sensitivities and progress in the 'programmed development' of the larvae may have a role in this physiological effect, but it seems highly probable that increased titres of free ecdysteroids also have a role.

Tritiated ecdysone was injected into premolt larvae and the extract assayed by radio TLC. The ^{3}H-ecdysone is efficiently converted to ^{3}H-20-hydroxyecdysone during an 8–14-h incubation period. Additional confirmation of the product was obtained by acetylation of the eluted ^{3}H-20-hydroxyecdysone by acetic acid-pyridine. TLC of the acetylation products gave a nice fingerprint of the 4 20-hydroxyecdysone acetates.

Discussion. The identification of 20-hydroxyecdysone in *Limulus polyphemus* larvae is based upon purification in TLC and HRLC systems and detection of the products by RIA and bioassay. The data from these experiments strongly implicate this compound as a significant free ecdysteroid in *Limulus*, although confirmation of structure requires mass spectral analysis.

The change in concentration of free 20-hydroxyecdysone during the molt cycle is in agreement with data from crustaceans[9], insects[10], and arachnids[11]. Although ecdysteroids may exist in conjugated form, as Bebbington et al.[12] suggest, it seems reasonable to conclude that the free ecdysteroid component, which occurs at ng/g levels and show increased concentrations during premolt, plays a major role in the molting physiology of *Limulus*.

Limulus larvae also exhibit the ability to rapidly convert ecdysone to 20-hydroxyecdysone as insects and crustaceans do[13]. Therefore it appears likely that the basic molting physiology and biochemistry of *Limulus* is similar to crustaceans, insects and probably all arthropods.

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Estrogen action in the male[1]

A. Rodríguez, M.A. Mena, C. Unda and A. Tchernitchin

Laboratory of Experimental Endocrinology, Department of Experimental Morphology, University of Chile Medical School-Santiago Norte, Casilla 21104, Santiago 21 (Chile), 2 August 1978

Summary. Estrogen administration induces a migration of eosinophil leukocytes to ductus deferens.

Estrogens are known to be synthetized in the mammalian testis[2–4]. Specific estrogen-binding proteins were identified in the male reproductive organs in several species[5–7], including man[8]. It was suggested that estrogens might be involved in modulatory mechanisms of testosterone synthesis[4], as well as in the regulation of its action in target organs[7]. However, the physiological role of estrogens in the male reproductive system still remains unclear.

In the mammalian female, estrogens induce migration of eosinophil leukocytes to the uterus[9], where they have been proposed to mediate some parameters of estrogen stimulation[10]. The aim of the present work is to investigate if similar mechanisms of estrogen action are also present in the male.

Material and methods. Mature male Sprague-Dawley rats were used in the present experiments. 60 µg estradiol 17 β/100 g b.wt in saline were injected s.c. and the animals were sacrificed 24 h after estrogen or vehicle injection. Testis, epididymis, prostate, ductus deferens and seminal

Estrogen-induced tissue eosinophilia in male sexual organs

Organ	Average number of tissue eosinophils/mm^2 ± SEM Control untreated rats	Rats 24 h after estrogen treatment	Variation in tissue eosinophils after estrogen treatment, expressed as Δ% over the controls
Testis	2.9 ± 0.7	1.3 ± 0.2	44.8 (n.s.)
Epididymis	5.7 ± 0.7	5.7 ± 1.2	100.0 (n.s.)
Prostate	8.3 ± 1.3	6.2 ± 0.9	74.6 (n.s.)
Ductus deferens			
Lamina propria	69.4 ± 7.8	263.5 ± 23.1	379.6 (*)
Muscular layer	15.2 ± 1.7	46.2 ± 4.4	303.9 (*)
Seminal vesicle			
Lamina propria	6.3 ± 0.8	10.7 ± 1.6	169.8 (n.s.)
Muscular layer	11.4 ± 1.4	17.0 ± 2.9	149.1 (n.s.)

* $p < 0.001$; n.s., not significant.

vesicle were fixed in 10% neutral formaline and histologically processed for the study of tissue eosinophilia[11].

