Aust. J. Biol. Sci., 1979, **32**, 581–6

Summer and Winter Cycles in Plasma Melatonin Levels in the Elephant Seal (*Mirounga leonina*)

D. Griffiths,[A] *R. F. Seamark*,[B] *and M. M. Bryden*[A]

[A] School of Anatomy, University of Queensland, St Lucia, Qld 4067.
[B] Department of Obstetrics and Gynaecology, University of Adelaide, G.P.O. Box 498, Adelaide, S.A. 5001.

Abstract

Plasma melatonin concentration of immature male elephant seals was determined by radioimmunoassay. Comparison of concentrations during two 24-h periods, one in midsummer and one in midwinter, showed that there was a marked circadian cycle in winter which was greatly modified during the long day length of summer. It is suggested that in summer there was sufficient ambient lighting during the night hours to depress the nocturnal rise in plasma melatonin. The complexity of pineal cycles in the natural environment is stressed, and in this regard the polar regions are of particular interest due to the extreme seasonal changes in day length there.

Introduction

The southern elephant seal (*Mirounga leonina*) has been studied extensively at Macquarie Island (55°S., 159°E.) by members of the Australian National Antarctic Research Expeditions (A.N.A.R.E.). In 1977 attempts were made to gain further understanding of the physiological controls of the elephant seals' annual breeding cycle.

Many mammals that inhabit polar regions have restricted breeding seasons (Asdell 1964). The southern elephant seal at Macquarie Island has a circumscribed breeding season from September to November (Carrick *et al.* 1962). The principal environmental signal entraining circannual activity in mammals in these temperate zones is the seasonal change in day length which mediates its effects via the pineal gland (Reiter 1975*a*). Many polar mammals, including pinnipeds, possess an inordinately large pineal (Cuello and Tramezzani 1969; Elden *et al.* 1971), but no data are available on pineal function in natural populations of mammals subjected to the marked seasonal changes in polar light.

Most investigations on pineal cycles have involved laboratory animals and simulated light conditions. Pineal cycles would be expected to be more pronounced in populations living nearer the polar regions, where changes in day length are very dramatic. In this study circadian changes in plasma melatonin concentration have been assessed in the southern elephant seal during the short day length of midwinter and the long day length of midsummer to examine whether changes in pineal activity could provide a basis for seasonal physiological events.

Materials and Methods

Experimental Animals

Plasma samples were obtained from seals over two 24-h periods, one during midwinter and one during midsummer, and measured for melatonin content by radioimmunoassay. Three sexually

immature male seals of age 1–2 years were used for each trial. Lengths and computed weights for each seal are as follows:

	Seal No.	Length (cm)	Weight (kg)
Winter	1	120	48
	2	160	115
	3	180	164
Summer	4	170	138
	5	180	164
	6	200	225

The seals were not weighed directly but weights were calculated from direct nose-to-tail lengths according to the formula of Ling *et al.* (1967). Both experiments were performed within a circular enclosure 15 m in diameter with steel walls 1·8 m high. In winter, seals were found close to the enclosure and were driven inside. In summer they were further away, and were sedated with ketamine hydrochloride (Ketalar, Parke-Davis; 2·5 mg/kg intramuscular injection), then transported to the enclosure. In both winter and summer the experiment was performed the day after penning.

Restraint for Blood Sampling

During the winter trial the seals were allowed to roam free in the enclosure and were immobilized by an intramuscular injection (1·3–1·5 mg/kg) of suxamethonium chloride (Ling *et al.* 1967) before taking each blood sample. Prior to the start of the summer trial, the seals were immobilized with suxamethonium chloride and strapped down to heavy planking with canvas firehose. Once restrained no subsequent chemical immobilization was necessary, and blood samples were collected every 3 h.

