Pineal function in the sheep: evidence for a possible mechanism mediating seasonal reproductive activity[1]

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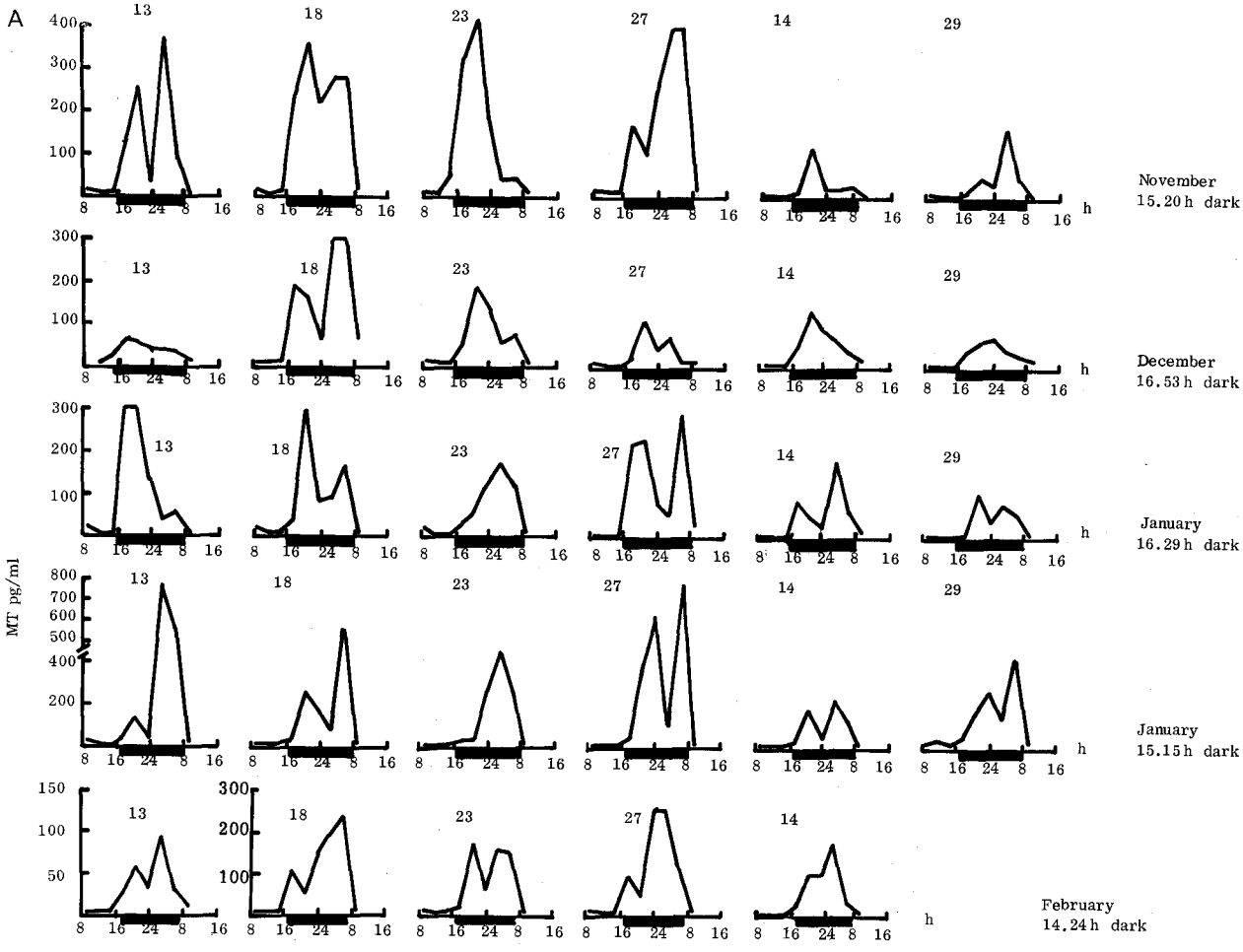
Summary. The 24-h profiles of plasma melatonin in the intact ewe in natural light indicate that a bimodal pattern of secretion is frequently present in oestrus, whereas a single dark-phase peak is characteristic of anoestrus. Based on these findings, a mechanism for the possible pineal-mediated control of seasonal breeding is proposed.

