

PLASMA MELATONIN LEVELS IN THE SCINCID LIZARD *TRACHYDOSAURUS RUGOSUS*

THE EFFECTS OF PARIETAL EYE AND LATERAL EYE IMPAIRMENT

BY B. T. FIRTH* AND D. J. KENNAWAY

*Department of Anatomy and Histology and Department of Obstetrics and Gynaecology,
University of Adelaide, Adelaide, South Australia, 5001, Australia*

(Received 13 July 1979)

SUMMARY

1. At a constant temperature of 24 °C there was a diel fluctuation in plasma melatonin concentration; highest levels occurring in the scotophase of a reversed daily light-cycle. Parietalectomy did not appear to affect melatonin titres under these conditions.

2. When lizards were subjected to a photoperiod together with a thermo-period (31 °C in the photophase, 24 °C in the scotophase), nocturnal plasma melatonin levels were almost twice as high as those in animals subjected to a photoperiod at constant temperature.

3. Capping the lateral eyes of *T. rugosus* under these conditions did not alter the phase or amplitude of the rhythm in plasma melatonin content. However, removal of the parietal eye abolished the rhythm, owing mainly to reduced levels during the mid-scotophase and elevated levels during the mid-photophase.

4. It is concluded that plasma melatonin levels are regulated extraretinally, and that the parietal eye may help to mediate environmental input to centres secreting melatonin. It is suggested that the parietal eye may mediate thermal as well as photic information.

INTRODUCTION

The pineal organ forms part of the diencephalic brain roof of most vertebrates. It may be directly photosensory, as in fishes, amphibians, and some reptiles, or it may receive photic information indirectly via sympathetic neural pathways, as in birds, mammals and certain reptiles (Collin, 1971; Kappers, 1971). Some frogs and lizards may, in addition to the pineal, possess an extracranial parapineal organ (frontal organ and parietal eye respectively), the photoreceptive nature of which has been determined from ultrastructural (Eakin, 1973) and neurophysiological (Dodt, 1973) studies.

* Present Address. Department of Zoology, University of New England, Armidale, N.S.W. 2351, Australia.

The function of the lizard parietal eye remains uncertain, but it has been suggested that it may be a radiation dosimeter (Stebbins & Wilhoft, 1966) or, more appropriately, an illuminometer (Packard & Packard, 1972). In support of this hypothesis, field and laboratory studies of lizards have shown that parietal eye impairment results in more frequent exposure to bright light, increased basking time and increased activity (Glaser, 1958; Stebbins, 1963, 1970; Palenschat, 1964; Packard & Packard, 1972; Stebbins & Cohen, 1973). The 'eye' therefore might help to regulate seasonal physiological cycles, such as reproduction and metabolic activity, which depend to some degree upon environmental light cues for their synchronization (Stebbins & Wilhoft, 1966). Indeed, there is evidence that reproductive cycles (Stebbins, 1970; Stebbins & Cohen, 1973) and seasonal thyroid activity cycles (Stebbins & Eakin, 1958; Stebbins & Wilhoft, 1966; Stebbins & Cohen, 1973) are accelerated by parietal eye impairment.

Recent studies suggest a thermoregulatory function for the parietal eye. Parietalectomy or parietal nerve disruption causes lizards to behaviourally select higher temperatures in thermal or photothermal laboratory gradients (Hutchison & Kosh, 1974; Engbretson & Hutchison, 1976; Roth & Ralph, 1976, 1977). Parietalectomy also depresses thermal tolerance, as measured by the critical thermal maximum (Kosh & Hutchison, 1972) and the threshold for thermal panting (Firth & Heatwole, 1976).

The vertebrate pineal organ appears to be the principal, though not the sole source of secretion of the indoleamine *N*-acetyl-5-methoxytryptamine, or melatonin (Ozaki & Lynch, 1976; Kennaway *et al.* 1977; Gern & Ralph, 1979; Gern, Owens & Ralph, 1978*a*). The levels of this substance vary rhythmically within the blood of fish (Gern, Owens & Ralph, 1978*b*), amphibians (Gern & Norris, 1979), lizards (Kennaway *et al.* 1977; Firth, Kennaway & Rozenbils, 1979), turtles (Owens, Gern & Ralph, 1978), birds and mammals (Ralph, 1976), with maximal levels occurring during the dark phase (scotophase) of a daily light cycle. Melatonin is believed to be a chemical transducer of the photic environment, and has been implicated in a number of functions, including reproduction (Reiter, 1978), circadian activity rhythms (Turek, McMillan & Menaker, 1976) and thermoregulation (Ralph *et al.* 1979).

