Table 2. Number of synaptic junctions of conventional (*A*) or ribbon (*B*) type with advancing age and under light (L) and darkness (D) conditions

	Day	L	D	p⩽
A	6	(0.2)	–	
	7	(0.1)	(1.1)	
	8	2.2 ± 0.8	1.0 ± 0.2	
	9	5.2 ± 1.9	1.2 ± 0.4	0.02
	10	4.2 ± 0.3	1.1 ± 0.3	0.001
	11	5.3 ± 0.6	2.8 ± 0.9	
	12	(4.6)	4.3 ± 2.1	
	13	5.5 ± 0.7	4.1 ± 1.4	
B	6	–	–	
	7	0.2	0.1	
	8	0.3 ± 0.1	0.7 ± 0.4	
	9	0.6 ± 0.2	0.7 ± 0.3	
	10	0.8 ± 0.3	0.2 ± 0.1	
	11	1.5 ± 0.1	0.5 ± 0.1	0.01
	12	(1.8)	1.0 ± 0.6	
	13	1.1 ± 0.4	1.8 ± 0.6	

Junctions have been counted over the greatest possible area in the central part of the retina on E-PTA stained sections[7], and calculated to 100 μm^2. Before day 8, the number of junctions is very low and no statistical operation was possible. For day 12, L-values are incomplete. p-values are only given when ⩽ 0.05.

with the exception of a sharp regression in the D-retina on day 10. The counting of synaptic vesicles in ultrathin sections of axon terminals revealed 3 groups of terminals; with <20, 20–50 and >50 vesicles per terminal section (details will be given in another paper in preparation). In the D-series, until day 7 only terminals with less than 20 vesicles per section were found. Terminals with more than 20 and more than 50 vesicles appeared on days 8 and 10 respectively. In the L-retina, terminals with 20–50 vesicles are represented already on day 6, those with more than 50 on day 7. Dense core vesicles of the inner plexiform layer do not occur before day 10 in the D-retina, when they are found in 6% of all terminals. This percentage will not change up to day 13. In the L-retina they appear already on day 8, account for 8% on day 10 and for 10% on day 12.

In the retina of embryos and larvae reared in light conditions, we observe at about day 8 an increase in the number of ganglion cell axons, thus augmenting tractus opticus thickness and lowering the number of ganglion cells per area, along with an increase of number and ramifications of ganglion cell dendrites. This latter increase may stimulate the formation of synaptic contacts and of synaptic vesicles and ribbons in interneuronal processes. In the D-retina, all this occurs some 2 or 3 days later, when the values for the L-retina are attained. The net increase for inner plexiform layer thickness, tractus opticus diameter and synaptic junction number between days 6 and 13 is nearly the same in both L- and D-series. Therefore one has to assume that continuous light, being the experimental situation for a normally dark reared animal, induces a precocious rather than a simply stimulated growth of retinal structures. The sequence of developmental processes seems not to be altered, and neither light nor darkness effects a disordered development.

At the time when first differences between L- and D-retinae arise, receptive and transmitting structures of the retina just start their development. Receptor cell synaptic vesicles and outer segment membranes appear on day 6[8], but it is doubtful whether the latter are capable of receiving light impulses at this date[9]. In the inner plexiform layer, first synaptic junctions are seen on day 7 and they are probably not mature before day 8, that is to say synchronously to first expressions of light-darkness-differences. Thus, the light seems to exert its effect not via the receptor cells, but directly onto the ganglion cells. There are several examples of a nonvisually mediated photosensitivity on developmental stages[10], presumably connected to hormonal action. It is, however, noteworthy that Hansson and Sourander[11] described a pronounced sensitivity to light of retinal ganglion cells in vitro. The question whether the effect is mediated by receptor cells of retina or pineal organ, or by the tectum opticum, is presently under study.

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Histochemical and ultrastructural modifications of mice endometrium, vagina and pituitary following zeranol treatment

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Summary. The histochemical and ultrastructural changes produced by zeranol treatment on the endometrium and the gonadotropic FSH cells of the adenohypophysis have been investigated in mice at the prepuberal and virginal stages. Modifications similar to those induced by estrogen treatment were observed. It is concluded that both estrogens and zeranol share the same activity on the tissues examined.

The aim of this study was to investigate the effect of a new compound, obtained from cultures of molds *Fusarium graminearum*[1] and used in clinical practice for treatment of menopausal symptoms[2], on the histology of mice ovaries and pituitary.

Mice at the prepuberal and virginal stages were injected i.p. with 50μg and 100μg of zeranol 6 (6–10-dehydroxyundecil) β-resorcylic acid μ lactone supplied by the Istituto Chemioterapico Italiano daily for a period of 7 days. Tissue slices from uterus, ovary, vagina and

adenohypophysis were obtained from each animal. The specimens were subdivided into different groups and fixed in: a) Susa, for ordinary light microscopy; b) Gendre, for the histochemical reactions with Schiff periodic acid, Alcian bleu (pH 3.5), and Halmi trichromic staining and c) cacodylate buffer, 1% osmium tetroxide (Palade), for electron microscopy. For the latter preparation, tissues were dehydrated with acetone and embedded either in Araldite or Vestopal. These sections of the plastic embedded material were then stained with lead-citrate according to Reynolds[3] and examined by a Philips EM 300 electron microscope.

