No testosterone or estradiol receptors could be demonstrated in the supernatant from the stages 24–29, with analytical procedures used. In tadpoles of stages 31–33 (climax), a testosterone receptor was found both in males and females. The binding specificity was proved by a high competition of unlabelled testosterone, 11-ketotestosterone and dehydrotestosterone; while no competition was observed with estrone, corticosterone, progesterone, cyproterone acetate (CPA) and I.C.I. (Table). These results agree with those obtained on the skin from adult frogs. In fact receptors were found only for androgens both in male (d'ISTRIA et al.[2]) and female (unpublished) frogs. It might be interesting to note that CPA does not compete with testosterone receptor as found in the adult frogs; in the thumb pad, however, this antiandrogen competes for testosterone receptor.

In all the stages examined, no retention curve was observed with ^{3}H-estradiol, but it is to be emphasized that in females of stages 31–33, unlabelled estradiol reduces the binding of H^3-testosterone by about 70% and in males by about 30%. The refractiveness of a testosterone receptor to cyproterone acetate and a displacement of testosterone from the receptor sites by estradiol-17β was observed also by GIANNOPOULOS[6] in the immature rat uterus.

The K_{ass} for testosterone varies from 1 to $2.79 \times 10^9 M^{-1}$ in various developing stages, while the number of sites ranges between $2.40–2.90 \times 10^{-10}$ in stages 31–32; $11.7–12.1 \times 10^{-10}$ in stage 33. In adult males, it has an average value of 12×10^{-10}, while in adult females it is in the order of 5.92×10^{-10}.

The results reported above indicate that the sex hormone receptors appear in the frog skin in a rather advanced stage of metamorphosis. A testosterone receptor has been found both in the male and female tadpoles, as earlier reported for adults of both sexes (d'ISTRIA et al.[2], and unpublished results). As far as the comparative aspect of the problem is concerned, the presence of sex hormone receptors has also been indicated in the skin of male crested newt (estradiol, d'ISTRIA et al.[2]) and in sebaceous gland of male hamster (dehydrotestosterone; ADACHI and KANO[5]).

If the presence of a receptor in the thumb pads of frog, the skin of the newt and the sebaceous gland of mammals, has a physiological significance since all are well known male SSC, the same cannot be implied for the skin of frog in as much as there is no information about the effects of sex hormones on this organ. On the other hand, for the skin of frog, the term receptors could be used even if this organ does not turn out to be responsive to sex hormones, as the case presented by SHYAMALA[7] for the unresponsive GR mouse mammary tumour.

The number of binding sites increases 4-fold from stage 31 to stage 33 both in male and female tadpoles. A similar observation has been made by CLARK and GORSKI[8]; these authors found that estradiol receptors in the uterus of immature rats increases from the first day post partum to the 10th day post partum.

The appearance of sex hormone receptors in frog tadpoles (stage 31–33) seems to precede the onset of sex hormone secretion by the gonads and the differentiation of secondary sex characters, and follows the sex differentiation of the gonads (stage 26–27)[9]. This fact further supports the hypothesis that embryonic sex inductors differ from adult sex hormones (CHIEFFI et al.[10,11]).

Summary. In tadpole skin of *Rana esculenta*, a specific testosterone receptor was detected during the climax in both males and females. The K_{ass} ranged between 1 and $2.79 \times 10^9 M^{-1}$.

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Reduction (%) in cytosol binding of H^3-testosterone

Competing hormone	Concentration (nM)	Stage 31–32		33	
		♂	♀	♂	♀
Testosterone	1.4	88	88	85	94
Dehydrotestosterone	1.4	70	70	65	80
11-Ketotestosterone	1.5	70	70	65	80
Cyproterone acetate	2.0	10	0	0	20
Estradiol – 17β	1.3	24	71	33	65

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Lack of Increase in 3′,5′-Cyclic AMP by Estrogens in Oviducts of *Coturnix* Quail

Evidence regarding the role of cyclic nucleotides in the mechanism of estrogenic action upon reproductive tissues has been conflicting[1–5]. PRESLOCK and HAMPTON[6] recently reported that both estrogens and 3′,5′-cyclic AMP stimulated ornithine decarboxylase in oviducts of *Coturnix* quail. As this enzyme has been correlated with increased RNA and protein synthesis[7,8], these data suggested a role for cyclic nucleotides in the estrogenic induction of protein synthesis in reproductive tissues. Since the demonstration of an increase in tissue levels of 3′,5′-cyclic AMP is necessary to establish a function for this substance as a second messenger in the induction of ornithine decarboxylase by estrogens in the quail oviduct, the purpose of the following experiments was to determine whether estrogens can elevate 3′,5′-cyclic AMP levels in oviducts of *Coturnix* quail.

Coturnix quail (*Coturnix coturnix japonica*) were obtained at 1 day of age from a local supplier and maintained in a gonad-stimulating photoperiodicity (16L:8D). Wayne Game Bird Starter (30% total protein) and water were provided ad libitum. Immature females were utilized at 28–30 days of age, as sexual maturity occurred near day 40 under our laboratory conditions.

