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The control of the frog *(Rana esculenta)* thumb pad[1]

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Summary. Extirpation of the pars distalis of the pituitary or castration in any period of the year cause thumb pad regression. Thumb pad development is regulated by an interaction of both temperature and androgenic hormones.

The thumb pad in male anurans represents an androgen-dependent secondary sexual characteristic[2]. Histological observations (height of the epidermis and glandular epithelium and mitotic rate of the epidermal cells) and biochemical studies (DNA, RNA and protein content and β-glucuronidase activity) have demonstrated that the thumb pad of the green frog, *Rana esculenta*, undergoes regression following hypophysectomy or castration; androgen therapy stimulates its development[3-7]. Its seasonal developmental cycle (regression in summer and development in autumn and early winter) has been correlated with annual cyclic fluctuations in the Leydig cell-secretory activity and plasma androgen levels[8-10]. In addition to this, Rastogi et al.[11] showed that high temperature (28 °C) cause thumb pad regression and low temperature (4 °C) tend to maintain, if not to stimulate, its integrity. Furthermore, in frogs housed at 28 °C exogenous testosterone does not stimulate the thumb pad. These facts led us to undertake a series of experiments in order to analyze better the interaction of hormonal and environmental factors in the control of thumb pad development.

Materials and methods. Experimental series 1: Pars distalis of the pituitary was removed in animals caught in February, June, August and October, as described earlier[12]. Animals were sacrificed at different time intervals. Pars distalis homogenate, in deionized water, was injected into the dorsal sac of operated frogs. Each treated frog received a total of 2 partes distales given in 6 equal fractions, each 3rd day.

Experimental series 2: Group A (January-April 1971). 75 days after castration the animals were treated with different doses of testosterone propionate (TP); group B (September-October, 1979). 28 days after castration the animals were treated separately with TP or dihydrotestosterone (DHT) at either 20 °C or 4 °C; group C (September-October, 1980). 30 days after castration all frogs were kept at 4 °C and treated separately with TP or DHT; group D (January-March, 1981). 28 days after castration the animals were treated separately with TP or DHT at 8 °C and 16 °C. The total dose mentioned here was given in 6 equal fractions. The i.m. injections (group A) were all in 0.1 ml amond oil, whereas the s.c. injections, in the dorsal sac,

Table 1. Influence of the removal of the pars distalis and homologous pars distalis homogenate on thumb pad development*

Treatments**	Thumb pad epidermis	Glandular epithelium
February initial control	100±9 (well developed)	100±13
PDX 50 days	60±5***	58±2***
PDX 90 days	30±1***	37±2***
PDX 90+PD	93±6	98±4
June initial control	100±12 (regressed)	100±7
PDX 40 days	89±8	92±5
PDX 100 days	60±9***	68±6***
PDX 100+PD	146±6***	137±6***
August initial control	100±8 (markedly regressed)	100±11
PDX 50 days	96±5	91±8
PDX 150 days	87±7	90±6
PDX 150+PD	164±11***	156±10***
October initial control	100±9 (developed)	100±10
PDX 40 days	71±3***	69±7***
PDX 100 days	47±2***	38±14***
PDX 100+PD	179±9***	162±15***

* Each control value is represented by 100 and that for treated groups is expressed as a percentage of the equivalent control value. ** All experimental animals were maintained at room temperature (in summer 18-22 °C; in winter 12-18 °C). PDX, pars distalis removed; PD, pars distalis homogenate. *** Significance of difference vs equivalent control $0.01 < p < 0.02$.

