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Immunoelectrophoretic determination of vitellogenin titre in the serum of Xenopus laevis

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Biologia. — *Immunoelectrophoretic determination of vitellogenin titre in the serum of* Xenopus laevis. Nota di FRANCO GIORGI (*), presentata (**) dal Socio M. BENAZZI.

RIASSUNTO. — Il titolo ematico della vitellogenina è stato determinato in femmine di *Xenopus laevis* trattate ormonicamente mediante impiego della tecnica di immunoelettroforesi quantitativa. La somministrazione di 17-*b*-estradiol in femmine mature di *Xenopus* causa un aumento lineare nel tempo del titolo vitellogenetico. D'altra parte a seguito del trattamento con gonadotropina, femmine di *Xenopus* presentano una variazione complessa del titolo di vitellogenina nel siero. Questa variazione include un primo aumento seguito da un lento declino verso il valore iniziale. I dati che emergono da questo studio sono in accordo con quelli di altri autori precedenti e sono per questo interpretati alla luce della corrente letteratura sulla vitellogenesi.

INTRODUCTION

It is now firmly established that yolk platelets in amphibian oocytes comprise two major molecules—lipovitellin and phosvitin—whose haematic precursor or vitellogenin is known to be synthesized in the liver (Wallace, 1978; Wallace and Jared, 1969). Because of its heterosynthetic nature, the vitellogenin titre in the serum is believed to depend upon both the rate of synthesis in the liver and that of pinocytotic activity in the ovary (Redshaw and Follett, 1971, 1974). Since each of these processes appears to be controlled by such hormones as gonadotropin and estrogen (Wittliff and Kenney, 1972; Holland and Dumond, 1975), the vitellogenin titre in the serum may simply result from the animal hormonal status. Therefore, the knowledge as to how the vitellogenin titre varies following a given hormonal treatment may provide useful information on the mechanisms controlling vitellogenesis.

So far the vitellogenin titre has been determined by making use of techniques such as isotopic determination (Wallace, 1970) or radio immunoassay (Redshaw and Follett, 1976) in aliquots of serum from specimens in different physiological conditions. The present study was undertaken to ascertain how the vitellogenin titre varies in a single animal subjected to a specific hormonal treatment. This end was achieved by determining vitellogenin concentrations is small blood volumes with quantitative immunoelectrophoresis.

MATERIAL AND METHODS

Specimens of the toad *Xenopus laevis* were maintained in laboratory conditions with a 12 h period of light/darkness alternance and fed weekly on vitamin-enriched liver. *Xenopus* females were either injected with 1 ml

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of 17–*b*–estradiol (Sigma) at a concentration of 10 mg/ml of propylene glycol or with 1 ml of human chorionic gonadotropin (hCG : 1000 I. U.) dissolved in OR2 solution. Ten microlitre blood samples were collected daily from the toe of each hormone treated specimen for a period of 14 days, centrifuged and stored at – 20 °C until used for immunoelectrophoretic analysis. Vitellogenin was isolated from the serum of estrogenized *Xenopus* males following the chromatographic procedure divised by Wallace (1965). Once purified, vitellogenin was used to raise antibodies in rabbits whose serum was used as such without any further treatment. Protein concentration was determined according to the procedure of Lowry *et al.* (1951). The vitellogenin titre in the blood samples was determined by using the quantitative immunoelectrophoretic analysis of Laurell (1966), Tris-citric acid buffer at pH 8.2 being used instead of veronal buffer.

Results

The photograph in Pl. I, *a* illustrates an example of immunoelectrophoretic analysis obtained by electrophoresing vitellogenin aliquots of known concentrations against a 1.5 % agarose gel containing vitellogenin antiserum

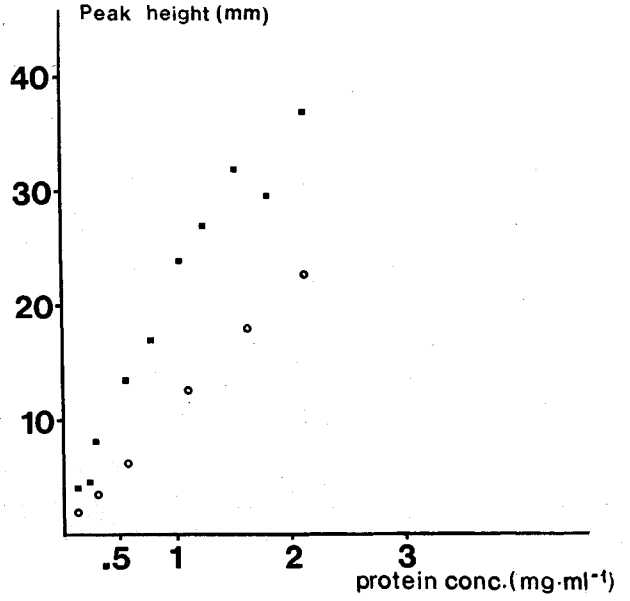

Fig. 1. – Linear relationship yielded by the peak height as plotted against the protein concentration after immunoelectrophoresis of vitellogenin aliquots at known concentration (see Pl. I*a*).
(■) calibration obtained with the antiserum of the first bleeding.
(○) calibration obtained with the antiserum of the second bleeding.

at a concentration of 100 µl/ml of gel. When the heights of the vitellogenin peaks are plotted against the protein concentration as shown in Fig. 1, it becomes evident how the two variables yield a linear relationship to each

other. On this basis, the peak height reached by each serum sample at equilibrium can be expressed as a function of the known vitellogenin concentration for any given antiserum titre (Table I).

