

## Metabolic Alterations in Hypothalamus and Limbic Structures in Female Diabetic Rats<sup>1</sup>

The resection of 95% of the pancreas has been shown to induce a reduction of fertility and to develop important alterations in further pregnancy, fetuses and placentae of female rats<sup>2,3</sup>.

The administration of alloxane has been reported to produce modifications in the genital tract of female as well as male rats<sup>4</sup>; the testicular tissue was particularly lesioned, as evidenced by histological examination<sup>5</sup>. In addition, in the immature rat this substance retards or suppresses the sexual development<sup>6</sup>. STUTINSKY and MIALHE<sup>7</sup> have also observed profound alterations in the morphology of the hypothalamus and of the pituitary of the pancreatectomized male rat. It has recently been demonstrated that the anterior hypothalamus of these animals shows a decrease in the oxygen uptake and an increase in the lactic production<sup>8</sup>. It is interesting to note that castration in the male rat has a similar effect on the same metabolic parameters<sup>9,10</sup>.

In view of this observation and of the fact that the oxidative metabolism of the hypothalamus<sup>11</sup> and of the limbic structures<sup>12</sup> undergo cyclic changes in relation to the different phases of the estrous cycle, it has been decided to study the oxidative metabolism of the hypothalamus, the amygdala and the hippocampus in the female rat submitted to pancreatectomy.

**Material and methods.** Wistar female rats were used. The animals were fed on the standard diet of Catedra 1<sup>a</sup> de Fisiología. The following groups of animals were studied: a) normal rats in diestrus; b) normal rats in estrus; and c) pancreatectomized rats in diestrus.

The surgical removal of the pancreas (partial pancreatectomy, 95%) was performed according to the method of FOGLIA<sup>13</sup>, when the body weight of the rat was between 80–100 g; blood sugar was measured at monthly intervals by the method of HAGEDORN and JENSEN<sup>14</sup>. The animals were used 8 months after the operation; their blood sugar levels were above 1.56 mg/100 ml. The average glycemia of the controls in diestrus and in estrus was 0.87 mg/100 ml.

The animals were decapitated and the hypothalamus, the amygdala and the hippocampus removed. Oxygen uptake in these structures was determined by Warburg manometry technique in vessels of 12–15 ml capacity, containing 3 ml of Krebs-Ringer phosphate buffer, pH 7.4 and 7.7 mM glucose; the central well contained 0.2 ml of saturated NaOH solution. The vessels were gassed for 5 min with O<sub>2</sub> 100%; 10 min were allowed for the equilibration and the observed period lasted 60 min. All incubations were performed at 37 °C and the vessels were shaken 120 times/min. Results expressed as  $\mu\text{l O}_2/\text{mg wet tissue/h}$  and analyzed for variance following the method of SNEDECOR<sup>16</sup>. The statistical significance of the data was determined according to TUKEY's method<sup>17</sup>. The minimal significant difference of the means was 0.1865 in the hypothalamus, 0.3437 in the amygdala and 0.2369 in the hippocampus.

**Results.** The Table reports the average of  $\text{QO}_2$  expressed in  $\mu\text{l O}_2/\text{mg wet tissue/h}$  for the hypothalamus, the amygdala and the hippocampus in the several groups studied and the statistical significance according to the multiple comparison test. The analysis of variance indicates that the differences in the several tissues are statistically significant with *P* value of 0.01 for each area.

The results shown in the Table show that the oxygen uptake of the hypothalamus and of the amygdala is very high during the estrus phase and low at diestrus. Pancreatectomy brings about a decrease in the oxidative metabolism of the hypothalamus to levels as low as those

found in diestrus. In contrast to this, the diabetes induced by pancreatectomy markedly stimulates the oxygen uptake in the amygdala of the diabetic rats, which have been always killed at diestrus, to levels as high as those found in the normal rats at estrus.

When the oxidative activity of the hippocampus is considered, the highest oxygen uptake is observed at diestrus and the lowest at estrus in the control group. Pancreatectomy does not induce any modification in the oxygen uptake of the hippocampus, as shown by the values which remain as high as those found in the control rats at diestrus.

**Discussion.** Our data seem to indicate that diabetes induced by partial pancreatectomy modifies the oxidative activity of the hypothalamus and of the amygdala. In the hypothalamus of the diabetic rats, the values of oxygen uptake were much lower than those shown by the controls in diestrus. This indicates the existence of profound alterations in this nervous tissue.

It has previously been demonstrated that the amygdala of normal female rat shows cyclic variations during the estrous cycle<sup>12</sup>. These cyclic changes are eliminated in the pancreatectomized rats which, although they are in diestrus, exhibit an oxygen uptake in the hippocampus of diabetic rats parallel to the different phases of estrous cycle, showing values at diestrus equivalent to those found in normal rats at diestrus<sup>12,18</sup>; i.e. the presence of diabetic syndrome does not influence this metabolic activity at the hippocampal level. This may indicate that the inverse relationship usually found in the oxidative metabolism<sup>12</sup> and electrical activity<sup>19</sup> between the amygdala and the hippocampus during the estrous cycle of normal female rats had disappeared.

It has been reported that the hypothalamus of diabetic male rats shows alterations in the oxidative and glycolytic metabolism<sup>8</sup>. This work presents data indicating the

<sup>1</sup> Supported by a grant of Fundación Marqués de Urquijo.

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<sup>5</sup> B. E. LEMA, V. G. FOGLIA and E. L. FERNANDEZ COLLAZO, *Revta Soc. argent. Biol.* 41, 197 (1965).