Coturnix oviducts (5–10 mg) were homogenized (10 strokes) in 1.0 ml ice colde 6% TCA, the homogenate centrifuged at 10,000 *g* for 15 min, and the supernatant

extracted with petroleum ether (3 × 5 ml). The extracts were pooled and dried under an atmosphere of nitrogen, the residue dissolved in 0.05 *M* sodium acetate buffer, pH 6.2, and immediately assayed for 3′,5′-cyclic AMP.

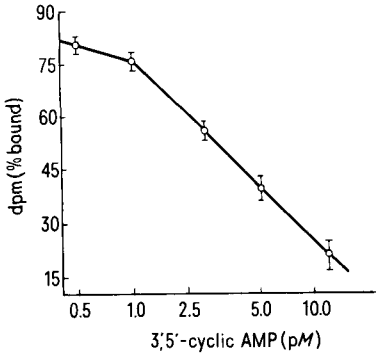
The assay for 3′,5′-cyclic AMP was carried out according to the double antibody radioimmunoassay method described by STEINER et al.[9]. This method, in brief, involves incubation of tissue extracts of cyclic nucleotide standard with anti-cyclic AMP antiserum (1:600 dilution) and I^{125}-cyclic AMP trimethylester (I^{125}-cAMP-TME) in 0.05 *M* sodium acetate buffer, pH 6.2. Incubations were carried out for 4 h at 4 °C, excess goat anti-rabbit IgG added to each tube, and precipitation of the antibody complex allowed to proceed overnight. Sodium acetate (2.0 ml) was added to each tube, the tubes centrifuged (4000 rpm) for 30 min at 4 °C, the buffer decanted and the precipitate counted in a gamma spectrometer. Counts per minute (cpm) were converted into picomoles (p*M*) and p*M* analyzed for significant differences between treatment group means by analysis of Covariance and Duncan's new multiple range test.

The standard curve for the radioimmunoassay of 3′,5′-cyclic AMP obtained by incubating varying quantities of unlabelled 3′,5′-cyclic AMP with anti-cyclic AMP and I^{125}-cAMP-TME is demonstrated in the Figure. The usable linear portion of the curve was between 0.50 and 10.0 p*M*, where the percent bound decreased from 91.1% bound to 20.1% bound. The coefficient of variation was from 3.43% to 10.5% within this range.

Levels of 3′,5′-cyclic AMP[a,b] in oviducts of *Coturnix* quail following administration of selected estrogens

	3′,5′-Cyclic AMP			
	Vehicle	Estrogens		
Time (min)	Controls	Dose (μg)	Diethylstilbestrol	17-β-estradiol
0.5	4.38	20	4.56	4.49
		50	4.64	4.37
		100	4.71	4.55
2.0	4.56	20	4.20	4.76
		50	4.40	4.98
		100	4.16	4.01
5.0	4.46	20	4.44	5.07
		50	3.99	4.31
		100	4.30	4.54

[a] Data are expressed as p*M* 3′,5′-cyclic AMP/mg oviduct, wet weight.
[b] Each value represents the mean of 8–10 separate determinations.



Standard curve (mean ± SE) for the determination of 3′,5′-cyclic AMP by radioimmunoassay. The linear portion of the curve was from 0.50 to 10.0 p*M* where the percent bound decreased from 91.1 % to 20.1% bound.

The experimental design utilized in these studies was a 3 × 3 × 2 factorial experiment replicated 3 times. There were 3 doses of estrogens (20, 50 and 100 μg), 3 time intervals (0.5, 2.0 and 5.0 min), and 2 estrogens administered (diethylstilbestrol, 17-β-estradiol) per replicate. Estrogens were dissolved in 0.2 ml saline:ethanol (3:2), and administered i.v. into the wing vein. Appropriate vehicle-injected and uninjected controls were utilized for each treatment. At appropriate time intervals the quails were sacrificed by decapitation, and their oviducts immediately removed and rapidly frozen in liquid nitrogen. Oviducts were maintained at −60 °C until thawing for weighing, extraction and assay.

The Table demonstrates that no detectable elevation of oviductal levels of 3′,5′-cyclic AMP resulted from estrogen administration. Neither diethylstilbestrol nor 17-β-estradiol increased levels above that of vehicle-injected controls within the time intervals utilized in this study.

These results suggest that the response of avian reproductive tissues to estrogens apparently does not involve an increase in tissue levels of 3′,5′-cyclic AMP. KISSELL et al.[3] and ROSENFELD and O'MALLEY[4] reported no increased 3′,5′-cyclic AMP in the chick oviduct from i.v. administration of diethylstilbestrol. Therefore, the scheme proposed earlier[6] whereby circulating estrogens increase oviductal 3′,5′-cyclic AMP during rapid sexual maturation, resulting in a stimulation of oviductal ormithine decarboxylase, is not supported by the present evidence. It is possible, however, that the sensitivity of the assay was not adequate to detect subtle changes in 3′,5′-cyclic AMP, or that the increase in ornithine decarboxylase by 3′,5′-cyclic AMP may represent a nonspecific reaction of the avian oviduct to this substance.

Summary. Different estrogen concentrations do not induce a change in oviductal 3′,5′-cyclic AMP of *Coturnix coturnix japonica.*

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