NaOH solution. The vessels were gassed for 5 min with O_2 100%; 10 min were allowed for the equilibration and the observation period lasted 60 min. All incubations were performed at 37 °C. Results expressed as µl O_2/mg wet tissue/h have been compared with Student's *t*-test accordingly with FISHER and YATES[6].

Results. The oxygen uptake of the amygdala, hippocampus and cerebral cortex from diestrus and estrus rats is summarized in the Table. As can be seen the oxygen uptake of the amygdala is higher during estrus than in diestus phase. On the other hand the oxidative activity of hippocampus is higher during diestrus than in estrus rats. No modifications were found in the respiration of cerebral cortex during sexual cycle.

Discussion. Several observations have demonstrated that the limbic system is one of the brain structures implicated in gonadal function. It was shown that electrical stimulation of the amygdala led to ovulation in the rabbit, the cat and the rat[7–9], and stimulation of the hippocampus induced ovulation in the rabbit[10]. It was also found that bilateral lesions of the hippocampus or amygdala in the adult rat altered the estrus cycle[11,12].

The results of the present paper showed cyclic changes in the oxidative activity of the amygdala and hippocampus. In the amygdala the highest values were observed during estrus and in the hippocampus during diestrus. Such results could be correlated with the observations performed by TERASAWA and TIMIRAS[13] who found that the electrical activity of hippocampus decreases in the morning of the estrus, while the medial part of amygdala decreases its electrical activity during diestrus.

Many functional mechanisms could be implicated from the cyclic changes in the metabolic activity of the limbic structures; nevertheless, taking into account the modifications of sexual hormones and gonadotrophins during the sexual cycle, it is probable that the limbic modifications are in some way connected with such hormonal variations. Further studies are needed before a conclusion can be reached in this respect.

Resumen. Se ha estudiado el consumo de oxígeno de la amigdala y del hipocámpo en ratas hembras durante el ciclo sexual. La corteza cerebral se utilizó como control. Los resultados obtenidos indican que ambas estructuras límbicas sufren cambios cíclicos. La mayor actividad metabólica de la amigdala fué observada durante el estro en cambio el hipocampo tiene su mayor consumo de oxígeno durante el diestro.

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Oxidative activity of limbic structures and cerebral cortex in female rats

	QO_2 (µl O_2/mg wet tissue/h) Amygdala	Hippocampus	Cerebral cortex
Diestrus	1.08 ± 0.11 (15)	1.43 ± 0.10 (26)	1.31 ± 0.12 (13)
Estrus	1.51 ± 0.14 (17)	1.18 ± 0.08 (24)	1.30 ± 0.11 (13)
P value	< 0.02	< 0.05	n.s.

[a] Mean ± standard error. Figures in parenthesis are the number of determinations; n.s., not significant.

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Immunochemical Studies of Ribosomal Proteins Using Passive Hemagglutination Techniques

Immunochemical analysis of ribosomal proteins could be a convenient method for the study of structural similarities between different fractions of ribosomal proteins from the same organism or between ribosomal proteins from different sources, but the use of these techniques has been hindered by the low solubility of ribosomal proteins in acqueous buffers. Unfractionated ribosomal proteins are soluble at fairly high concentrations (> 2 mg/ml) only in urea, in high salt solution, or at extreme values of pH, i.e. under conditions which interfere with the antigen-antibody reaction.

Total ribosomal proteins from *Neurospora crassa*, labelled with ^{14}C dissolved in pyrophosphate buffer at pH 8.5, were assayed in quantitative precipitin reactions, and shown to be all antigenically active, although a considerable fraction of the antigen-antibody complex remained soluble under the conditions of assay[1], therefore preventing any meaningful estimation or comparison.

In this paper we report passive hemagglutination techniques which allow circumvention of some of the difficulties indicated above.

Methods. Ribosomes were isolated from *Neurospora crassa* mycelia and from *Saccharomyces cerevisiae* cells according to a procedure shown to yield ribosomes free from cytoplasmic contaminations[2]. Ribosomes from potato tubers were isolated in the same way, except that $2 \times 10^{-4} M$ natrium diaethyldithiocarbammicum (DIECA) was added to block polyphenoloxidase activities[3] and to avoid the formation of inhibitory quinones[4]. Ribosomes

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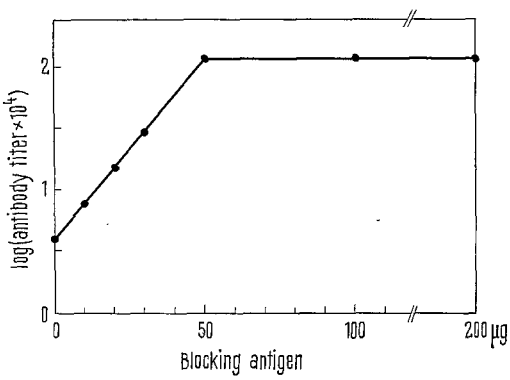
Passive hemagglutination of erythrocytes coated with ribosomal proteins by antisera against *Neurospora crassa* ribosomal proteins