It has been suggested that there is a functional interaction between the parietal eye and pineal organ of lizards. Neuropharmacological studies show that in *Crotaphytus collaris* the electrical activity of the parietal eye is modified by efferent feedback from the pineal organ (Engbretson & Lent, 1976). Also, in *Sceloporus occidentalis*, the activity of pineal hydroxyindole-O-methyltransferase (HIOMT), the final enzyme in the melatonin biosynthetic pathway, is altered by parietlectomy (Quay *et al.* 1971; Bethea & Walker, 1978).

The present study examines the role of the parietal eye and the lateral eyes in mediating the photothermal regulation of diel rhythms in plasma melatonin levels in the scincid lizard, *Trachydosaurus rugosus*.

MATERIALS AND METHODS

General

Lizards (*Trachydosaurus rugosus* Gray) were collected in the vicinity of Adelaide, South Australia, in the spring of 1976 and were housed in enclosures, partly exposed to the natural environment. Animals were marked by toe-clipping, and the sex, snout-vent length (S-V) and weight recorded.

Prior to each experiment, lizards were acclimated in an environmentally controlled, light-proof box. The photothermal regimen for each experiment is specified below. Blood sampling and anaesthetic procedures have been described previously (Firth *et al.* 1979).

Lizards were parietectomized under tribromoethanol anaesthesia, according to the following procedure. The interparietal scale was removed, and the bony border surrounding the exposed parietal eye was pared away with a dental drill. The 'eye' was removed with watchmaker forceps, care being taken not to puncture the underlying meninges. Sham parietectomy consisted of drilling a small wound 1 mm lateral to the interparietal scale.

Photic input to the lateral eyes was eliminated by capping the eyes with surgical tape overlaid with aluminium foil, and sealed at the edges with contact cement. The control animals for this experiment had contact cement placed above the eyes.

Melatonin levels were determined by radioimmunoassay. This involved incubation with an antibody raised against *N*-acetyl serotonin-BSA, following extraction in borate buffer and chloroform and in columns of Lipidex 5000 (see Kennaway *et al.* 1977, and Firth *et al.* 1979 for details).

Experiment 1

Twenty lizards were parietectomized and sham-parietectomized (five males and five females in each treatment group) in November. They were acclimated for 12 days at a constant 24 °C, with a photoperiod of 13 h light, 11 h dark (13L:11D; photophase, 05.30–18.30 h, 240 lx incandescent). On the 12th night of acclimation, blood samples were taken around midnight from half of the lizards in each treatment group. The remaining half were sampled on the 13th night of acclimation. Precautions were taken to prevent the second group of lizards from being exposed to light while sampling the first group.

Experiment 2

Twenty-eight lizards were parietectomized and sham-parietectomized in April (the Austral Autumn). They were acclimated to a constant 24 °C and a reversed photoperiod of 13L:11D (photophase, 17.30–06.30; 240 lx incandescent) for 12 days. In early May one-half of each treatment group was sampled for blood around mid-photophase (24.00 h) and the remaining half around mid-scotophase (12.00 h).

The lizards were then returned to their enclosures for 2 weeks, following which they were reacclimated to the above conditions for 12 days. The animals were subjected to a similar blood sampling procedure as before, except that those previously sampled at 12.00 h were sampled at 24.00 h and vice versa.

Experiment 3

Male and female *T. rugosus* were divided into four treatment groups: parietectomized (P), lateral eyes shielded (E), parietectomized and lateral eyes shielded (PE) and a control group (C) which was subjected to both sham-parietectomy and sham eye-shielding.

Beginning in November, the lizards were acclimated for 12 days to a 13L:11D photoperiod delayed by 6 h (photophase, 11.30–00.30 h.) The intensity of the in-

candescent light source was increased to 830 lx, and the temperature in the photophase elevated to 31 °C. The scotophase temperature remained at 24 °C.

Blood samples were collected over a period of 1 h bracketing each of the following times: 06.00 h, 12.00 h, 18.00 h and 24.00 h. Because of the large number of animals sampled, the experiment was staggered over a period of 4 weeks (6 Nov. to 6 Dec.).

Statistical procedures

Experiment 1 was analysed by a factorial two-way analysis of variance with sex and experimental treatment as the factors.

In experiments 2 and 3, a three-way factorial analysis of variance was performed on \log_{10} transformed data. Time, experimental treatment and sex were the factors. Where appropriate, a Tukey's multiple comparison test was applied (Sokal & Rohlf, 1969).

An analysis of covariance was administered on all data to test for the effect of size on plasma melatonin levels.

RESULTS

In no experiment was a significant sexual difference ($P > 0.05$) in plasma melatonin concentrations evident. Similarly, the covariate of size (snout-vent length) did not significantly affect melatonin levels ($P > 0.05$).

