matographed on a Sephadex G100 column (2.3 × 13 cm) and the middle peak pooled as unaggregated PRL.
Milk samples were assayed at 25–200-fold dilutions, as more concentrated samples either did not yield a dilution curve parallel to that of the standards or reduced the total bound value when assayed in the presence of excess antibody.

Results and discussion. Inter- (6.9%) and intra-assay (7.7%) variations, when calculated from a series of milk samples were slightly higher than those reported for serum[8]. There was no incubation damage by milk samples diluted 25-fold or greater and the dose response curve for milk (10–100-fold dilution) was parallel to the purified PRL standards (fig. 1).
Prolactin concentrations in mouse milk increased from the day of parturition to give an early peak of 230 ng/ml on days 2 and 3 of lactation (fig. 2). Levels then decreased to 140 ng/ml by day 6 and were maintained at approximately this level until weaning.

This pattern of milk PRL concentrations differs from that reported for bovine and human milk[9,10], where PRL levels are high in colostrum and decrease dramatically down to relatively low levels in mature milk. However, it is similar to the reported pattern of EGF levels in mouse milk[3].
The observed peak of PRL on days 2 and 3 may be directly due to variations in the concentration of circulating PRL[11] and/or the number of PRL receptors expressed on the mammary epithelial cells[12], assuming that these receptors are involved in the receptor-mediated transcellular transport of PRL.
The prolactin concentrations reported for mouse milk are higher that those found in human[10], goat, sheep or cow's milk[9], but are lower than the values recorded for bovine colostrum[9]. Whether milk PRL plays a role in the neonate is not known. However, plasma levels of PRL are very low in suckling rats[15] and it has been shown that PRL can cross the mucosa of the neonatal gut[13,14].

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Melatonin in the plasma of growing sheep subjected to short and skeleton long photoperiods

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Summary. The levels of melatonin in plasma were measured at hourly intervals for 24 h in 8 sheep, 4 under 8L:16D (short day) and 4 under 7L:10D:1L:6D (skeleton long day) after 38 days of exposure. Mean concentrations did not differ significantly between treatments (52 pg/ml under short days; 91 pg/ml for skeleton long days), but the levels were more stable during 24 h in the SD treatment. Under skeleton long days there were 3 peaks during the 10D scotophase, with low levels during the 6D scotophase and the 7L photophase.

A daily rhythm of the pineal hormone melatonin has been demonstrated in rodents[1] and sheep[2–4] with highest blood levels during the scotophase of the daily cycle. However, the amount of melatonin secreted does not reflect proportionately the duration of darkness and sheep kept under long photoperiods show higher night-time plasma concentrations than do sheep kept under short photoperiods[4]. In sheep under natural, changing photoperiods the situation is complicated by the extremely pulsatile nature of melatonin secretion[3,5]. Sheep are more active during the day than during the night, the reverse of the situation in the rat, even though the diurnal rhythms of melatonin are similar in the 2 species.
The melatonin rhythm will continue in sheep kept in constant darkness, but under constant illumination melatonin secretion is suppressed[2]. Rollag et al.[6] showed that at the onset of darkness melatonin concentrations in plasma rose to their maximal level within 10 min and fell to basal levels within 10 min of the lights being switched on again. However, the changes at dusk and dawn are not always so rapid as this[7].
In rats, brief exposure to light during the night causes a rapid decline in the activity of pineal N-acetyl transferase, the first specific enzyme in the pathway to melatonin, and a decline in pineal and plasma melatonin concentrations. As little as 1 min of exposure to light is sufficient to cause a marked inhibition for at least 5 h even though the rats were maintained in darkness after exposure to the flash of light[8]. The administration of melatonin has been shown to mimic the effects of photoperiod on the reproductive processes of hamsters[1] and sheep[9].
The present study describes the patterns of melatonin secretion in sheep during short (8 h light:16 h dark – 8L:16D) and skeleton long (7L:10D:1L:6D) photoperiods which both have 8 h of light per day but have been shown to have different effects on both reproductive paradigms[10] and prolactin secretion[11,12].
Materials and methods. Animals. 8 Suffolk ♂ × Greyface ♀ crossbred lambs (4 males castrated neonatally and 4 females) born during March were weaned to a concentrate diet on 3 May. On 10 May they were transferred from natural photoperiodic conditions to individual pens in 2 exactly similar rooms of a lightproofed building (2 males and 2 females to each room) and subjected to a photoperiodic regime of 12L:12D for 12 days. At the end of this time the lighting in one room was changed to 8L:16D and in the other to