Table 2. Effect of androgens and temperature on the thumb pad development*

Experimental groups		Thumb pad epidermis	Glandular epithelium
A	Intact control	100±4	100±10
	75-day castrated	40±2**	36±1**
	75-day castrated control	100±5	100±5
	+TP 60 µg	110±11	107±14
	+TP 150 µg	133±7**	119±3**
	+TP 300 µg	152±9**	132±7**
	+TP 600 µg	189±11**	194±18**
B	Intact control	100±8	100±7
	28-day castrated	68±4**	63±4**
	28-day castrated control	100±7	100±3
	+DHT 300 µg; 20 °C	149±11**	156±10**
	+DHT 300 µg; 4 °C	112±16	103±6
	+TP 300 µg; 20 °C	126±2**	107±3
	+TP 300 µg; 4 °C	109±7	104±4
C	Intact control	100±12	100±8
	30-day castrated	50±6**	56±4**
	30-day castrated control	100±11	100±8
	+DHT 480 µg; 4 °C	106±7	106±5
	+TP 480 µg; 4 °C	103±10	89±17
D	Intact control	100±8	100±10
	28-day castrated (8 °C)	67±8**	74±6**
	28-day castrated (16 °C)	55±2**	62±4**
	8 °C-28-day castrated control	100±3	100±4
	+DHT 480 µg	119±3**	107±5
	+TP 480 µg	145±11**	115±2**
	16 °C-28-day castrated control	100±10	100±8
	+DHT 480 µg	169±6**	139±5**
	+TP 480 µg	171±7**	151±6**

* Same as in table 1. ** Significance of difference vs equivalent control: $0.01 < p < 0.05$.

were all in 0.2 ml deionized water. During hormonal treatment animals in groups B, C and D were housed in thermo-photo-static chambers. A photoperiod of 12 h of light daily was constantly maintained. Animals were killed within 24–48 h after the last injection.

1 thumb pad from each frog was processed for histological examination. The height of the epidermis and that of the glandular epithelium was measured in arbitrary units with the help of an oculometer (using an eye piece 8 times and an objective 10 times). The mean values, based on 30–40 measurements for each thumb pad, for treated animals were expressed as the percentage of the equivalent control value. The statistical significance of the difference was calculated by Student's t-test. Each batch of animals was composed of 10 frogs.

Results and discussion. Independent of the period of the year in which the operation was done, removal of the pars distalis caused thumb pad regression (table 1). This regression, progressive in time in all cases, was slower in winter and faster in summer. Moreover, the magnitude of regression was higher if the frogs were operated on when they had well-developed thumb pads, i.e. in the winter period, than when they had a seasonally-regressed thumb pad i.e. in summer. Administration of homologous pars distalis homogenate stimulated thumb pad development.

The results expressed in table 2 show that castration caused thumb pad regression and the androgen-replacement therapy stimulated the regressed organs. In group-A animals the thumb pads showed a clear dose-dependent response to TP. In group B both DHT and TP stimulated the regressed thumb pads. Stimulation with DHT was slightly greater than with TP for the epidermis and was significant only with DHT for the glandular epithelium. In addition to this, a significant stimulation of thumb pad was obtained only in frogs maintained at 20 °C and not in those kept at 4 °C. This result was consistent with that obtained 1 year later in the same period and under similar conditions (group C). Group-D animals showed that thumb pad regression due to castration was slightly greater at 16 °C than at 8 °C. A similar androgen-therapy caused a significantly greater stimulation at 16 °C than at 8 °C (TP 8 °C vs 16 °C $0.02 < p < 0.05$; DHT 8 °C vs 16 °C $p < 0.05$). In this experiment, however, DHT was less potent than TP at 8 °C. In an earlier publication it was found that TP, 2α-methyl-DHT and 2α,17α-dimethyl-DHT are equally potent in stimulating the regressed thumb pads[7].

Thus it seems clear that the magnitude of thumb pad regression depends on the period of operation and the post-operation interval, whereas the magnitude of stimulation depends mainly on the quantity and not on the quality of the 2 androgens injected. In conclusion, the present data demonstrate that both factors, androgens and temperature, work in concert to regulate thumb pad development. In fact at very low (4 °C) and at very high (28 °C) temperatures androgens are practically ineffective and the optimal temperature range for thumb pad development apparently lies somewhere between 10 and 20 °C.

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