(A) Effect of parietectomy on plasma melatonin levels at constant temperature

Fig. 1A shows plasma melatonin levels measured at midnight (mid-scotophase) in sham-parietectomized and parietectomized *T. rugosus* subjected to normal 13L:11D photoperiod. Parietectomy did not significantly alter melatonin levels ($P > 0.05$).

Reversing the photoperiod resulted in a concomitant shift in plasma melatonin concentration, mid-scotophase (midday) levels being higher than those at mid-photophase (midnight) ($P < 0.001$). Fig. 1B represents the data for the sampling periods of May and June combined, since analysis of variance with repeated measures indicated no significant difference ($P > 0.05$) between the two sets of data.

There was no significant difference ($P > 0.05$) in plasma melatonin concentration between parietectomized and sham-parietectomized lizards either at mid-scotophase or at mid-photophase.

(B) Effect of parietectomy and lateral eye-shielding on plasma melatonin levels in a fluctuating photothermal environment.

Fig. 2 illustrates the plasma melatonin concentrations of *T. rugosus* subjected to various experimental treatments and exposed to a delayed 13L:11D photoperiod accompanied by a thermoperiod of 31 °C (in the photophase) and 24 °C (scotophase). Analysis of variance on control and eye-shielded (C and E) lizards indicated that there was a diel fluctuation in plasma melatonin levels ($P < 0.001$), the peak of which coincided with the middle of the scotophase and the trough of the thermoperiod. Further analysis with a *Q* test (Sokal & Rohlf, 1969) revealed that the melatonin levels at 06.00 h were greater than at all other times ($P < 0.05$) and that the levels at

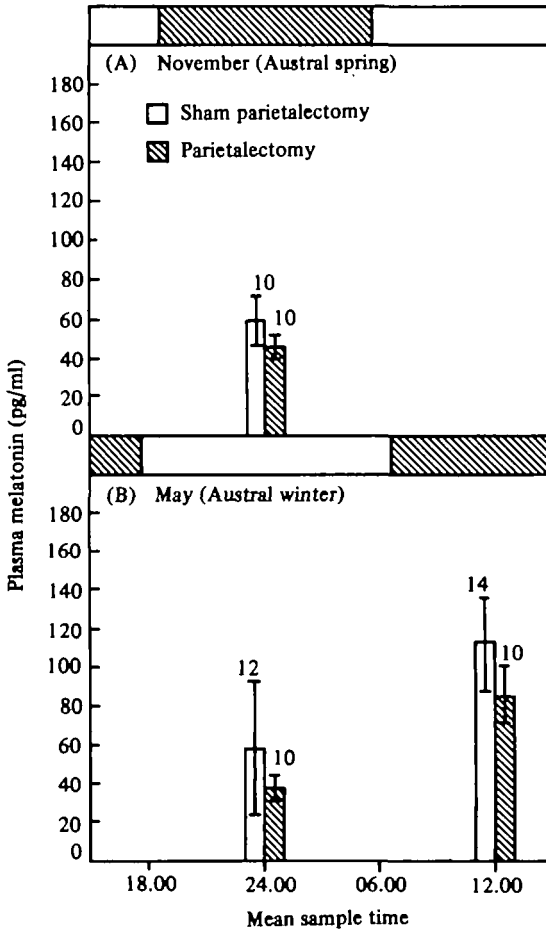


Fig. 1. Mean plasma melatonin levels of sham-parietalectomized and parietectomized *T. rugosus* in 13L:11D, in (A) normal photoperiod and (B) reversed photoperiod. The vertical lines represent 1 s.e.m. and the numbers above them the sample size, *N*. Shaded areas at the top of the figure represent the dark period of the lighting cycle.

24.00 h were higher than those at 12.00 h ($P < 0.05$). A similar analysis of the parietal-ectomized and parietectomized-eye shielded (P, PE) groups, however, did not indicate any diel fluctuation in melatonin concentrations ($P > 0.1$). The analysis also demonstrated that experimental treatment did not significantly affect melatonin levels when separately considering the C and E groups ($P > 0.7$) and the P and PE groups ($P > 0.3$). However, a grouping of the data based on the presence or absence of the parietal eye (i.e. C and E animals combined and P and PE animals combined) showed that parietectomy significantly lowered ($P < 0.01$) mid-scotophase levels of plasma melatonin and elevated them (although not significantly so) at 18.00 h (Fig. 3).

