of the isolated and control group data (Table) revealed that the isolated-nonfighter mice displayed the greatest increase in adrenal weights and plasma corticosterone levels and the greatest degree of thymic involution. The respective differences were statistically significant when compared to the isolated-fighter and the paired-nonfighter mice. Although the paired-nonfighter animals had smaller adrenal weights, lower plasma corticosterone levels, and larger thymic weights than the isolated-fighters, the differences were not statistically significant. In contrast, evaluation of the adrenal catecholamine levels revealed that in the isolated mice, the fighters had significantly higher catecholamine levels than the isolated-nonfighters. The paired-nonfighter animals had values intermediate between the two groups. Statistical analyses of the higher adrenal catecholamine levels in the paired-nonfighters when compared with the isolated-nonfighter group, although not significant yielded a low P value of 0.08.

In comparing the total isolated vs paired female data, the alterations in behavior, open-field activity, WBC counts and organ weights agree with previous reports of increased excitability, aggressiveness and adrenocortical activity due to isolation[1-3, 12, 13]. While evaluation of the total population of isolated ($n = 46$) vs paired mice ($n = 44$) showed no significant differences in the respective adrenal catecholamine levels, analyses of the data based on fighting behavior indicated that the significantly higher levels in adrenal catecholamine concentrations of the isolated-fighter mice were apparently counterbalanced by the isolated-nonfighter group. The intermediate although higher catecholamine values observed in the paired-nonfighter vs isolated-nonfighter groups (P 0.08) would suggest that animal interaction present in paired populations stimulated adrenal medullary activity to a certain degree even though it was not accompanied by aggression. It is of interest that the catecholamine levels of the total population of paired mice ($n = 44$), which included even aggressive animals, were significantly higher than the concentrations observed in the isolated-nonfighter group (P 0.01). The present findings with female mice consequently agree in general with previous studies involving males which indicated that isolated fighters had higher sympathetic-adrenal activity than isolated-nonfighter mice[4, 5, 15, 16]. In contrast, measures of adrenocortical activity (adrenal weights, thymic involution, and plasma corticosterone) indicated that isolated nonfighters had significantly higher pituitary-adrenal activity than either the isolated-fighters or the paired-nonfighters and suggested that heightened adrenocortical activity was not directly related to agression. Recent reports have indicated that increased aggressiveness due to isolation was associated with low ACTH titers and reduced aggressiveness with high plasma ACTH[23-25]. The present investigation indicates that isolation induced aggression in female mice was directly related to increased sympathetic-adrenal activity. Conversely, no direct relationship was noted between adrenocortical activity and aggressiveness.

Résumé. Lorsque des souris femelles soumises à l'isolement sont groupées selon leur tempérament belliqueux et non-belliqueux, on observe une augmentation de l'activité médullossurrénalienne chez les animaux isolés belliqueux. Au contraire, les souris isolées non-belliqueuses ont montré des taux de catécholamines inférieurs et une secrétion cortico-surrénale nettement augmentée.

R. SCHWARTZ[26], A. M. SACKLER and A. S. WELTMAN

Laboratories for Therapeutic Research, Brooklyn College of Pharmacy, Long Island University, 600 Lafayette Avenue, Brooklyn (New York 11216, USA), 29 June 1973.

[23] A. I. LESHNER, Hormones *5*, 272 (1972).
[24] P. F. BRAIN, N. W. NOWELL and A. WOUTERS, Physiol Behav. *6*, 27 (1971).
[25] P. F. BRAIN, Nature, Lond. *233*, 489 (1971).
[26] Submitted by RALPH SCHWARTZ to the faculty of Richard L. Connelly College, Long Island University in partial fulfillment of the requirements for the Degree of Masters of Science.

Effect of Oestradiol on Ovarian Progesterone and 20α Hydroxypregn-4-en-3-one Secretion During Pseudogestation in the Rat

Having demonstrated that the ovarian secretion of progesterone in the pseudopregnant rat follows a nychthemeral rhythm[1], we proceeded to examine and analyse the effects induced by different doses of oestradiol during the various phases of this circadian secretory cycle, in an attempt to elucidate the role of oestrogens in the overall regulation of luteal function under different endocrine conditions.

