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*.

1 This work was supported by CNRS, Grant No. 1918, and by DGRST, No. 1425. We thank Prof. M.I. Seligman for helpful comments on the manuscript.
2 G. Fraenkel and C. Hsiao, J. Insect Physiol. *11*, 513 (1965).
3 R.R. Mills and D.J. Nielsen, J. Insect Physiol. *13*, 273 (1967).
4 D. Von Knorre, M. Gersch and T. Kusch, Zool. Jb., Abt. allg. Zool. Physiol. *76*, 434 (1972).
5 I.M. Seligman and F.A. Doy, Israel J. Ent. *7*, 129 (1972).
6 R.D. Vanderberg and R.R. Mills, J. Insect Physiol. *20*, 623 (1974); *21*, 221 (1975).
7 G. Fraenkel, Am. Zool. *15* (suppl. 1), 29 (1975).
8 G. Fraenkel, A. Blechl, J. Blechl, P. Herman and M.I. Seligman, Proc. nat. Acad. Sci. USA *74*, 2182 (1977).
9 M. I. Seligman, A. Blechl, J. Blechl and G. Fraenkel, Proc. nat. Acad. Sci. USA *74*, 4697 (1977).
10 J. Delachambre, J.P. Delbecque, A. Provansal, J.P. Grillot, M.L. De Reggi and H.L. Cailla, submitted to Insect Biochem.
11 J.P. Grillot, J. Delachambre and A. Provansal, J. Insect Physiol. *22*, 763 (1976).
12 M.A. Delaage, D. Roux and H.L. Cailla, in: Molecular Biology and Pharmacology of Cyclic Nucleotides, p. 155. Ed. G. Folco and R. Paoletti. Elsevier, Amsterdam 1978.
13 D.L. Whitehead, Nature *224*, 721 (1969).
14 L.C. Post, Biochim. biophys. Acta *290*, 424 (1972).

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

Lorraine R. Herrenkohl and R.R. Gala

Psychology Department, Temple University, Philadelphia (PA 19122, USA) and Physiology Department, Wayne State University School of Medicine, Detroit (MI 48201, USA), 1 February 1979

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-

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

Parameter (mean ± SE)	Normal lactating females (n = 11)	Prenatally stressed females Maintain young (n = 9)	Did not maintain young (n = 8)
Postpartum day 1			
Number of pups	13.45 ± 0.48	12.20 ± 0.39	5.50 ± 2.50*
Weight per pup (g)	7.54 ± 0.22	7.94 ± 0.38	6.87 ± 0.23*
Postpartum day 10			
Number of pups	13.36 ± 0.41	12.00 ± 0.48	0*
Weight per pup (g)	19.85 ± 0.69	18.94 ± 0.67	0*
At Autopsy**			
Number of implantation sites	14.27 ± 0.59	12.60 ± 0.50	10.63 ± 0.46*
Serum prolactin (ng/ml)	112.02 ± 16.33	121.15 ± 20.96	13.66 ± 1.56*

* $p < 0.001$, analyzed by a 1-way analysis of variance followed by the Duncan's multiple range test for paired comparisons. In each case, the prenatally-stressed group that did not maintain young differed significantly from each of the other groups; ** Postpartum day 10.

der some conditions resemble those of the androgen-sterile female, may be related in part to gonadotropic secretory deficiencies in adulthood[11,12]. The present experiment was conducted to determine whether a relationship exists between serum prolactin levels and postpartum maintenance of progeny by prenatally-stressed female offspring.

Materials and method. 24 primiparous, pregnant Sprague-Dawley rats, weighing about 250 g were obtained from Zivic Miller (Allison Park, Pa.) 1 week before stressing. They were housed singly under a standard 12 h light/dark cycle and maintained on food and water ad libitum. On day 14 of gestation, 12 pregnant females were selected at random and subjected to 3 45-min stress sessions per day through day 21. Each 45-min stress session was followed by a rest period of equal duration in the home cage. The stress consisted of simultaneous bright light, heat and restraint administered according to methods modified from Ward[1]. Specifically, females to be stressed were placed individually in 18 × 8 cm semicircular Plexiglas restraining cages grouped in rows under 4 bright incandescent lights which produced a surface illumination of more than 400 ft candles and surface temperature of 34 °C. The remaining 12 pregnant females were left unhandled in their home cages. Females were observed daily for the day of birth after which they were left undisturbed for 3 weeks to rear young. At 21 days of age, litters were weaned, segregated by sex and housed 2 per cage in 24 × 32 × 16 cm Fiberglas observation cages with San-i-cel bedding under a standard 12 h light/dark cycle beginning at 08.00 h and maintained on an ad libitum diet of Purina chow and water. At 90 days of age, the estrous cycles of a randomly-selected sample of nonstressed and prenatally-stressed females were examined daily by the method of vaginal lavage. At 105 days of age, the 71% and 33% of the 2 samples that exhibited normal estrous cycles (proportions = 15/21, 40/121, respectively) were placed into the home cage of stud males for 2 h of mating. After confirmation of pregnancy by vaginal lavage, the 11 nonstressed and 17 prenatally-stressed females that maintained pregnancy became the subject of the present report (the animals remaining either did not become pregnant or spontaneously aborted young). On the day of parturition, litters were counted, weighed and left undisturbed until postpartum day 10, when they were counted and weighed again. On our about postpartum day 10, after it was determined that the females were in vaginal diestrous, they were sacrificed and uteri were examined for implantation sites. A 1.0 ml sample of blood was allowed to clot at room temperature for 3–4 h and the serum recovered by centrifugation. Serum was assayed for prolactin by a double antibody radioimmunoassay at two dilutions each in duplicate[13].

