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The pineal gland is very large and active in newborn antarctic seals[1]

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Summary. The pineal gland of newborn elephant seals and Weddell seals is larger than in adult females. The gland is considerably larger at birth in Weddell seals than in elephant seals. The former experience greater extremes of temperature. Plasma melatonin concentrations in excess of 2000 pg/ml were recorded in the first days of life, compared with 20–50 pg/ml in adults.
Key words. Pineal gland; melatonin; thermoregulation.

Considerable emphasis has been placed on the role of the pineal gland in mediating the influence of the photoperiod on the neuroendocrine-reproductive axis[2,3]. At times this has been assumed to be the primary role of the gland, but it appears that the role may be more general than this: as currently perceived, the function of the pineal gland is to serve as an intermediary between the external environment, especially the photoperiod, and the organism as a whole[4,5].

For several reasons, the pineal gland may be expected to be small and relatively inactive in newborn seals. In those mammals whose pineal ontogeny has been investigated, the gland is small and lacks organization at birth, but undergoes considerable enlargement postnatally[6,7]. Plasma and pineal melatonin concentrations have been measured in only a few species during early postnatal development, but in all they are lower than in adults[8,9]. Pineal activity in subadult male southern elephant seals, *Mirounga leonina* is greatest during periods of long scotophase, as judged by organ size, size of pinealocytes and plasma melatonin concentration[10,11], but the peak of births of elephant seal and Weddell seal *(Leptonychotes weddelli)* pups occurs in October when scotophase is very short (less than 10 h for elephant seals and virtually nonexistent for Weddell seals). Nonetheless, the pineal gland of foetal (G.C. Liggins, pers. comm.) and newborn seals is very large[12] (fig. 1). We report observations on the pineal gland of neonatal elephant and Weddell seals, revealing that it is also an extremely active gland. We conclude that it may play an important role in thermoregulation about the time of birth and may be vital to survival in the harsh environments in which these seals live. The Weddell seal occurs in greatest abundance near the coast of Antarctica[13], and experiences the greatest degree of cold of any mammal, while the southern elephant seal inhabits mainly subantarctic waters[14].

Pineal glands of seven elephant seal pups from birth to 45 days of age, and two Weddell seal pups seven and 23 days old, were weighed during dissection. Each pineal was hemisected, and fixed in Bouin's fixative or 10% neutral buffered formalin, dehydrated, and embedded in wax. A 5 µm median section from each half gland was stained by the Heidenhain Azan technique. The pinealocyte densities were estimated using the method of Aherne[15]. In adults, densities were determined for both cortex and medulla[12] since, unlike in pups, these regions have different pinealocyte concentrations. The pinealocyte population of the total gland was estimated from a consideration of total gland weight, pinealocyte density, and volumetric proportions for cortex and medulla.

Venous blood was collected from neonatal elephant seals from birth to three weeks, and neonatal Weddell seals from birth to five weeks of age. Collections were made between 11.00 and

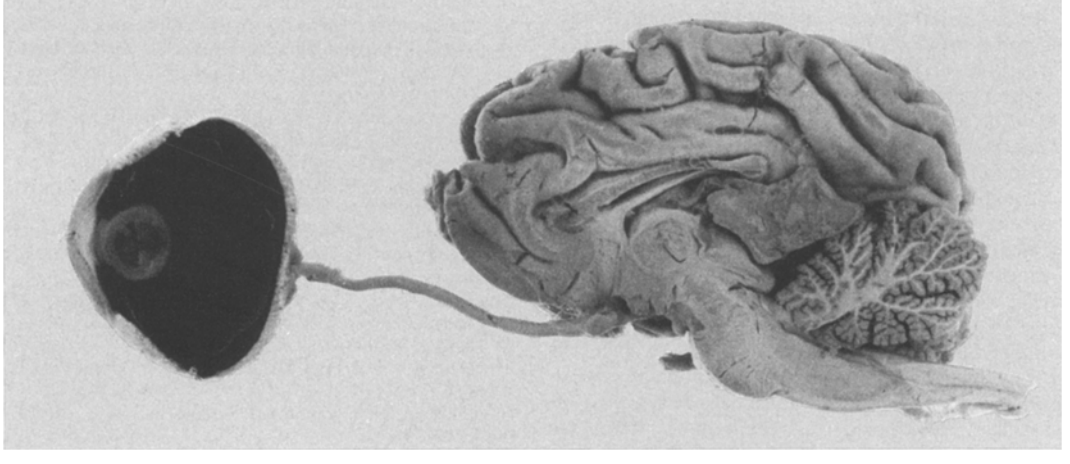

Figure 1. Median section of the brain of a newborn Weddell seal, with the orbit attached by the optic nerve. The pineal gland (p), which is extraordinarily large, lies between the cerebrum and the cerebellum. The gland is very active at birth, and subsequently is influenced by daily and seasonal variation in light intensity.