Materials and methods. Adult female rats (Ivanovas, Kisslegg, Germany) weighing 200–250 g, that had been kept under standard laboratory conditions in respect of temperature, humidity and lighting (light from 06.00 until 20.00 h daily), were mated with vasectomized males on the evening of pro-oestrus. The onset of pseudogestation was confirmed by the presence of a vaginal plug on the following morning (subsequently verified by vaginal smears). The rats were then divided into groups of 10 (5 in each cage) and treated daily from the 1st until the 6th day of pseudogestation with various doses of 17β-oestradiol, dissolved in sesame oil and administered s.c. Controls received the solvent only. Where appropriate, 20α-hydroxypregn-4-en-3-one (20α-OH-P) levels were measured by a fluorimetric method.

On the 7th day, between 08.00 and 12.00 or 15.00 and 17.00 h, a cannula was inserted in the renal vein near the left ovarian vein according to the method described by FAJER and BARRACLOUGH[2]. Blood samples (0.5 ml collected in 3–5 min) taken from each animal were subjected to chromatography, and the progesterone content of the eluate was measured by a slightly modified version of the protein-binding assay of NEILL et al.[3].

Results. The Figure shows the variations in progesterone secretion in relation to the doses of oestradiol on the 7th day of pseudogestation in intact rats. The administration of oestradiol in doses of 0.01, 0.03 or 0.1 μg/kg daily for the first six days of pseudogestation resulted in a statistically significant decrease in the progesterone content of the ovarian venous blood cannulated on the 7th day, between 08.00 and 12.00 h (Figure), i.e.

[1] P. BISCHOF, C. KRÄHENBÜHL and P. A. DESAULLES, Experientia *29*, 615 (1973).
[2] A. B. FAJER and C. A. BARRACLOUGH, Endocrinology *91*, 617 (1973).
[3] J. D. NEILL, E. D. B. JOHANSSON, J. K. DATTA and E. KNOBIL, J. clin. Endocr. *49*, 120 (1965).

Effects of oestradiol on the secretion of progesterone and 20α-OH-P in the rat on the 7th day of pseudogestation

Time of sampling (7th day)	N	Treatment doses (μg/kg/day)	Secretion rate (μg/h + SE) Progesterone	20α-OH-Prog.	Progestins
08.00–12.00 h	7	Control	13.4 ± 1.9	9.8 ± 1.8	23.2 ± 3.5
	12	Oestradiol 0.03	3.9 ± 0.6	16.9 ± 1.8	20.8 ± 3.1
	10	Oestradiol 30	16.0 ± 3.1	11.6 ± 1.1	27.6 ± 3.6
15.00–17.00 h	5	Control	5.5 ± 1.2	4.0 ± 1.2	9.6 ± 2.6
	7	Oestradiol 0.03	4.1 ± 0.8	5.7 ± 1.8	9.9 ± 2.2
	7	Oestradiol 30	5.8 ± 1.0	7.9 ± 2.1	13.7 ± 1.9

at the peak of the nychthemeral secretion of progesterone[1], in comparison with the levels noted in controls treated with sesame oil only. The average rate of progesterone secretion recorded in the solvent-treated controls was 15.4 ± 2.0 μg/h, as against 3.9 ± 0.6 μg/h in the rats treated with 0.03 or 0.1 μg/kg of oestradiol ($p < 0.001$). The inhibitory effect of 0.01 μg/kg oestradiol daily was less marked, but still significant (7.2 ± 1.1 μg/h; $p < 0.05$). By contrast, the injection of higher doses of oestradiol (0.3–100 μg/kg) had no significant effect on progesterone secretion.

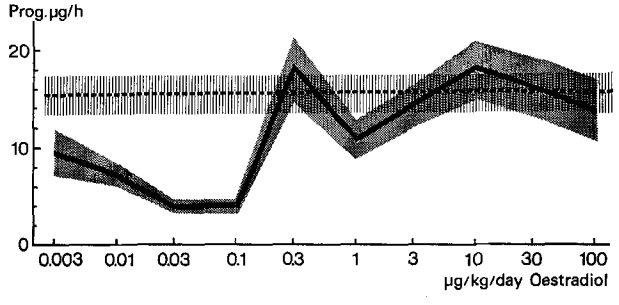
Under the same conditions, the administration of 0.3 μg/kg of oestradiol daily produced a statistically significant ($p < 0.05$) increase in the secretion of 20α-OH-P. 30 μg/kg daily of the same oestrogen was completely ineffective, as is shown in the Table.