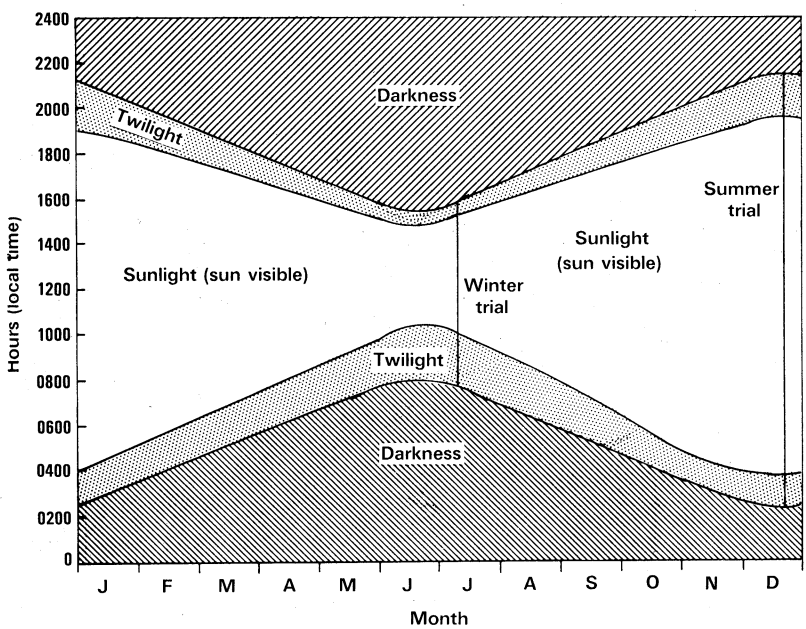

Fig. 1. Seasonal lighting fluctuation for Macquarie Island.

Procedure for Bleeding

Blood was drawn from the extradural vertebral venous sinus (Hubbard 1968, p. 346) using a 9 cm by 1·6 mm (16-gauge) needle. 50–60 ml of blood were collected into screw-topped plastic vials containing 100 mg EDTA (winter) or a small drop of heparin (summer), capped and gently rotated to mix the anticoagulant. The erythrocytes were found to be very fragile and any rough handling or delay in centrifugation caused severe haemolysis. After centrifugation at 2000 rpm, the plasma was poured into plastic centrifuge tubes and stored at −20°C until assay.

Environmental Lighting

Seasonal lighting fluctuation for Macquarie Island is shown in Fig. 1. The winter trial was conducted on July 10–11, 1977. On these two days the day length (sunrise to sunset, plus twilight) was from 0745 to 1545 h local time, or 8 h. The summer trial was conducted on 21–22 December 1977 when day length lasted from 0230 to 2130 h local time, or 19 h.

Melatonin Assay

Plasma melatonin level was determined by radioimmunoassay following the method of Kennaway *et al.* (1977). The smallest quantity of melatonin detectable with this method was 30 pg, and 50% inhibition was produced by 225 pg of melatonin. When samples were expected to contain little melatonin, 4 ml of elephant seal plasma were mixed with an equal volume of 0·5 M borate buffer (pH 10·0), and extracted with 30 ml of chloroform for 30 min. When samples were expected to be high in melatonin, 2-ml units were extracted with 16 ml of chloroform. Four reference standards of charcoaled elephant seal plasma containing 300 pg melatonin were included in each assay to check for melatonin recovery. Test samples were routinely assayed using duplicates of differing volumes of plasma, as a test for parallelism. Intra-assay variation ranged from 7 to 22%, and inter-assay variation was 18%. Very good agreement was shown by samples tested using different amounts of plasma.

No estimates of pineal indole concentrations or of pineal gland enzyme levels were made.