Results. Prenatally-stressed females fell equally divided into 2 groups, those that did and did not maintain young. Compared to the normal lactating animals and the prenatally-stressed group that did maintain young, prenatally-stressed females that did not maintain young gave birth to significantly fewer live offspring that were significantly lower in birthweight (table). None of the litters born to those prenatally-stressed females survived to the tenth postpartum day (table). Upon autopsy, it was observed that a) those prenatally-stressed females that did not maintain young had more than twice as many uterine implantation sites than the number of fetuses they bore and b) their serum prolactin levels had fallen significantly below those of the other 2 groups (table).

Discussion. The findings that prenatal stress significantly reduced the number of progeny born to female offspring and increased the number of low birthweight young support our previous findings and indicate that prenatal stress consequences are 'all-or-none': females either can or cannot maintain young[7]. Moreover, the data partially confirm the findings of Euker and Riegle[14] that prepartal stress significantly increased embryonic mortality in about half of their pregnant rats. Neonatal mortality may be caused by lactational dysfunctions (e.g., biochemical changes in milk, involutional mammary glands, failure of milk ejection, etc.) or by poor nursing behavior[15]. Behavioral accounts in our previous prenatal stress experiments suggest that disturbances in maternal behavior may be secondary to those caused by lactational disruptions[7]. Therefore lactational disorders may be the likely cause of neonatal mortality in the present experiment.

Additional support for lactational dysfunction as the likely cause of neonatal mortality is derived from the present observation that prolactin levels were markedly reduced in prenatally-stressed females that did not maintain young compared with those in normal lactating females or in the group of prenatally-stressed females that did maintain young. Although it is not yet clear whether the reduction in serum prolactin was the cause or the effect of the litter mortality (serum prolactin levels characteristically decline in the absence of the suckling stimulus), the low implantation count in the latter treatment at autopsy suggests that major hormonal deficiencies (possibly prolactin-related) were present even before giving birth. Exogenous administration of prolactin, in conjunction with other treatments, has been reported to allow normal implantation and the maintenance of pregnancy in neonatally-androgenized females that otherwise as adults would remain infertile[10]. It is important to determine whether similar treatment might repair gestational or postpartum disorders in prenatally-stressed females. Prenatal stress may create hormonal imbalances beginning in the fetal stage which produce a variety of reproductive dysfunctions depending not only upon the primary hormonal deficiency but also the particular reproductive stage in which it is expressed.

1 I.L. Ward, Science *175,* 82 (1972).
2 J.A. Moyer, L.R. Herrenkohl and D.M. Jacobowitz, Brain Res. *121,* 385 (1977).
3 J.A. Moyer, L.R. Herrenkohl and D.M. Jacobowitz, Brain Res. *144,* 173 (1978).
4 J.A. Politch, L.R. Herrenkohl and R.R. Gala, Physiol. Behav. *20,* 91 (1978).
5 S.M. McCann, P. Kalra, A. Donoso, W. Bishop, H. Schneider, C.P. Fawcett and L. Krulich, in: Brain - Endocrine Interaction, p.224. Ed. K. Knigge, D. Scot and A. Weindl. Karger, Basel 1975.
6 L.R. Herrenkohl and J.A. Politch, Experientia *34,* 1240 (1978).
7 L.R. Herrenkohl, The American Psychological Association. Paper presented at the 86th Annual Convention, Toronto 1978.
8 R.A. Gorski and C.A. Barraclough, Anat. Rec. *139,* 304 (1961).
9 R.A. Gorski in: Frontiers in Neuroendocrinology, p.237. Ed. L. Martini and W.F. Ganong. Oxford University, New York 1971.
10 D.W. Hahn and J.L. McGuire, Endocrinology *102,* 1741 (1978).
11 R.E. Harlan and R.A. Gorski, Endocrinology *101,* 741 (1977).
12 R.E. Harlan and R.A. Gorski, Endocrinology *101,* 750 (1977).
13 E.Y.H. Kuo and R.R. Gala, Biochim. biophys. Acta *264,* 462 (1972).
14 J.S. Euker and G.D. Riegle, J. Reprod. Fert. *34,* 343 (1973).
15 L.R. Herrenkohl, Physiol. Behav. *13,* 495 (1974).