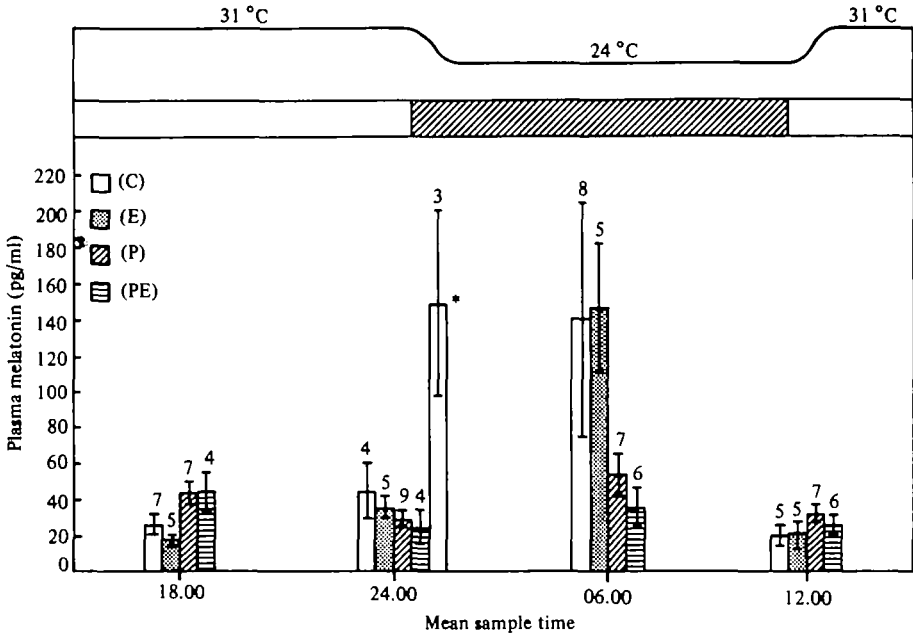


Fig. 2. Mean plasma melatonin levels of control, eye-capped, parietectomized and eye capped-parietectomized *T. rugosus* subjected to 13L:11D photoperiod delayed 6 h, and a thermo-period of 31 and 24 °C. The sample at 24.00 h indicated by an asterisk represents a control group of lizards sampled immediately following 'lights-off'. C, Control; E, eyes capped; P, parietectomized; PE, eyes capped and parietectomized. Other conventions as in Fig. 1.

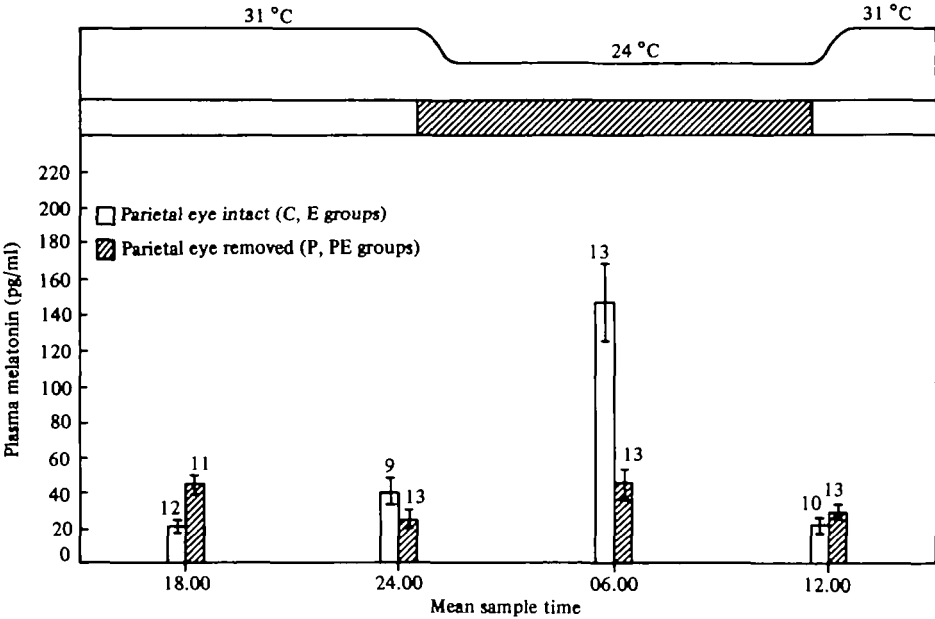


Fig. 3. Mean plasma melatonin levels of *T. rugosus*. Aggregated data based on Fig. 2, grouped according to the presence or absence of the parietal eye. Conventions as Figs. 1 and 2.