7L:10D:1L:6D. All artificial photoperiods began at 08.00 h and feeding, at a restricted level according to individual liveweight, took place between 08.00 and 08.30 h. Illumination was by 60 watt incandescent bulbs giving a light intensity of between 80 and 100 lx at the head level of the animals.

After 38 days of the 2 artificial photoperiod treatments blood samples were withdrawn hourly for 24 h through previously inserted jugular catheters. The blood was transferred to an ice bath, centrifuged within 2 min, and aliquots of plasma frozen and stored at −20 °C until assayed.

Radioimmunoassay. The assay method used was that of Rollag and Niswender (1976) using rabbit anti-melatonin serum R1055 which was a gift from Dr G. Niswender, Colorado State University. The method for the extraction of melatonin from ovine plasma and modifications to the assay procedure have been described[13]. Blank values (with assay buffer being subjected to the extraction procedure followed by the radioimmunoassay) gave values which were below the detection limit of the assay (9 pg/ml^{-1}; zero binding ± 2 SD). Parallelism between doubling dilutions of 2 plasma samples and also the iodinated ligand (^{125}I-N-3-(4-hydroxyphenyl)propionyl-5-methoxytryptamine) and melatonin standard solutions were demonstrated. Intra-assay variance for 6 replicates was 11.0% and inter-assay variance for 4 assays was 17.9%.

Analysis. All hormone data were subjected to logarithmic transformation to equalize the variance between compared data before parametric statistical analysis. For each animal the 24-h mean value was calculated from the $\log_{10}$ hormone values and the 24-h mean for the 4 animals in each group was calculated from these individual means. Analysis of hour to hour fluctuations in hormone concentration was based on the non-parametric sign test[14] as used by Lincoln et al.[4], for detecting consistent changes in the hormone levels related to the time of day; the individual values for each hormone were assigned as being above, below, or equal to the median value for the hormone during the sampling period. The times at which all four animals in the same group had hormone values above or below their individual medians were determined.

Results. Radioimmunoassay. The parallelism between ligand extracted from plasma samples, standard melatonin and iodinated melatonin analogue is shown in figure 1. This shows that the binding characteristics under the conditions of the assay were similar for the three ligands used in the assay.

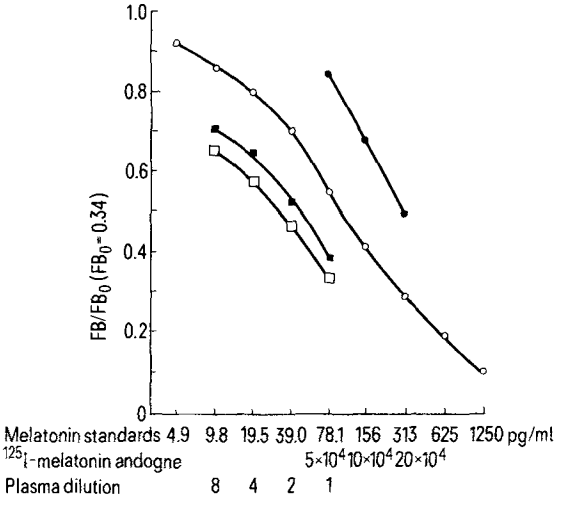

Figure 1. Melatonin standard curve (6 replicates of each dilution) showing parallelism with dilutions of 2 plasma samples subjected to the extraction and assay procedure (3 replicates of each dilution) and of ^{125}iodinated melatonin analogue.

