practically free of normal L-chain[14]. In the Figure 3 is shown that the B-J protein, rather homogeneous by electrophoresis, is separated into 5 bands, 3 of them being intensely stained.

Our results show that the isoelectric heterogeneity of the normal and pathological L-chains is considerably grater than is indicated by electrophoresis.

The isoelectric heterogeneity of the L-chains could arise from the alteration of some labile groups on its surface, during the reduction of the disulphide bonds, as AWDEH et al.[15] recently suggested. Because the B-J protein analyzed was not subjected to any reduction, it is not impossible that the charge properties alteration of this protein take place in blood or urine, after its secretion by the cells. AWDEH et al.[16] have demonstrated that, immediately after its synthesis, the mouse myeloma IgG_2 becomes very susceptible to alteration of its charge properties and both inside and outside the cells the IgG_2 presents an increased isoelectric heterogeneity.

The IEF is able to separate those L-chain variants which present differences in their isoelectric points and it seems possible, using this technique, to show differences of heterogeneity between different L-chain preparations[17].

Zusammenfassung. Die Kaninchen-L-Kette wurde bei der Elektrophorese in 7–8 und bei der Isoelektrofokusierung in 20 Fraktionen aufgetrennt. Die mielomatöse L-Kette wurde durch Elektrofokusierung bei pH 3–10 in 5 und bei pH 5–8 in 8–9 Fraktionen aufgetrennt. Fünf Fraktionen weist das bei gewöhnlicher Elektrophorese homogen erscheinende Bence-Jones-Protein bei pH 5–8 auf.

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'Hypothalamic Deafferentation' and Gonadotropin Secretion

FRASCHINI and MOTTA[1] reported in 1967 that in adult, normal, male rats of the Sprague-Dawley strain caged under standard light conditions (14 h of light beginning at 06.30 h, 10 h of dark) there is a diurnal cycle in the levels of gonadotropins stored in the anterior pituitary. A peak of follicle stimulating hormone (FSH) and of luteinizing hormone (LH) concentrations was observed to occur in the afternoon, between 16.00 h and 18.00 h. Several reports of the same laboratory have subsequently confirmed these findings[2,3]. This diurnal cycle apparently does not exist in adult, male rats of the Holtzman strain[4].

It has been proposed that the diurnal variations in the concentrations of pituitary gonadotropins found in male animals might be due to the influence of pineal principles[3]. It is known that the biosynthesis of the pineal principles melatonin and 5-methoxytryptophol is cyclic in nature, and is strictly regulated by the light-dark schedule to which the animals are exposed[5]: the synthesis of pineal methoxyindoles is inhibited during the day and is activated during the night, because light inhibits the activity of the enzyme hydroxy-indole-*o*-methyl-transferase (HIOMT) which is essential for introducing the methoxy group on the indole molecule. Moreover, melatonin seems to be a specific inhibitor of the synthesis of LH[3,6–8], while 5-methoxytryptophol specifically blocks the formation of FSH[3,7–9].

It has recently been demonstrated that rats submitted to a complete 'hypothalamic deafferentation' according to HALÁSZ[10] technique do not show the diurnal cycles of plasma corticosterone levels[10,11] and of pituitary ACTH concentrations[10,12] which are usually found in normal animals. 'Deafferented' rats lose also the ability of releasing gonadotropins cyclically[10].

It was deemed of interest to investigate whether adult, normal male rats of the Sprague-Dawley strain, kept in conditions identical to those described in the papers by the Milan group[1–3], might retain the diurnal cycle in pituitary gonadotropin concentration 8 days after being submitted to a complete 'hypothalamic deafferentation'.

Materials and methods. Mature, Sprague-Dawley male rats were used in this study. They were allowed a standard rat pellet diet and water ad libitum. A complete 'hypothalamic deafferentation' was performed on 4 groups of rats (12 animals per group) using the technique by HALÁSZ[10,11] and a Stoelting stereotaxic instrument. At time of autopsy (8 days following the operation) each group was subdivided into 2 sub-groups: the first one was killed at 10.00 h; the second was sacrificed at 16.00 h.

