Seasonal changes in the pineal gland related to the reproductive cycle in the male hare, *Lepus europaeus*

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Summary. During the autumn months, the gonads and reproductive tract of adult male hares (*Lepus europaeus*) are regressed and circulating gonadotrophin levels are low. At this time the pineal glands are most active as judged by the organ size, and the nuclear and cytoplasmic size of the pinealocytes. There was an inverse relationship between the size of the pineal gland and the weight of the testis, the plasma and testicular testosterone levels, and possibly also the plasma LH levels.

Many clinical and experimental observations suggest a functional link between the pineal gland and the gonads of mammals (see Wurtman, Axelrod & Kelly, 1968). Antigonadotrophic factors have been found in the pineal glands of several species and the organ probably modifies the activity of the gonads by influencing the secretion of gonadotrophins from the pituitary by its action on the hypothalamus (Fraschini, Collu & Martini, 1971; Vaughan, Reiter, Vaughan, Bigelow & Altschule, 1972).

The suppressive effect of the pineal gland on reproduction is of particular interest in seasonally breeding animals in which there are conspicuous changes in the reproductive tract during the year and the pineal appears to play some role in mediating the environmental effect of light on reproduction (Herbert, 1972; Reiter, 1973). Histological studies of wild animals indicate that the pineal gland may be most active when the gonads are regressed (Mogler, 1958; Quay, 1956; Millar, 1972; Pevet & Saboureau, 1973). The purpose of the present study was to extend these observations to the brown hare, *Lepus europaeus*, which experiences seasonal regression of the gonads and reproductive tract (Lincoln, 1974).

Thirty-four adult male hares estimated to be >12 months of age were shot during 1971 on arable land near Guestwich in Norfolk (52°48'N, 0°59'E) for a separate study (Lincoln, 1974). At the time of death a blood sample was taken and stored on ice until the plasma could be separated 1–4 hr later. The left testis was placed on solid CO₂ and later weighed, and the posterior portion of the skull, including the brain tissue surrounding the intact pineal gland, was fixed in formol saline.

Plasma LH levels were measured by radioimmunoassay as described by Lincoln & MacKinnon (1976), and the testosterone levels in the testes and plasma were measured by the radioimmunoassay of Rowe *et al.* (1974), with a single chromatographic separation for the testicular tissue (Lincoln, 1974). The fixed pineal organs were dissected from the adjoining tissue, weighed, and paraffin-wax sections cut at 5 µm and stained in haematoxylin and eosin. Nuclear diameters of 20 randomly selected pinealocytes were measured in each of 2 animals from each month, and the cytoplasmic extent of these cells assessed by counting the number of nuclei in 10 random high-power microscope fields for each animal. Correlation coefficients were calculated by least square analysis and the probabilities determined by Student's *t* test.

There was a clearly defined seasonal cycle in all reproductive parameters measured. During the non-breeding season in September and October, LH values were low, the testes were small and blood and testicular testosterone levels were minimal (Table 1). The peak in reproductive activity occurred from March to June when the circulating levels of LH were high, the testes fully developed, and the blood and testicular levels of testosterone were maximal.

The weight of the pineal gland also varied throughout the year, being 71 % larger in September and October than in May and June. The nucleus of the pinealocytes was increased in diameter, the nucleoli were more conspicuous, and the cytoplasm more expansive in the pineals of animals killed

between June and October, compared to those of animals killed between December to April (Table 1). There was an inverse relationship between the weights of pineals and the weights of the testes ($r=-0.721$; $P<0.001$), the testosterone content of the testes ($r=-0.526$; $P<0.02$) and the testosterone concentration of the blood ($r=-0.530$; $P<0.02$) of hares shot at the different seasons. There was also an inverse relationship between pineal weight and plasma LH level, although this was only significant ($P<0.05$) if 3 animals with greatly elevated LH values (>30 ng/ml) were omitted. These high values were thought to be due to episodic LH secretion and not to reflect closely the mean circulating levels.

Table 1. Characteristics (mean $\pm$ S.E.M.) of the pineal gland and reproductive tract of adult male hares killed at different times of year

	Jan./Feb.	March/April	May/June	July/Aug.	Sep./Oct.	Nov./Dec.
No. of animals	5	4	7	8	5	5
Wt of left testis (g)	9.0 $\pm$ 0.2	9.7 $\pm$ 0.7	10.3 $\pm$ 0.3	7.3 $\pm$ 0.8	1.7 $\pm$ 0.1	3.8 $\pm$ 0.6
Wt of seminal vesicles (g)	2.1 $\pm$ 0.1	2.6 $\pm$ 0.2	3.1 $\pm$ 0.3	2.2 $\pm$ 0.2	0.7 $\pm$ 0.1	0.9 $\pm$ 0.2
Plasma LH (ng NIH-LH-S13/ml)	2.4 $\pm$ 0.5	3.7 $\pm$ 0.4	5.4 $\pm$ 0.7*	2.1 $\pm$ 0.4†	1.3 $\pm$ 0.1	2.0 $\pm$ 0.2
Plasma testosterone (ng/100 ml)	151 $\pm$ 23	349 $\pm$ 78	414 $\pm$ 54	179 $\pm$ 30	74 $\pm$ 20	105 $\pm$ 42
Testicular testosterone (ng/testis)	450 $\pm$ 206	914 $\pm$ 130	863 $\pm$ 171	220 $\pm$ 45	23 $\pm$ 2	75 $\pm$ 7
Pineal wt (mg)	20.8 $\pm$ 0.8	18.3 $\pm$ 1.5	16.1 $\pm$ 1.9	18.3 $\pm$ 4.1	27.4 $\pm$ 3.5	22.8 $\pm$ 2.5
Diam. of pinealocyte nucleus (μ m)	6.4 $\pm$ 0.06	6.4 $\pm$ 0.06	6.7 $\pm$ 0.07	6.5 $\pm$ 0.5	6.7 $\pm$ 0.07	6.2 $\pm$ 0.04
No. of pinealocyte nuclei/high-power microscope field	69 $\pm$ 3	62 $\pm$ 2	60 $\pm$ 1	58 $\pm$ 2	49 $\pm$ 1	58 $\pm$ 3

Values for *2 hares and †1 hare omitted (see text).

The present results are consistent with the premise that the pineal gland has a suppressive effect on the function of the reproductive system. An increase in the overall size of the pineal gland, and an increase in the nuclear volume and the staining reaction of the nucleolus, have been claimed to indicate enhanced pineal activity (Fiske, Bryant & Putman, 1960; Roth *et al.*, 1962), and there is therefore evidence that increased pineal activity is associated with decreased gonadal activity in the hare. Since photoperiod is thought to control the timing of the reproductive cycle in the hare (Lyman, 1943; Davis & Meyer, 1972), it is probable that the pineal gland plays a role in mediating the day-length effect as described in the hamster (Reiter, 1973). Thus, changing daylength following the summer solstice in June leads to an increase in pineal activity, and the secretion of an unknown factor which suppresses gonadotrophin secretion and leads to gonadal regression. After October the reverse changes occur and with the removal of the suppressive effect of the pineal the male hare regains its fertility.

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