Oxidative Metabolism of the Limbic System in Androgenized Rats

It has been demonstrated that the limbic system in the rat presents variation in the oxygen consumption in relation to the sexual cycle. In the amygdala the uptake increases during the estrous and decreases during the diestrous phase, whereas the contrary variations occur in the hippocampus[1]. These variations are eliminated by ovariectomy and then the oxygen consumption is high in both structures[2]. A relationship between oxygen consumption in the limbic system and the pituitary gonadotropins has been suggested by in vitro studies[3].

The absence of corpora lutea in the ovary[4] and alterations in the oxidative metabolism of the hypothalamus[5] in rats treated postnatally on the 5th day presents a condition of permanent sterility in which a study of the oxidative metabolism of the limbic system would appear worthy of interest.

Materials and methods. To this end groups of Wistar rats were studied in estrus, diestrus and in permanent estrus following 5th day postnatal s.c. administration of 1.25 mg testosterone propionate in olive oil. The animals were sacrified by decapitation at 6–7 months and the amygdala and hippocampus removed by dissection. The oxygen consumption was determined by Warburg manometry in 12–15 ml vessels containing 3 ml. Krebs-Ringer pH 7.4 phosphate buffer and 7.7 m*M* glucose, with 0.2 ml saturated aqueous NaOH solution in the central well. The test solutions were gassed for 5 min with 100% O_2 and, after equilibration of the system for 10 min, measurements were made at 37°C, 120 beats/min for 1 h. Results were expressed as µl O_2/mg tissue wt./h. The data were analyzed using Student's *t*-test, variance analysis, and the Pairwise test[6].

Results. Table I expresses the oxygen consumption per amygdala and hippocampus in the different groups of animals. During the estrous phase, the oxygen consumption per amygdala is significantly higher than that per hippocampus. In the diestrous phase, the oxygen consumption of the hippocampus exceeds that of the amygdala, but no difference is noted between the oxygen consumption of amygdala and hippocampus in the permanent estrous phase.

Table II shows the variance of data and the significance of differences of averages as demonstrated by the Pairwise test. The data are significant for amygdala where the oxygen consumption differs in the 3 groups of animals. Differences are evident between rats in estrus and diestrus, in estrus and permanent estrus, but not between those in diestrus and permanent estrus. Although the oxygen consumption by the hippocampus is greater in diestrus than in estrus and very similar in diestrus and permanent estrus, the data for the hippocampus are not statistically significant.

Discussion. Participation of the limbic system in the regulatory processes of the gonadal function has been suggested by various observations. Thus, stimulation of the amygdala provokes ovulation in rats with permanent

[1] O. SCHIAFFINI, B. MARIN and A. GALLEGO, Experientia *25*, 1255 (1969).
[2] O. SCHIAFFINI and B. MARIN, Neuroendocrinology *7*, 302 (1971).
[3] O. SCHIAFFINI, F. PIVA and L. MARTINI, in press.
[4] C. A. BARRACLOUGH, Endocrinology *68*, 62 (1961).
[5] J. MOGUILEVSKI and L. RUBINSTEIN, Neuroendocrinology *2*, 213 (1967).
[6] T. YAMANE, *Statistics, an Introductory Analysis* (Harper and Row, New York 1970).

Table I. Oxygen consumption per amygdala and hippocampus by rats in estrous, diestrous, and permanent estrous phase following postnatal administration of testosterone propionate

Organ	Estrus	Diestrus	Permanent estrus
Amygdala	1.57[a] ± 0.12 (11)[b]	1.07 ± 0.10 (15)	1.26 ± 0.12 (19)
Hippocampus	1.21 ± 0.08 (21)	1.41 ± 0.09 (24)	1.46 ± 0.08 (20)
	$P < 0.05$	$P < 0.05$	N.S.

[a] Average value. [b] Standard deviation of oxygen consumption expressed in µl O_2/mg tissue/h. The numbers in parentheses indicate the number of animals utilized.

Table II. Analysis of variance

Source of variance	d.f.	Sum of squares	Mean square	F ratio	*P* value
Amygdala					
Between groups	2	1.95	0.975	4.06	0.05
Within groups	47	11.46	0.240		
Total	49	13.41			
Hippocampus					
Between groups	2	0.69	0.345	2.31	N.S.
Within groups	60	9.27	0.149		
Total	62	9.96			

Pairwise test for averages corresponding to amygdala. $P < 0.05$ between E and P; < 0.01 between E and D; N.S. between P and D. E, estrous; D, diestrous; P, permanent estrous phase.

estrus[7], elevates the plasmatic level of gonadotropins[8], and increases the production of progesterone by the ovary[9]. Otherwise, after 3 weeks, destruction of the amygdala brings about a decline in the levels of FSH-RF[10] and elevation of those of LH-RF[11]. These activities seem to be mediated by the stria terminalis[8] which connects the amygdala and hypothalamus. On the contrary, stimulation of the hippocampus impedes ovulation in cyclic rats[12].

The limbic system has cyclic modifications in its oxidative metabolism which disappear with ovariectomy[2], experimental diabetes[13], and on postnatal treatment with testosterone. In addition to their state of permanent estrus, the androgenized rats present a picture similar to that of diestrus. Experimental data similar to those obtained in androgenized rats have been obtained with male animals. These alterations in the limbic system may be due to hormonal disequilibration consequent on androgenization, or possibly may be a direct effect of the postnatal treatment.

