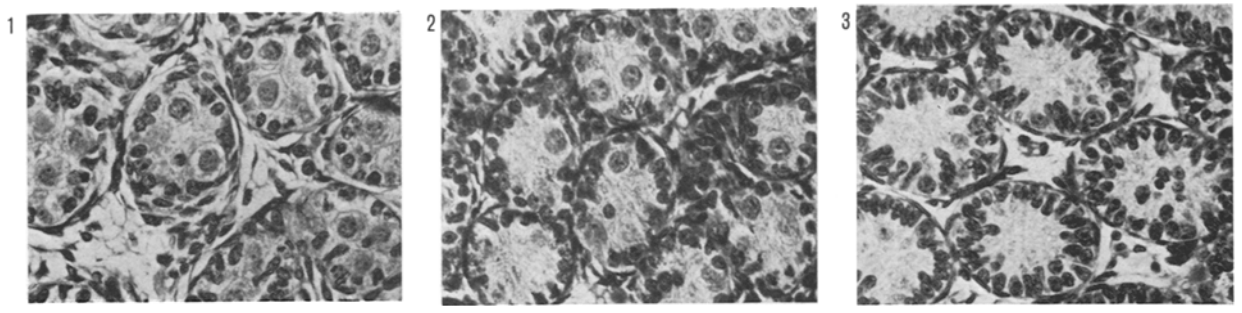

Fig. 1. Control 1-day-old rat testis. The seminiferous tubules show gonocytes and supporting cells. Occasional Leydig cells. HE, × 320.
Fig. 2. LH-RH treated, 1-day-old rat testis. Similar cell types. HE, × 320.
Fig. 3. Control 11-day-old rat testis. The tubular diameter and the number of supporting cells has increased. Primitive type A spermatogonia are seen. He, × 320.

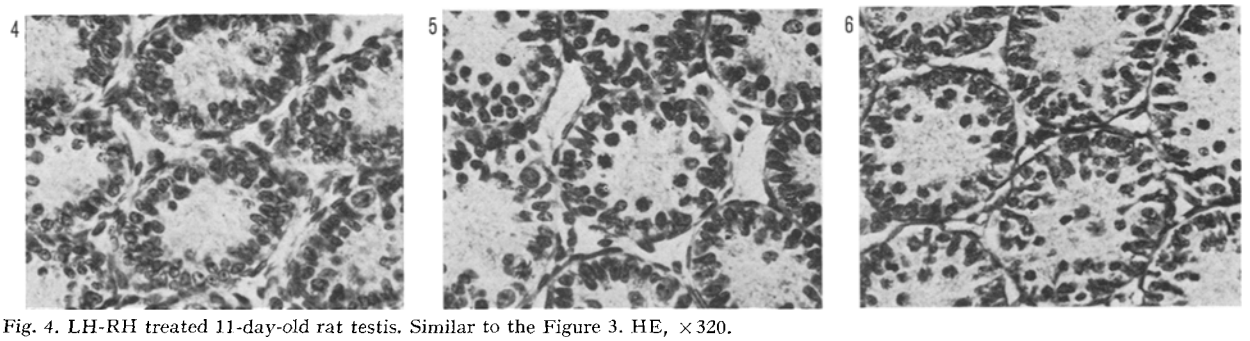

Fig. 4. LH-RH treated 11-day-old rat testis. Similar to the Figure 3. HE, × 320.
Fig. 5. Control 13-day-old rat testis. Tubular diameter increased. Adult type spermatogonia and primary spermatocytes are present in the germinal epithelium. HE, × 320.
Fig. 6. LH-RH treated 13-day-old-rat. Testes similar to the control. HE, × 320.

reported only slight stimulation of the testis. RAMIREZ and MCCANN[9] have suggested that the onset of 'puberty' in the rat is determined by a change in the hypophysis sensitivity to releasing hormones rather than by changes of the gonadotropin content of the glands. In human, ROTH et al.[4] stated that pubertal and adult testes appears to be more responsive than the immature testis in releasing testosterone after the administration of LH-RH. Also SANDOW and BABEJ[10] speculated that chronic LH-RH treatment may have a negative feed-back effect on pituitary sensitivity to the gonadotropin releasing hormones.

Our results showed that administered synthetic LH-RH is neither able to initiate per se the development of spermatogenesis and maturation of Leydig cells, nor to accelerate the normal rhythm of the natural evolution of the gonads. It is quite possible that other mechanisms might be involved in the onset of puberty. On the other hand, we had no evidence of a negative feed-back induced by the injected LH-RH, since both treated and untreated animals showed neither qualitative nor quantitative histological differences in the testis. The significance of the higher number of spermatogonial cells in untreated 14-day-old rats is open to question.

Resumen. Ratas machos inmaduros tratados crónicamente con factor liberador sintético de LH desde el primero hasta el 15 dia de edad a las dósis de 5 μg subcutáneos cuatro veces al dia cada cuatro horas por animal, fueron incapaces de iniciar o acelerar el proceso de maduración testicular estudiando el proceso espermatogénico por contages celulares diferenciales en secciones transversales y el desarrollo de las células de Leydig maduras.