TABLE I.

Calibration of vitellogenin titre against its specific antiserum.

Bleeding	Peak height (mm)	Protein (μg)
I°	4.0	.12
	4.5	.18
	8.0	.24
	13.5	.51
	17.0	.75
	24.0	1.05
	27.0	1.20
	32.0	1.50
	29.0	1.80
	37.0	2.10
II°	2.0	.12
	3.0	.24
	6.0	.51
	13.0	1.05
	18.0	1.56
	22.5	2.10

Pl. I,*b* and I,*c* show the variations of vitellogenin titre in serum samples collected from *Xenopus* females treated with either gonadotropin or estrogen. As can be seen in Pl. I *b*, the vitellogenin titre in the serum shows a slight increase soon after injection of gonadotropin and seems to decline around the initial value within a two week period. In contrast with the gonadotropin treatment, estrogen injection into *Xenopus* females causes an overall increase in the vitellogenin titre which is linear with time for at least a two week period. With reference to the calibration curve in Fig. 1, the peak height of each electrophoresed blood sample can be converted into a known vitellogenin concentration. Table II reports the value of the vitellogenin titre measured in a number of specimens subjected to either estrogen or gonadotropin treat-

TABLE II.

Vitellogenin titre (mg/ml) *in* Xenopus *females treated with either estrogen* (E) *or gonadotropin* (H) *over a period of time of 14 days.*

SPECIMEN	0	1	2	3	4	5	6	7	8	9	10	12	14
ICE1-7		17	10	21	32	36	35	48					
ICH1-14		2.1	3.2	4.0	4.6	6.8	7.8	8.6			7.2		8.3
IDE1-10		8.6	14	21	27	32	34	43	55	87	98		
IDH1-14		6.2	5.4	6.6	7.6	8.2	8.4	8.2			5.1		3.9
IIAEo-14	8.3	10	17	19	28	34	36	42	44	47		58	53
IIAHo-14	1.8	2.0	1.6	2.6	3.0	3.2	3.0	3.2	3.0	1.7		3.2	2.9
IIBEo-14	6.6	8	14	26	28	34	40	49	51	58		66	56
IIBHo-14	1.6	1.8	1.8	2.1	3.0	2.9	3.4	3.7	3.6	3.0		1.8	2.4

ment. The patterns exhibited by the vitellogenin titre for a given hormonal treatment can be better appreciated when these data are plotted together as a function of time (Fig. 2).

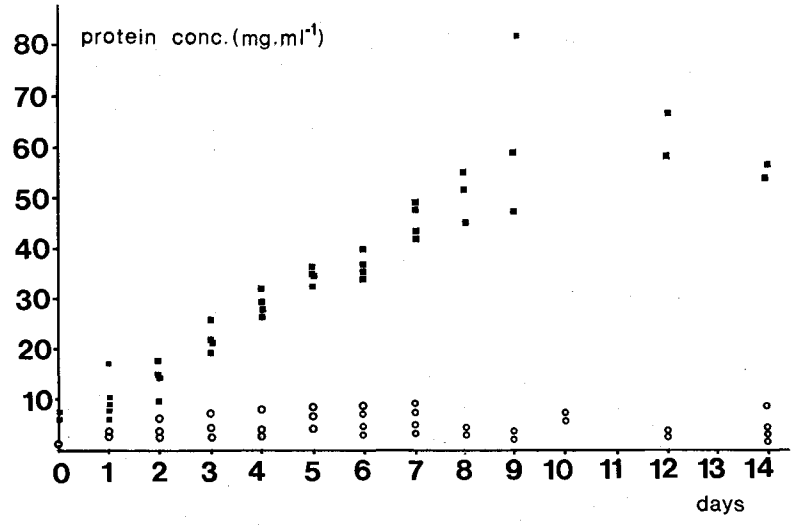

Fig. 2. – Relationship yielded by vitellogenin titre over a time period of 14 days obtained by plotting together the data of serum samples collected from all *Xenopus* females subjected to either estrogen (■) or gonadotropin (○) treatment.

Although periods of treatment longer than two weeks have not been considered, it appears that within the period of time studied the vitellogenin in the serum of estrogenized females reaches a plateau at about 67–70 mg/ml. Conversely, the gonadotropin treatment causes the vitellogenin titre to stabilize around the value of 3–4 mg/ml.

