

## **Circulating Levels of Melatonin following Its Oral Administration or Subcutaneous Injection in Sheep and Goats**

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### *Abstract*

The effect of differing doses and routes of administration of melatonin on plasma melatonin levels in sheep and goats has been examined. Melatonin injected subcutaneously in a saline or oil vehicle caused high transitory peaks in plasma melatonin whereas oral administration, in either saline solution or adsorbed onto pelleted foodstuff, resulted in sustained elevated blood levels for periods exceeding 7 h. Oral dosages of about 2 mg proved adequate to raise the normal daytime plasma levels in both sheep and goats to levels within the normal night-time range.

It was concluded that with ruminants the oral route of administration provides a convenient and practical way of administering melatonin for physiological study.

### **Introduction**

Since the discovery of the indoleamine derivative melatonin in the pineal gland by Lerner *et al.* in 1958 there have been many attempts to determine its physiological role(s) in vertebrates (see Minneman and Wurtman 1975). Particular emphasis has been given to the possibility that it mediates the antigonadotrophic role of the pineal because, in rats, melatonin injection during the pro-oestrous phase of the cycle will suppress the LH surge and thus prevent ovulation (Ying and Greep 1973). More recently melatonin has been implicated in the control of seasonal gonadal regression in hamsters (Tamarkin *et al.* 1976). These findings, together with the development of radioimmunoassays for circulating blood levels of melatonin in many species (Arendt *et al.* 1975; Rollag and Niswender 1976; Kennaway *et al.* 1977), have resulted in an upsurge in interest in how melatonin and the pineal gland exert their influence on gonadal function. Many of the early experiments in which injection of melatonin was used to evoke physiological or endocrine changes can be criticized not only on the basis of administration at inappropriate times (Tamarkin *et al.* 1976) but also on the basis that pharmacological rather than physiological doses of melatonin were used (Minneman and Wurtman 1975). An essential prerequisite to our intended studies of the endocrine effects of melatonin in the larger domesticated animals (sheep, goats) was therefore to examine the effects of different dosages and routes of administration of melatonin with the aim of determining a protocol that would result in increases in circulating melatonin levels which would remain within the normal physiological range.

### **Materials and Methods**

The studies were carried out using two adult Merino crossbred ewes and eight female goats which had been acclimatized to the animal house conditions [temp. 21°C, lighting regime 12 h

light : 12 h dark (sheep) or 16 h light : 8 h dark (goats)] for at least 3 weeks prior to the experimental period (16 October–7 November, 1979). The animals were maintained on a lucerne chaff diet and were fed at 1600 h.

The melatonin was administered to the sheep in various formulations allowing at least 2 days between each treatment:

- (1) orally, either (a) in aqueous solution [10 ml of a water–ethanol (8:2 v/v) mixture containing 10 mg melatonin] or (b) added to 100-g lots of pelleted foodstuff (lucerne chaff). The melatonin (in amounts of 2 and 10 mg) was added to the preformulated pellets in alcohol, the solvent being allowed to evaporate before feeding; untreated pellets were offered to animals serving as controls. In both groups pellets were eaten within 4 min of being offered.
- (2) by injection (subcutaneously) either in (a) aqueous solution [1 mg in 2 ml of a saline–ethanol (9:1 v/v) mixture] or (b) oil solution (100 µg melatonin in 1 ml peanut oil). All trials commenced at 0900 h, which was 2 h after lights on, and samples collected by jugular venepuncture for up to 7 h.

Four goats were fed 2 mg melatonin adsorbed onto pelleted food on five consecutive days at 1600 h (12 h after lights on) and four other goats were fed untreated pellets as controls. Both groups were bled at 1630 h.