The geometric mean levels of melatonin in plasma under short photoperiod was 52 pg/ml and under skeleton long photoperiods, 91 pg/ml (SEM ± 1.7); there was no significant difference between these means and no effect of sex on the melatonin concentrations.

The 24 h hormone profiles for the means for 4 animals are shown in figure 2. The geometric mean concentration during the photophase (8L) of the short photoperiod treatment was 48 pg/ml (SEM ± 5.0), whereas the mean level during the scotophase (16D) (96 ± 8.9) was significantly higher ($p < 0.001$). There were also significantly lower levels in the first photophase (7L) of the skeleton long photoperiod treatment (54 ± 7.2) than in the first scotophase (10D; 170 ± 24.4; $p < 0.001$), which were in turn, higher than the scotophase under short photoperiod ($p < 0.01$). During the 1L 'flash' melatonin levels declined and remained low (44 ± 9.5) during the second (6D) scotophase; this latter level was not significantly different from the 7L photophase concentration but was significantly less than that during the 10D scotophase ($p < 0.01$).

Discussion. The lack of significant difference between the mean melatonin levels of the lambs in the 2 photoperiods supports the results of Lincoln et al.[4] who found no difference between photoperiods of 8L:16D and 16L:8D in the plasma melatonin concentration of Soay rams. In both reports it is interesting to note that the mean levels tended to be somewhat higher in the long or skeleton long photoperiods than in the short photoperiods.

The shapes of the melatonin profiles in skeleton long photoperiod and short photoperiod in the present experiment were also similar to those described by Lincoln et al.[4] in Soay rams in long and short photoperiods. High levels were found during the first dark period (10D) of the 7L:10D:1L:6D lighting pat-

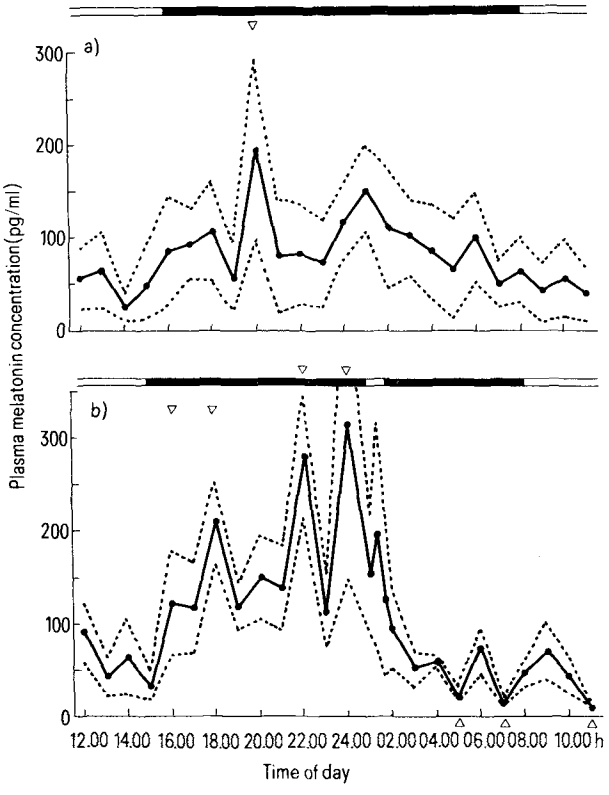

Figure 2. Mean and distribution (± 1 SE) of melatonin concentrations in plasma of lambs on a) short and b) skeleton long photoperiods. The arrowheads indicate times when all 4 animals had levels higher (▽) or lower (△) than their individual 24 h medians. The bar along the top indicates when the lights were on (white) and off (black).

tern with low levels from the 'flash' (1L), through the second (6D) scotophase, and continuing until the end of the 7L photoperiods. As in the rat[7], it appears that the changes induced by a short period of light, in this case 1 h, have effects on melatonin secretion which last through the succeeding 6-h period of darkness. Thus, it is the length of the first scotophase which is critical in determining the secretory pattern of melatonin. This agrees with the conclusions of Ravault and his colleagues[15] who found that the effects of a 1-h 'flash' of light on prolactin secretion in sheep were dependent on the length of time between the end of the main photophase and the start of the 'flash' – the period during which, in our experiment, melatonin levels were elevated.

