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]

J. W. Siebers[2], J. Schmidtke and W. Engel

Universitäts-Frauenklinik, Hugstetterstrasse 55, and Institut für Humangenetik und Anthropologie der Universität, D-7800 Freiburg i. Br. (Federal Republic of Germany, BRD), 1 October 1976

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

1 This work was supported in part by the Deutsche Forschungsgemeinschaft (SFB 46). Purified HCG was a gift of Schering AG, Berlin-West. We thank Dr F. Peters for performing the iodination of HCG.

2 Address reprint requests to JWS, Universitäts-Frauenklinik, Hugstetterstrasse 55, D-7800 Freiburg, German Federal Republic, BRD.

3 S. R. Ojeda and V. D. Ramirez, Endocrinology *90*, 466 (1972).
4 K. D. Döhler and W. Wuttke, Endocrinology *94*, 1003 (1974).
5 J. Frowein, W. Engel and H.-C. Weise, Nature New Biol. *246*, 148 (1973).
6 W. Engel and J. Frowein, Cell *2*, 75 (1974).
7 W. Engel and J. Frowein, Nature *251*, 146 (1974).
8 J. Presl and V. Figarová, Workshop Meeting on the Development and Maturation of the Reproductive Organs and Functions in Female, Copenhagen, 3–5 July 1972.
9 J. Presl, V. Figarová and Z. Krabec, Čslká Gynek. *35*, 27 (1970).
10 J. Presl, J. Jirásek, J. Horský and M. Henzl, J. Endocr. *31*, 283 (1965).
11 J. Presl, J. Herzman and J. Horský, J. Endocr. *45*, 611 (1969).
12 R. L. Goldenberg, E. O. Reiter and T. Ross, Fert. Steril. *24*, 121 (1973).
13 S. A. Lamprecht, U. Zor, A. Tsafriri and H. R. Lindner, J. Endocr. *57*, 217 (1973).
14 C. Y. Lee and R. J. Ryan, Biochemistry *23*, 4609 (1973).

Binding of ^{125}I-HCG to rat ovary homogenates at various stages of development

Age	Number of animals	pg HCG bound/ 5 mg wet weight*		Number of binding sites**		K_D ($\times 10^{10}$)
		Total	Non-specific	moles/mg wet weight ($\times 10^{15}$)	moles/ovary ($\times 10^{15}$)	
Prenatal	40	40.8	35.4	0	0	–
Newborn	250	45.0	40.6	0	0	–
5-day-old	250	40.7	33.4	0	0	–
10-day-old	100	113.0	37.0	2.36	1.65	4.37
21-day-old	12	94.5	27.0	2.59	15.18	6.18
31-day-old	10	115.0	38.5	5.55	61.05	11.66
Spleen adult		41.3	36.5			

*Determined with 4 ng ^{125}I-HCG in the incubation assay. **Determined by Scatchard[22] analysis.

HCG was determined from samples containing a 1000fold excess of unlabelled hormone. The specific uptake of HCG was calculated from the difference between the radioactivity bound by samples incubated with ^{125}I-HCG alone and the radioactivity bound nonspecifically. Iodination of purified HCG (biological activity of 11,000 IU/mg) was performed according to the modified method C of Leidenberger and Reichert[15] with a time of exposure to chloramine-T of 20 sec. Specific radioactivity of the ^{125}I-HCG was 30–50 μCi/μg.

Results and discussion. As can be seen from the table, ovarian tissues of prenatal, newborn and 5-day-old rats do not specifically bind radioactively labelled HCG. Specific binding of HCG is first observed in ovaries of 10-day-old animals. These results are supported by the observation of Presl and Figarová[8] that accumulation of injected radioactively labelled HCG is only demonstrable in ovaries of rats older than 7–8 days. The number of binding sites, as well as the dissociation constants (K_D) of the receptor-hormone complex, are similar in 10–21-day-old rats. Twice the number of binding sites as in the 21-day-old rat were demonstrable in rats 31 days of age. The values for K_D and number of binding sites in the 31-day-old rat are largely consistent with those obtained by Lee and Ryan[14] in 35-day-old rats. It might be assumed that the lack of HCG binding in ovarian tissue of rats younger than 10 days is due to masking of the receptors by endogenous LH. This assumption can be abandoned, since specific HCG receptors are first demonstrable in ovarian tissue when the endogenous LH level has attained its maximum[3, 4, 16, 17].

