A PRELIMINARY ASSESSMENT OF THE SOURCE OF OESTROGEN WITHIN THE OVARY OF THE DOMESTIC FOWL, *GALLUS DOMESTICUS*

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(Received 15th August 1974)

Summary. Oestradiol was measured by radioimmunoassay in blood draining individual preovulatory follicles and in different ovarian tissues of the hen. The concentration of oestradiol in blood from follicles 20 to 50 hr before ovulation ranged from 66 to 264 pg/ml and was less than that in peripheral blood collected concurrently, suggesting a net uptake rather than secretion of oestradiol by the follicle at these times.

In one bird approximately 6 hr before ovulation, the highest content of oestradiol in tissue (35 ng) was in the small (<5 mm) follicles and ovarian stroma; this represented 87.5% of the total ovarian content. Only 0.86 to 1.02 ng oestradiol (2.0 to 2.5%) was found in the large preovulatory follicles and 2.22 ng (5.5%) in the postovulatory follicles. High concentrations of oestradiol (2.4 ng/g) were also found in the liver.

These results suggest that the small follicles and/or ovarian stroma are the main site of oestradiol production in the fowl; they do not exclude the possibility that oestradiol is secreted at a high rate by the mature follicle for a short period immediately before ovulation, thus influencing the release of LH.

INTRODUCTION

In the domestic fowl, progesterone may be the ovarian hormone that causes the release of LH required for ovulation (Fraps & Dury, 1943; Fraps, 1955, 1961; Ralph & Fraps, 1959, 1960; Furr, 1969, 1973; Peterson & Common, 1971; Kappauf & van Tienhoven, 1972; Furr, Bonney, England & Cunningham, 1973) though, in mammals, oestrogens may induce the LH surge (Vande Wiele & co-authors, 1970). A similar rôle for oestrogens in the hen has not been fully investigated. During the ovulatory cycle of the hen, maximum concentrations of oestrone and oestradiol are known to occur in peripheral plasma 4 to 6 hr before ovulation (Peterson & Common, 1972; Senior, 1974a) and the increase in the concentration of oestradiol precedes that of LH by 2 hr (Senior & Cunningham, 1974). If this preovulatory oestrogen peak is involved in the mechanism of ovulation, it might result from an increase in the rate of steroidogenesis in the preovulatory follicle similar to that which occurs in mammalian

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species. The purpose of the present work was to determine the site of oestrogen secretion within the hen ovary by studying the concentration of oestradiol in blood draining individual follicles and by estimating the levels of oestradiol in different ovarian tissue elements.

MATERIALS AND METHODS

Solvents and reagents

The organic solvents and reagents used for oestradiol radioimmunoassay are described by Glencross, Munro, Senior & Pope (1973).

Experimental birds

White Leghorn laying hens were caged singly, subjected to a 14-hr light/10-hr dark regimen and fed on a commercial layers' ration.

Collection of follicular venous blood

A polyvinyl cannula was inserted (Furr, 1973) into individual follicles of five anaesthetized laying hens. Heparin (2000 U) in 2 ml of 0.9% sodium chloride solution was injected through a brachial vein cannula to prevent blood clotting during collection. Follicular vein blood samples were collected over 10-min intervals (0.5 to 1.0 ml blood/sample). During this collection, a sample of peripheral blood was withdrawn from the brachial vein cannula. In one bird (F2), 1 µg ovine LH (NIH-LH-S11) was injected into the brachial vein during collection of follicular blood.

At the end of the experiment, the hens were killed and the ovaries examined. From the position of the follicle under investigation in the hierarchy of ovarian follicles, and from the position of any eggs in the oviduct, the approximate time at which follicular blood was sampled relative to the expected time of ovulation of the follicle was estimated for each bird.

Plasma was immediately separated by centrifugation and stored at -20°C until assay.

Extraction of oestradiol

A blood sample (10 ml) was taken from the brachial vein of a laying hen approximately 6 hr before the predicted time of ovulation (estimated from the previous laying record and the presence of a hard-shelled egg in the shell gland) and the bird was immediately killed by cervical dislocation. The largest follicle, the second largest follicle, the third, fourth and fifth follicles, two post-ovulatory follicles, the rest of the ovary consisting of small (<5 mm) follicles and stroma, a piece of liver, and a piece of pectoral muscle were quickly dissected out and placed on ice. A distilled water blank was also included. The five large follicles were punctured and the yolk discarded since it does not contain large quantities of oestrogens (Hertelendy & Common, 1965).

