role this tripeptide plays in the protection against oxidative stress.

Materials and methods. 25 GSH-normal and 25 GSH-deficient Merino sheep were selected on the basis of GSH level[5]. The methods used for the preparation of haemolysates and measurements of enzyme activities were those of Beutler[6] (for catalase and GSH-Px) and McCord and Fridovich[7] (for SOD). The assays were carried out at 37 °C in a cuvette having a light path of 1 cm and a final volume of 1 ml, using a Carl Zeiss recording spectrophotometer. All the reagents were purchased from Sigma Chemical Co., St. Louis, USA.

Results and discussion. The level of GSH in the red blood cells of GSH-deficient sheep were about 20% of the normal sheep (table). However, there were no significant differences in any of the three enzyme levels measured in the 2 groups of sheep (table). We also analyzed our data to find if there was some relationship between GSH level and the enzyme activity or between one enzyme level and the other. None of these correlations reached the level of significance. No report appears to have been made on catalase and SOD and so we cannot compare our results. Reports on GSH-Px are conflicting[5].

Andrewartha[8] found that GSH-deficient sheep had about 30% lower activity of GSH-Px than normal sheep and also confirmed a previous report[9] that the administration of selenium (Se) level decreases GSH in the red blood cells implying that higher levels of Se somehow reduces GSH levels, which in turn is associated with low GSH-Px activity. Although we did not measure Se level, the fact that all sheep were taken from one flock indicate that Se level would be, at least on theoretical grounds, similar.

In summary, these results in confirmation of our previous results and those of others[5] suggest that in spite of large differences in GSH levels, the red blood cells from GSH-deficient and GSH-normal Merino sheep appear to have similar response to oxidative stress against which GSH is credited to play a major role. GSH deficiency per se is not sufficient to cause cell damage but that additional chemical challenges are required as suggested by Kosower and Kosower[10].

After submitting this abstract, we have also come across a paper by Atroshi et al.[11] which suggests that GSH-Px activity in Finn sheep is genetically determined. Low GSH-Px animals show better performance such as larger weight gain and wool production and lower mortality rate of their offspring. In confirmation of our results presented here, they also reported an absence of correlation between GSH-Px activity and GSH.

Levels of GSH, GSH-Px, catalase and SOD in the red blood cells of GSH-normal and GSH-deficient sheep

		GSH-normal sheep	GSH-deficient sheep	P
GSH	μmole/gHb	9.44 ± 0.34	1.90 ± 0.34	-
	mg/dl RBC	97.1 ± 4.7	20.6 ± 3.6	-
GSH-Px	IU/g Hb	89.58 ± 14.14	105.17 ± 18.34	NS
Catalase	$\times 10^4$ IU/g Hb	1.662 ± 0.147	1.745 ± 0.141	NS
SOD	$\times 10^3$ units/g Hb	2.404 ± 0.377	2.744 ± 0.286	NS

Values are means ± SD; NS, not significant.

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Dissociation of estrous cycle and activity rhythm in rats

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Summary. When rats were kept in continuous light there was a time-lag between the onset of activity arrhythmia and that of persistent estrus. When animals showing both arrhythmias in continuous light were kept under a LD 21:3 photoperiod, the activity rhythm returned to normal but the estrous cycle did not.

In continuous light (LL), many circadian rhythms in nocturnal mammals initially show a free-running rhythm followed by a loss of apparent rhythmicity, a state known as periodicity fade-out[1,2]. Many kinds of animal behavior, for example wheel running (WR), show a circadian rhythm. The general activity in nocturnal animals usually continues in LL as a free-running rhythm with a circadian period and terminates in arrhythmias[1,3-5]. Since the rat's estrous cycle is known to be based on the circadian rhythm[6,7], exposure to LL leads first to a free-running rhythm and then to a fading out of the estrous cycle[2,8]. The latter condition is eventually characterized by persistent estrus (PE), cystic follicles, and anovulation.

In the normal light cycle (LD 12:12 or 14:10), the behavioral and estrous cycles are maintained and entrained to the light cycle and hence are synchronized with each other. The present study was designed to determine whether activity arrhythmia and persistent estrus develop simultaneously or

independently in LL, and whether the states of activity arrhythmia and of persistent, estrous states induced by LL return to normal simultaneously or independently in an atypical light cycle (LD 21:3).

Material and method. General. All animals used were female Wistar strain rats. They were provided with food and water ad libitum. 5 animals were used in each experiment.

Vaginal smears were taken at random in the morning (09.00-12.00 h) to avoid providing a nonphotic 24 h time signal. Prolonged estrus was defined as less than 6 consecutive vaginal cornified days, and persistent estrus as 7 or more.

The wheel was 60 cm in diameter and 6 cm in width. The side cage, in which food and water were available, was 15×20×20 cm in size. One rotation of the wheel was recorded as one. The number of rotations were integrated and printed every 30 min (Integrating indicator, Tosoku Co., Tokyo). Onset of activity arrhythmia was defined as the day when activity became spread, or was not observed during one day (00.00-24.00 h) when the activity record of each animal was examined.