Results

Winter Trial

The blood levels of melatonin during the winter trial show abrupt changes at the onset of daylight and darkness (Fig. 2). It can be seen from Table 1 that the night levels of melatonin were significantly higher than the day levels ($P < 0{\cdot}01$, Wilcoxon

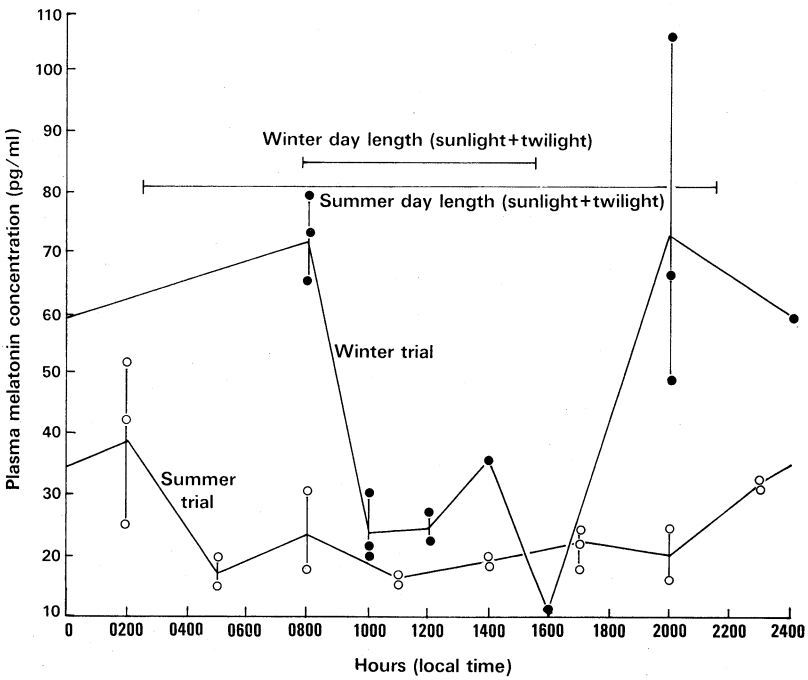

Fig. 2. Plasma melatonin concentration in elephant seals versus day length for midwinter and midsummer.

rank sum test, Snedecor and Cochran 1967). These levels were of the same magnitude as those of the rats used in the artificial light experiments of Ozaki *et al.* (1976) and Wilson *et al.* (1978).

Summer Trial

During summer there was no significant difference between mean day and night levels of melatonin (Table 1; Fig. 2). Thus the marked circadian fluctuation in plasma melatonin observed during the winter trial was not present during the long day length of summer.

There was no significant difference in the mean daylight levels of melatonin between winter and summer, but night levels in winter were higher than those in summer.

Table 1. Comparison of daytime and nighttime mean plasma melatonin levels in summer and winter

	Melatonin levels (pg/ml)	
	Day	Night
Summer	$19{\cdot}4 \leftarrow P > 0{\cdot}05^{A} \rightarrow 32{\cdot}6$	
	$\uparrow$	$\uparrow$
	$P > 0{\cdot}05^{A}$	$P < 0{\cdot}01$
	$\downarrow$	$\downarrow$
Winter	$24{\cdot}5 \leftarrow P < 0{\cdot}01 \rightarrow 70{\cdot}7$	

[A] No significant difference.

Discussion

The seals used in this experiment, even though young, were large (see tabulation, p. 582) and extremely strong and it was impossible to immobilize them manually for venipuncture.

In the winter trial the skeletal muscle relaxant suxamethonium chloride was used for restraint, for several reasons. It was found to be a safe drug, usually taking effect within 2 min, having a short duration of 5–10 min, and a moderately quick recovery time. Being non-depressant centrally, it should not have influenced blood melatonin levels during the experiment. Ling *et al.* (1967) recommended a dose of 2·0 mg/kg deeply intramuscular, but while producing complete paralysis, this dose also produced apnoea for up to 10 min. In our experiment a dose rate of 1·3–1·5 mg/kg produced nearly complete immobility but with little or no apnoea. The additional restraint necessary for bleeding was easily provided manually.

After several bleedings the seals became very difficult to approach for injection, and attempts at immobilization became less and less successful. For this reason it was decided to immobilize the summer seals for the duration of the experiment, by strapping them to planks whilst under suxamethonium immobilization. It is highly unlikely that this change of procedure contributed to the differences between summer and winter melatonin levels.