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Effect of Castration on the Rat Pineal Gland: a Fluorescence Histochemical and Biochemical Study

Earlier investigations[1-3] demonstrated that some pinealocytes of the rabbit, rat, hedgehog and mole contain a yellow autofluorescent substance. In rabbit and rat pinealocytes, this material appeared to be a tryptophan-rich protein[1,2] the function of which is still unknown. It might possibly be a carrier protein of pineal hormones[2]. Considering this hypothesis, it seemed of interest to determine, in the rat pineal gland, the quantity of cells containing yellow autofluorescent material under varying

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Table I. Number of autofluorescent cells in the pineal gland after castration (cells/mm²)

4 days		1 week		3 weeks	
Controls	Castrated	Controls	Castrated	Controls	Castrated
158 ± 15	147 ± 23	137 ± 15	216 ± 44	160 ± 37	287 ± 40

Table II. Quantity of serotonin in the pineal gland of rat after castration (γ/g of pineal tissue)

4 days		1 week		3 weeks	
Controls	Castrated	Controls	Castrated	Controls	Castrated
47.8 ± 2.3	43.9 ± 3.6	45.1 ± 4.6	46.8 ± 5.4	46.4 ± 4	45.3 ± 4

experimental conditions related to the function of the epiphysis in the regulation of the hypothalamo-hypophyseo-gonadal system. Because SMITH[2] demonstrated an inverse relation in different circumstances between the amount of autofluorescent material and that of serotonin in the rat pineal, it was necessary to study the serotonin concentration under the same experimental conditions.

The present investigation concerns the determination of the number of cells containing yellow autofluorescent material and the quantity of pineal serotonin, respectively, in adult castrated male rats as compared to those in controls over a period of 3 weeks after castration.

Material and methods. 84 male wistar rats (weight 200 g) were used for this study (30 for the fluorescence histochemical study, 54 for the biochemical study). They were kept in cages at 25 °C and constant humidity. The animals were exposed to 12 h light daily (from 07.00 h to 19.00 h). They received tap water and standard rat pellets ad libitum.

Fourty-two rats were castrated by the abdominal route, the others were sham-operated. 28 rats (14 castrated – 5 for fluorescence histochemical study, 9 for biochemical study – and 14 sham-operated) were killed 4 days after castration and the 56 others, respectively 1 and 3 weeks after the operation.

Fluorescence histochemical study. After decapitation (without anaesthesia ,between 09.00 and 10.00 h), the pineal was removed within 1 min and frozen in freon-12, cooled to −150 °C by liquid nitrogen. Sections of 15 µm were cut on a cryostat at −18 °C. From each pineal gland, approximately 20 frontal sections were cut as described above. 10 alternating sections were used for the study of autofluorescent material. After cutting, the sections were transported to a freeze-dryer. In a vacuum chamber, the cryostat sections were frozen-dried at −30 °C for 45 min. During this period, the pressure decreased to 0.01 Tor. Then the sections were brought within 10 min to room temperature in vacuo. For 2 h, the frozen-dried sections were placed at 80 °C (technique of FALCK et al.[4], as modified by HEENE[5], without formaldehyde vapour treatment. For more details see SMITH[2,6]).

For the demonstration of autofluorescence, a Leitz fluorescence microscope equipped with a mercury lamp (Hb0200) was used.

The quantification of yellow autofluorescent cells was determined by counting, in the different sections, the number of these cells present in a determined area (0.03 mm²). Countings were made in 10 to 15 areas per section, the number of areas studied depending on the size of the section. The standard error was calculated in the results obtained in all rats of the group studied (5 rats per group).

Biochemical study. For the determination of the quantity of serotonin in the pineal gland, pools were made. For each group (4 days, 1 and 3 weeks after castration; 4 days, 1 and 3 weeks after sham-operation) 3 determinations were made, and for 1 determination 3 pineal glands were pooled. All rats were sacrificed between 09.15 h and 10.30 h. As this period was relatively long, castrated and control rats were sacrificed alternatively. If there is an effect of the circadian rhythm on the serotonin concentration of the pineal gland during this time lapse, the effect is identical in the two groups, so that it is possible to compare these two groups.

Isolation of serotonin (5-HT) is based on Amberlite CG50 column chromatography. Pineal glands were removed, pooled and extracted in ice-cold 10 ml of 0.4 *M* perchloric acid, containing 0.1 ml ascorbic acid (2%) and 0.2 ml of 10% EDTA. The extracts were adjusted to a pH of 5.6 with 5 *N* K_2Co_3, centrifuged and the supernatants were put on Amberlite CG50 columns (4.5 × 50 mn)

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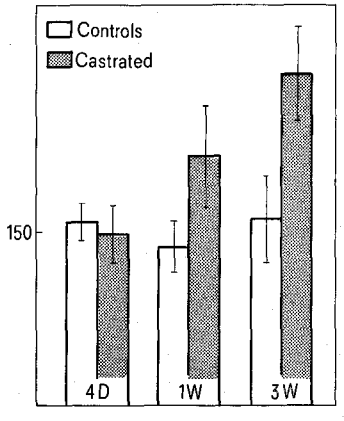