	Antibody titer									
	$^1/_{10}$	$^1/_{20}$	$^1/_{40}$	$^1/_{80}$	$^1/_{160}$	$^1/_{320}$	$^1/_{640}$	$^1/_{1280}$	$^1/_{2560}$	$^1/_{5120}$
Etns + IS	—	—	—	—	—	—	—	—	—	—
Ets NC + IS	+++	+++	+++	+++	+++	+++	+++	+++	++	—
Ets NC + NIS	—	—	—	—	—	—	—	—	—	—
Ets P + IS	—	—	—	—	—	—	—	—	—	—
Ets Y + IS	—	—	—	—	—	—	—	—	—	—

Abbreviations used: Etns, erythrocytes tanned not sensitized; Ets NC, erythrocytes sensitized with *Neurospora crassa* ribosomal proteins; Ets P, erythrocytes tanned sensitized with potato ribosomal proteins; Ets Y, erythrocytes tanned sensitized with yeast ribosomal proteins; IS, anti *Neurospora crassa* ribosomal proteins serum; NIS, non-immune serum. Experimental outline: The first well of a Takatsy plate contained 0.9 ml of saline B, pH 7.2, and 0.1 ml of antisera. 0.5 ml of this reaction mixture was transferred to the second well containing 0.5 ml of saline B, and so on in a serial way for 15–20 wells. To each of the serial dilutions of antibody, 0.05 ml of erythrocytes suspension was added. The plate was gently shaken and left covered at room temperature overnight before scoring.

were dissolved in 0.05 *M Tris* (Hydroxymethyl aminomethane) chloride pH 7.8 containing 60 m*M* KCl, 7 m*M* $Mg(C_2H_3O_2)_2$, and 20 m*M* β-mercaptoethanol to the final concentration of 40 O.D. at 260 nm/ml. To 1.0 ml of the ribosomal solution, 1.2 ml of a solution of 4 *M* LiCl and 8 *M* urea were added. The RNA was discarded by centrifugation and the supernatant containing the ribosomal proteins was used[1]. Antisera were obtained from rabbits immunized with *Neurospora crassa* ribosomal proteins in LiCl-urea, as previously described[1]. Formalinized sheep erythrocytes, obtained through the courtesy of Dr. E. CLERICI, were washed with saline solution A (containing 0.45% NaCl in 75 m*M* sodium potassium phosphate buffer, pH 7.2). To 1 ml of the packed red cells, 5 ml of 1/10,000 tannic acid solution were added. The suspension, retained at room temperature for 10–30 min, was centrifuged and the sediment washed once with saline A. To the tanned erythrocytes 2 ml of saline solution A and 5 ml of antigen solution were added. The antigen solution was a LiCl-urea preparation of ribosomal proteins, containing about 600 µg proteins/ml. After standing at 37 °C for 30 min with occasional stirring, the red cells were packed, washed twice with saline solution B (saline solution A containing 1% of fresh rabbit serum heated at 56 °C for 5 min) and once saline A. Packed sensitized red cells were suspended in 20 vol. of saline A. The passive hemagglutination test was run in Takatsy plates with serial dilution[5].



Relationship between the amounts of blocking antigen (*Neurospora crassa* ribosomal proteins) and the corresponding antibody titers. The amounts of blocking antigen added to the first well are plotted on the abscissa; the logarithm of the antibody titers ($\times 10^4$) are plotted on the ordinate. Experimental outline: as described in the Table, except increasing amounts of blocking antigen in 0.05 ml of 4 *M* urea were added to the first wells.

Results and discussion. As shown in the Table, erythrocytes coated with *Neurospora crassa* ribosomal proteins were agglutinated by homologous antisera at fairly high titers. There was no positive reaction when non-immune sera or tanned non-sensitized erythrocytes were used. Red cells coated with potato or yeast ribosomal proteins were not agglutinated by antisera against *Neurospora crassa* ribosomal proteins, thus indicating the lack of cross-reaction between these unrelated species.

The ability of homologous proteins to block the reaction of agglutination was investigated. A preliminary control showed that the addition of 0.05 ml of 4 *M* urea to the first well of a serial dilution did not affect either the hemagglutination reaction or the antibody titer. Increasing amounts of *Neurospora crassa* ribosomal proteins, in a final volume of 0.05 ml of 4 *M* urea to the reaction mixture were added 20–30 min before the addition of sensitized erythrocytes. As shown in the Figure, the inhibition of the hemagglutination occurred even at very low protein concentrations. The linear relationship between the amount of antigen added and the logarithm of the antibody titer shown in the early part of the graph could be used to determine the amount of antigen present and to express in a quantitative manner the extent of cross-reaction between different ribosomal proteins.

The passive hemagglutination techniques described above appear quite convenient for immunochemical studies of ribosomal proteins: the sensitization of the erythrocytes can be achieved using proteins dissolved in urea; the antigen-antibody reaction occurs in normal saline and gives indication of species specificity; the blocking reaction observed after addition of small amounts of antigen to the system can be expressed in a quantitative manner.

Riassunto. Emazie di montone sensibilizzate con proteine ribosomali di *Neurospora crassa* sono agglutinate da anticorpi specifici, mentre emazie sensibilizzate con proteine ribosomali di lievito o di patata non sono agglutinate da questo stesso siero. Inibizione della reazione di emoagglutinazione si ottiene in presenza di concentrazione molto basse di antigene specifico.

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