Results. Estrogen administration induced an increase in the number of tissue eosinophils in both the lamina propria and muscular layer of ductus deferens (table). In other organs, estrogen-induced changes in tissue eosinophilia were not statistically significant (table).

Discussion. The estrogen-induced migration of eosinophil leukocytes to ductus deferens, similar to that described in the uterus[12], would be explained by the close ontogenic development of Wolffian and Müllerian ducts in mammalian embryo[13]. Possibly estrogen-induced migration of eosinophils to ductus deferens is mediated by similar mechanisms proposed for the uterus[11].

The role of eosinophils in the male reproductive system is unknown. It was previously proposed that enzymes released from eosinophil leukocytes play a role in sperm capacitation in the female genital tract[14]. It is possible to speculate that eosinophils migrating to ductus deferens release enzymes which may play a similar role in sperm capacitation at this level. Further work is necessary to ascribe this or other functions to eosinophil leukocytes in the male reproductive system.

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Influence of light on the plasma gonadotropin concentrations in the newborn rat

B. Lemaître and C. Dreux[1]

Laboratoire de Biochimie de l'Hôpital Saint-Louis, 2, place du Dr Fournier, F-75475 Paris Cédex 10, and Laboratoire de Chimie Clinique et Biologie Moléculaire, 15, rue de l'école de médecine, F-75270 Paris Cédex 06 (France), 10 July 1978

Summary. The physiological increase in plasma gonadotropin (LH and FSH) levels in newborn rats is indisputably influenced by light. Permanent illumination accentuates this increase, whereas darkness decreases it in 16-day-old female rats. In male rats of the same age, only permanent illumination was tested with the same results.

We have recently shown[2] that an increased plasma level of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) appears in premature born female children treated by light during their first days of life, due to icterus neonatorum[3]. In order to see if light alone was responsable for the increase of gonadotropins, we compared the plasma FSH and LH levels in light-treated groups of male and female newborn rats with those of control groups.

Material and methods. 90 newborn Wistar CF rats (40 males and 50 females) were grouped in tens and kept in transparent plastic cages with a nursing mother. The groups were sacrified at 7 days (average weight 13 g) and at 16 days (average weight 22 g). The experiment was carried out in August as follows (figure):

a) 2 control groups A (males and females) were maintained under natural light conditions for 7 days (98 h of light and 70 dark). b) 2 control groups C were maintained under natural light conditions for 16 days (224 h of light and 160 dark). c) 4 groups (2 groups B and 2 groups D) were submitted to constant artificial illumination for the first 7 days of life (168 h). The rats were placed in a cage without bedding and received light from five 20-W fluorescent tubes[4] placed 70 cm above the cage. The light intensity at the level of the skin was between 2800 and 3300 lx. 2 groups B were sacrified after 7 days of these treatment (168 h artificial light). 2 groups D were kept for a further 9 days after treatment under laboratory light (168 h artificial light, 126 h of natural light and 90 h dark). d) Finally, a group E of rats were kept 16 days in complete darkness (364 h). Blood samples were taken by cardiac puncture in rats that had been anaesthetized with fluothane[5].

Blood, collected on heparin, was immediately centrifuged and the plasma was frozen at −20 °C. The measurements of LH (in all cases) and of FSH (when the quantities remaining permitted, that is to say in females of groups D, E and males of groups C, D) were measured by radioimmunoassay under the same conditions[6]. Statistical analysis of the results was made using the nonparametric test of Kolmogorov-Smirnov[7].

Results. Individual values of FSH and LH concentrations are given in the figure and the mean and the SD of the different groups in the table.

Males. In the 4 groups of male rats, the LH concentrations are less than the limit of detection (20 ng/ml) and no significant variation appears in the different groups. On the other hand, a significant increase in FSH ($p < 0.02$) appears in the light-treated group D in comparison with the control group C.

Females. In the control group A the LH concentrations are low and less than 40 ng/ml: In the group B also the concentrations are low and not significantly different to A ($p > 0.1$). In the group C the concentrations vary from 26 to 130 ng/ml and differ significantly from those observed in the control group A ($p < 0.05$). However, the values ob-