control rats were similarly injected with equal amounts of the vehicle. The animals were sacrificed 6 h after the injection and the uteri excised. The right uterine horn was used for biochemical studies and the left uterine horn was used for histological studies.

The following parameters were measured in the uterus of each animal: wet weight, DNA[6], RNA[7], protein[8] and glycogen[9] content, and the total number of uterine eosinophils[10]. The effects of the estrogen stimulation were assessed individually in each animal. The mean value ($\pm$ SEM) of each parameter of estrogen stimulation were calculated by pooling the results obtained in each of the 8 to 12 animals used for each experimental condition. The uterine wet weight ,RNA/DNA, protein/DNA and glycogen/DNA increases were expressed as percent change over the controls. The uterine eosinophilia was expressed as the total number of eosinophils in the uterus.

Results. The Figure shows the increases in the total number of uterine eosinophils, in the uterine wet weight and in the uterine RNA, protein and glycogen content 6 h after the i.v. injection of estradiol-17β or estriol. Estriol is a stronger estrogen than estradiol for the uterine eosinophilia and the uterine wet weight responses (Figure a and b). Estradiol is a stronger estrogen than estriol for producing increases in the uterine RNA and protein content (Figure c and d). Both hormones present similar potencies to induce the increase in the uterine glycogen content, except at a dose of 0.1 µg/100 g body weight, dose at which estriol produces a slightly stronger response than estradiol (Figure e).

Discussion. The present investigation shows that estradiol-17β is a stronger estrogen than estriol for the genomic response of estrogens, that is, the 6 h increases in the uterine RNA and protein contents. It is well established that estradiol-17β has a higher affinity than estriol for the uterine cytosol-nuclear receptor system[11]. Comparing the affinity data of estradiol-17β and estriol for the cytosol-nuclear receptor system with the dose-response of these estrogens, it is clear that the genomic response of estradiol-17β and estriol correlates with the affinities of these estrogens for the cytosol-nuclear receptor system. This correlation provides further support of the evidence for the mediation of the genomic response of estrogens by the uterine cytosol-nuclear receptor system.

A different situation occurs with the estrogen-induced uterine eosinophilia and the 6 h increases in the uterine wet weight. Estriol is a stronger estrogen than estradiol-17β for inducing these two parameters of estrogen stimulation. We have previously shown that estriol has a higher affinity than estradiol-17β for the receptors in the uterine eosinophils[1]. Comparing the affinity data of estradiol-17β and of estriol for the eosinophil receptors with the dose-response data of these estrogens, it is clear that the uterine eosinophilia and the 6 h wet weight responses produced by estradiol-17β or estriol correlate with the affinities of these estrogens for the eosinophil receptors. This correlation supports the hypothesis of the mediation of the uterine eosinophilia and the 6 h wet weight responses by the eosinophil receptors[1,3,12].

Summary. Estradiol-17β is a stronger estrogen than estriol for the genomic response of estrogens. Estriol is a stronger estrogen than estradiol-17β for the estrogen-induced uterine eosinophilia and the 6 h increase in the uterine wet weight[13].

A. TCHERNITCHIN, X. TCHERNITCHIN and P. GALAND[14]

Laboratory of Molecular Endocrinology, Department of Cellular Biology and Genetics, University of Chile-Santiago Norte, Correo 21, Casilla 21104, Santiago (Chile), and Biology Unit, Laboratory of Nuclear Medicine, Free University of Brussels Medical School, 115 Boulevard Waterloo, Bruxelles 1000 (Belgium),
30 January 1975.

[6] K. BURTON, in *Methods in Enzymology* (Eds. L. GROSSMAN and K. MOLDAVE; Academic Press, New York 1968), vol. 12, p. 163.
[7] Z. DISCHE, in H. CHARGAFF and J. N. DAVIDSON, *The Nucleic Acids* (Academic Press, New York 1955), vol. 1, p. 301.
[8] O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR and R. J. RANDALL, J. biol. Chem. *193*, 265 (1951).
[9] R. MONTGOMERY, Arch. Biochem. Biophys. *67*, 378 (1957).
[10] A. TCHERNITCHIN, J. ROORIJCK, X. TCHERNITCHIN, J. VANDENHENDE and P. GALAND, Nature, Lond. *248*, 142 (1974).
[11] E. E. BAULIEU, Annls. Endocr. *29*, Suppl. 131 (1968).
[12] A. TCHERNITCHIN, J. ROORYCK, X. TCHERNITCHIN, J. VANDENHENDE and P. GALAND, Molec. cell. Endocr. *2*, in press (1975).
[13] Acknowledgments. This work was supported by a contract of the Ministère de la Politique Scientifique, within the framework of the Association Euratom – University of Brussels – University of Pisa. P. GALAND is a Maître de Recherches of the Fonds National de la Recherche Scientifique.
[14] Biology Unit, Laboratory of Nuclear Medicine, Free University of Brussels Medical School, 115, Boulevard de Waterloo, B-1000 Bruxelles (Belgium).