The pineal gland appears to be of importance in the mediation of photoperiodic responses in sheep. Pinealectomy removes some photoperiod-related hormonal changes[3,4] and superior cervical ganglionectomy (which effectively denervates the pineal, together with other sympathetically innervated cranial structures), eliminates the ability to respond to artificial photoperiod changes[5]. The absence of the pineal may not be of importance in the short-term control of reproductive activity[6], nevertheless its presence may assure long-term synchronisation with season as has been shown for the ferret[7].

Well documented evidence from various species indicates that the pineal gland produces a biochemical response to changes in light and dark: light inhibits and dark stimulates synthesis of the indoleamine melatonin in all species studied to date, whether nocturnal or diurnal[8]. Furthermore, pinealectomy and superior cervical ganglionectomy both greatly reduce circulating melatonin[9,10]. Melatonin itself is known to have both antigonadotrophic and progonadotrophic effects in the hamster, in vivo, dependent on the dose, the time and the light regime[11,12] and in view of the control mechanisms of its synthesis, it is clearly a candidate for the transmission of information concerning the light-dark phase to the neuroendocrine axis.

Circulating immunoreactive melatonin in the ewe has a 24-h rhythm, being elevated during darkness[13,14]. The rhythm is eliminated in continuous light[13] and the duration of melatonin secretion appears to monitor length of darkness[15]. We have sought to determine the 24-h profiles of circulating immunoreactive melatonin in oestrous and anoestrous ewes under natural photoperiodic conditions.

4–6 Suffolk-cross ewes were used. The animals were aged approximately 6 months at the beginning of the experiment and were kept permanently in natural light. Diet consisted



A Plasma melatonin (pg/ml) in 5–6 individual sheep (Nos 13, 18, 23, 27, 14, 29) at 3-h intervals throughout 24 h at different times of oestrus. Darkness is indicated by the black bar. 21 out of 29 profiles show evidence of a bimodal secretion pattern.

