Effect of mating on Monarch butterfly oogenesis[1]

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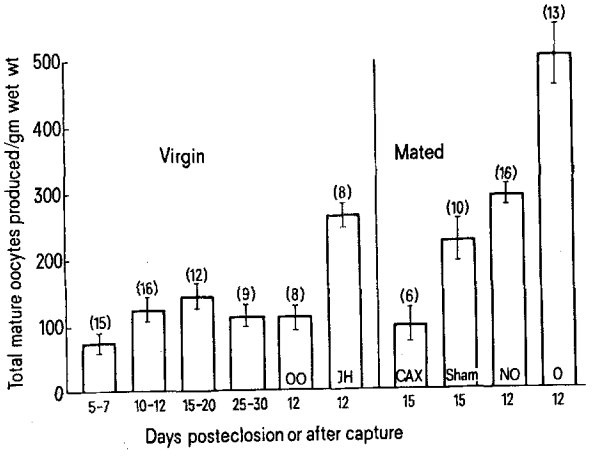
Summary. Oogenesis ceases in virgin Monarch butterflies at about 10 days posteclosion, but an enhanced production of mature oocytes occurs after mating. Juvenile hormone injections into virgins, and allatectomies of mated females, indicate that the observed fluctuations in oogenesis are due to periods of corpora allata activity and inactivity.

As part of our continuing study of the reproductive neuroendocrinology of Monarch (Danaus plexippus) butterflies[3–7], we recently noticed that virgins seemed to produce far fewer oocytes than did mated females. This casual observation suggested that mating might exert a profound influence on the activity of the Monarch neuroendocrine system. We therefore decided to examine this question in greater detail. The results of our studies, demonstrating arrested oogenesis in virgins and enhanced oogenesis in mated Monarchs and suggesting cyclic activity of the corpora allata as a causal factor, are described below.

Monarchs were either obtained from pupae reared in the field or captured as adults. Some females were held in glassine envelopes in incubators programmed at 25 °C with a 16-h-photophase; such animals never oviposite. Females allowed to oviposite were placed with fresh daily milkweed (Asclepias syriaca) and held in cages outdoors. Animals were reared, captured, and used for our studies during June, July and August of 1975–6. All butterflies were fed 30% honey daily after eclosion or capture. Allatectomies were performed and controlled as described elsewhere[3]; the juvenile hormone was the Ayerst mixture[3–7] dissolved in 10 µl olive oil. At the completion of each experiment animals were weighed, the bursa copulatrix examined for the presence (= mated) or absence (= virgin) of spermatophores and all mature oocytes counted. In some studies we also wieghed[4] the colleterial glands. Total mature oocytes produced were determined as oocytes oviposited (if any) plus oocytes contained at dissection. Data, presented as mean ± SEM, was analyzed both as mature oocytes/animal and mature oocytes/gm animal wet weight. The conclusions were the same with both methods; the latter, which reduced variation, was used in this report. Data were examined with Student's t-test; the term significance herein denotes statistical significance at the 5% level or better.



Effects of various treatments on oogenesis in Monarch butterflies. *OO* injected with 10 µl olive oil; *JH* injected with 100 µg/animal juvenile hormone; *CAX* allatectomized mated females; *Sham* sham-operated controls for allatectomies; *NO* animals not allowed to oviposite; *O* animals allowed to oviposite.

Our initial studies determined the time-course of oogenesis in virgins held at 25 °C from eclosion until the day of dissection. The totally undeveloped ovaries of newly emerged females[3] undergo rapid growth; by 10 days posteclosion they have produced about 125 mature oocytes/g animal. By contrast, during the next 20 days additional mature oocytes are apparently not produced, since the mean number of such oocytes present does not significantly change during that period (see figure). The static condition of the ovaries observed from 10 to 30 days posteclosion could result from a combination of oogenesis and oocyte resorption, but the latter process was never clearly evident. The colleterial glands, which like the ovaries are regulated by juvenile hormone in this species[3,4], exhibit a developmental time-course in virgins comparable to that of ovaries, i.e., they attain an apparently maximal mean size of about 7 mg wet weight in 10–12-day posteclosion virgins, and show no further significant size increases during the next 3 weeks.

2 further experiments, one using laboratory-emerged animals and one using wild-caught females, examined oogenesis in mated Monarchs. 5–7 days after eclosion laboratory-emerged animals were placed outdoors in cages with milkweed and mature males; about 50% of such animals normally mated within 24 h. Half the mated animals were then allowed to oviposite; the remaining females were held at 25 °C. Those females that did not mate within 24 h in the mating cages served as controls, as did virgins never exposed to males. Wild-caught females have normally mated prior to capture. We therefore separated such animals into 2 groups, 1 held outdoors with milkweed and 1 held at 25 °C. Animals in these studies were dissected either 12 days after eclosion and 5–7 days after mating (laboratory-emerged) or 12 days after capture (wild-caught).

