taining pseudopregnancy (HCG or LH induced) in rabbits on the basis of too low E2 levels to maintain corpus luteum. Although ovulation will occur, particularly when induced by the exogenous administration of HCG or LH, there is evidence that a certain level of E2 is required to maintain the corpus luteum[11]. There is also some evidence[12] that, for an optimal response of endometrium to progesterone, a critical priming dose of estrogen is needed. Finally, the present observations indicating a lack of relationship between the appearance of the external genitalia and the circulating E2 levels emphasize the importance of redefining an estrous rabbit.

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Induction of epidermal cyclic AMP by bursicon in mealworm, *Tenebrio molitor*[1]

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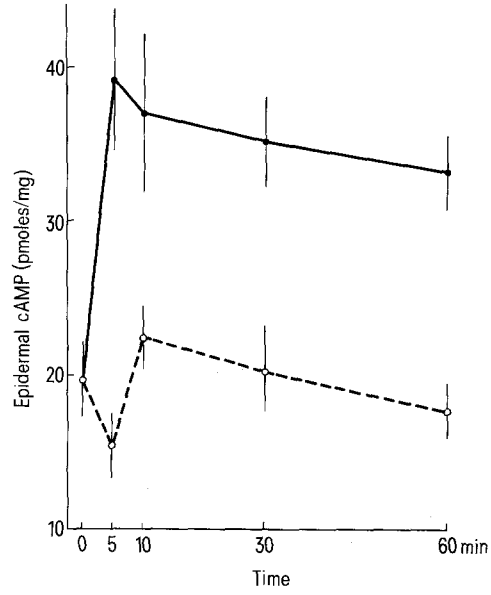
Summary. Bursicon active hemolymph of *Tenebrio*, injected into host pupae deprived in bursicon, induces a 2-fold increase of cyclic AMP in the epidermis of the hosts 5 min after the injection. No increase is observed by injecting bursicon inactive hemolymph or insect Ringer. From these experiments it can be concluded that cyclic AMP is a second messenger of bursicon.

Cuticle sclerotization (tanning) of insects is controlled by a peptidic hormone: bursicon[2,3]. Several studies have suggested that cyclic AMP is implicated in the regulation of cuticle tanning: tanning is induced by injection of cyclic AMP in newly emerged adult flies deprived of bursicon by neck ligation[4,5]. Similar results have been obtained in cockroaches after dibutyryl cyclic AMP injections[6] and, more recently, it was reported that cyclic AMP mimics the action of the puparium tanning factor (PTF), a protinaceous hormone that initiates hardening and darkening of dipterian puparium specially[7-9]. Thus cyclic AMP has been postulated to be the second messenger of these 2 hormones. Data that would strongly favor this hypothesis are: a) correlated changes of cyclic AMP in the target tissue and levels of circulating hormone, and b) induced changes of intracellular levels of cyclic AMP after injecting hormonal preparations.

Cyclic AMP content in the epidermis of the pharate adult mealworm is clearly correlated with bursicon activity of the hemolymph[10]. In this paper, we will show that injecting bursicon-containing hemolymph induces a specific rise of cyclic AMP concentration in the epidermis of mealworm pupae deprived of hormone by a thoracic ligation, which prevents the release of bursicon into the hemolymph and consequently tanning of the adult cuticle.

Materials and methods. The methods of selecting donors and hosts of appropriate age for injections have been already described[11]. Host pupae were ligated between pro- and mesothorax 1 or 2 h after pupal ecdysis. At room temperature, they became old pharate adults 10 or 12 days after ligation. This stage corresponds to the rise of bursicon activity in the hemolymph of nonligated animals[10,11], and



Epidermal cyclic AMP content of *Tenebrio* host pupae after injection with 3 µl bursicon active hemolymph (●) or bursicon inactive hemolymph (○). Each $HClO_4$ extract (150 µl) was neutralized with 30 µl 9 M KOH and the insoluble salts removed by centrifugation. 150 µl of the supernatant was acetylated with 6 µl pure acetic anhydride, 10-fold diluted with 9 volumes 0.1 M citrate buffer, pH 6.2, and cyclic AMP was measured by RIA. Bound and free nucleotides were separated by equilibrium dialysis[12]. Each point is a mean of 10 replicates with ±SEM shown as vertical bars. Noninjected animals: t_0.

was thus chosen for injecting bursicon active or nonactive hemolymph from donors. Bursicon active hemolymph was obtained from newly emerged adults and bursicon inactive hemolymph from young pharate adults[11] by cutting off the legs. The same glass calibrated micropipette was used to take 3 µl hemolymph from donor and to inject the host immediately.

