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Electrophoretic pattern of aspecific esterases and soluble proteins of the lizard, Podarcis s. sicula, during the annual cycle

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Fisiologia. — *Electrophoretic pattern of aspecific esterases and soluble proteins of the lizard,* Podarcis s. sicula, *during the annual cycle* (*). Nota di VIRGILIO BOTTE, presentata (**) dal Corrisp. G. CHIEFFI.

RIASSUNTO. — Nella lucertola, *Podarcis s. sicula,* le esterasi e le proteine estratte dall'ovidutto presentano caratteristiche variazioni dei pattern elettroforetici collegabili ad alcune fasi del ciclo riproduttivo.

Le esterasi aspecifiche, frazionate mediante electrofocusing, si risolvono in varie bande la cui attività relativa aumenta significativamente nell'ovidutto maturo. Due varianti enzimatiche, inoltre, sono sotto il controllo dell'estradiolo in quanto scompaiono nella lucertola ovariectomizzata e vengono indotte dal trattamento con questo ormone.

L'elettroforesi in SDS delle proteine solubili permette di individuare alcune bande migranti nelle aree dell'ovalbumina e delle proteine del guscio. Anche queste frazioni proteiche sono controllate dagli ormoni dell'ovario: le prime dall'estradiolo seguito dal progesterone; le seconde dal progesterone.

Le indagini riportate indicano che sia le esterasi aspecifiche che alcune proteine possono essere utilizzate come bersagli negli studi sui meccanismi di regolazione ormonale dell'ovidutto.

In the lizard, *Podarcis s. sisula,* the oviduct undergoes peculiar morphological modifications during the reproductive period (from April to June). These consist mainly in the hypertrophy of its different zones and in the activation of tubal and uterine glands secreting respectively the albumen and the organic components of egg shell (Botte, 1973 a). Less known, however, are the biochemical events which are coupled to the morphological modifications. Some observations indicate that during the breeding season there is an increase of total RNA, proteins and of the activity of several hydrolytic enzymes (Botte, 1973 b).

As a prerequisite to the understanding of the mechanism of oviduct regulation it is imperative to study the various biochemical modifications of the organ. Here we report the modifications of the electrophoretic patterns of aspecific esterases and soluble proteins in the lizard oviduct during the year and under different experimental conditions.

The aspecific esterases have been chosen because in several vertebrates their electrophoretic pattern modifies during growth and differentiation of organs (Masters and Holmes, 1975; Shaklee and Whitt, 1977). The study of soluble proteins, on the other hand, could be useful to identify and evaluate the secretion of albumen and shell proteins which are the more clear end point of oviduct function during reproduction.

(*) Lavoro eseguito presso l'Istituto e Museo di Zoologia dell'Università di Napoli.
(**) Nella seduta del 12 gennaio 1980.

MATERIAL AND METHODS

Adult females of *Podarcis s. sicula*, were obtained every month from the outskirts of Naples. For experimental studies lizards captured in February were utilized. They were kept in terraria with natural photoperiod and a temperature of about 26 °C and fed on meal worm and fresh vegetables. These animals were in part ovariectomized and then included in 4 experimental groups treated as follows: (A) injected with 200 μg of estradiol, dissolved in 200 μl of almond oil, divided into four doses each given every three days, (B) as in A with progesterone, (C), treated with three doses of estradiol followed by a single dose of 0.5 mg of progesterone, and (D) injected with solvent. Three days after the last hormonal injection the animals were killed and used as indicated below.

Oviducts from the animals obtained during the year (then adults each month) and from the experimental ones were removed, weighed and homogenized in cold distilled water (50 mg fresh tissue/ml water). The homogenates were centrifuged at 15,000 rpm in the cold for 1 h and the supernatants were adjusted to 20 μg of protein/ml after protein determination by the method of Lowry *et al.* (1951). The protein solutions were used for separation of esterases (15 μl) and proteins (500 μl).

The electrofocusing of esterases was carried out according to the method of Lancieri and Botte (1977), using a pH gradient of 3.5 to 9.0. Separation was accomplished by applying a potential difference of 80 V for 14 h at 4 °C. The gel plates were stained for the demonstration of enzymatic activity after preincubation in 8 mM Tris–HCl buffer pH 7.4, for 20 min at 37 °C. The plates were then transferred to 200 ml incubation medium consisting of fresh buffer, 80 mg Fast Blue RR salt and 20 mg α-naphthyl acetate as substrate (previously dissolved in 4 ml acetone). Control incubations were carried out in media lacking the substrate.

The plates were incubated for 20 min at 37 °C, washed with tap water and fixed in 30 % ethanol in water.

