

---

ATTI ACCADEMIA NAZIONALE DEI LINCEI  
CLASSE SCIENZE FISICHE MATEMATICHE NATURALI  
**RENDICONTI**

---

MICHELA D'ISTRIA, FRANCA CITARELLA, GIOVANNI DEL RIO

**Radioimmunoassay of plasma estriol in males and females of *Rana esculenta***

*Atti della Accademia Nazionale dei Lincei. Classe di Scienze Fisiche, Matematiche e Naturali. Rendiconti, Serie 8, Vol. 64 (1978), n.3, p. 315–317.*  
Accademia Nazionale dei Lincei

<[http://www.bdim.eu/item?id=RLINA\\_1978\\_8\\_64\\_3\\_315\\_0](http://www.bdim.eu/item?id=RLINA_1978_8_64_3_315_0)>

L'utilizzo e la stampa di questo documento digitale è consentito liberamente per motivi di ricerca e studio. Non è consentito l'utilizzo dello stesso per motivi commerciali. Tutte le copie di questo documento devono riportare questo avvertimento.

---

*Articolo digitalizzato nel quadro del programma  
bdim (Biblioteca Digitale Italiana di Matematica)  
SIMAI & UMI*

<http://www.bdim.eu/>



**Biologia.** — *Radioimmunoassay of plasma estriol in males and females of Rana esculenta* (\*). Nota di MICHELA D'ISTRIA, FRANCA CITARELLA e GIOVANNI DELRIO, presentata (\*\*) dal Socio G. MONTALENTI.

RIASSUNTO. — La concentrazione plasmatica di estriolo in maschi e femmine di *Rana esculenta* è stata determinata col metodo radioimmunologico durante il ciclo annuale. L'estriolo è presente solo nelle femmine nel mese di dicembre ( $300 \pm 105$  pg/ml).

Estrogens have been measured in amphibian blood plasma by several Authors using the fluorimetric method or gas-chromatography (Gallien and Chalumeau Le Foulgoc, 1960; Cedard and Ozon, 1962; Polzonetti Magni *et al.*, 1970). d'Istria *et al.* (1974) performed the radioimmunoassay of testosterone, estradiol-17 $\beta$  and estrone in the plasma of both sexes of *Rana esculenta* (Fig. 1) but did not assay estriol since a specific antiserum for this hormone was not easily available at that time.

The present report completes the annual cyclic pattern of plasma estrogens in *Rana esculenta* and in fact describes the determination of estriol by RIA in individual plasma samples from males and females during the sexual cycle; the method used was that of Youssefnejadian and Sommerville (1973).

Blood from 15 males and 15 females of *Rana esculenta* captured monthly in the surroundings of Naples was collected from the *conus arteriosus* in heparinized microtubes. H<sup>3</sup>-estriol (S.A. 85 Ci/mol.) from the Radiochemical Center Amersham (England) was used. The chemicals were analytical grade from Baker (U.S.A.). An estriol-6-carboximethyloxima-BSA antiserum, purchased from SORIN (Saluggia, Italy) was used. Aliquots of 200  $\mu$ l or 400  $\mu$ l of plasma were used for each assay. The plasma plus 2000 dpm of H<sup>3</sup>-estriol was extracted twice with 10 ml of diethyl ether; the extracts, transferred to a pointed tube, were dried at 50 °C under a stream of nitrogen. The residues were removed with four drops of benzene-methanol 85 : 15 (v/v) containing a yellow dye isatin (0.1 %) and transferred to a column (Pasteur pipette) of Sephadex LH 20 equilibrated with benzene-methanol 85 : 15 (v/v). Elution was performed with the same solvents. The yellow fraction containing estradiol was discarded and immediately a fraction of 4 ml was collected from the column in a counting vial, dried under nitrogen at 40 °C and redissolved in acetone. Appropriate aliquots were removed for assay and the remainder was subjected to liquid scintillation to determine the recovery.

(\*) Research performed under CNR project "Biology of Reproduction".

(\*\*) Nella seduta dell'11 marzo 1978.

Standard curve (10-200 pg) and unknown samples were prepared in duplicate, dried under nitrogen at 50 °C, cooled to room temperature and equilibrated with 100 µl of diluted antiserum (1/10,000) in phosphate buffer pH 7 for 10'. After addition of 100 µl of H<sup>3</sup>-estriol (20,000 dpm) in phosphate