Fluctuations in blood melatonin levels in response to a light–dark transition have been demonstrated in the sheep (Rollag and Niswender 1976), the rat (Ozaki *et al.*

1976) and in man (Pelham *et al.* 1973). The present work has established that in *M. leonina* blood melatonin levels exhibit a circadian variation, which is much more marked in winter (approximately 16 h of darkness) than in summer (approximately 5 h of darkness). There was a rapid increase in blood melatonin concentration in the midwinter trial to a maximum level, attained 4 h after the onset of darkness. Although each trial lasted only 1 day, it suggests strongly that there is a repetitive circadian cycle of blood melatonin during the winter, and that the cycle is attenuated during the long day length of summer. Because Elliott *et al.* (1972) demonstrated that very short periods of total light or dark could influence blood melatonin levels, it is unlikely that the suppression of the rise in plasma melatonin can be explained by the short duration of darkness in summer. During midsummer at Macquarie Island there was rarely full darkness, due to the dull glow of the sun from the southern horizon, and probably this faint lighting was enough to inhibit the nocturnal rise in blood melatonin. However, it is not possible with the low numbers of animals used in this experiment, to say that the circadian rhythm was totally abolished during summer. Had more animals been used, the rise in plasma melatonin concentration observed during the summer darkness may have been significant.

Almost without exception, investigators of pineal-associated rhythms have subjected animals to controlled periods of artificial lighting with abrupt changes in illuminance. In a natural environment the situation is more complex. The changes in illuminance are not abrupt, but merge from light to twilight to dark over a period of several hours. There may be a cut-off point in illuminance below which melatonin production is maximal, which may vary among species, so that different species in the same environment could have different circadian rhythms in pineal activity. Natural darkness is rarely completely dark. If pineal activity is proportional to ambient illuminance there should be low nocturnal melatonin levels over the full moon, during periods of auroral activity or in populated areas. In addition the wavelength of ambient lighting may influence pineal activity, and it is important to compare the wavelength of lighting in laboratory experiments with natural daylight. The sharp rise in plasma melatonin concentration with darkness and equally sharp fall with the onset of morning twilight in midwinter, even in a natural situation, suggests that there is some cut-off value for ambient lighting below which pineal activity is maximal and above which it is minimal, rather than being proportional to strength of illumination.

The functional significance of melatonin is obscure. It has been proven beyond doubt to be a hormone and, except in the golden and Djungarian hamsters (Orts *et al.* 1975; Reiter *et al.* 1975), is a potent antigonadotropin. The fact that it is present in the blood of immature seals in concentrations similar to those in sexually mature mammals of other species indicates either that the primary function of melatonin is not antigonadotropic, or that the mechanism for gonadal control is present in the animal long before it reaches sexual maturity. It is now clear that the pineal indole 5-methoxytryptophol is also an important antigonadotropin and that there are at least two polypeptide pineal antigonadotropins 60–1000 times more potent than melatonin (Matthews and Benson 1973; Reiter 1975*b*). Once the complete spectrum of pineal hormones is identified, melatonin may be found to be of minor importance in the control of gonadal function, and other substances may be more realistic indicators of pineal function.

Acknowledgments

We thank the Tasmanian National Parks and Wildlife Service, and the Antarctic Division, Department of Science and the Environment, for their support of this project. The following members of the 1977 A.N.A.R.E. assisted in experimental work: Dr Jean Ledingham, Dr Trevor Tierney, Mr Keith Blundell and Mr Tony Everett. Miss Bronwyn Parsons, Queen Elizabeth Hospital, Adelaide, supervised the melatonin radioimmunoassay.

References

Asdell, S. A. (1964). 'Patterns of Mammalian Reproduction.' 2nd edn. (Cornell University Press: Ithaca, New York.)