The samples used for protein separation were diluted (1 : 1 v/v) with 50 mM Tris-HCl buffer, pH 7.5, 2 % SDS, 5 % mercaptethanol and then boiled for 2 min. After the addition of a drop of bromophenol blue, the samples containing around 80 μg of proteins were applied to an acrylamide electrophoresis disc, prepared according to the method of Weber and Osborn (1966). Two concentrations (5 and 7 % of acrylamide) were used, very similar results being obtained. After electrophoresis the gels were stained in 0.25 % Comassie Blue in 1 % acetic acid, for 30 min at 37 °C. The excess dye was eliminated by several washings in 7 % acetic acid.

Results

The aspecific esterases show multiple variants (about 9) when separated by electrofocusing. These become very evident during the breeding season (Pl. I, Fig. 1, *c* and *d*), but are less active when the oviduct starts its spring recrudescence (Pl. I, Fig. 1, *a* and *b*). Two of the variants, focused respectively at pH 5.9 and 6.3, are not evident in the quiescent oviduct; they seem to be first synthesized in March and disappear in ovariectomized females (Pl. I, Fig. 1, *e*). In these animals the injection of estradiol or estradiol plus progesterone induces the synthesis of the two variants, whereas progesterone alone has no effect (Pl. I, Fig. 1, *f*, *h* and *i*).

The electrophoretic pattern of soluble proteins changes during the sexual cycle. In spring, two groups of bands can be easily distinguished. The first one, formed by high molecular weight components, corresponds as R_f to the migration of the main proteins extracted from egg shell (Pl. I, Fig. 2, *b* and *d*, arrow G). The second behaves like ovalbumin (Pl. I, Fig. 2, *b* and *c*, arrow OV). The proteins of the first group disappear in the postovulatory oviducts (Pl. I, Fig. 2, *c*).

The extracts obtained from ovariectomized females show a minor amount of both albumen and shell proteins. Ovalbumin production can be induced by estradiol followed by progesterone, whereas this hormone alone seems to be active in stimulating shell protein synthesis.

Discussion and Conclusions

In the oviduct of the lizard, *Podarcis s. sicula*, the two parameters studied, i.e. apecific esterases and soluble proteins, show peculiar modifications during the reproductive period.

The electrophoretic patterns of esterases indicate that this enzyme system has in the oviduct a certain degree of multiplicity which recalls that reported for another tissue (liver) of the same species (Botte and Basile, 1976). Two variants, focused at pH 5.9 and 6.3 respectively, seem to be synthesized only during the breeding season and are under the control of estradiol, since they disappear in ovariectomized adult females and can be induced by estradiol administration (Pl. I, Fig. 1). It is difficult, at present, to correlate the synthesis of these two hormone-induced enzyme variants with any peculiar function of the oviduct. It must be considered, however, that the functions *in vivo* of esterases are not well known since there are few indications of natural substrates of these enzymes (cf. Deimling and Bocking, 1966). The presence of esterase variants regulated by gonadal hormones is common in several animal tissues, but the interpretation of this aspect is still waiting for a satisfactory explanation (cf. Botte and Basile, 1976).

The electrophoretic separation of soluble proteins allowed the identification of the main secretory products of oviduct during the reproductive period: ovalbumin and shell proteins. The experimental treatments, like ovariectomy and sex hormone administration, indicate that ovalbumin secretion is under the control of estradiol followed by progesterone. This mechanism is very similar to that already reported for avidin secretion in the same species (Botte *et al.*, 1974 a; Botte and Granata, 1977 a).

The egg shell proteins, which seem to show a high molecular weight, are not demonstrable in lizards which have ovulated. Experimentally, the synthesis of these proteins is induced only by progesterone administration in ovariectomized animals.

In conclusion, our study indicates new biochemical parameters which can be usefully utilized in the understanding of the mechanism of endocrine regulation of the oviduct in the oviparous lizards. At present, it has been observed that in the oviduct cytosol specific receptor molecules for sex steroids are present (Botte *et al.*, 1974 b); these molecules increase during the breeding period (Botte and Granata, 1977 b). In ovariectomized females, moreover, sex hormones induce a significant increase of RNA and protein synthesis (Botte and Granata, 1977 a). All these findings support the hypothesis that the mechanism of oviduct regulation is very similar to that found in sex-hormone target organs of higher vertebrates.