DISCUSSION

The present study confirms two previous observations in connexion with plasma melatonin levels in *T. rugosus* (Kennaway *et al.* 1977; Firth *et al.* 1979). At constant temperature there was (i) a light-dependent diel rhythm, with scotophase levels exceeding those in the photophase and (ii) an apparent seasonal fluctuation, with scotophase levels in spring being lower than those at other seasons. Nocturnal melatonin levels described here for winter (May; around 100 pg/ml) are slightly higher than spring levels (November; 60–80 pg/ml) but not as high as those reported by Kennaway *et al.* (1977) for mid-summer (January; 240 pg/ml). A similar seasonal rhythm in melatonin content (with peak levels in mid-summer and lower levels in spring) has been found in the pineal gland of the tortoise, *Testudo hermanni* (Vivien-Roels, Arendt & Bradtke, 1979).

It is well documented that, in birds and mammals, melatonin secretion is closely coupled to the environmental lighting cycle (Ralph, 1976). The present demonstration of a photoperiod-dependent shift in plasma melatonin levels in *T. rugosus* confirms that this is true for lizards. Under a reversed photoperiod, there is a complete phase reversal of melatonin titres within 12 days. A similar light-dependent rhythm in pineal HIOMT activity in the lizard *Lampropholis guichenoti* has been shown to become entrained to an altered light-cycle within 5 days (Joss, 1978) and in rats a 12 h shift in environmental lighting has been shown to re-entrain the melatonin excretion rhythm within 5–7 days (Adler, Lynch & Wurtman, 1979).

The low nocturnal levels of plasma melatonin in *T. rugosus* in November (Fig. 1 A) were comparable to those previously recorded for that month (Firth *et al.* 1979). However, the nocturnal melatonin titres of control and eye-shielded lizards exposed simultaneously to a photoperiod and thermoperiod in November (Figs. 2, 3) were almost double those subjected to a photoperiod at constant temperature during the same month. Such elevated nocturnal melatonin concentrations may have been due to one or both of two factors: (i) the higher intensity of illumination during the photophase (830 lx *v.* 240 lx) or (ii) the higher photophase temperature (31 °C versus 24 °C). As to the first possibility, constant light intensity has been shown to be inversely related to pineal HIOMT activity (Betha & Walker, 1978), but no studies have been conducted to test whether photophasic light intensity may influence scotophasic pineal or plasma melatonin content. However, there is some precedent for suggesting that ambient temperature may influence either the secretion, release or degradation of melatonin. For example, the activity of pineal HIOMT in field populations of lizards (*Sceloporus occidentalis*) has been observed to be lower on cold days than on warm days (Quay *et al.* 1971). Similarly, Eichler & Moore (1975) showed that diencephalic HIOMT activity in the frog *Rana pipiens* was temperature-sensitive. Under a photoperiod and a thermoperiod (12 h light, 27 °C: 12 h dark, 22 °C), a diel rhythm in HIOMT activity was present, but this rhythm was abolished if the same lighting conditions were imposed at a constant 22 °C. In rats, the activities of two of the enzymes involved in pineal melatonin synthesis, HIOMT and *N*-acetyltransferase (NAT) are depressed by a high temperature of 33 °C (Nir, Hirschmann & Sulman, 1975; Nir & Hirschmann, 1978). Furthermore, in 12-day-old suckling rats, the activity of pineal NAT is more sensitive to temperature changes than to light

fluctuations, although pineal HIOMT activity is unresponsive to both environmental variables (Ulrich *et al.* 1973). Such thermal sensitivity may be related to the poikilothermic nature of suckling rats, since artificial cycles in environmental temperature do not affect the daily rhythm of melatonin excretion in adult animals (Adler *et al.* 1979).

The observation that an alteration of photophase temperature affects melatonin levels in the scotophase (Figs. 2, 3) suggests the possibility of a photoperiod-temperature interaction in the regulation of rhythms in plasma melatonin concentration in *T. rugosus*. Recent studies on plasma melatonin levels of salamanders subjected to a variety of photothermal regimens suggest that such a phenomenon is true for ectothermic vertebrates in general. W. A. Gern & D. O. Norris (personal communication) have found in *Ambystoma tigrinum* that the rhythm in serum melatonin concentration was most prominent when the temperature was highest (20 °C) during the photophase. As in *T. rugosus*, the diel rhythm in melatonin levels was present in the absence of a temperature cycle. However, this was only obvious at low, constant temperature (10 °C).

Shielding the lateral eyes of *T. rugosus* did not significantly alter the rhythm in plasma melatonin content from that of controls. It is unlikely that such lateral eye impairment simulated the effect of constant darkness, since the latter appears to inhibit the rhythm of plasma melatonin concentration in this species (Firth *et al.* 1979). The most probable explanation is that, in *T. rugosus*, melatonin synthesis and/or release is extraretinally mediated. Studies in optic tract-sectioned trout have similarly shown persistent plasma melatonin rhythms, indicating a direct photosensory input to the melatonin-secreting source(s) (Gern *et al.* 1978a). These observations are consistent with the view that, in many ectothermic vertebrates, the pineal organ, which is presumed to be the major source of melatonin secretion, perceives light directly (Collin, 1971; Kappers, 1971) rather than via accessory optic tracts as in mammals (Moore, 1978).