The combined results of the 2 experiments (see figure) clearly showed that both mating and oviposition strongly stimulated oogenesis. In both experiments mated animals produced mean values more than twice those observed in virgins. Additionally, in both experiments mated animals allowed to oviposite produced significantly more mature oocytes than mated females not allowed to oviposite. Wild-caught virgins, virgins held with males, and virgins held without males (all lacking spermatophores) produced mature oocytes in a quantity insignificantly different from the mean values of virgins stored at 25 °C from 10 to 30 days. Again, colleterial gland wet weights paralleled the number of oocytes produced, e.g., 10.6 ± 1.9 mg and 7.4 ± 0.8 mg for laboratory-emerged mated versus virgin. The above data, especially when combined with previous results[3–7], strongly suggested that reduced or absent

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corpora allata activity in 10–30-day posteclosion virgins and increased or renewed activity of corpora allata after mating, could be responsible for the observed changes in both oogenesis and colleterial gland weights. 2 types of experiments were designed to test this possibility. In the first we examined the effects of juvenile hormone injections (100 µg/animal) into 7-day posteclosion virgins. The results of this experiment were clear; animals given hormone and held at 25 °C until 12 days posteclosion produced oocytes at a rate nearly that of mated animals not allowed to oviposite, while controls (either intact or injected with hormone vehicle) behaved as normal virgins (see figure). In the second experiment we examined the effect of corpora allata ablations on the increased oogenesis noted in mated animals. The results were again clear cut; mated animals allatectomized within 24 h after mating showed no increase in mature oocyte production, while sham-operated and intact controls produced oocytes at a rate comparable to that of intact mated animals and JH injected virgins (see figure).

It is clear from our results that after a period of intensive mature oocyte production, lasting about 10 days at 25 °C, virgin Monarch butterflies enter a period of oogenesis arrest lasting for at least 20 days or until mating occurs. Since juvenile hormone injections overcome this static situation, we believe that it is probably due to corpora allata inactivity. Mating clearly results in renewed mature oocyte production; since allatectomy of mated females eliminates this response we conclude that activation of a dormant corpora allata is probably required. Oviposition also increases mature oocyte production. The characterization of the mechanisms involved in this apparently complex regulatory process is currently being attempted.

HCG-insensitivity of the postnatal rat ovary is due to the lack of a specific receptor[1]

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Summary. Rat ovary is insensitive to luteinizing hormone and human chorionic gonadotrophin till to the 8th to 10th day of postnatal development. This insensitivity is due to the lack of a specific hormone receptor.

It is well established that testicular as well as ovarian function in sexually mature animals is controlled by the hypophyseal hormones luteinizing hormone (LH) and follicle stimulating hormone (FSH). However, during early postnatal development, despite high LH levels[3,4], both target organs are insensitive to LH and likewise do not respond to exogenous human chorionic gonadotrophin (HCG). In the rat testis, hormone sensitivity is lost after birth and restored at the end of the 2nd week of life[5]. HCG insensitivity of the postnatal testis is due neither to the lack of the specific HCG receptor, nor to the absence of the enzyme adenyl cyclase, both of which are prerequisites of HCG action[5,6]. Since injections of hydrocortisone into newborn male rats maintain HCG sensitivity of the postnatal testis, Engel and Frowein[7] suggested that LH refractoriness of the testis during the first 2 weeks after birth is due to the lack of glucocorticoids during this developmental period.

The rat ovary is insensitive to LH or HCG till the 8th to 10th day of postnatal development[8]. Only at that time does ascorbic acid concentration in the ovary decrease[9], and ovarian 3β-hydroxysteroid dehydrogenase activity[10], as well as estrogen in the peripheral blood[11], are demonstrable for the first time. Exogenous gonadotrophins in doses which produce dramatic weight increases of ovaries of 21-day-old female rats elicit only minimal ovarian weight gain when given in rats younger than 8 days of age[12]. Lamprecht et al.[13] observed an increase of LH inducible adenyl cyclase activity in rat ovaries for the first time in 8–10-day-old animals. The LH insensitivity of the postnatal rat ovary has not found an explanation so far. The present study was undertaken to test if the HCG insensitivity of the early postnatal rat ovary is due to the lack of specific HCG receptors.

Material and methods. The age and the number of animals used for the experiments are given in the table. The binding studies with radioactively labelled HCG were performed essentially as described by Lee and Ryan[14]. The ovaries were pooled and homogenized 1:10 (w/v) in 40 mM Tris-HCl 5 mM $MgSO_4$ buffer, pH 7.4. The homogenate was centrifuged at 100 × g for 20 min. Aliquots of the supernatant corresponding to 5 mg tissue wet weight were incubated in duplicate in the homogenization buffer containing 0.1% bovine serum albumine (BSA) and varying amounts of labelled and unlabelled HCG, in a final volume of 1 ml, at 37 °C for 30 min. The reaction was stopped by the addition of 1 ml ice-cold buffer and the incubates were immediately filtered with suction through cellulose acetate filters (pore size 0.45 µm, Sartorius, Göttingen, Federal Republic of Germany) previously washed with 10 ml 4% BSA. The nonspecific binding of

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