The endometrium of the zeranol-treated animals showed a significant increase in its thickness and in the number of tubular glands lined by a pseudostratified epithelium. The basal lamina exhibited a weak PAS positive reaction, whereas the apical portion of the epithelium stained strongly with PAS. These changes were most striking in the virginal mice. In addition, edema of the endometrial stroma was observed as being more pronunced in mice at the prepuberty stage (figures 1 and 2).

In vagina of all treated animals, a striking cheratinization of the more superficial cells was observed. Similar modifications of the endometrium and vagina have been described after estrogen administration[4–6].

The FSH cells of the adenohypophysis increased in size as early as 24 h after zeranol injection, their cytoplasm showed a strong green reaction with Halmi's method. On the other hand, STH, LTH, ACTH, TSH and gonadotropic LH cells did not show any clear modification.

In the FSH cells at the ultrastructural level, numerous secretory dense granules (2400 Å in diameter) were seen in the central and peripheral regions of the cytoplasmic reticulum among the secretory granules. The Golgi apparatus was slightly enlarged and exocytosis poor. These changes suggest that in the FSH cells the ability to synthesize the specific product was associated with an inhibited expulsive capacity of the material accumulated into the secretory granules. In conclusion, our data demonstrate that the changes induced by zeranol on both mice endometrium and adenohypophysis are similar to those observed after estrogen treatment[7–10].

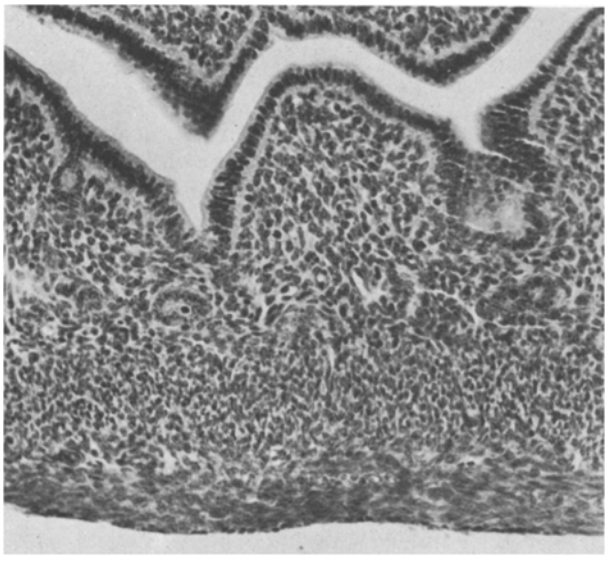

Fig. 1. Uterus of control mouse. ×30.

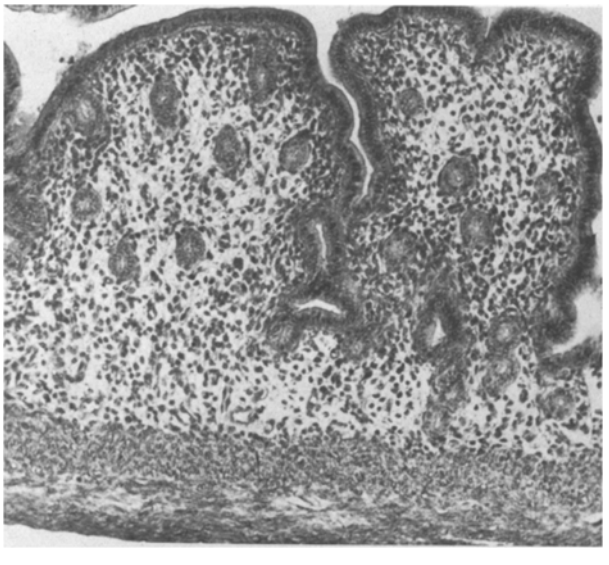

Fig. 2. Uterus of mouse at the prepuberal stage sacrificed after treatment with 100 γ/cc of zeranol daily for a period of 6 days. In the tunica mucosa, gross edema with glandular tubules, mostly tortuous, are seen. ×25.

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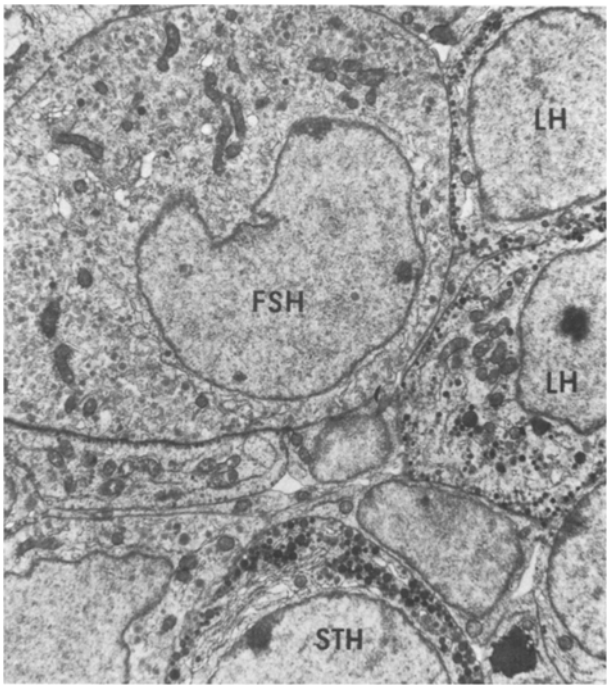

Fig. 3. Adenohypophysis of mice at the prepuberal stage sacrificed after treatment with 50 γ/cc of zeranol. The gonadotropic FSH cells show numerous secretory dense granules. LH = Gonadotropic luteotropic cells. STH = Somatotropic cells. ×8000.