Our study indicates that, in *T. rugosus*, at least one extraretinal photoreceptive structure, the parietal eye, may help to mediate environmental information for the regulation of rhythms in plasma melatonin content. The exact nature of the environmental information mediated by the parietal eye for this purpose requires further investigation. However, the observation that parietectomy altered plasma melatonin titres only under fluctuating ambient temperatures suggests that the parietal eye may mediate changes in plasma melatonin levels that are induced photothermally or thermally. At least two studies indirectly support the notion that the parietal eye might mediate photothermal factors influencing melatonin production. Parietectomy in *S. occidentalis*, subjected to constant light, has been shown to elevate pineal HIOMT activity, especially at high light intensities (Betha & Walker, 1978). In a natural population of the same species, Quay *et al.* (1971) showed that pineal HIOMT activity was lowered by parietectomy on cold days, but not on warm days. However, since pineal melatonin levels may not identically parallel pineal HIOMT activity (Klein & Weller, 1970; Binkley, 1976), no firm conclusions can be drawn from these studies with respect to plasma melatonin. It would also need to be presumed that the parietal eye exerts its main influence over the pineal organ rather than other possible

melatonin sources, although pharmacological studies indicate a parietal-pineal interaction (Engbretson & Lent, 1976). The possibility also remains that the parietal eye itself produces melatonin, since high HIOMT activity has been demonstrated in the parietal eye of *Iguana iguana* (Quay, 1965).

The above data point to the possibility that the parietal eye acts as a relay for the processing of photothermal environmental information, and that melatonin may be a chemical transducer of such information. The ultrastructural appearance of the 'eye' (Eakin, 1973) and certain behavioural studies (Stebbins, 1970) have led to the conclusion that this structure is purely photoreceptive. On the other hand, studies on thermoregulatory behaviour (Hutchison & Kosh, 1974; Engbretson & Hutchison, 1976; Roth & Ralph, 1976, 1977) and thermal tolerance (Kosh & Hutchison, 1972; Firth & Heatwole, 1976) indicate that the parietal eye may be sensitive to thermal as well as photic information.

Licht (1972) has stressed the importance of temperature, particularly in the spring, in regulating reptilian seasonal physiological cycles. Indeed, in some species there is a complex interaction between temperature and photoperiod in determining gonadal cycles (Licht, 1967*a, b*, 1969). Consequently, factors such as the apparent seasonal sensitivity of the parietal eye (Firth & Heatwole, 1976) and its possible mediation of thermal as well as photic input should be considered in the design of future experiments concerning the seasonal aspects of this organ's function.

We are grateful to Marilyn Campion for statistical advice and to Dr R. F. Seamark for his interest in this study. We also thank Mr J. S. Turner, and Drs W. A. Gern, M. Kavaliers and C. L. Ralph for critically reading the manuscript. This work was supported by a University of Adelaide Postdoctoral Fellowship (to B. T. F.) and the Sir John Gellibrand Memorial Scholarship (to D. J. K.). Some of the manuscript costs were covered by NIH grant NS 12257 to Dr C. L. Ralph.

REFERENCES

- ADLER, J., LYNCH, H. J. & WURTMAN, R. J. (1979). Effect of cyclic changes in environmental lighting and ambient temperature on the daily rhythm in melatonin excretion by rats. *Brain Res.* **163**, 111-120.
- BETHEA, C. L. & WALKER, R. F. (1978). Parietal eye-pineal gland interactions in the lizard *Sceloporus occidentalis* (Reptilia, Lacertilia, Iguanidae). *J. Herpetol.* **12**, 83-87.
- BINKLEY, S. (1976). Comparative biochemistry of the pineal glands of birds and mammals. *Am. Zool.* **16**, 57-66.
- COLLIN, J. P. (1971). Differentiation and regression of the cells of the sensory line in the epiphysis cerebri. In *The Pineal Gland* (ed. G. E. W. Wolstenholme and J. Knight), pp. 79-125. London/Edinburgh: Churchill-Livingstone.
- DODT, E. (1973). The parietal eye (pineal and parapineal organs) of lower vertebrates. In *Handbook of Sensory Physiology* (ed. R. Jung), vol 7/3B, pp. 113-140. Heidelberg: Springer-Verlag.
- EAKIN, R. M. (1973). *The Third Eye*. Berkeley: University of California Press.
- EICHLER, V. B. & MOORE, R. Y. (1975). Studies on hydroxyindole-O-methyltransferase in frog brain and retina: enzymology, regional distribution and environmental control of enzyme levels. *Comp. Biochem. Physiol.* **50C**, 89-95.
- ENGBRETSON, G. A. & HUTCHISON, V. A. (1976). Parietalectomy and thermal selection in the lizard *Sceloporus magister*. *J. exp. Zool.* **198**, 29-38.
- ENGBRETSON, G. A. & LENT, C. M. (1976). Parietal eye of lizard-neuronal photoresponses and feedback from pineal gland. *Proc. natn. Acad. Sci. U.S.A.* **73**, 654-657.
- FIRTH, B. T. & HEATWOLE, H. (1976). Panting thresholds of lizards: the role of the pineal complex in panting responses in an agamid, *Amphibolurus muricatus*. *Gen. comp. Endocr.* **29**, 388-401.