For the assays of LH and FSH, the anterior pituitary lobes of each sub-group of animals were pooled and homogenized. LH was assayed by the ovarian ascorbic acid depletion method of PARLOW[13] as modified by

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SCHMIDT-ELMENDORFF and LORAINE[14] using 30-day-old female recipients primed with PMS-HCG. Ovarian ascorbic acid was determined according to ROE and KUETHER[15]. A 2 + 2 design was used against a standard of LH provided by the National Institutes of Health (NIH-LH-S-14-ovine). 4 assay animals were used for each point.

FSH was evaluated according to the ovarian augmentation test of STEELMAN and POHLEY[16]. A 2 + 2 design was used against a standard of FSH provided by the National Institutes of Health (NIH-FSH-S-8 ovine).

Results and discussion. The results of the determination of the concentrations of FSH in the pituitaries of the 8 sub-groups of adult male rats submitted to a complete 'hypothalamic deafferentation' are shown in Table I. It appears quite clearly that there is no significant difference between the amounts of FSH present in the pituitaries of the animals killed in the morning and that present in the glands of animals killed in the afternoon.

Table I. Effect of 'hypothalamic deafferentation' on the concentration of FSH in the anterior pituitary of adult male rats

Groups	Pituitary FSH (μg/mg)[a]	Fiducial limits (95%)
Animals killed in the morning		
1	39.27	26.44–62.44
2	29.97	25.85–34.84
3	20.18	20.98–32.63
4	19.45	15.52–24.03
Mean	27.21 ± 4.67	
Animals killed in the afternoon		
1	17.14	13.69–20.90
2	20.75	17.05–24.84
3	35.80	26.08–49.59
4	23.74	20.31–29.80
Mean	24.35 ± 4.04	

[a] Microgram equivalents of NIH-FSH-S-8 Ovine per mg wet weight of pituitary tissue.

Table II. Effect of 'hypothalamic deafferentation' on the concentration of LH in the anterior pituitary of adult male rats

Groups	Pituitary LH (μg/mg)[a]	Fiducial limits (95%)
Animals killed in the morning		
1	1.10	0.70–1.77
2	2.02	1.33–3.20
3	0.87	0.27–2.44
Mean	1.33 ± 0.35	
Animals killed in the afternoon		
1	0.63	0.33–1.05
2	1.30	0.75–1.84
3	2.22	1.36–3.92
Mean	1.38 ± 0.46	

[a] Microgram equivalents of NIH-LH-S-14 Ovine per mg wet weight of pituitary tissue.

Table II summarizes the data obtained when LH was measured in the pituitary glands of the same sub-groups of 'deafferented' animals. Pituitary stores of LH measured in the afternoon do not appear to be different from those measured in the morning.

These data clearly indicate that the diurnal cyclicity, previously reported to occur in the concentrations of pituitary gonadotropins of normal male rats[1–3], is lost if the animals are submitted to a complete 'hypothalamic deafferentation'. It appears from the data that the interruption of all pathways impinging on the hypothalamus disrupts also this diurnal rhythm. The disappearance of the diurnal cyclicity of the activity of the pituitary adrenal axis[10–12] and of cyclic release of gonadotropins in female rats[10] had been previously reported. The data are strongly in favor of the hypothesis that the rhythmicity of the functions of the hypothalamic-pituitary complex is regulated by extrahypothalamic influences[17,18].

The interpretation provided in the preceding paragraph is not in conflict with the suggestion that the pineal gland might be involved in the control of rhythmic phenomena[3,6,8]. It has recently been postulated that pineal principles might operate on endocrine processes by interacting with the monoaminergic systems whose cell bodies are located in the midbrain. This postulation is supported by the observation that pineal principles lose their inhibitory activity on the pituitary-adrenal axis following reserpine-induced depletion of cerebral amines[19]. In addition, the administration of exogenous melatonin is followed by significant changes in the stores of monoamines of the brain stem[20]. If pineal principles need to interact with monoaminergic pathways originating in the midbrain, it is obvious that they should become completely ineffective in 'deafferented' animals, in which all such pathways are completely interrupted[21].

Résumé. Le contenu en FSH et en LH a été évalué à 10.00 h du matin et à 16.00 h de l'après-midi dans les hypophyses de rats mâles après que tout influx nerveux arrivant à l'hypothalamus avait été interrompu. Dans ces conditions expérimentales, la fluctuation journalière que l'on rencontre chez les animaux normaux disparaît.

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