*. (1994) have shown that ovariectomized specimens of *B. germanica* produce low levels of juvenile hormone during the first 9 days of imaginal life, whereas Martín *et al*. (in press) described that ovariectomized females produce huge amounts of vitellogenin which accumulate in the haemolymph (see also Kunkel, 1981). All these data suggest that the Juvenile Hormone cycle (Bellés *et al*., 1987; Maestro *et al*., 1994) does not modulate that of vitellogenesis (Martín *et al*., 1995a)….'. We suspected and subsequently found that the CA of ovariectomised females produced abundant JH, much more than reported by Maestro *et al*. (1994). Indeed, by the fourth day of adulthood, when the fat body is producing large amounts of vitellogenin (Martín *et al*., 1996), the CA of ovariectomised and intact virgin females were synthesizing hormone at a similar rate (Holbrook *et al*., 2000). We concluded, therefore, that the synthesis of JH and vitellogenin are linked in the German cockroach. We did find that after day 4 the CA of ovariectomised virgin females produced less hormone than those of intact, virgin females. However, in mated, ovariectomised females the CA were as active as those in intact mated females (Gadot *et al*., 1991; Holbrook *et al*., 2000), in sharp contrast to what had been previously found in blaberids and in a blattid. In any event, our findings lent support to the thesis that changing rates of JH

biosynthesis regulate vitellogenin synthesis in the German cockroach.

The Bellés group takes exception to some of our conclusions. For example, they state they have never excluded JH as an important regulator of vitellogenesis, yet their published comments do not bear this out. The foregoing passage from Martín *et al*. (1995a) is illuminating in this regard, as are later comments by Martín *et al*. (1996): 'Our previous studies have shown that virgin ovariectomized specimens of *B. germanica* produce low levels of JH during the first 9 days of imaginal life (Maestro *et al*., 1994). In spite of this, the present results indicate that ovariectomized females steadily increase the production of vitellogenic proteins during these days. This suggests that rising rates of JH synthesis observed during the gonadotropic cycle of intact females (Bellés *et al*., 1987; Gadot *et al*., 1989; Maestro *et al*., 1994) are not necessary to modulate the parallel increase in the production of vitellogenic proteins.'. However, existing data do support the parsimonious conclusion that changing rates of JH biosynthesis regulate vitellogenesis in ovariectomised *B. germanica*. We found that the CA in ovariectomised virgin females attained a level of activity sufficient to stimulate the production of prodigious vitellogenin in intact females (see discussion in Holbrook *et al*., 2000). Additionally, recent research from Bellés and co-workers showing a dose-dependent effect of JH on vitellogenin synthesis (Comas *et al*., 1999) lends great credence to the hypothesis that a high rate of production of JH is responsible for the synthesis of abundant vitellogenin in ovariectomised females. We consequently reject the view that 'rising rates of JH synthesis… are not necessary to modulate the parallel increase in the production of vitellogenic proteins.'

Bellés *et al*. apparently do not accept a reappraisal within Holbrook *et al*. (2000) of previous work (Gadot *et al*., 1991) and point out what appears to be, at least to them, an internal contradiction. Gadot *et al*. (1991) found that the activity of the CA was consistently low in ovariectomised females that failed to mate. Bellés *et al*. misconstrue this as meaning that JH synthesis remains low in ovariectomised virgin females. Such an interpretation is, however, incorrect, because females that refuse to mate distinctly differ from the virgin females examined by Holbrook *et al*. (2000). Female *B. germanica* become sexually receptive only after their CA have produced considerable JH (see Schal *et al*., 1997), and although females with ovaries almost always mate within 6 days of eclosing, a cohort of ovariectomised females usually contains some that remain non-receptive for more than 30 days; the CA of the latter produce little JH compared with those of the former. In our recent investigation (Holbrook *et al*., 2000), we did not screen for female receptivity and pooled all ovariectomised females when measuring JH synthesis. If we had, in fact, eliminated the few females that would not have mated, we may very well have found smaller differences in the activities of CA from intact and ovariectomised females. Nevertheless, the issue is, to some extent, irrelevant in that neither sham-operated nor ovariectomised virgin females would ever mate in the first 4 days of adulthood, during which the fat body synthesizes a large amount of vitellogenin.

An issue addressed in the counterposing correspondence is the extent to which the results of radiochemical assays *in vitro* adequately reflect activities of the CA *in vivo*. We, too, believe this issue to be pertinent and concur with Bellés *et al*. that differences in incubation conditions may fully explain disparities between our results. They point out that we add roughly five-fold, more probably, about three-fold, more calcium to the culture medium than they do and propose that this 'high' concentration of calcium stimulates JH synthesis in our assays. We would, however, argue that they are using non-physiological conditions detrimental to gland activity. Bellés *et al*. use medium 199, which contains in most formulations 1.8 mM calcium but in some just 1.3 mM. As it turns out, the concentration of calcium in cockroach haemolymph, 4 mM in adult *P. americana* (King *et al*., 1986) and slightly higher in adult *Leucophaea maderae* (Todd, 1958), far exceeds these levels. So we are within reason using 5 mM calcium, especially as cockroach CA in 3–5 mM calcium produce similar amounts of JH (Kikukawa *et al*., 1987). It is notable that Kikukawa *et al*. (1987) also found CA activity to be greatly inhibited in subphysiological concentrations of calcium.

Bellés *et al*. state that many expressed differences between our results and theirs are 'more apparent than real.' We contend that real differences in opinion exist. Whether they believe, as we do, that JH synthesis rises and falls (cycles) in ovariectomised females remains unclear. They cannot have it both ways in claiming CA activity increases and decreases but does not significantly change. This issue aside, other significant differences exist. Above all, we find a much smaller disparity than they do in the activity of the CA of ovariectomised and intact females.

Can common ground be reached between our two laboratories? The clarifications provided by Bellés *et al*. of their hypotheses seem to leave no serious disagreement on the important role that JH plays in vitellogenesis in the German cockroach. Likewise, there has not been, nor is there now, reason to dispute the main thesis of our earlier research that in the German cockroach, unlike in other cockroaches, the CA become highly active in the absence of ovarian factors. Of course, real differences in opinion may persist, but this should not be judged in a negative light. The effort to find 'regularities and agreements, not discrepancies', can at times impede elucidation of differences among species, for example, among *B. germanica*, *N. cinerea*, *D. punctata* and *P. americana*. Certainly, knowledge of such differences is fundamental to understanding the evolution of mechanisms regulating endocrine function.

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