- FIRTH, B. T., KENNAWAY, D. J. & ROZENBILDS, M. A. M. (1979). Plasma melatonin in the scincid lizard, *Trachydosaurus rugosus*: diel rhythm, seasonality, and the effect of constant light and constant darkness. *Gen. comp. Endocr.* **37**, 493-500.
- GERN, W. A. & NORRIS, D. O. (1979). Plasma melatonin in the neotenic tiger salamander (*Ambystoma tigrinum*): effect of photoperiod and pinealectomy. *Gen. comp. Endocr.* (in the Press).
- GERN, W. A., OWENS, D. W. & RALPH, C. L. (1978a). Persistence of the nycthemeral rhythm of melatonin secretion in pinealectomized or optic tract-sectioned trout (*Salmo gairdneri*). *J. exp. Zool.* **205**, 371-376.
- GERN, W. A., OWENS, D. W. & RALPH, C. L. (1978b). Plasma melatonin in the trout: day-night changes demonstrated by radioimmunoassay. *Gen. comp. Endocr.* **34**, 453-458.
- GERN, W. A. & RALPH, C. L. (1979). Melatonin synthesis by the retina. *Science, N.Y.* **204**, 183-184.
- GLASER, R. (1958). Increase in locomotor activity following shielding of the parietal eye in night lizards. *Science, N.Y.* **128**, 1577-1578.
- HUTCHISON, V. H. & KOSH, R. J. (1974). Thermoregulatory function of the parietal eye in the lizard *Anolis carolinensis*. *Oecologia* **16**, 173-177.
- JOSS, J. M. P. (1978). A rhythm in hydroxyindole-O-methyltransferase (HIOMT) activity in the scincid lizard, *Lampropholis guichenoti*. *Gen. comp. Endocr.* **36**, 521-525.
- KAPPERS, J. A. (1971). The pineal organ: an introduction. In *The Pineal Gland* (ed. G. E. W. Wolstenholme and J. Knight), pp. 3-34. London/Edinburgh: Churchill-Livingstone.
- KENNAWAY, D. J., FRITH, R. G., PHILLIPOU, G., MATTHEWS, C. D. & SEAMARK, R. F. (1977). A specific radioimmunoassay for melatonin in biological tissue and fluids and its validation by gas chromatography-mass spectrometry. *Endocrinology* **101**, 119-127.
- KLEIN, D. C. & WELLER, J. L. (1970). Indole metabolism in the pineal gland: a circadian rhythm in *N*-acetyltransferase. *Science, N.Y.* **169**, 1093-1095.
- KOSH, R. J. & HUTCHISON, V. H. (1972). Thermal tolerances of parietectomized *Anolis carolinensis* acclimatized at different temperatures and photoperiods. *Herpetologica* **28**, 183-191.
- LICHT, P. (1967a). Environmental control of annual testicular cycles in the lizard *Anolis carolinensis*. I. Interaction of light and temperature in the initiation of testicular recrudescence. *J. exp. Zool.* **165**, 505-516.
- LICHT, P. (1967b). Environmental control of annual testicular cycles in the lizard *Anolis carolinensis*. II. Seasonal variations in the effects of photoperiod and temperature on testicular recrudescence. *J. exp. Zool.* **166**, 243-254.
- LICHT, P. (1969). Environmental control of annual testicular cycles in the lizard *Anolis carolinensis*. III. Temperature thresholds for photoperiodism. *J. exp. Zool.* **172**, 311-322.
- LICHT, P. (1972). Problems in experimentation on timing mechanisms for annual physiological cycles in reptiles. In *Hibernation and Hypothermia, Perspectives and Challenges* (ed. F. South), pp. 681-711. New York: American Elsevier.
- MOORE, R. Y. (1978). The innervation of the mammalian pineal gland. In *The Pineal and Reproduction. Progress in Reproductive Biology* (ed. R. J. Reiter), vol. 4, pp. 1-29. Basel: Karger.
- NIR, I. & HIRSCHMANN, N. (1978). Pineal *N*-acetyltransferase depression in rats exposed to heat. *Experientia* **34**, 1645-1646.
- NIR, I., HIRSCHMANN, N. & SULMAN, F. G. (1975). The effect of heat on rat pineal hydroxyindole-O-methyltransferase activity. *Experientia* **31**, 867-868.
- OWENS, D. W., GERN, W. A. & RALPH, C. L. (1978). The effects of pinealectomy and sham pinealectomy on serum melatonin in the green sea turtle. *Am. Zool.* **18**, 625 (Abst.).
- OZAKI, Y. & LYNCH, H. J. (1976). Presence of melatonin in plasma melatonin and urine of pinealectomized rats. *Endocrinology* **99**, 641-644.
- PACKARD, G. C. & PACKARD, M. J. (1972). Photic exposure of the lizard *Callisaurus draconoides* following shielding of the parietal eye. *Copeia*, 1972, pp. 695-701.
- PALENSCHAT, D. (1964). Beitrag zur lokomotorischen Aktivität der Blindschleiche (*Anguis fragilis* L.) unter besonderer Berücksichtigung des Parietalorgans. Ph.D Dissertation, Göttingen University.
- QUAY, W. B. (1965). Retinal and pineal hydroxyindole-O-methyltransferase activity in vertebrates. *Life Sci.* **4**, 983-991.
- QUAY, W. B., STEBBINS, R. C., KELLEY, T. D. & COHEN, N. W. (1971). Effects of environmental and physiological factors on pineal acetylserotonin methyltransferase activity in the lizard *Sceloporus occidentalis*. *Physiol. Zool.* **44**, 241-248.
- RALPH, C. L. (1976). Correlations of melatonin content in pineal gland, blood, and brain of some birds and mammals. *Am. Zool.* **16**, 35-43.
- RALPH, C. L., FIRTH, B. T., GERN, W. A. & OWENS, D. W. (1979). The pineal complex and thermoregulation. *Biol. Rev.* **54**, 41-72.
- REITER, R. J. (1978). Interaction of photoperiod, pineal and seasonal reproduction as exemplified by findings in the hamster. In *The Pineal and Reproduction, Progress in Reproductive Biology* (ed. R. J. Reiter), vol. 4, pp. 169-190. Basel: Karger.