Table 1. Weight, pinealocyte density, and total number of pinealocytes in the pineal glands of neonatal and adult elephant and Weddell seals

		Gland weight (g)	Pinealocyte density (cells, cu $mm^{-1} \times 10^6$)	Total pinealocyte (cells $\times 10^9$)
Elephant seal				
Neonatal,	mean	1.55	1.296	1.80
	S.D.	0.52	0.114	0.62
Adult F,	mean	0.70	–	–
	S.D.	0.20	–	–
Adult M,	mean	1.90	Cortex 1.308 Medulla 0.117	0.04
Weddell seal				
Neonatal	1	3.50	0.583	2.04
	2	3.60	0.457	1.65
Adult F,	mean	0.50	Cortex 0.924 Medulla 0.014	0.023

13.00 h, into bottles containing EDTA or heparin, and plasma was separated and frozen for subsequent assay. Plasma melatonin concentrations were determined by radioimmunoassay[16].

Pieces of pineal tissue (approximately 30 mg) were homogenized in 1 ml 0.5 M NaOH, mixed with an equal volume of 0.5 M borate buffer at pH 10.0, and extracted with 16 ml redistilled chloroform. The aqueous phase was aspirated and discarded, and the chloroform phase was evaporated at 37 °C under a stream of nitrogen.

The residue was redissolved in 0.5 ml chloroform:petroleum spirit (1:1), and transferred to a column (3.2 × 175 mm) of Lipidex 5000 (Packard Instrument Co., Illinois) along with 7.5 ml chloroform:petroleum spirit (1:1). The first 3 ml eluate was discarded, and the remainder, containing the melatonin, collected into siliconized glass tubes (12 × 100 mm). This quantity was evaporated at 37 °C under a stream of nitrogen and radioimmunoassayed.

The pineal gland was relatively and absolutely larger in newborn elephant seals (b.wt. approximately 35 kg) than adult females whose body weight was approximately 350 kg (table 1). Neonatal gland weight was only a little less than that of adult males, which in this trial weighed an average 1350 kg. There was no trend suggestive of a reduction in pineal gland weight between birth and 45 days of age. In *Leptonychotes* the situation appears to be similar, as gland weights of two pups were 3.5 and 3.6 g, in contrast to adult females 0.50 g ± 0.19 (n = 5).

Small, amorphous, noncellular structures, histologically identical to structures described as concretions containing calcium salts in other species[17], were scattered throughout the periphery of most glands examined. These structures reacted negatively to the Von Kossa and Alizarin Red S tests for calcium, and their chemical composition remains unknown. Small, irregularly-distributed calcium-positive granules were observed in the tunica adventitia of blood vessels near the centre of some glands.

The total number of pinealocytes in the pineal gland of elephant seal pups was approximately 50 times greater than that in adult males, and in Weddell seals this ratio between pups and adult females is of the order of 100 to 1 (table 1). The density of pinealocytes was similar in pups and adults, indicating that these cells are functional in neonatal animals.

Figure 2 shows the midday concentrations of melatonin in the plasma of elephant seal and Weddell seal pups. It is seen that plasma levels are extremely variable, but high values are present in some individuals. Mean levels in day old elephant seal pups are 80 times greater than in adult males (table 2). Similar figures for Weddell seals are unavailable because the upper limit of plasma melatonin concentration in the pups was not measured, but the trend was similar. Total gland melatonin was greater in newborn elephant seals than in adult males (table 2).

These observations clearly demonstrate that the pineal gland of neonatal elephant and Weddell seals is extremely active at birth, and remains variably but highly active for at least several weeks postpartum. These findings contrast with earlier observations of enlarged but inactive endocrine glands other than the pineal in newborn seals[18]. Most endocrines are enlarged at birth in seals, due to proliferation of interstitial cells, presumably under the influence of maternal and placental hormones. While these or-