The tissue samples were weighed (Table 2) and then homogenized in 3 vols distilled water using a Silverson homogenizer. The homogenate was added to a flask containing [³H]oestradiol-17β (2 × 10⁵ d/min; 250 pg) and extracted overnight with 10 vols acetone. The precipitate was then removed by filtration

and was washed with a further two 3-*vol* aliquots of acetone. The combined filtrate was evaporated to dryness and the weighed and dried extract was submitted to chromatography on silica gel. A slurry in light petroleum (B.P. 60° to 80°C) of silica gel (British Drug Houses, 60 to 120 mesh) previously activated at 105°C for 10 min was used to pack a column, 26 cm × 1.5 cm i.d. The extract was transferred to the column with two 2-ml aliquots of toluene. Elution of the column with toluene removed a large proportion of the lipid; a fraction containing steroids was then eluted with acetone.

The acetone was distilled off and the extract was submitted to chromatography on the strongly basic anion exchange resin Dowex 1 (Sigma Chemical Co.) using a modification of the procedure of Eberlein (1969). A suspension in distilled water of the resin in the bicarbonate form was pipetted into a glass column, 10 cm long × 1 cm i.d. to a height of 4 cm. The column was equilibrated by passing 30 ml methanol through it. The extract was transferred to the column with two 2-ml aliquots of methanol, and neutral lipids and steroids were eluted with a further 25 ml methanol; phenolic steroids were then eluted with 15 ml of a mixture of 4% glacial acetic acid in methanol.

The phenolic steroids were subjected to chromatography on a 15-cm column of Sephadex LH-20 with the solvent system toluene:methanol (85:15 v/v), as previously described (Senior, 1974a) and a fraction containing oestradiol was obtained. The dried oestradiol fraction was dissolved in 1 ml redistilled toluene and 100 µl of the solution were removed. The radioactivity was counted to estimate procedural losses.

Oestradiol assays

Oestradiol in blood plasma was measured by radioimmunoassay (Senior, 1974b).

The tissue extracts were assayed at several levels, using the same procedure as for plasma.

RESULTS

Oestradiol in follicular venous blood plasma

Samples collected from two of the five birds contained barely detectable levels of oestradiol. The remaining three birds provided twenty-six samples of follicular venous blood. Table 1 shows that the concentrations of oestradiol in these samples ranged from 66 to 264 pg/ml and appeared to be lower than the levels in peripheral blood taken at the same time.

Oestradiol in ovarian tissue

The mean total content of oestradiol in each tissue examined was calculated from the values obtained at all levels of the extract; the results for each ovarian tissue are also expressed as a percentage of the total ovarian content (Table 2). The highest levels of oestradiol were found in the liver and in the small follicles and ovarian stroma. All tissue levels were significantly higher ($P < 0.001$) than the blank value of 134 pg.

The oestradiol concentration in the peripheral plasma was 159 pg/ml.

Table 1. Concentrations of oestradiol in the venous plasma from the ovarian follicles of laying hens

Bird No.	Tissue	Oestradiol ($\mu\text{g/ml}$)
F2	Follicle at least 50 hr before ovulation	145
		116
		133
	1 μg NIH-LH injected $\longrightarrow$	225
		264
		183
		143
	Peripheral plasma	229
		194
		1077
F3	Follicle at least 40 hr before ovulation	180
		162
		130
		174
		125
		121
		191
	Peripheral plasma: 100- μl aliquot	218
		315
		200- μl aliquot
		344
F5	Follicle at least 20 hr before ovulation	300- μl aliquot
		339
		80
		96
		89
		66
		157
	Peripheral plasma: 300- μl aliquot	88
		81
		174
	600- μl aliquot	235
		211
		171

Samples were collected over 10-min intervals and a sample of peripheral blood was also drawn from each bird.