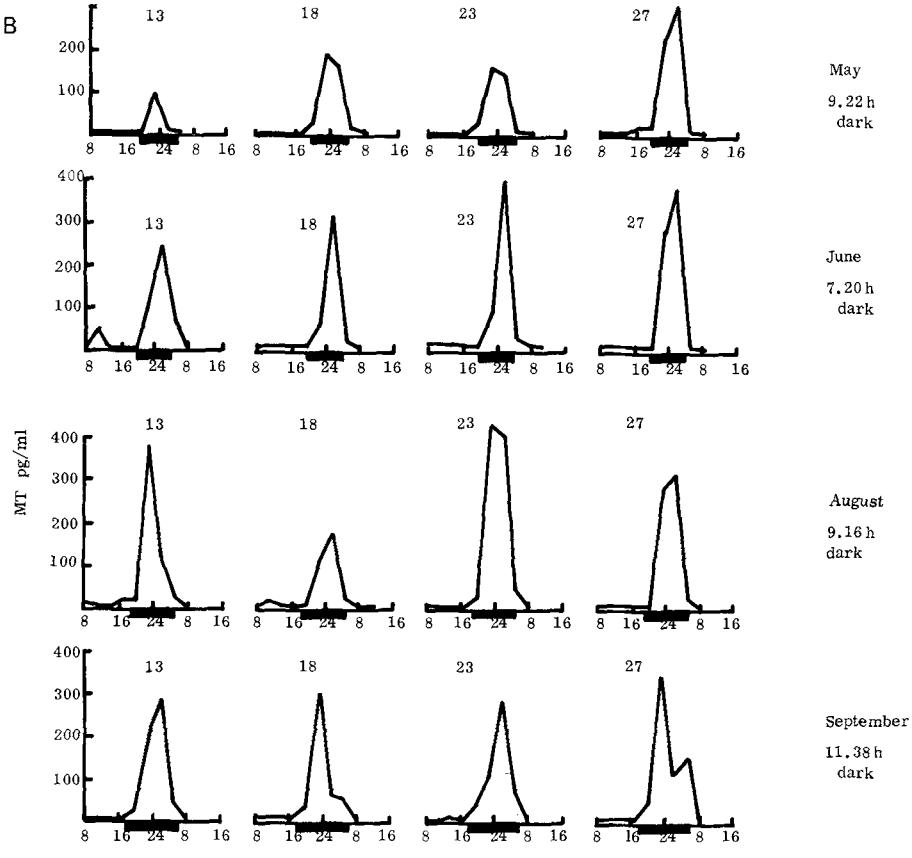
of 2 kg hay and 1 kg ewe and lamb nuts (B.P. Nutrition (U.K.) Ltd, Witham, Essex) per ewe per day. Animals were frequently handled to avoid experimental stress as far as possible. For melatonin determinations blood was sampled from the jugular vein using vacutainer tubes (lithium heparin anticoagulant, Becton and Dickinson Ltd) at 3-h intervals throughout 24 h at 2-week to 3-month intervals for 1 year. All dark-phase samples were taken with the help of a dim red torch and avoiding light in the animals eyes. Samples were taken according to clock hour, starting and finishing at 09.00 h. During the months from May to September this was equivalent to starting and finishing at 08.00 h. Throughout the study the blood was also sampled, as previously described, 2–3 times per week between 09.00 and 12.00 h in order to determine reproductive status by progesterone levels. Plasma was either decanted immediately or blood was stored for a maximum of 6 h at 0–4 °C before separation, and plasma was kept deep frozen until assayed.

Melatonin was determined by radioimmunoassay as previously described for human serum[16] using antibody K244: 1 or 2 ml of plasma were extracted and samples were assayed in duplicate. This assay was validated for sheep plasma as follows: parallelism of pooled extracted, day and night-time sheep plasma with the standard curve was demonstrated; TLC identity of extracted immuno-reactivity with standard melatonin was shown in 2 different systems at 5 different times of year, using pooled day- and night-time plasma (silica gel G plates, chloroform:methanol, 9:1 and n-butanol solvents); recovery of melatonin ^{3}H was 88.5% (7.4% coefficient of variation = CV; recovery of 100 and 300 pg melatonin was 88.2% (19.8% CV) and 92.6% (16.9% CV) respectively; inter-assay variability during this series of experiments was 16.8% and 20.2% at 33 and 101 pg respectively; intra-assay variability in 16.4%, 7.8% and 11.1% at 15, 45 and 93 pg respectively; estimated detection limit of the assay varied from 14 to 29 pg/ml plasma. All samples from any particular 24-h experiment were determined in the same assay. Progesterone was assayed by radioimmunoassay[17] using antiserum HP/S/53-IIC (Guildhay Antisera, Guildford).

Length of oestrus was defined as the number of days from the end of the first clear progesterone peak to the end of the last clear progesterone peak. Length of oestrous cycle was defined as the number of days between peaks of progesterone secretion. Darkness was taken as the time from sunset to sunrise and thus includes twilight.

Progesterone determinations indicated that all ewes were undergoing regular 16–18 day oestrous cycles from mid-November to late January, and no ewes were cycling from May to September. No relationship was found between stage of oestrous cycle and plasma melatonin levels, as previously observed[15].