Homologous pieces of ventral epidermis of the host were cut off 5, 10, 30 and 60 min after the injection, rapidly rinsed to avoid fat body and hemolymph contamination, then homogenized by sonication in 200 µl 0.95 N $HClO_4$, and centrifuged at 15,000×g for 5 min to remove the insoluble fraction. The cyclic AMP content of each epidermal supernatant was determined by RIA[12]. Cyclic AMP content was expressed as pmoles/mg of pure epidermis. It was not meaningful to measure the total protein content of samples because of excessive contamination with cuticular protein. The fresh weight of pure epidermis was estimated histologically. According to this approximation, the fresh weight of each epidermal sample was about 50 µg.

Results and discussion. The figure clearly shows that, 5 min after the injection of bursicon active hemolymph, a significant increase ($p < 0.001$) of epidermal cyclic AMP is evident. Thereafter, the cyclic AMP content decreases slowly, but even 1 h after, the cyclic AMP content is still higher than in the epidermis of noninjected pupae. Bursicon inactive hemolymph or insect Ringer do not promote any significant increase.

This experiment confirms the clear temporal relationship between the increase of bursicon activity in hemolymph and the increase of the cyclic AMP concentration in the epidermis as indicated previously[10], and shows that the epidermis is, like hemocytes[13,14], a target tissue for bursicon.

The kinetics of the cyclic AMP response in the epidermis is similar to those observed in a cyclic nucleotide mediated system, i.e. a rapid rise followed by a slow decrease. While the hosts were not under normal physiological conditions (thoracic ligation for at least 10 days), the amplitude of the epidermal response is equivalent to that observed when cyclic AMP is measured during bursicon release in nonligated animals[10]. These data are entirely consistent with the hypothesis that cyclic AMP is a second messenger of bursicon in *Tenebrio*.

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Serum prolactin levels and maintenance of progeny by prenatally-stressed female offspring

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Summary. Prenatal stress significantly reduced the number of progeny born to 47% of the female offspring and significantly increased the incidence of low birthweight young. None of these litters survived by the tenth postpartum day when serum prolactin levels were significantly reduced. Upon autopsy, these females had twice as many uterine implantation sites than the number of fetuses they bore, suggesting that a) the reduced postpartum serum prolactin most likely was the cause rather than the effect of the neonatal mortality and b) major hormonal deficiencies (possibly gonadotropic-related) were present even before giving birth.

Evidence is accumulating that prenatal stress adversely alters the neuroanatomical or biochemical organization of the male brain beginning in the fetal stage to cause aberrations in reproductive-related neurohormonal responses in adulthood[1-4]. Evidence is also accumulating that prenatal stress adversely affects hormonally-mediated reproductive functions in female offspring. Stress during gestation markedly increases concentrations of the neurotransmitter dopamine in the hypothalamic arcuate nucleus of female offspring[3]. Marked alterations in arcuate dopamine have been associated with abnormalities in gonadotropic hormone release from the anterior pituitary gland[5]. With respect to female offspring, prenatal stress has been reported to a) disrupt estrous cycling[6]; b) increase the incidence of high risk pregnancies through spontaneous abortions and vaginal hemorrhages[7]; and c) elevate the incidence of stillbirths and neonatal mortality among subsequent progeny[7].

Both the maintenance of pregnancy and the survival of progeny are dependent upon a variety of gonadotropic hormones, important among which is the luteotropic hormone prolactin. It is well known that androgen treatment of newborn female rats results in persistent vaginal cornification and anovulation, a condition also associated with hypothalamic disorders[8,9]. If neonatal females are only lightly-androgenized, they evidence reproductive cycles for some time following puberty and then lose cyclic functioning. Under certain conditions, the administration of either exogenous progesterone or prolactin to androgenized females allows normal implantation and maintenance of pregnancy[10]. Thus the possibility arises that reproductive dysfunctions in the prenatally-stressed female[7], which un-