These results strongly suggest that the HCG insensitivity of the early postnatal rat ovary is due to the lack of specific receptors. With the first appearance of the LH receptors in the ovary, the animals become sensitive to LH. Thus, in the female rat, in contrast to the male rat[18], LH receptor is not a constitutive protein. This difference between male and female is well understandable from a biological point of view. While steroidogenic activity of the testis is an absolute prerequisite for the development of the male phenotype, feminine differentiation occurs in the absence of gonadal steroids[19, 20]. Only during the postnatal development do ovarian steroids become necessary. From the observation that in 10-day-old female rats only the interstitial tissue of the ovary is steroidogenic[21], one might assume that interstitial cells are the first cells in the ovary to become endowed with LH receptors. The higher number of binding sites in the ovaries of 31-day-old rats compared with ovaries of 10-day-old rats might indicate that besides interstitial cells also granulosa and theca cells are equipped with LH receptors at this developmental time.

15 F. Leidenberger and L. E. Reichert, Jr, Endocrinology *91*, 135 (1972).
16 N. B. Schwartz, C. H. Anderson, L. G. Nequin and C. A. Ely, in: Control of the Onset of Puberty, p. 367. Ed. M. M. Grumbach, G. D. Grave and F. E. Mayer. John Wiley and Sons, New York 1974.
17 S. M. McCann, S. Ojeda and A. Negro-Vilar, in: Control of the Onset of Puberty, p. 1. Ed. M. M. Grumbach, G. D. Grave and F. E. Mayer. John Wiley and Sons, New York 1974.
18 J. Frowein and W. Engel, Nature *249*, 377 (1974).
19 A. Jost, Phil. Trans. Roy. Soc., Lond. *259*, 119 (1970).
20 S. Ohno, U. Tettenborn and R. Dofuku, Hereditas *69*, 107 (1971).
21 W. D. Odell and R. S. Swerdloff, in: Control of the Onset of Puberty, p. 313. Ed. M. M. Grumbach, G. D. Grave and F. E. Mayer. John Wiley and Sons, New York 1974.
22 G. Scatchard, Ann. N. Y. Acad. Sci. *51*, 660 (1949).

The effect of epinephrine and the hyperglycemic factor of the scorpion's cephalothoracic ganglionic mass (CTGM) on the phosphorylase activity of hepatopancreas of the scorpion, Heterometrus fulvipes C. Koch

K. Raghavaiah[1], M. Sreeramachandramurthy, R. Ramamurthi, P. Satyam and V. Chandrasekharam

Department of Zoology, S. V. University and S. G. S. Arts College, Tirupati 517 501 (India), 8 September 1976

Summary. Injection of epinephrine and CTGM extract showed different effects on hepatopancreatic phosphorylase activity and levels of total carbohydrate and glycogen in Heterometrus fulvipes. The former hyperglycemic principle involves phosphorolysis of glycogen whereas the latter does not.

The role of neuroendocrine system in the metabolite regulation has been explored in Arachnida only to a limited extent[2]. A hyperglycemic principle has been identified in the cephalothoracic ganglionic mass of the South Indian scorpion Heterometrus fulvipes[3, 4]. The vertebrate hormone, epinephrine also has been found to cause hyperglycemia in Heterometrus fulvipes.

The present communication examines the effect of epinephrine and scorpion's CTGM extract on the levels of total carbohydrates, glycogen and phosphorylase activity