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<sup>11</sup> J. A. MOGUILLEVSKY and R. M. MALINOW, *Am. J. Physiol.* 206, 855 (1964).

<sup>12</sup> O. SCHIAFFINI, B. MARIN and A. GALLEGO, *Experientia*, in press.

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<sup>14</sup> H. C. HAGEDORN and B. N. JENSEN, *Biochem. Z.* 135, 46 (1923).

<sup>15</sup> W. W. UMBREIT, R. H. BURRIS and J. STAUFFER, *Manometric Techniques and Tissue Metabolism* (Burgess Publishing Co., Minnesota 1959).

<sup>16</sup> G. W. SNEDECOR, *Statistical Methods* (Iowa University Press, Ames 1956).

<sup>17</sup> K. W. TUKEY, *The Problems of Multiple Comparisons* (Trans. N.Y. Acad. Sci. Series II, 16, 1953).

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<sup>19</sup> E. TERESAWA and P. S. TIMIRAS, *Endocrinology* 83, 207 (1968).

existence of a similar alteration in the hypothalamus of the female rat with diabetes. These results and the abundant literature existing on reproductive and gonadal alteration in the diabetic male<sup>4,5</sup> and female rats<sup>2-4</sup> may suggest that an impairment in the synthesis and/or the release of pituitary gonadotropins may occur. A relationship between the gonadotropin levels and the oxidative metabolism of the hypothalamus<sup>20-22</sup> has already been suggested. Evidence is accumulating which indicates that the oxidative activity of the hypothalamus is also associated with the 'short' feed-back mechanism<sup>23</sup> controlling the secretion of FSH<sup>24</sup> and of LH<sup>25</sup>. VELASCO and TALEISNIK<sup>26</sup> have also shown that the limbic structures are functionally associated and participate in the ovulatory processes. However, more data are needed in order to establish whether the metabolic alterations which have been observed in the diabetic rats are produced by an impairment at the central (hypothalamic) level or at the peri-

pheral (gonadal) level of the hypothalamic-hypophyseal-gonadal axis.

**Resumen.** Se determinò el consumo de oxígeno de hipotálamo, amígdala e hipocampo en ratas hembras a las que se les había producido una diabetes experimental por pancreatectomía sub-total (95%). Se usaron las ratas diabéticas durante la fase de diestro y como controles se utilizaron animales normales en distintas etapas del ciclo sexual. Los animales diabéticos en hipotálamo presentaron valores de consumo de oxígeno inferiores a los más bajos registrados en los animales controles (diestro). La amígdala, de las ratas diabéticas, mostró un metabolismo oxidativo similar al presentado en diestro por los controles normales, a pesar de encontrarse en la fase diestro en el momento del sacrificio. Por el contrario el consumo de oxígeno del hipocampo de los animales diabéticos no mostró modificaciones en comparación con los controles normales.

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Oxidative metabolism of hypothalamus, amygdala and hippocampus in diabetic rat

Groups	QO <sub>2</sub> (μl O <sub>2</sub> /mg wet tissue/h)		
	Hypo- thalamus	Amygdala	Hippocampus
D-diestrus	1.30 ± 0.07 (14)	1.09 ± 0.09 (19)	1.49 ± 0.08 (29)
E-estrus	1.59 ± 0.08 (17)	1.59 ± 0.11 (28)	1.21 ± 0.06 (35)
P-pancreatectomized	0.98 ± 0.08 (15)	1.45 ± 0.06 (12)	1.54 ± 0.06 (15)
Analysis of variance			
F ratio	16.62	6.38	5.98
P value	0.01	0.01	0.01
Multiple comparisons test			
P < 0.05 between	D vs. E D vs. P E vs. P	D vs. E D vs. P —	D vs. E — E vs. P

Mean ± S.E. Figures in parentheses are number of determinations.

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## Transport of Whole Protein Molecules from Blood to Saliva of a Plant-Bug

An unresolved problem in the transmission of plant viruses in the saliva of Homoptera is how the virus penetrates cellular barriers in the insect: whether as whole particles or as components that are eventually reassembled. A number of metabolites are transferred rapidly to the saliva when injected into the haemolymph of plant-bugs<sup>1</sup>, and the haem group from haemoglobin finds its way into the salivary glands of the blood-sucking bug *Rhodnius prolixus* (Heteroptera: Reduviidae)<sup>2</sup>. But the protein of the salivary 'haemalbumin' of *Rhodnius* is of unknown origin and history; and, to the knowledge of the present writers, no evidence has yet been presented of the direct transfer of particles as large as protein molecules from the haemolymph to the saliva of Hemiptera.

Horseradish peroxidase is a highly stable enzyme with a mol wt. of about 40,000; and it is easily detectable with a benzidine reagent<sup>3</sup>. Peroxidases occur naturally in insects<sup>4</sup>, and in the present study were found in the salivary

'sheath material' (the solidifying fraction of the saliva<sup>5</sup>) of the rose aphid, *Macrosiphum rosae* (Homoptera: Aphididae) and in the haemolymph, watery saliva<sup>1</sup>, and sheath material of the peanut trash bug, *Elasmolomus sordidus* (Heteroptera: Lygaeidae). On the other hand, the fifth instar larva of *Eumecopus punctiventris* (Heteroptera: Pentatomidae) had barely detectable amounts of peroxidase in its haemolymph, and no detectable enzyme in its sheath material. This insect did not produce watery

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