Plasma melatonin concentration throughout 24 h is shown for each individual ewe at different times of oestrus and anoestrus in figure A and B. As previously reported[13–15], melatonin levels are elevated during the dark phase and low to undetectable during the light phase of the day. Marked individual differences were found in quantity and timing of melatonin secretion during the dark phase of the day, notably in oestrus. In anoestrus a single homogenous peak of melatonin is evident. In June, with 7.2 h dark, all ewes showed maximum values at 03.00 h in late dark phase. The most consistent overall difference between oestrus and



B Plasma melatonin (pg/ml) in 4 individual sheep (Nos 13, 18, 23, 27) at 3-h intervals throughout 24 h at different times of anoestrus. Darkness is indicated by the black bar. 15 out of 16 profiles show a single peak of secretion.

anoestrus values is the frequent presence of 'shoulders' or frank double peaks of secretion during the oestrous phase of the annual cycle. 21 out of 29 profiles showed such evidence of a bimodal secretion pattern, whereas during anoestrus only 1 out of 16 24-h profiles appear to have a 2nd peak. Although clearly the presence of more peaks cannot be excluded with the sampling times used, it is theoretically possible to detect up to 3 peaks in December and January and 2 peaks in May, August and September. A similar bimodal pattern of melatonin secretion has been previously reported in 6 out of 8 castrated ram-lambs in 12L 12D sampled at frequent intervals[9].

A previous report by Rollag et al.[15] of plasma melatonin variations at different stages of the annual reproductive cycle in the ewe indicated that no overall statistical differences were present with regard to melatonin secretion, with the exception that the duration of elevated melatonin concentration corresponded to length of photoperiod and hence was longest during oestrus. Our experimental conditions differed from those of Rollag et al.[15], inter alia in that sheep were both maintained and sampled in natural light, as opposed to being sampled in artificial light. The melatonin assay employed here appears to give lower basal values in sheep than that of Rollag et al.[13,15]. The data reported by Rollag et al., particularly for ewes in luteal phase in November (mid-oestrus)[15] is nevertheless suggestive of a bimodal pattern of secretion, although values from individual sheep are not shown.

Both unimodal and bimodal patterns of physiological phenomena such as activity and hormone concentrations have been frequently observed and may depend on photoperiod length[3,18-20]. Melatonin secretion in the ewe is likely to depend principally on photoperiodic conditions: experiments in castrates would however be essential to differentiate photoperiodic effects from any effects due to gonadal steroids.

Whether, in natural conditions, melatonin secretion is an important determinant of reproductive activity in the ewe remains an open question, however, the association of double peaks or extended secretion with reproductive activity suggests a possible mechanism for photoperiodic control. Assuming a single, short-duration, dark-phase peak of melatonin secretion, corresponding to short nights, is inhibitory to reproductive function, and that melatonin receptors in the sheep are subject to reduced sensitivity after exposure to melatonin (or 'down-regulation') as recent evidence suggests in the hamster[21,22], then the presence of 2 peaks, or extended secretion, indicative of long-nights, could hypothetically cancel the inhibitory effect of 1 peak of short duration, allowing reproductive resurgence or restraining reproductive atrophy. This theory would predict that melatonin administration in late light-phase in anoestrus would induce early oestrus and that inhibition of melatonin secretion in early dark-phase in oestrus would induce early anoestrus, although clearly allowance must be made for a possible underlying endogenous annual rhythm of reproductive competence.

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Effect of diet on osmotic water flow across rat colon mucosa[1]

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Summary. Osmotic water flow across colon mucosa was increased in rats adapted to a high protein diet (HP) compared to rats fed a high carbohydrate diet (HC). The diet-induced change of the osmotic permeability of the colon is probably a manifestation of a regulatory mechanism controlling intestinal water absorption.

We have shown in former work[3,4] that Na, Cl and water absorption from the colon ascendens is elevated in rats adapted to a high protein diet (HP) compared to rats fed a high carbohydrate diet (HC). Moreover in these experiments the ratio between net water absorption and net absorption of solutes was higher in HP-rats than in HC-rats, indicating that the osmotic permeability of the colon epithelium might be affected by the diet. In the present study we have therefore investigated whether the osmotic water flow across colon epithelium is influenced by feeding