Fig. 1. Number of autofluorescent cells in the pineal gland after castration (cells/mm²)

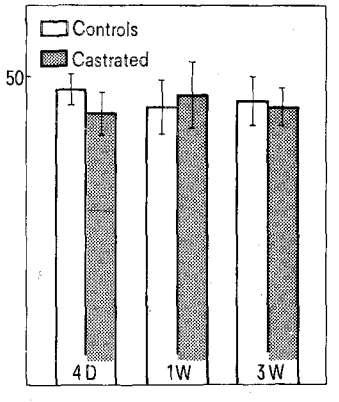

Fig. 2. Quantity of serotonin in the pineal gland after castration (γ/g of pineal tissue)

at a rate not exceeding 0.5 ml/mn. Then the columns were washed with 10 ml 0.02 *M* phosphate buffer (pH 6.5) containing 0.2% EDTA. Elution was performed with 1 *N* HCl. The first 6 ml of the elution contained 5-HT. Serotonin was determined fluorimetrically[7].

The results were expressed in γ/g of pineal tissue, and the standard error is measured for each 3 determinations.

Results. 4 days after the operation, no statistically significant differences in the quantity of autofluorescent cells (Figure 1 and Table I) and the quantity of serotonin (Figure 2 and Table II) between the castrated and sham-operated rat pineal glands were observed.

One week after the castration, the quantity of autofluorescent cells in the pineal gland of the operated rats was about 37% increased in respect to the sham-operated rat group (Figure 1, Table I). No statistically significant differences have been observed in the serotonin concentration in the castrated and sham-operated rat pineal glands (Figure 2, Table II).

Three weeks after the operation, the quantity of autofluorescent cells in the pineal gland of the castrated rats was increased, this increase being larger than after 1 week (about 66% in respect to the sham-operated group) (Figure 1, Table I). However, no statistically significant differences were observed between the serotonin concentration of the castrated and sham-operated rat pineal gland (Figure 2, Table II).

Discussion. Pineal response to castration appeared to be very slow. 4 days after the operation we have not observed any effect on the number of autofluorescent cells and the 5-HT levels. After 1 week, a small increase of the quantity of autofluorescent cells was observed, while after 3 weeks this increase is larger. This slowness of the pineal response is possibly due to the parameters chosen. Indeed, in other experiments using different parameters (metabolic compounds[8]), we have observed a quick response of the pineal gland to castration.

The fact that there was no modification in serotonin levels in the pineal gland of orchidectomized adult rats is interesting but difficult to interprete. It is possible that the castration does not effect the production of pineal indole derivatives but it is also possible that, after castration, the 5-HT turnover changes without any change in the 5HT levels in tissue.

The increase of quantity of cells containing autofluorescent material demonstrated that orchidectomy has an effect on the pineal gland. Just after castration, an abrupt drop of androgen plasma levels occurs. This causes a rapid increase in plasma gonadotropin[9–11]. At that moment it is not possible to determine whether the effect of castration, described in the present paper, is due to the decreasing plasma androgen level or to the increase of FSH and LH levels. Perhaps both may have an effect on the pineal gland. It is interesting that, some time after castration, a notable increase was observed of pineal protein synthesis, i.e., increase of the quantity of autofluorescent material. Intensified pineal protein synthesis is also known to occur in estrogen-treated immature female rats. This is caused by increased LH secretion by the adenohypophysis[12]. As castration also provokes an increase of LH secretion[9–11], it may therefore be that the increase of autofluorescent pinealocytes after castration is caused by increased LH secretion.

It is possible that the yellow autofluorescent material represents an active protein compound (pineal hormone ?) different to pineal indole derivatives. Under these experimental conditions, castration may be restricted to the synthesis of these active protein compounds[13].

Summary. The orchidectomy of the adult rat induces an increased quantity of cells containing autofluorescent material (proteinaceous material[1,2]).

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Suppression of Pupal Esterase Activity in *Aedes aegypti* (Diptera: Culicidae) by an Insect Growth Regulator

Considerable interest has been generated by the suggestion that insect populations may be controlled by use of analogues and mimics of natural juvenile hormone[1,2]. These insect growth regulators (IGR) are believed to alter the normal hormonal balance and thus interfere with post-embryonic development; however, the precise mode of action has not yet been resolved. Slade and Wilkinson[3] have indicated that, because many IGR's are structurally dissimilar to the natural hormone, it is unlikely that their effect is mediated directly through an interaction with the natural hormone receptor. They propose that the IGR's stabilize the natural hormone by inhibiting the normal degradation pathways. One enzyme responsible for catabolism of endogenous juvenile hormone is a carboxyesterase which is induced within 30 min of the appearance of juvenile hormone[4]. The present study was undertaken to determine the effect of one IGR (isopropyl 11-methoxy-3, 7, 11-trimethyl-dodeca-2, 4- dienoate, Altosid ®) (ZR 515) on the esterases of the mosquito, *Aedes aegypti* (L.), an insect species which is particularly sensitive to the effects of IGR's[5].

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