Resumen. Se ha estudiado el metabolismo oxidativo de amígdala e hipocampo en ratas en fase de estro, diestro y androgenizadas postnatalmente con testosterona. En estos animales desaparecen las variaciones observadas en animales cíclicos, presentando unos valores semejantes a los de diestro.

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[7] J. P. Bunn and J. W. Everett, Proc. Soc. exp. Biol. Med. *96*, 369 (1957).
[8] M. E. Velasco and S. Taleisnik, Endocrinology *84*, 132 (1969).
[9] M. Kawakami, K. Seto and K. Yoshida, Jap. J. Physiol. *16*, 254 (1966).
[10] B. E. Eleftheriou and M. L. Pattison, J. Endocrin. *39*, 613 (1967).
[11] B. E. Eleftheriou and A. J. Zolovick, J. Reprod. Fertil. *14*, 33 (1967).
[12] M. E. Velasco and S. Taleisnik, Endocrinology *84*, 1154 (1969).
[13] O. Schiaffini, B. Marin and V. G. Foglia, Experientia *26*, 610 (1970).

Multiple Lactate Dehydrogenase Alleles in the Lizard *Agama stellio*

Agama stellio is probably the most conspicuous reptile of Israel. The species is widely distributed from the eastern Mediterranean region to Iraq[1]. Standard techniques of horizontal starch-gel electrophoresis were utilized to examine the mobilities of the lactate dehydrogenase (LDH) isozymes from 347 specimens of this species. We used buffer systems of pH 6.0[2], and 8.6[3]; and the histochemical staining procedure of Fine and Costello[4]. As among many vertebrates the LDH's of many lizards, including *Agama stellio*, are the products of two different genetic loci which may be termed H (heart) and M (muscle)[5,6]. The active enzyme is a tetramer and the products of the H and M loci can form hybrid tetramers. If the organism was homozygous at both loci, 5 isoenzymes could be seen on gel electrophoresis. An organism heterozygous at one of the two loci could express 15 isoenzymes[7].

We utilized red blood cells lysed with distilled water; and skeletal muscle and heart tissue from which the LDH was extracted not by the conventional method of tissue grinding but by soaking 1 volume of tissue in 4 volumes of 2% 2-phenoxyethanol in a 0.25 *M* sucrose solution[8].

Virtually all of the specimens were collected from areas administered by Israel (Table). The great majority had 5 equally spaced LDH bands in the red cell extracts. The anodally fasted migrating band predominated in heart tissue, the slowest band in skeletal muscle. At pH 8.6 all bands migrate toward the anode, at pH 6.0 the slow bands exhibit cathodal migration (Figure 1). No variation in electrophoretic mobility was seen among the 5-isoenzyme homozygous lizards.

There were 21 specimens that differed from the typical pattern, exhibiting, in all, 3 different electrophoretic phenotypes. All were 15 banded heterozygotes, with the heterozygosity involving the *H* locus[9]. The alleles were arbitrarily called LDH-H^n ('normal' or commonest), LDH-H^s ('slowest'), LDH-H^f ('fast'), and LDH-H^v ('very fast'). At pH 8.6 distinct differences could be seen between the mobilities of the heterozygotes *Hnf* and *Hnv*. At pH 6.0 the mobility appears tobe the same (Figure 1). The lower pH provides superior resolution of the expected 15 isoenzymes.

All the heterozygotes were concentrated in a relatively limited geographic area (Figure 2, Table). The allele H^v was found at all major collecting sites within the area encompassed by the presence of heterozygotes, but nowhere was it common (Table). Thus it is not surprising that no *Hvv* homozygotes were found. The other alleles were even rarer, and again homogzyotes would not have been expected. The alleles H^f and H^s were limited in distribution, the former to a single area in the Golan and the latter at 2 sites separated by a linear distance of about 50 km (Figure 2).

[1] S. Daan, Beaufortia *14*, 109 (1967).
[2] G. Bailey and A. C. Wilson, J. biol. Chem. *243*, 5843 (1968).
[3] I. Smith, *Chromatographic and Electrophoretic Techniques*, 2nd. edn. (William Heinemann Medical Books, Ltd., Pitman Press, Bath, England 1968), vol. 2, p. 524.
[4] I. H. Fine and L. A. Costello, in *Methods in Enzymology* (Eds. S. P. Colwick and N. O. Kaplan; Academic Press, New York 1963), vol. 6, p. 938.
[5] A. C. Wilson, N. O. Kaplan, L. Levine, A. Pesce, M. Reichlin and W. S. Allison, Fedn. Proc. *23*, 1258 (1964).
[6] S. N. Salthe, C. P. Chilson and N. O. Kaplan, Nature, Lond. *207*, 723 (1965).
[7] S. H. Boyer, D. C. Fainer and E. J. Watson-Williams, Science *141*, 642 (1963).
[8] M. Nakanishi, A. C. Wilson, R. A. Nolan, G. C. Gorman and G. S. Bailey, Science *163*, 681 (1969).
[9] Heterozygosity at the *H* locus of *Agama stellio* was suspected by G. Gorman, M. Nakanishi and A. C. Wilson, Syst. Zool. *20*, 167 (1971). They also demonstrated that *A. stellio* H LDH is immunologically related to H LDH of the lizard *Iguana iguana*.