Carrick, R., Csordas, S. E., Ingham, S. E., and Keith, K. (1962). Studies on the southern elephant seal, *Mirounga leonina* (L). III. The annual cycle in relation to age and sex. *CSIRO Wildl. Res.* **7**, 119–60.

Cuello, A. C., and Tramezzani, J. H. (1969). The epiphysis cerebri of the Weddell seal: its remarkable size and glandular pattern. *Gen. Comp. Endocrinol.* **12**, 154–64.

Elden, C. A., Keyes, M. C., and Marshall, C. (1971). The pineal body of the northern fur seal (*Callorhinus ursinus*): a model for studying the probable function of the mammalian pineal body. *Am. J. Vet. Res.* **32**(4), 639–47.

Elliott, J. A., Stetson, M. H., and Menaker, M. (1972). Regulation of testis function in golden hamsters: A circadian clock measures photoperiodic time. *Science* **178**, 771–3.

Hubbard, R. (1968). Husbandry and laboratory care of pinnipeds. In 'The Behaviour and Physiology of Pinnipeds'. (Eds R. Harrison, R. Hubbard, R. Peterson, C. Rice and R. Schusterman.) pp. 299–358. (Appleton Century Crofts: New York.)

Kennaway, D. J., Frith, R. G., Phillipou, G., Matthews, C. D., and Seamark, R. F. (1977). A specific radioimmunoassay for melatonin in biological tissue and fluids and its validation by gas chromatography—mass spectrography. *Endocrinology* **101**(1), 119–27.

Ling, J. K., Nicholls, D. G., and Thomas, C. D. B. (1967). Immobilization of southern elephant seals with succinylcholine chloride. *J. Wildl. Manage.* **31**(3), 468–79.

Matthews, M. J., and Benson, B. (1973). Inactivation of pineal antigonadotropin by proteolytic enzymes. *J. Endocrinol.* **56**, 339–40.

Orts, R. J., Kocan, K. M., and Wilson, B. (1975). Inhibitory action of melatonin on a pineal antigonadotropin, *Life Sci.* **17**, 845–50.

Ozaki, Y., Lynch, H. J., and Wurtman, R. J. (1976). Melatonin in rat pineal, plasma, and urine: 24 hour rhythmicity and effect of chlorpromazine. *Endocrinology* **98**(6), 1418–24.

Pelham, R. W., Vaughan, G. M., Sandock, K. L., and Vaughan, M. K. (1973). Twenty-four-hour cycle of a melatonin-like substance in the plasma of human males. *J. Clin. Endocrinol. Metab.* **37**, 341.

Reiter, R. J. (1975*a*). The pineal gland and seasonal reproductive adjustments. *Int. J. Biometeor.* **18**, 282–8.

Reiter, R. J. (1975b). Endocrine rhythms associated with pineal gland function. In 'Biological Rhythms and Endocrine Function'. (Eds L. Hedland, J. Franz and A. Kenney.) pp. 43–78. (Plenum Press: New York.)

Reiter, R. J., Vaughan, M. K., Blask, D. E., and Johnson, L. Y. (1975). Pineal methoxyindoles: New evidence concerning their function in the control of pineal-mediated changes in the reproductive physiology of male golden hamsters. *Endocrinology* **96**, 206–13.

Rollag, M. D., and Niswender, G. D. (1976). Radioimmunoassay of serum concentrations of melatonin in sheep exposed to different lighting regimens. *Endocrinology* **98**(2), 482–9.

Snedecor, G. W., and Cochran, W. G. (1967). 'Statistical Methods.' pp. 130–1. (Iowa State University Press: Ames, Iowa.)

Wilson, B. W., Lynch, H. J., and Ozaki, Y. (1978). 5-Methoxytryptophol in rat serum and pineal: Detection, quantitation, and evidence for daily rhythmicity. *Life Sci.* **23**, 1019–23.

Manuscript received 23 April 1979, accepted 25 September 1979