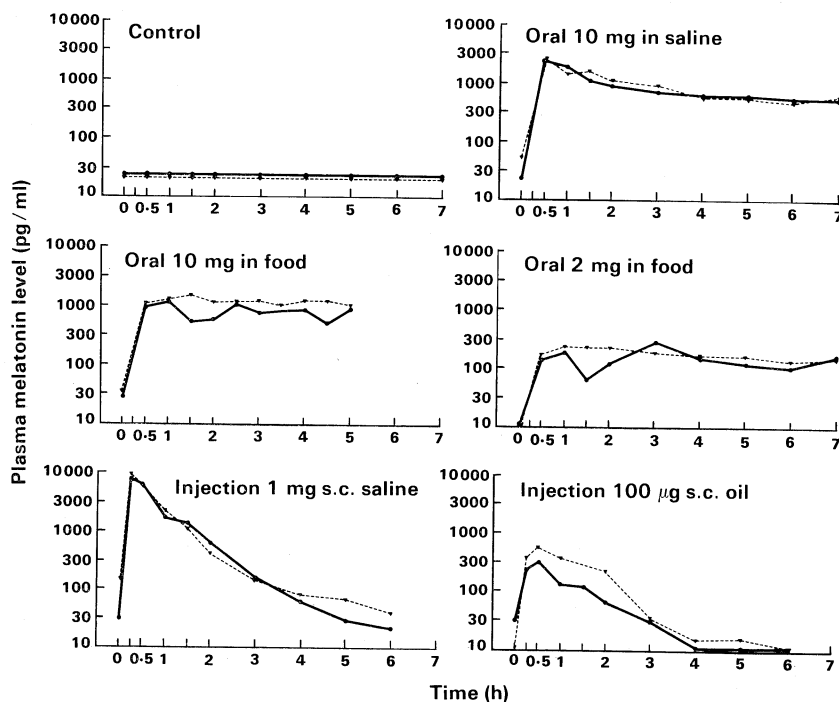


Fig. 1. Plasma melatonin levels following melatonin administration to two Merino cross-bred sheep.

Plasma melatonin was determined by a radioimmunoassay (Kennaway *et al.* 1977). After the addition of borate buffer (0.5 M; pH 10; 1 ml), 0.1–2 ml of plasma was extracted with dichloromethane and the organic extract chromatographed on Lipidex 5000 columns with dichloromethane:hexane (1:1 v/v) as eluant. The melatonin fraction was collected and subjected to assay. Sensitivity of the assay was 18 pg/ml and 50% inhibition was produced by 140 pg of melatonin. Recovery of added melatonin was 75% and intra- and interassay coefficients of variation less than 10 and 20% respectively. The radioimmunoassay showed no cross reactivity (<0.002%) with any known melatonin metabolites likely to be circulating in blood plasma following melatonin administration.

## Results

Fig. 1 summarizes the results obtained for melatonin administration to the sheep. Melatonin was not detected ( $<20$  pg/ml) in daytime samples taken prior to experiment or during a period following control feeding of untreated pellets but reached high levels following all treatments with melatonin. Subcutaneous injection of melatonin, whether in a saline or oil vehicle, resulted in an extremely rapid rise in plasma hormone titres to peak within 15 min, the subsequent decay indicating an apparent half-life for plasma melatonin of about 30 min. Four hours after injection, blood levels had returned to non-detectable values. Oral administration of melatonin in either saline solution or adsorbed onto food pellets resulted in blood melatonin levels which rose within 30 min to a plateau which was sustained for at least 7 h.

The results of oral administration of 2 mg melatonin in pellets to four goats are shown in Table 1. Melatonin was apparent in blood for all the treated goats within 30 min at concentrations similar to observed night-time values (Kennaway and Seamark, unpublished data), whereas no melatonin was detectable in blood taken from the control animals. At 0900 h on the morning following melatonin feeding, plasma melatonin was again at undetectable levels in all goats.

**Table 1. Plasma melatonin levels in goats**

Levels measured 30 min after oral administration of 2 mg melatonin adsorbed onto 100 mg pelleted food at 1600 h (see text for details). Plasma melatonin levels in four control goats determined 30 min after feeding untreated pellets were all below 30 pg/ml

	G131	Melatonin level (pg/ml)		G282
		G81	G270	
Day 1	231	144	150	61
Day 2	212	175	97	45
Day 3	200	200	240	115
Day 4	368	287	100	144
Day 5	165	259	115	184
Mean	235	213	140	110
s.e.	35	26	26	26

## Discussion

Recent studies in the sheep (Kennaway *et al.* 1977, 1978; Arendt *et al.* 1979) indicate that during the day plasma melatonin levels are rarely above 30 pg/ml but with the onset of darkness rapidly rise tenfold and remain at these elevated values throughout the dark period. By contrast Rollag *et al.* (1978a, 1978b) have reported higher mean daytime melatonin levels (200 pg/ml) but found a similar tenfold increment during the night-time period.