In vitro inactivation of corpora allata of the bug *Oncopeltus fasciatus* by precocene II

P.J. Müller, P. Masner, Maria Kälin and W.S. Bowers[1]

Dr R. Maag Ltd, CH-8157 Dielsdorf (Switzerland), and Department of Entomology, New York State Agriculture Experiment Station, Geneva (New York 14456, USA), 25 October 1978

Summary. Corpora allata from *Oncopeltus fasciatus* incubated in vitro in medium containing $10^{-5.35}$ M (1 µg/ml) of precocene II lose their ability to secrete juvenile hormone when reimplanted into last instar larvae.

Precocenes were the first substances discovered with antiallatotropic activity in insects[2]. Larvae of the large milkweed bug, *O. fasciatus,* reared in contact with sublethal doses of precocene II, undergo a precocious metamorphosis and moult into diminutive forms with adultoid characters. Freshly hatched females exposed to precocene remain sterile for the rest of their life. Their corpora allata (CA) are inactive[3] and do not regain their activity when transplanted into last instar larvae[4]. This indicates that precocene somehow inactivates the CA and thus blocks biosynthesis of juvenile hormone (JH). Submicroscopical changes and degeneration of CA were observed in treated *O. fasciatus*[5] and *Locusta migratoria*[6].

Precocene was found to be a modest inhibitor of JH biosynthesis in cultures of CA from cockroaches[7,8]. On the other hand, the morphogenetic and sterilant effects in these insects are negligible (Masner, unpublished results). We have examined the in vitro effect of precocene on the CA of *O. fasciatus* where the in vivo activity is very high.

Material and methods. A sunflower strain of the large milkweed bug, *O. fasciatus,* was reared at 18 h daily illumination, 30 °C and 60% relative humidity[9]. Fertile (copulating) females containing ripe eggs were used as donors of corpora allata-cardiaca complexes (CA). The animals were surface sterilized in a 3% sodium hypochlorite solution and dissected in sterile Ringer solution at an airflow sterile bench.

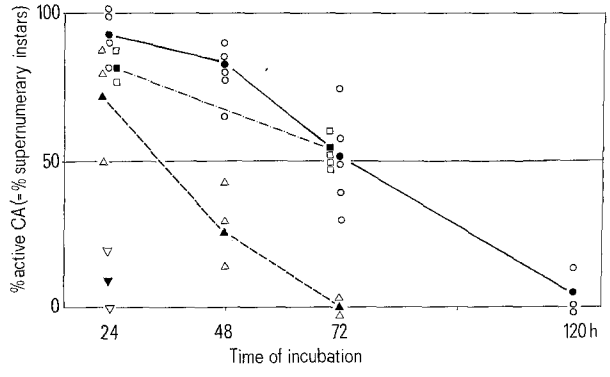
CA were incubated for 1-10 days at 30 °C in Leighton tissue culture glass tubes (Bellco, Biological Glassware, USA). Fully synthetic medium S_{20F}[10] or Shields medium[11] without serum or haemolymph were used (1 ml or 0.18 ml per flask). All solutions were filter-sterilized. The cultures which showed contamination (colour change of phenol red indicator) were discarded. Precocene II (6,7-dimethoxy-2,2-dimethyl-2H-1-benzopyran)[2] or the chemically closely related β-asarone ((Z)2,4,5-trimethoxy-1-propenyl benzene)[12] were dissolved in absolute ethanol and added to the culture medium. An equivalent amount of ethanol (concentration $\leqslant 10^{-3.6}$ M) was added to the control medium.

The activity of the CA was assayed by transplantation of the gland into young fifth (last) instar larvae anaesthetized by submersion in water[13]. The outcome of the ensuing ecdysis of the host provided evidence about the morphogenetically active JH output of the gland. The percentage of supernumerary instars was considered to be proportional to the percentage of active glands. The inactive CA allowed normal adult development of the hosts[4].

Results. In the first series of experiments CA were incubated in the medium S_{20F}. More than 90% of glands incubated

Percent of morphogenetically active CA after incubation for 5 or 10 days in Shields and Sang's medium, to which precocene II, β-asarone or equivalent of ethanol (untreated) was added

Substance	Dose 10^{-x} M	Percent of active CA after incubation for 5 days	n	10 days	n
Precocene II	4.35	13	16		
	5.35	64	28	20	20
	6	89	9		
β-Asarone	4.32	100	19		
Untreated	-	94	18	90	33



Percent of morphogenetically active CA after incubation for 24-120 h in medium S_{20F}. The number of transplantations performed in 2-5 series (empty symbols) varies between 17 and 45. The full symbols indicate the mean values. Incubation in control medium (●——●), in medium with $10^{-4.35}$ M (▼), $10^{-5.35}$ M (▲——▲) and 10^{-6} M (■–·–■) of precocene II.