Table 2. The concentration of oestradiol in tissues from a laying hen

Tissue	Net wt (g)	Recovery of radioactive tracer (%)	Oestradiol content (μg)		
			Whole tissue (mean $\pm$ S.E.M.)*	Per g tissue	% of ovarian total
L	6.640	78.1	15,930 $\pm$ 348	2,399	—
M	7.241	73.2	669 $\pm$ 42	92	—
F ₁	3.563	86.9	858 $\pm$ 92	241	2.1
F ₂	1.898	79.9	1,024 $\pm$ 86	540	2.5
F _{3/5}	4.867	70.8	975 $\pm$ 98	200	2.4
POF	0.539	25.3	2,218 $\pm$ 638	4,115	5.5
O	3.962	64.4	34,405 $\pm$ 662	8,936	87.5
B	—	49.5	134 $\pm$ 20	—	—

L, liver; M, muscle; F₁, largest ovarian follicle; F₂, second largest follicle; F_{3/5}, combined third, fourth and fifth largest follicles; POF, postovulatory follicles; O, remainder of ovary (stromal tissue and small follicles < 5 mm diam.); B, distilled water blank.

* Six or ten determinations at three or five levels of each tissue extract.

DISCUSSION

The specificity of the assay method for measuring oestradiol in tissues requires some comment since, particularly in the ovary and liver, high concentrations of a large range of steroids are likely to be present. The anion-exchange column separated phenolic from neutral steroids but, in addition, the antiserum bound non-phenolic steroids to an extent which was $<0.01\%$ of the binding of oestradiol- 17β . Although oestrone and oestriol were 80% and 10%, respectively, as effective in binding to the antiserum as oestradiol- 17β , they were clearly separated from the oestradiol epimers on Sephadex LH-20, as most probably were the oestriol epimers and 16-oxo-oestrone and 16-oxo-oestradiol- 17β . Of the steroid oestrogens secreted by the hen (Mathur & Common, 1968), oestradiol- 17α probably contributes to the value measured. We have therefore stated in the results that concentrations of oestradiol, rather than oestradiol- 17β , are measured.

The concentration of oestradiol in the peripheral plasma of laying hens varies during the ovulatory cycle in a manner congruent with the concept that oestrogens are involved in the mechanism controlling the release of LH required for ovulation (Senior, 1974a; Senior & Cunningham, 1974). It would seem logical that the signal which informs the hypothalamo-pituitary system that the follicle is ready to ovulate should come from the follicle itself but no net secretion of oestradiol could be detected by maturing follicles 20 to 50 hr before ovulation. Rather, the follicles appeared to be accumulating oestradiol from the peripheral plasma. This agrees with earlier work on the uptake of [^3H]oestradiol by the follicle wall (Hawkins, Heald & Taylor, 1969a, b). The very high value found in the peripheral plasma of bird F2 exaggerates the difference between peripheral and follicular vein levels but this may be due to the treatment of this hen with ovine LH. Certainly oestradiol concentration increased twofold in follicular venous blood 20 min after this treatment.

The follicular vein cannulation procedure will have some influence on blood flow through the follicle, but it is not likely that this will significantly affect the steroid output since progesterone was present in high concentrations in the same plasma samples that contained low levels of oestradiol (Furr, 1973). The conclusion that oestradiol is not an important secretion of these large follicles is also supported by the finding that all such follicles together account for only 7.0% of the total ovarian oestradiol content.

The largest quantity of oestradiol was present in the numerous small (<5 mm) follicles and ovarian stroma. This conclusion agrees closely with that of earlier work in which Allen, Whittset, Hardy & Kneibert (1924) and Marlow & Richert (1940) found that oestrogenic activity, judged by cornification of the vagina of spayed rats, was confined to the small follicles. In the present investigation, no attempt was made to determine whether the follicles or the stroma were responsible for the high levels of oestradiol found. Boucek & Savard (1970) demonstrated the presence of the enzymes Δ^5 - 3β -hydroxysteroid oxidoreductase and 17β -hydroxysteroid oxidoreductase in ovarian stromal cells.

All these results indicate that, unlike progesterone (Furr, 1969, 1973), oestradiol is not secreted by the major follicles, at least up to 20 hr before the

time of ovulation, and that the basal secretion of oestradiol during the hen's ovulatory cycle is probably derived from the small follicles or ovarian stroma (or both). Without undue trauma to the preparation, however, it was not found possible to identify and cannulate follicles at much closer times to ovulation when the peripheral plasma rise in oestradiol level occurs (Senior & Cunningham, 1974). Certainly, the major follicles contain enzymes necessary for oestrogen biosynthesis (Chieffi & Botte, 1965; Boucek & Savard, 1970) and further investigations are essential to determine whether a large increase in oestradiol output by the follicles occurs 4 to 8 hr before ovulation.

ACKNOWLEDGMENT

We gratefully acknowledge the financial support of the British Egg Marketing Board.

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