Time-Dependence of Estradiol Effects on Protein Synthesis in the Rat Neurohypophysis

The participation of estrogens in the modulation of the oxytocin-releasing reflex is now well documented[1,2]. Estrogens can alter this reflex by acting at any of several sites along the arc. One of possible sites of action is the neurohypophysis itself, i.e., estrogens could modify the responsiveness of the posterior lobe to nervous signals triggering oxytocin release. In a recent work we have observed that the neurohypophysis of the spayed female rat is able to concentrate and retain estradiol from the blood stream, and contains a cytoplasmic macromolecular component that binds estradiol with high affinity[3]. Estradiol uptake varies diurnally and seems to be dependent upon melatonin secretion from the pineal gland[3]. The present experiment was undertaken to examine the time-dependency for estrogens effects on the posterior lobe by measuring changes in ^{3}H-leucine incorporation into neurohypophyseal proteins as a function of time of injection of estradiol.

Material and methods. Wistar female rats were kept in the light from 07.00 to 21.00 daily and were given access to food and water ad libitum. Rats castrated 3 weeks earlier received a single dose of 0.3 µg estradiol or vehicle at 06.00 or 14.00 h. 24 h later the rats were sacrificed and pools of 2 neurohypophyses were incubated for 1 h at 37 °C in Krebs-Ringer bicarbonate buffer containing 1.5

[1] J. S. ROBERTS and L. SHARE, Endocrinology *84*, 1076 (1969).
[2] J. S. ROBERTS, Endocrinology *89*, 1029 (1971).
[3] E. PEDROZA-GARCÍA, D. P. CARDINALI, N. P. LABORDE, W. GARCÍA-BIENERE, C. A. NAGLE and J. M. ROSNER, Neuroendocrinology *14*, 174 (1974).

μCi of L-4,5-^{3}H-leucine (spec. act. 5 Ci/mmol) under a 95% oxygen: 5% carbon dioxide atmosphere. ^{3}H-leucine incorporation into proteins was estimated by measuring the radioactivity present in the trichloroacetic acid-insoluble residues[4]. Proteins concentration was measured according to LOWRY et al.[5].

Results and discussion. The administration of a single dose of estradiol caused a significant increase 24 h later of ^{3}H-leucine incorporation into neurohypophyseal proteins (Table). The effects of estradiol depended largely upon time of injection. Rats injected at 06.00 h, i.e., at the end of the dark period, exhibited a 74% increase in protein synthesis, whereas rats injected at 14.00 h, i.e. at the middle of the light period, showed only a 30% increase in protein synthesis.

Limited information is available concerning the possibility that estradiol treatment modifies the function of the neurohypophysis. In female rats, estrogens increase oxytocin content of the posterior lobe and gonadectomy reduces it slightly[6,7]. Data reported herein reveal that neurohypophyseal protein synthesis increases significantly after estradiol treatment. In addition, the time of day when estradiol was injected has a remarkable influence on the extent of stimulation of labelled amino acid incorporation into proteins. This may be related to the previously reported diurnal variations in estradiol uptake by the posterior lobe[3].

Time-dependence of the effects of estradiol on protein synthesis of the rat neurohypophysis

Treatment[a]	^{3}H-leucine incorporation into proteins (dpm/mg of protein)	
	06.00 h	14.00 h
Vehicle	612.0 ± 34.2[b]	591.1 ± 51.6
Estradiol	1065.3 ± 60.7[c]	769.8 ± 32.8[d]
Increase (%)	74.0	30.2

[a] Rats received a single injection of 0.3 ug of estradiol or vehicle at 06.00 or 14.00 h and were killed 24 h later. [b] Mean ± SE $n = 8$ in each group. [c] $p < 0.01$, Student's t-test. [d] $p < 0.05$.