The total progestin level (progesterone and 20α-OH-P) remained unchanged after both treatments.

When on the 7th day of pseudogestation the ovarian vein was cannulated between 15.00 and 17.00 h, i.e. during the phase of basal progesterone secretion[1], the rate of secretion found in the controls was 5.5 ± 1.8 μg/h (cf. Figure). Under these circumstances, the daily administration of oestradiol in a dose of 0.03 μg/kg, i.e. a dose that reduces the progesterone peak between 08.00 and 12.00 h, or 30 μg/kg, a dose that has no effect on the progesterone peak (cf. Figure), did not cause any change in the secretion of progesterone.

The rate of secretion of 20α-OH-P was lower in the afternoon (15.00–17.00 h) than during the morning (08.00–12.00 h) of the 7th day of pseudogestation (see Table). Neither 0.03 μg/kg nor 30 μg/kg of oestradiol daily, injected during the 6 first days of pseudopregnancy, significantly modified the production of 20α-OH-P as recorded between 15.00 and 17.00 h on the 7th day.

Discussion. In a recent publication[1] we have shown that in the pseudopregnant rat (7th day), the secretion of progesterone by the ovary follows a nychthemeral rhythm: a peak is reached between 09.00 and 11.00 h, while throughout the rest of the day the progesterone level remains low. Considering especially, among the data reported here, the opposite effects (decrease of progesterone secretion and increase in 20α-OH-P secretion) exerted by the low doses (0.01 to 0.1 μg/kg daily) of oestradiol, it could be deduced that the reduced levels of progesterone are not due to an inhibition of its production but rather to its accelerated metabolization in 20α-OH-P.



Cannulization of ovarian vein on 7th day of pseudogestation between 08.00 and 12.00 h. Effects of different doses of oestradiol on ovarian secretion of progesterone (μg/h ± SE). ---, controls; —,treated rats.

20α-hydroxysteroid dehydrogenase being an enzyme of exclusively luteal origin, as has been demonstrated by PUPKIN et al.[4], we can conclude that morphological origin of this phenomenon can reasonably be attributed to the corpus leteum.

Assuming that a substance is luteolytic if it significantly decreases progesterone production of luteal origin, 0.03 μg/kg daily may be described as a luteolytic dose of oestradiol under the foregoing experimental conditions.

The fact that very low doses of oestradiol exerted a luteolytic effect in this experimental model suggests that oestrogens may play an important part in the regulation of the luteal function. The low doses of oestradiol could exert their luteolytic effect by different mechanisms, such as a modification of the activity of prolactin at the ovarian level, a stimulation of the secretion of a luteolytic factor of an uterin origin, an inhibition of the release of prolactin and consequent abolition of luteotropic support, a promotion of the production of LH, the luteolytic properties of which have already been described (ROTHCHILD[5]). Experimental studies now in progress with hypophysectomized, hysterectomized or anti-LH treated pseudopregnant rats suggest that the luteolytic effect described here is due to an increased production of LH. They will be published next.

Résumé. Des doses faibles d'oestradiol (0,01–0,1 μg/kg/jour) administrées à des rattes les 6 premiers jours de la pseudogestation, produisent au 7ème jour une chute du taux veineux ovarien de progestérone et une augmentation de la 20α-OH-P. Les doses plus élevées (0.3–100 μg/kg) restent sans effet. Ces résultats prouvent l'action luteolytique de l'oestradiol à faibles doses.

P. BISCHOF, C. KRÄHENBÜHL and P. A. DESAULLES[6]

Biological Research Laboratories of the Pharmaceuticals Division of Ciba-Geigy Limited, CH-4002 Basel (Switzerland), 14 September 1973.

[4] M. PUPKIN, H. BRATT, C. W. LLOYD and K. BALOGH, Endocrinology *79*, 316 (1966).

[5] I. ROTHCHILD, Vitams. Horm. *23*, 209 (1965).

[6] Acknowledgements. The authors wish to express their appreciation to Dr. L. SCHENKEL for her helpful advice during this project.