DISCUSSION

The aim of the present study was to ascertain the feasibility of measuring the vitellogenin titre in minute volumes of blood collected from hormone treated females of *Xenopus laevis*. Quantitative immunoelectrophoresis proved to be an adequate technique for monitoring variations in the vitellogenin content differing in the order of micrograms. By using this kind of analysis it has also been possible to show how the vitellogenin titre varies in a single specimen following a given hormonal treatment. The graphs obtained by plotting together the data of several specimens subjected to the same hormonal treatment are comparable to those previously reported by Wallace and Jared (1969) who determined the vitellogenin titre by measuring the serum phosphorous content. In addition, the use of the immunoelectrophoretic analysis on a single specimen has proved particularly useful in a detailed analysis of the time course of vitellogenin variations. Estrogen treated females, although

differing in the absolute values attained by the vitellogenin titre, all exhibit a linear increase with time. As such the pattern followed by the vitellogenin titre after estrogen tratment appears to relate only to the processes of hepatic synthesis and release into the blood. This is in fact what should be expected if the ovarian function of pinocytosis were, as indeed is the case, inhibited by treatment with estrogen (Holland and Dumont, 1975). On the other hand, when blood samples were analyzed in a single specimen after gonadotropin treatment, the vitellogenin titre appeared to vary according to a composite function. The time course followed by vitellogenin in these conditions is consistent with the interpretation that gonadotropin stimulates both synthesis in the liver and uptake into the oocyte (Wallace and Bergink, 1974). However, the observation that within a two week period the vitellogenin titre declines around the initial values suggests that the two processes are controlled in a different manner by the hormone. One possibility could be that gonadotropin initially stimulates vitellogenin synthesis in the liver and only subsequently affects the pinocytotic activity in the ovary. Alternatively, one could also assume that while gonadotropin might stimulate both processes as soon as injected, the times at which each process is maximally affected by the hormone are out of step with each other.

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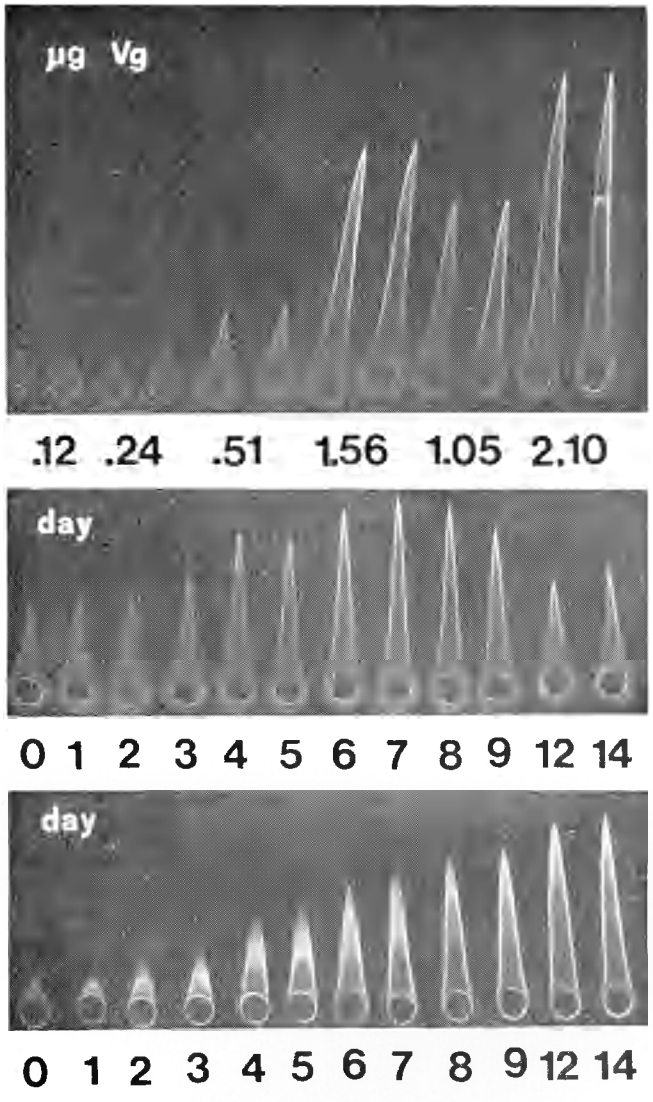

Immunoelectrophoretic analyses of (a) vitellogenin aliquots of known concentration tested against vitellogenin antiserum diluted 1/20 with 1.5% agarose in Tris-citric acid buffer at pH 8.2; (b) vitellogenin titre in serum samples at a dilution of 1/10 collected over a period of 14 days from gonadotropin treated *Xenopus* females; (c) vitellogenin titre in serum samples at a 1/100 dilution collected over a period of 14 days from estrogen treated *Xenopus* females. (b) and (c) have the same electrophoretic conditions as (a).

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