Plasma samples were also assayed for prolactin and cortisol in the current experiment and the results are reported elsewhere[16]. Prolactin secretion was stimulated by skeleton long photoperiod ($P < 0.001$), with no obvious circadian rhythm. Plasma cortisol was significantly lower in skeleton long photoperiods than in short days ($P < 0.05$), but again there was no clear circadian rhythm. Thus, the changes in plasma levels of melatonin which are reported in the current work do not immediately affect the secretion of prolactin or cortisol, although the pineal gland is involved in their control; pinealectomy blocks the effect of skeleton long photoperiod on prolactin and cortisol in growing sheep[17].

The results of this experiment support current evidence that it is the temporal pattern of melatonin secretion rather than the overall amount of melatonin present over 24 h which may be involved in the photoperiodic mechanisms in sheep[8].

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Effects of intraventricular administration of insulin on thyrotropin secretion in rats

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Summary. Intraventricular administration of insulin stimulates increases in the levels of thyrotropin-releasing hormone and thyrotropin in rats.

Insulin is found in the rat hypothalamus[3] and stimulates increases in the levels of growth hormone and corticotropin[4,5]. Studies on the effect of insulin on thyrotropin (TSH) secretion have revealed that the peripheral administration of insulin induced elevation of plasma TSH levels in rats[6,7]. However, sites of the effects on TSH secretion are unclear at present. Therefore, the authors investigated intraventricular administration of insulin on TSH release in rats.

Materials and methods. Animals. Male (Wistar strain) rats weighing 200 g were employed. They were housed in temperature (22 °C) – and humidity (60%) – controlled quarters and fed a diet of laboratory chow and water ad libitum. *Drugs.* Ovine insulin was kindly supplied by Shimizu Pharm. Co., Ltd. (Japan)[2]. Synthetic thyrotropin-releasing hormone (TRH) was purchased from the Protein Research Foundation (Japan). *Experimental design.* All experiments were conducted in a temperature-controlled room (22 °C). After overnight fasting, the rats were anesthetized with pentobarbital (50 mg/kg). One hundred nU of insulin was dissolved in 1 µl of saline, and injected intraventricularly using a 10 µl Hamilton syringe as described earlier[8]. The rats (5 at a time) were then decapitated with a guillotine at 5, 10, 30, 60 and 90 min after the insulin injection. Trunk blood was collected in heparinized tubes kept on ice. The hypothalami were obtained by the method previously described[9]. For the control, saline was injected. *Assay methods.* TRH, thyroxine (T_4) and 3,3',5-triiodothyronine (T_3) were measured by means of a specific radioimmunoassay for each[10,11]. TRH content in the hypothalamus was expressed with reference to a total amount of dissected hypothalamic section. TSH was determined by the rat TSH radioimmunoassay kit[12] supplied by the National Institute of Arthritis, Diabetes and Digestive and Kidney diseases Research Materials Distribution Program (NIAMDD). Blood glucose levels were measured by the autoanalyser method. *Statistics.* Mean and standard error of the mean was calculated for each group. Student's t-test was used to evaluate the differences between the control and experimental groups.

Results (table). The hypothalamic immunoreactive TRH (ir-TRH) content decreased significantly after insulin injection, whereas its plasma concentration increased significantly. The plasma TSH levels increased significantly with a zenith at 30 min after the injection. The plasma T_3 levels increased signi-