The wheel and side cage were illuminated by 20 W fluorescent tubes. The centers of the floor of both the wheel and side cage were illuminated at an average of 250 lux.

Experiment 1. Animals, 40 days old, were left 10 days in

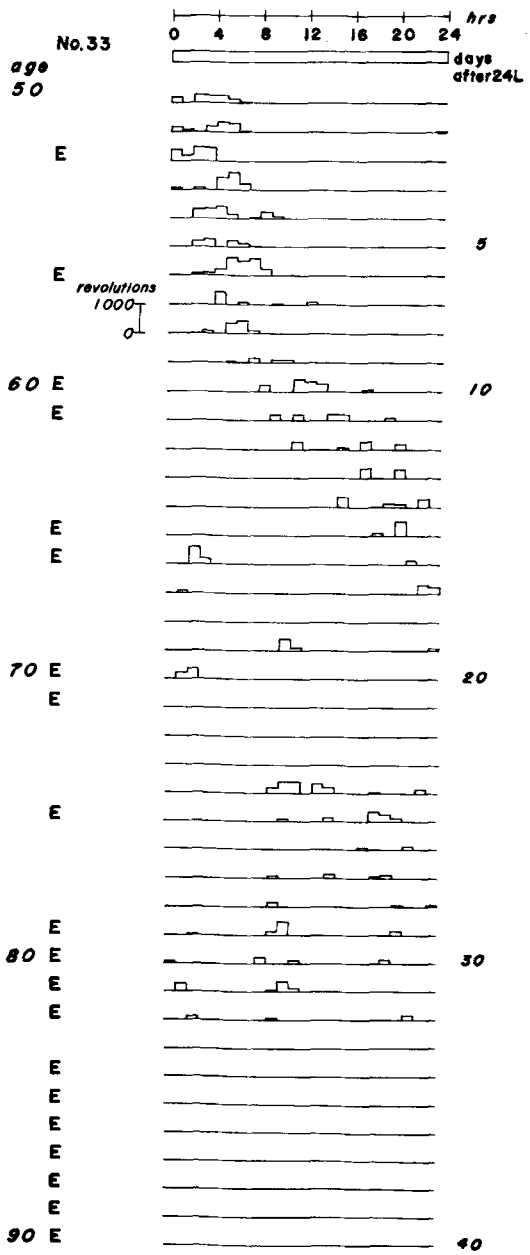

Figure 1. The wheel-running rhythm of a representative animal in continuous light (24 L) from 50 to 96 days of age. From 60 to 83 days of age, prolonged estrous state. After 84 days of age, persistent estrous state. The arrhythmical state in wheel running activity started at 68 days of age. The 1 line indicates 1 day (00.00-24.00 h). White bar, lighting period; E, vaginal estrous day.

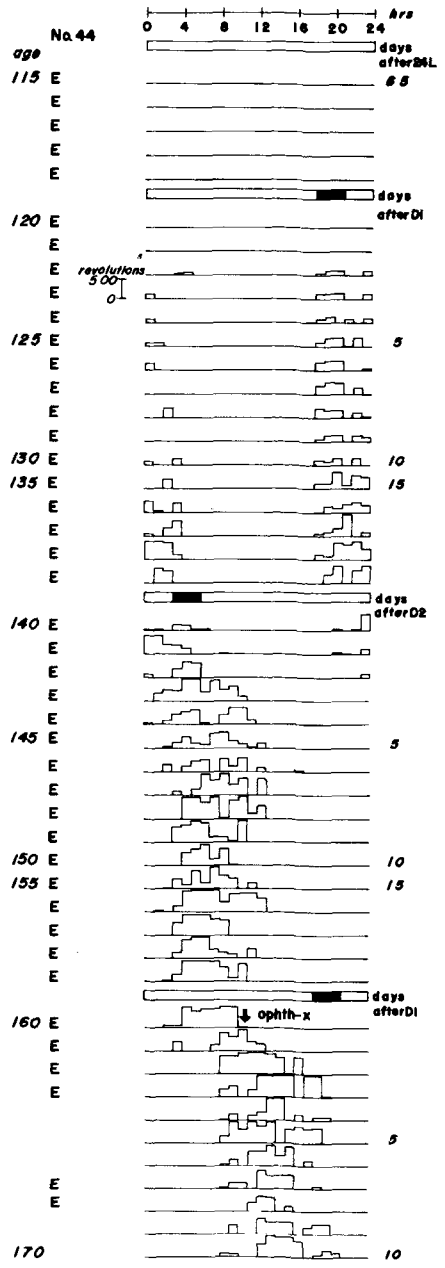

Figure 2. The wheel-running rhythm of a representative persistent estrous animal after turning from LL (24 L) to LD 21:3 (D_1 and D_2). Dark time; D_1, 18.00-21.00 h; D_2, 03.00-06.00 h (black bar). White bar, lighting period. Ophth x, bilateral ophthalectomy at 160 days of age. The 1 line indicates 1 day (00.00 to 24.00 h). E, vaginal estrous day.

their cages in LD 12:12 (06.00 h on) and then were exposed to LL from 50 days of age.