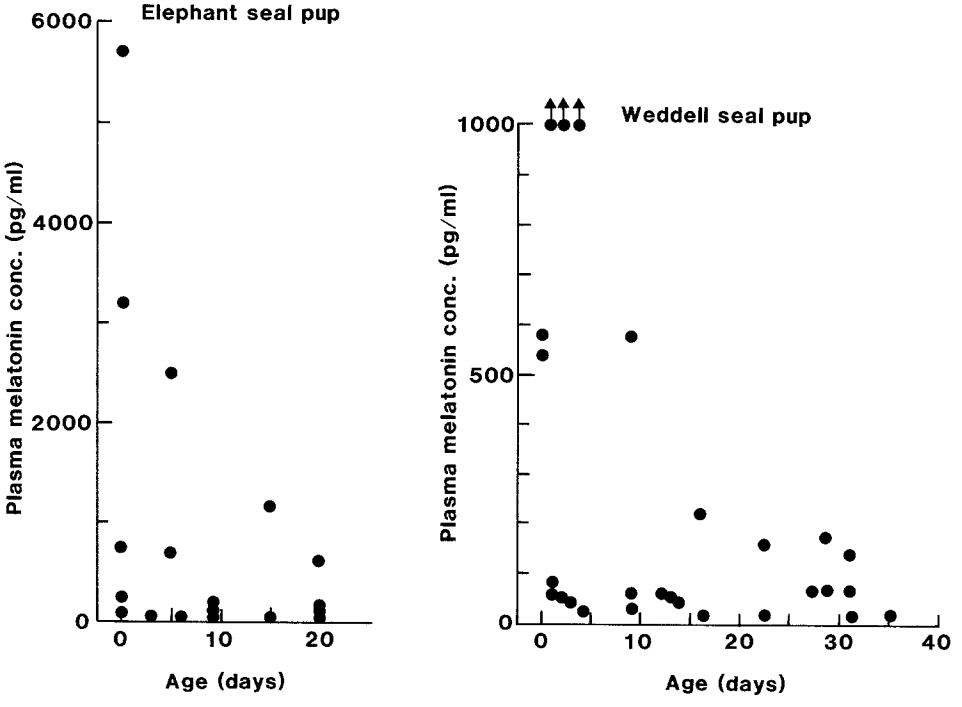

Figure 2. Midday plasma melatonin concentrations in neonatal elephant and Weddell seals.

Table 2. Concentrations of plasma and pineal melatonin in five newborn and three adult male elephant seals during periods of short scotophase (long days, > 13 h between sunrise and sunset), October to November

		Midday plasma melatonin (pg/ml)	Total gland melatonin (ng)
Newborn,	mean	2031	10.01
	S.D.	2451	7.79
Adult male,	mean	26	2.96
	S.D.	1	2.14

gans are inactive and involute rapidly after birth, this is not true of the pineal gland.

We have demonstrated that the pineal in newborn seals is relatively, and often absolutely, greater than in adults of the same species. Further, in the early postnatal period the gland is relatively, and absolutely, larger in the Weddell seal than the elephant seal. The evidence is circumstantial, but strongly suggestive of a thermoregulatory role for the pineal gland in neonatal seals. First, the newborn Weddell seal, with its larger pineal, is subjected to temperature gradients at birth, exceeding 60 °C on occasions, whereas the gradient rarely exceeds 40 °C for elephant seals at Macquarie Island. Secondly, the pineal gland is large and most active in the first few days of life, when physical insulation of the animal is least[19]. We are by no means the first to suggest a thermoregulatory role for the pineal gland in mammals[20], but the size and level of activity of the pineal in newborn seals is unequalled among mammals examined so far. It has been shown that, in the foetal sheep, melatonin accumulates within the pineal gland, and there is a rise in activity of hydroxyindole–O–methyltransferase (HIOMT), in the last few days of gestation[9,21]. Kennaway et al.,[9] have considered a possible relationship between these parameters and the endocrine changes preceding parturition. However the inordinately large pineal and very high plasma melatonin concentrations in newborn seals suggests a function in addition to, or other than, the initiation of parturition in Weddell and elephant seals.

Our findings extend previous reports in that the assumed thermoregulatory role played by the pineal in our animals seems to be different from that in laboratory mammals reported previously. There is increasing evidence that the pineal gland exerts an antithyrotrophic effect by suppression of the release of TRH from the hypothalamus[22–24]. However, in newborn Weddell seals there is ultrastructural (G.J. Little and M.M. Bryden, unpublished observations) and physiological[25] evidence that the thyroid gland is extremely active, despite the very high plasma melatonin concentrations. Exogenous melatonin treatment has been shown to elicit a thermotrophic response in Djungarian hamsters, this effect being brought about by stimulation of brown adipose tissue[26,27]. A similar effect was not demonstrable in rats[28,29]. However, brown adipose tissue could not be found in neonatal Weddell seals[25], and it seems likely that in this species the main source of thermogenesis is increased metabolic rate, controlled by the thyroid gland whose activity possibly is modified by the action of the pineal gland. The chemical nature and the pathway of transmission of information from the pineal to thermoregulatory effectors are unclear.

Several important principles result from the observations reported in this paper. First, in the conditions experienced by neonatal seals the pineal gland is able to maintain an extraordinarily high level of activity for at least several days in almost continuous natural light. Previous investigations have shown that under experimental conditions, continuous light suppresses the activity of the gland[30–32]. Nonetheless, in adult Weddell seals there is a circadian fluctuation in plasma melatonin concentration, even in continuous natural light (D.J. Griffiths, M.M. Bryden and D.J. Kennaway, unpublished observations), so we recognize that these results, based as they are on single time point analyses, should be treated with some caution. Secondly, in neonatal seals the activity of the gland probably is not controlled by external factors such as photoperiod, but it is genetically programmed to perform a vital function at birth. Thirdly, the principal role of the gland seems to alter as growth of the animal proceeds. Initially it is primarily involved in thermoregulation, whereas in adult seals it is primarily involved in photoperiodic control of gonadotrophic activity of the hypothalamo-hypophyseal system[11].

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