the quantity of secretion accumulated. The effect of the secretion on the corona cells might depend on the concentration of the dispersion factor.

The secretion obtained from rats in the metoestrus and di-oestrus phases did not dissolve the gel cementing the follicular cells; the surface areas found in these cases were smaller. The secretion from rats in pro-oestrus increased the surface area of the ovum to nearly twice the initial area, but the corona cells remained attached to the ovum. The patterns of corona-cell-dispersing activity produced by the uterine secretion from rats in each of the various phases of the sexual cycle were similar, regardless of the lenght of time for which it has been accumulated.

Discussion. From the vaginal smears it may be concluded that surgical closure of both extremities of the uterus caused a disturbance of the sexual cycle of the rat, such as might be produced by any surgical stress; later, however, regular cycles recommenced, indicating that the ovaries continued to function normally.

In the second part of this experiment, it was demonstrated that the ovarian function involves modifications, not only in the vaginal epithelium and in the weight of the uterus (Astwood[7]; Head et al.[8]) but also in the uterine fluid. These variations were particularly clear-cut after 21 days, when the regularity of the sexual cycle was already fully reestablished.

It has been suggested (Blandau[9]) that the fluid observed in the uterus of the normal rat at pro-oestrus – oestrus escapes after oestrus because the effects of progesterone produced by the ovaries at this time relax the neck of the uterus. Our results showed that the accumulation of fluid during this period is significantly diminished since the cervix is closed by electrocauterization and the fluid cannot escape, it appears likely that in metoestrus resorption of the aqueous content of the fluid also takes place, which may explain the increase in its viscosity at this time.

Finally, the last experiment demonstrates that not only the physical properties but also the chemical composition of the uterine secretion of the rat undergoes some modification from one stage of the sexual cycle to the next. Complete dispersion of the corona cells in vitro was only observed after incubation in accumulated secretion extracted on the day of oestrus, suggesting that this phenomenon might be oestrogen-dependent.

All these observations seem to correlate with the modifications induced at the different stages of the sexual cycle by endogenous steroid sex hormones (Brown-Grant et al.[10]; Barraclough[11]). The fact that a similar pattern was observed in each phase of the sexual cycle when the uterine fluid was allowed to accumulate for different periods (7, 14 and 21 days) shows that these modifications in uterine fluid are regular, constant and cyclic, in conformity with a hypothalamic-hypophyseal-gonadal rhythm. The fluid accumulating in the closed uterus is not really a physiological uterine secretion, but it seems to retain in solution the secretion of the endometrial glands.

Summary. It was found that closure of the uterus disturbed the first 2 cycles after the operation; thereafter the normal cycle was resumed. The quantity of uterine fluid was increased at pro-oestrus and oestrus and reduced at met-oestrus and di-oestrus. Slight inverse changes in viscosity were observed. There was no significant difference in the pH. The corona-cell dispersing factor seems to be an oestrogen-dependent constituent of uterine secretion.

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Appearance of Sex Hormone Receptors in Frog (*Rana esculenta*) Tadpole Skin During Metamorphosis

The problem of mechanism of action of sex hormones has been receiving ever increasing attention in recent years. A good deal of reserach has already demonstrated the importance of nuclear and cytoplasmic 'receptors' in numerous target organs.

The gonadal sex hormone secretion begins at different stages of vertebrate development and varies according to the species. Generally their appearance coincides or precedes the differentiation of secondary sex characters (SSC). Few studies were carried out on the appearance of the sex hormone receptors in these organs.

Delrio and d'Istria[1] have recently demonstrated the presence of an androgen receptor in the $105,000 \times g$ supernatant not only in the thumb pad (a male SCC) of *Rana esculenta*, but also in the skin taken from other parts of the body (legs, dorsal, ventral and lower jaw; d'Istria et al.[2]).

This work on the appearance of receptors has been done on the skin of the entire body of tadpoles and not on the thumb pads, since the anterior legs grow later in the course of metamorphosis of frog.

Rana tadpoles in the stages 24, 27, 28, 29, 31, 32 and 33 (according to the table of Witschi[3]) were procured from the surroundings of Naples. Tadpoles of stages 31–33 could easily be separated according to sex by observing their gonads under a binocular. Skin taken from these was homogenized in *Tris*-HCl buffer pH 7.4 and centrifuged at $600 \times g$ for 10 min; the supernatant was recentrifuged at $105,000 \times g$ for 1 h in a I.E.C. B–60 centrifuge. H^3-testosterone (S.A. 84 Ci/m*M*) and H^3-estradiol-17β (S.A. 85 Ci/m*M*) from Radiochemical Center, Amersham, England were used. For further details of the method used we refer to the works by Fang and Liao[4], Adachi and Kano[5] and Delrio and d'Istria[1].

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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]).

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

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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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