Experiment 2. Animals were exposed to LL for 70 days from 50 days of age. On the 120th day, they were given 3 h darkness (D_1, LD 21:3, dark time; 18.00-21.00 h) for 20 days. Afterwards, the lighting schedule was changed to D_2 (LD 21:3, dark time; 03.00-06.00 h) and continued for 20 successive days (140 to 160 days of age) and then changed again to D_1 (160 to 180 days of age). In order to determine whether light information is conveyed only via the retina, bilateral ophthalectomy was performed under ether anesthesia in the morning (09.00-10.00 h) at 160 days of age.

Result. The WR rhythm of a representative animal in experiment 1 was shown in figure 1. In the early prolonged estrous state from 50 to 67 days of age, the WR thythm with a free running rhythm of approximately 25.5-h period was observed. In the late prolonged estrous state, from 68 to 83 days of age, there was hardly any WR rhythm. After this WR arrhythmia, PE followed. A time lag between the onset of both arrhythmias existed (9.6 ± 1.7 day); the WR rhythm disappeared earlier than did the estrous cycle in LL.

As shown in figure 2, there was hardly any WR rhythm in the complete PE state. This was coincident with experiment 1 (fig. 1). When an animal showing both arrhythmias was given 3-h darkness (D_1), the WR rhythm reappeared in this short dark period within at least 3 days. The reappearance of this WR rhythm was found at off-time (18.00 h) and entrained with an approximately 24.0-h period to D_1. The WR activity period was about 10 h. In order to check whether the reappearance of the rhythm is driven by internal controlling elements, the dark time was shifted 9 h later (D_2). The WR rhythm shifted with an approximately 25.5-h period and then entrained with a 24.0 h period to D_2; the reappearance of the rhythm seems to be driven by internal controlling elements. Although the WR rhythm was clearly produced by an atypical light cycle, the PE state continued up to the time of the ophthalectomy. After the ophthalectomy, a free-running period of the WR rhythm was about 24.0 h. These blinded animals could not entrain again to the new lighting schedule, indicating that light informtion could be conveyed only via the retina.

Discussion. During the early prolonged estrous state in LL, the WR rhythm clearly showed a free-running pattern and its period was about 25.5 h. During the late prolonged estrous state, however, there was scarcely any WR rhythm. After this arrhythmicity of WR, the PE state was invariably observed. There was an apparent time lag (average 10 days) between the onset of arrhythmicity of WR and that of PE. This time lag was also found in atypical light cycles[9]. Conversely, when animals showing both arrhythmias in LL were exposed to a LD 21:3 photoperiod, the activity rhythm apparently returned to normal but the estrous cycle did not. These results suggested that 2 circadian rhythms might be separable in LL and in LD 21:3.

Recent reviews[10-12] have shown that the suprachiasmatic nucleus (SCN) might act as a central circadian controlling element. When it is destroyed or isolated, both the behavioral and the estrous rhythms become arrhythmical in nocturnal mammals[13-15]. An intact circadian system, as revealed by a regular activity rhythm, does not, however, guarantee a regular estrous rhythm because this is apparently disrupted in LD 21:3. The dissociation of the 2 rhythms in LL and in LD 21:3 suggests that a central circadian controlling element, probably in the SCN, might couple more strongly with the elements controlling the activity rhythm than with those controlling the estrous cycle.

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Synthesis of [1-Aib]-angiotensin II, an analogue with higher potency than [1-Asn,5-Val]-angiotensin II[1]

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Summary. [1-Aib]-angiotensin II was synthesized by Merrifield's solid-phase procedure. The analogue, tested on rabbit aorta strips, showed intrinsic activity $\alpha_E = 1$, and when tested on rat blood pressure it gave a pD_2 of 8.06; a 3.2 ± 1.3-fold higher potency than the Ciba-Hypertensin standard.

It is recognized that the residue in position 1 of angiotensin II contributes primarily to the duration of action through 2 possible mechanisms: a) it makes the molecules more-or-less resistant to aminopeptidases; b) it increases the binding affinity of the peptide for the receptor[3-5]. Moreover, the basicity of an α-nitrogen at the N-terminus is important for maximum agonistic properties of angiotensin II. Thus, [1-Sar]- and [1-diMeGly]- angiotensin II were found to be respectively 1.5 and 1.7 times as active as angiotensin II as pressor agents[6,7]. On the other hand, model compounds containing α-methyl-amino acids indicate that these compounds are resistant to chemical hydrolysis and to enzymatic attack by both endopeptidases and exopeptidases[8,9]; moreover, α,α-dialkyl-amino acids showed, on the basis of theoretical analysis, considerable restriction of conformational freedom of peptides[10]. Recent studies indicate that