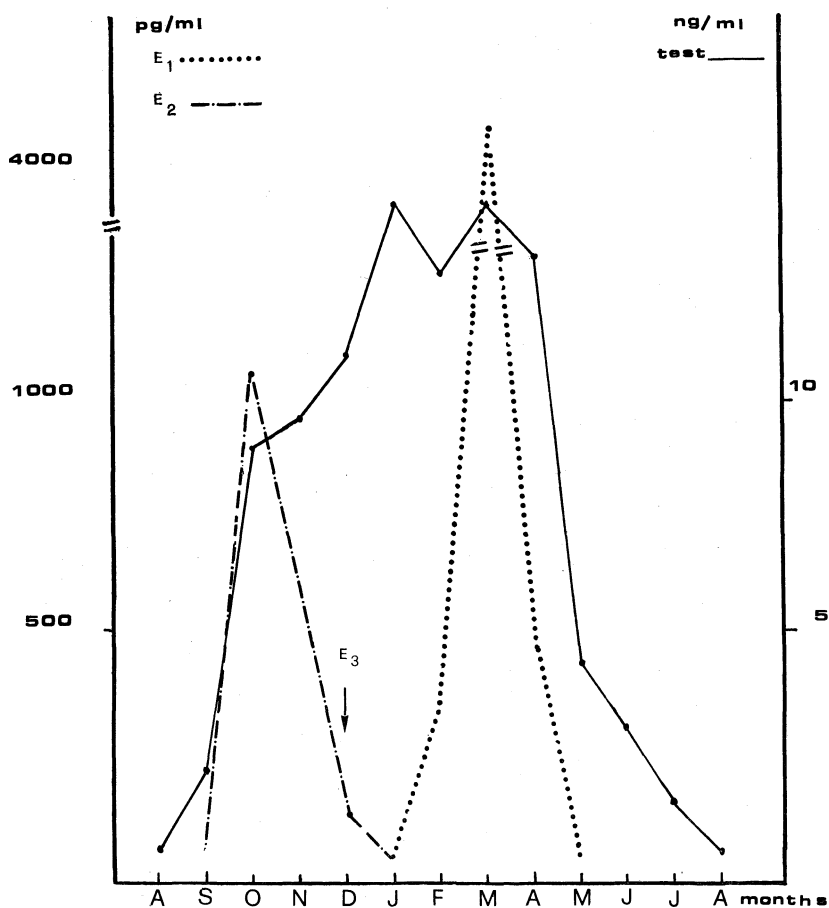


Fig. 1. - Plasma levels of testosterone, estradiol-17 $\beta$  and estrone in the female of *Rana esculenta*. The arrow indicates the estriol presence in December (300 pg/ml); this hormone was not detectable in other periods of the year. E<sub>1</sub> = estrone; E<sub>2</sub> = estradiol-17 $\beta$ ; E<sub>3</sub> = Estriol test = testosterone.

buffer, the solutions were mixed and equilibrated at 4 °C overnight. The free steroid was removed by the addition of dextran-coated charcoal. The accuracy, precision, sensitivity and specificity were controlled using amphibian plasma. Specific quantities of estriol were added to samples containing a known amount of hormone. The range of estriol added was 80-1500 pg and the recovery % (mean  $\pm$  S.D.) was  $95 \pm 9$ . The lower limit of sensitivity was 5 pg, whilst the precision (coefficient of variation % limits) was 9.4-14.

The antiserum had a cross reaction of less than 0.0005 % with testosterone, progesterone, corticosterone, cortisol, cholesterol and estrone, and less than 0.005 % with estradiol-17  $\beta$ .

Estriol was never detected in male plasma. In females it was detectable just in December (13 animals out of 15) with a mean value of  $300 \pm 105$  pg/ml, whereas in other periods of the year the amount is below the sensitivity of the method.

At this juncture the data obtained on the same species, using a gas chromatographic method by Polzonetti *et al.* (1970) must be brought into discussion because there is some perplexity as to the plasma value of estriol (110-360 ng/ml) reported by these Authors. This is a very high amount of estriol and, besides, it is strange that in the ovarian tissue extract we never detected the presence of this hormone using gas-chromatographic analysis (unpublished data). The value obtained by RIA is more credible, especially if compared with that obtained for estriol-17  $\beta$  and estrone in the plasma of the same species.

#### REFERENCES

- CEDARD L. and OZON R. (1962) - *Teneur en oestrogènes du sang de la grenouille rousse* (*Rana temporaria*), «Comp. Rend. Soc. Biol.», 156, 1805-1806.
- D'ISTRIA M., DELRIO G., BOTTE V. and CHIEFFI G. (1974) - *Radioimmuno-assay of testosterone, 17  $\beta$ -estradiol and estrone in the male and female plasma of Rana esculenta during sexual cycle*, «Steroids and Lipids Research», 5, 42-48.
- GALLIEN L. and CHALUMEAU-LE FOULGOC M. T. (1960) - *Mise en évidence des stéroïdes oestrogènes dans l'ovaire juvénile de Xenopus laevis Daudin, et cycle des oestrogènes au cours de la ponte*, «Compt. Rend. Acad. Sci. Paris», 251, 460-462.
- POLZONETTI MAGNI A., LUPO DI PRISCO C., RASTOGI R. K., BELLINI-CARDELLINI L. and CHIEFFI G. (1970) - *Estrogens in the plasma of females of Rana esculenta during the annual cycle and following ovariectomy*, «Gen. Comp. Endocrinol.», 14, 212-213.
- YOUSSEFNEJADIAN E. and SOMMERVILLE I. F. (1973) - *Radioimmunoassay of plasma oestriol*, «J. Ster. Biochem.», 4, 659-664.