- ROTH, J. J. & RALPH, C. L. (1976). Body temperature of the lizard (*Anolis carolinensis*): effect of parietal-ectomy. *J. exp. Zool.* **198**, 17-28.
- ROTH, J. J. and RALPH, C. L. (1977). Thermal and photic preferences in intact and parietal-ectomized *Anolis carolinensis*. *Behav. Biol.* **19**, 341-348.
- SOKAL, R. R. & ROHLF, F. J. (1969). *Biometry*. San Francisco: Freeman.
- STEBBINS, R. C. (1963). Activity changes in the striped plateau lizard with evidence on influence of the parietal eye. *Copeia* 1963, 681-691.
- STEBBINS, R. C. (1970). The effect of parietal-ectomy on testicular activity and exposure to light in the desert night lizard (*Xantusia vigilis*). *Copeia*, 1970, pp. 261-269.
- STEBBINS, R. C. & COHEN, N. W. (1973). The effect of parietal-ectomy on the thyroid and gonads in free-living western fence lizards, *Sceloporus occidentalis*. *Copeia*, 1973, pp. 662-668.
- STEBBINS, R. C. & EAKIN, R. M. (1958). The role of the 'third eye' in reptilian behavior. *Am. Mus. Nov.* no. 1870, pp. 1-40.
- STEBBINS, R. C. & WILHOFT, D. C. (1966). Influence of the parietal eye on activity in lizards. In *The Galapagos* (ed. R. I. Bowman), pp. 258-268. Berkeley: University of California Press.
- TUREK, F. W., McMILLAN, J. P. & MENAKER, M. (1976). Melatonin: effects on the circadian locomotor rhythms of sparrows. *Science, N. Y.* **194**, 1441-1443.
- ULRICH, R., YUWILER, A., WETTERBERG, L. & KLEIN, D. (1973/4). Effects of light and temperature on the pineal gland in suckling rats. *Neuroendocrinology* **13**, 255-263.
- VIVIEN-ROELS, B., ARENDT, J. & BRADTKE, J. (1979). Circadian and circannual fluctuations of pineal indoleamines (serotonin and melatonin) in *Testudo hermanni* Gmelin (Reptilia, Chelonia). 1. Under natural conditions of photoperiod and temperature. *Gen. comp. Endocr.* **37**, 197-210.