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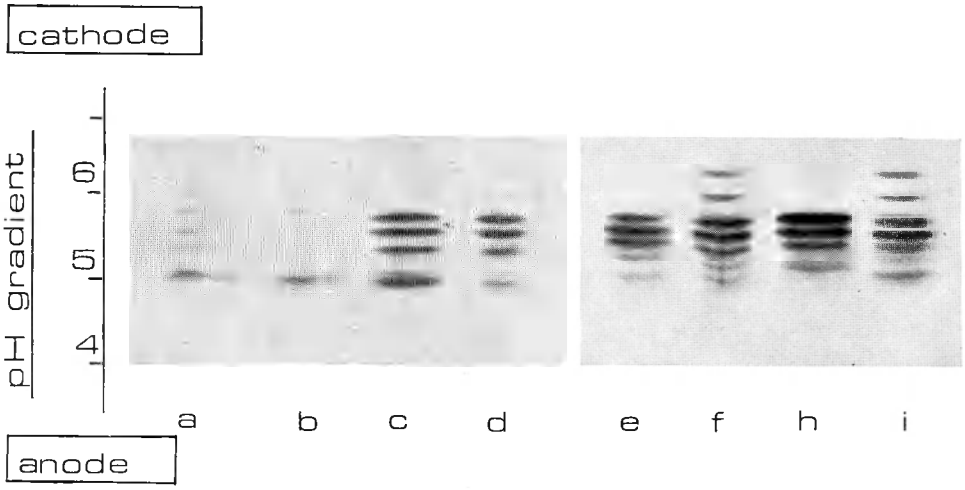
EXPLANATION OF PLATE I.

Fig. 1. – Electrofocusing of aspecific esterases extracted from the oviduct of *Podarcis s. sicula*. Legend: *a* to *d*, lizards captured at the beginning (*a* and *b*), during (*c*) and at the end of (*d*) the reproductive period. *e*, ovariectomized adult females; *f*, *h*, and *i*, ovariectomized females treated respectively with estradiol, progesterone and estradiol plus progesterone.

Fig. 2. – Electrophoretic separation of soluble proteins extracted from the *Podarcis s. sicula* oviduct before (*a*), at the beginning of (*b*) and during (*c*) the reproductive period. *d*, separation of the proteins extracted from the shell; some contamination with albumen was not avoided. G, R_f of shell proteins; *OV*, R_f of albumin.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pH gradient
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5
4
a
b
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d
e
f
h
i
anode

Fig. 1.

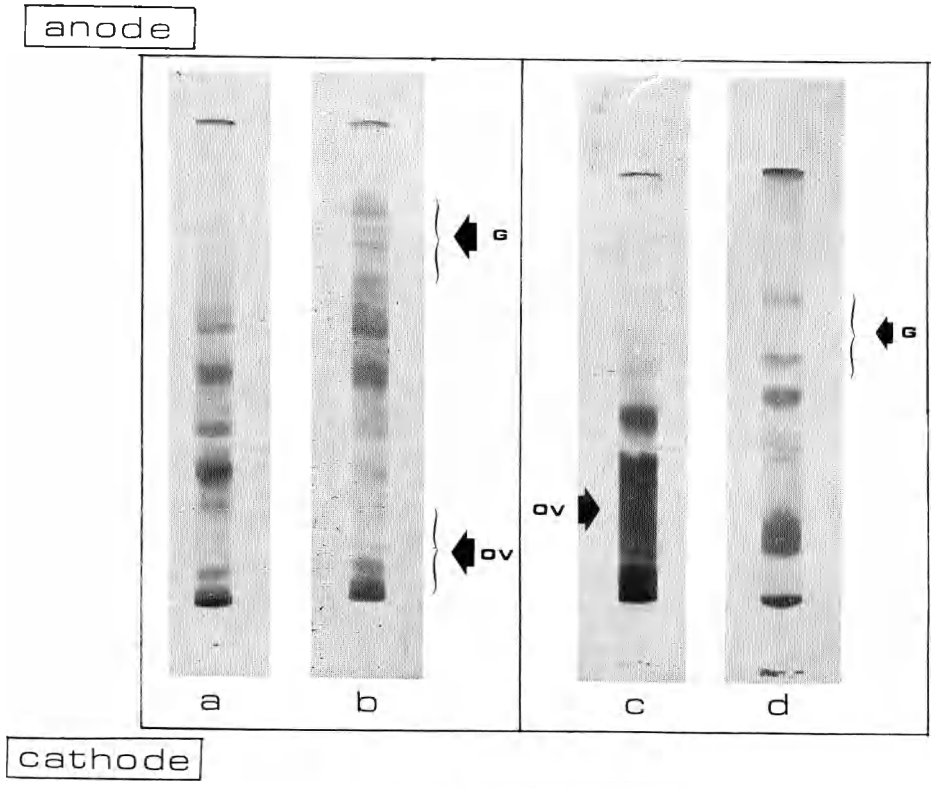
anode
G
OV
G
OV
a
b
c
d
cathode

Fig. 2.