This nocturnal rise is most readily mimicked by oral feeding. In the present study, oral dosages as low as 2 mg melatonin resulted in a period of at least 7 h when plasma melatonin levels were sustained at high night-time values. This pattern is in marked contrast to the transitory peak in plasma melatonin obtained as a result of feeding high dosages of melatonin to the human (Wetterberg *et al.* 1978) and is probably the result of the mixing of the administered melatonin with the contents of the commodious rumen and its subsequent slow release; the contents themselves have

a half-time of 9 h for the fluid and 19 h for solid fraction (Faichney 1975). Absorption, once the melatonin has left the rumen, is probably rapid and could occur either in the omasum, abomasum or upper intestine. One impression gained from these observations is that melatonin is not actively metabolized in the rumen, but in view of the likely variation in rumen fauna and flora the generality of this supposition would need to be examined further.

Melatonin given by injection was rapidly cleared. This is consistent with our other studies in the sheep and in man using heavy isotope (deuterium) labelled melatonin (A. J. Fellenberg, G. Phillipou and R. F. Seamark, unpublished data). Studies of urinary production in man (Fellenberg, Phillipou and Seamark 1980) and more recently in sheep (same authors, unpublished observations) indicate daily production rates in both species of about 30  $\mu\text{g/day}$ . Thus an oral dosage of 2 mg necessary to mimic the nocturnal rise still represents a considerable overdose. Oral administration, however, is a convenient way of administration and this technique is presently being used to examine the effects of melatonin on reproductive function in sheep and goats both in the laboratory and field environment.

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### References

- Arendt, J., Paunier, L., and Sizonenko, P. C. (1975). Melatonin radioimmunoassay. *J. Clin. Endocrinol. Metab.* **40**, 347–50.
- Arendt, J., Symons, A. M., and Marston, A. (1979). Annual variations of radioimmunoassayable melatonin (RIA-MT) in the ewe. *Acta Endocrinol. Suppl.* **225**, 239.
- Faichney, G. J. (1975). The use of markers to partition digestive function within the gastro-intestinal tract of ruminants. In 'Digestion and Metabolism in the Ruminant'. Proceedings of the IVth International Symposium on Ruminant Physiology held in Sydney, Australia, 1974. (University of New England Publishing Unit: Armidale.)
- Fellenberg, A. J., Phillipou, G., and Seamark, R. F. (1980). Specific quantitation of urinary 6-hydroxy melatonin sulphate by GC-MS. *Biomed. Mass Spectrom.* (In press.)
- 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 spectrometry. *Endocrinology* **101**, 119–27.
- Kennaway, D. J., Porter, K. J., and Seamark, R. F. (1978). Changes in plasma tryptophan and melatonin content in penned sheep. *Aust. J. Biol. Sci.* **31**, 49–52.
- Lerner, A. B., Case, J. D., Takahashi, Y., Lee, T. H., and Mori, W. (1958). Isolation of melatonin, the pineal gland factor that lightens melanocytes. *J. Am. Chem. Soc.* **80**, 2587.
- Minneman, K. P., and Wurtman, R. J. (1975). Effects of pineal compounds on mammals. *Life Sci.* **17**, 1189–99.
- Rollag, M. D., and Niswender, G. D. (1976). Radioimmunoassay of serum concentrations of melatonin in sheep exposed to different lighting regimens. *Endocrinology* **98**, 482–9.
- Rollag, M. D., O'Callaghan, P. L., and Niswender, G. D. (1978a). Serum melatonin concentrations during different stages of the annual reproductive cycle in the ewe. *Biol. Reprod.* **18**, 279–85.
- Rollag, M. D., Morgan, R. J., and Niswender, G. D. (1978b). Route of melatonin secretion in sheep. *Endocrinology* **102**, 1–8.
- Tamarkin, L., Westrom, W. K., Hamill, A. I., and Goldman, B. D. (1976). Effect of melatonin on the reproductive systems of male and female Syrian hamsters: a diurnal rhythm in sensitivity to melatonin. *Endocrinology* **99**, 1534–41.

- Wetterberg, L., Eriksson, O., Friberg, Y., and Vangbo, B. (1978). A simplified radioimmunoassay for melatonin and its application to biological fluids. Preliminary observations on the half-life of plasma melatonin in man. *Clin. Chim. Acta* **86**, 169-77.
- Ying, S-Y., and Greep, R. (1973). Inhibition of ovulation by melatonin in the cyclic rat. *Endocrinology* **92**, 333-5.

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