Effects of estrogens on the neurons of the paraventricular nuclei are supported by neurophysiological[8] and autoradiographic[9] data. To what exent changes in oxytocin output reflects an effect of estradiol on hypothalamic or neurohypophyseal sites remains to be established. However, the present results, together with previous data[3], seem to argue in favour of the view that estrogens could affect the release of oxytocin in part by acting directly on the neurohypophysis.

Summary. The incorporation of ^{3}H-leucine into neurohypophyseal proteins was measured in vitro, 24 h after the administration of a single dose of estradiol (0.3 μg) to castrated female rats. Estradiol treatment caused a significant increase of ^{3}H-leucine incorporation into proteins of the posterior lobe. The effects of estradiol depended largely upon time injection. Rats injected at 06.00 h, i.e., at the end of the dark period exhibited a 74% increase in protein synthesis, whereas rat injected at 14.00 h, i.e., at the middle of the light period only showed a 30% of increase.

C. A. NAGLE, D. P. CARDINALI and J. M. ROSNER[10]

Instituto Latinoamericano de Fisiología de la Reproducción (ILAFIR), C.C.10, S. Miguel (P.B.A., Argentina), 10 March 1975.

[4] D. P. CARDINALI, C. A. NAGLE and J. M. ROSNER, Life Sci. *13*, 823 (1973).
[5] O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR and R. J. RANDALL, J. biol. Chem. *193*, 265 (1951).
[6] K. FENDLER, Acta physiol. hung. *20*, 89 (1961).
[7] L. BARNAFI and H. CROXATTO, Acta endocr., Copenh. *52*, 3 (1966).
[8] H. NEGORO, S. VISESSUWAN and R. C. HOLLAND, J. Endocr. *59*, 559 (1973).
[9] W. E. STUMPF, Am. J. Anat. *129*, 207 (1970).
[10] These studies were supported by grants from the Comisión Nacional de Estudios Geo-Heliofísicos, Argentina and the World Health Organization. D.P.C. and C.A.N. are established investigators of the Comisión Nacional de Estudios Geo-Heliofísicos. J.M.R. is an established investigator of the Consejo Nacional de Investigaciones Científicas y Técnicas, Argentina.

Endocrine Control of Mating Instinct in *Dysdercus koenigii* (Hemiptera: Pyrrhocoridae)

In course of a study to investigate the endocrine pathway of the events occurring in the reproductive cycle of *Dysdercus koenigii*, a hemimetabolous insect pest in India on the common ladies finger plant (*Hibiscus esculentus*), a very interesting and remarkable relationship was observed between the neurosecretory cells of the brain and the urge for mating in the female.

Six paired groups of neurosecretory cells (NSC) occur in the protocerebrum of the brain of *Dysdercus koenigii*. Apart from the median neurosecretory cells (MNSC) forming 3 pairs of dorsal and 2 pairs of ventral groups, there is a lateral group of 3 neurosecretory cells (LNSC) on either side. The females mate within 24 h after emergence, may continue the act for 110 to 114 h and lay eggs about 120 h after commencement of mating. The receptivity of the female appears to be 'recognized' by the male which moves in the vicinity of the former and exhibits a courting behaviour. It has also been observed that mating must continue for at least 96 h for normal oocyte development and oviposition. During the first 96 h, the mating individuals are inseparably united and any interference during this period results in the failure of the females to lay eggs. Interruption of mating 96 h after commencement does not affect maturation and normal ovulation of eggs. Histological examination of paraldehyde – fuchsin stained sections passing through the protocerebrum to show the secretory activity of the brain NSC in various phases of the reproductive cycle has revealed that the LNSC are full of secretory material prior to, and at the time of commencement of mating, but after 24 h they are seen to be empty showing only a compact layer of secretory material at the periphery of the cells. There is no secretory activity in the MNSC of pars intercerebralis upto 72 h after commencement of mating. The activity of the LNSC completely subsides after mating and these cells have not been seen to enter a secretory phase even 10 days after oviposition. The insects probably never mate again after laying the first batch of eggs (no mating occurred in the females kept under observation for over a month, although they were quite active and feeding voraciously on the leaves of *Hibiscus*