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Effects of Photoperiod and Feeding on Plasma Corticosteroid Concentrations and Maximum Corticosteroid-binding Capacity in Pigs

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Abstract

The effects of time of feeding and photoperiod on the circadian rhythm of total and free plasma corticosteroid concentrations and the maximum corticosteroid-binding capacity (MCBC) were measured in pigs. There were four photoperiod treatments: natural long and short daylengths (light:dark, 14:10; 10½:13½, respectively), artificial short daylength and reverse artificial short daylength (light:dark, 9:15).

Photoperiod had a significant effect on corticosteroid concentrations. The corticosteroid concentrations on long daylength showed a major increase in the morning (from an average of 8 ng ml^{-1} at 0700 h to a peak level of 53·5 ng ml^{-1} at 0910 h). The amplitude of the morning peak was lower in the other treatments. Apart from this increase, corticosteroid levels at other times of day for pigs in all treatments were generally low. The MCBC varied greatly between animals ($< 25 - > 50$ ng ml^{-1}) but showed no circadian rhythm. There was no change in corticosteroid levels directly attributable to feeding.

It was concluded that, in experiments where the effect of acute stressors on corticosteroid levels is examined, blood sampling should be performed in the afternoon–evening period because the between-animal variation in hormone levels is less and the hormonal levels are more stable, and also that time of feeding did not affect the rhythm of corticosteroids. A blood-sampling regime to define the circadian rhythm in corticosteroid concentration and to determine the MCBC is described.

Introduction

Man and sheep exhibit a circadian rhythm in plasma cortisol which is composed of a number of secretory episodes which vary both in frequency and amplitude throughout the day (Krieger 1975; Fulkerson 1978; Fulkerson and Tang 1979). This circadian rhythm can be influenced in the mouse and rat by season (Haus and Halberg 1970) and the anticipation of feeding (Morberg *et al.* 1975; Morimoto *et al.* 1977). Also, there is an inverse relationship between plasma corticosteroid and transcortin concentrations in man (Angeli *et al.* 1978), and the level of this binding protein may influence the biological activity of plasma corticosteroids during the day. In the pig, there is a marked 24-h rhythm in plasma corticosteroid levels (Bottoms *et al.* 1972; Baldwin and Stephens 1973; Edqvist *et al.* 1980) but neither corticosteroid-binding capacity nor factors influencing the rhythm have been examined. The frequency of taking blood samples to obtain meaningful estimates of corticosteroid levels will be determined by variation in the frequency and amplitude of episodes of corticosteroid secretion.

This study was designed to determine the effects of daylength and time of daily feeding on the total and free plasma corticosteroid levels and the maximum corticosteroid-binding capacity (MCBC, as a measure of transcortin concentration) in pigs to establish appropriate blood-sampling regimes for future experiments on the effect of possible environmental stressors on the pig.

Materials and Methods

Animals

Two experiments were conducted using mature ovariectomized non-parous pigs ($\frac{3}{4}$ Large White × $\frac{1}{4}$ Berkshire).

Blood Sampling and Corticosteroid Assay

Total and free corticosteroid concentrations and the maximum corticosteroid-binding capacity were determined in plasma samples (see Barnett *et al.* 1981) obtained via indwelling venous cannulae inserted into the jugular vein 2 weeks before sampling under full anaesthesia (Christison and Curtin 1969). Patency of the cannulae was maintained by daily flushing with heparin–saline (125 units/ml). A 1% (v/v) solution of male dog plasma was the source of binding protein for the corticosteroid assay. The intra-assay coefficient of variation of a control plasma pool was 4·5% and the interassay coefficient of variation of this pool was 9·5%. The sensitivity of the assay was 125 pg tube^{-1} (equivalent to 3 ng ml^{-1} of plasma). In samples below this sensitivity, an assay using a 0·5% (v/v) solution of dog plasma gave a sensitivity of 30 pg tube^{-1} (equivalent to 0·6 ng ml^{-1} plasma).

Experimental Procedure

Experiment 1

Two pigs were maintained on a natural light (L):dark (D) regime (L:D, $11\frac{1}{2}$:$12\frac{1}{2}$) for 2 months in individual pens prior to blood sampling on 11 April 1979. Except for a 10-min exposure to a boar 1 week prior to blood sampling, the pigs were without visual or tactile contact with other pigs. They were fed daily at 0800 h and blood samples (8 ml) were taken at 10-min intervals between 0800 and 1600 h (all times are Eastern Standard Times and periods of light and dark are based on times of sunset and sunrise). These samples were assayed for total corticosteroid concentrations.

Experiment 2

Nine pigs were allocated to three treatment groups in January 1980 as follows:

Treatment 1: natural summer daylength (L:D, 14:10);

Treatment 2: artificial short daylength (lights on at 0700 and off at 1600 h; L:D, 9:15);

Treatment 3: artificial reverse short daylength (lights on at 2300 and off at 0800 h; L:D, 9:15).

The pigs on artificial daylength (illumination, 250–300 lux) were housed in light-proof rooms fitted with fluorescent lights controlled by time switches. Pigs on natural daylength were housed indoors in a 'grower shed' (illumination, 100–300 lux). They were kept in individual wire-mesh pens measuring 78 by 175 cm and had visual and tactile contact with other pigs. They were fed between 0720 and 0740 h each day.

In late January 1980, blood samples were collected from each pig over two 24-h periods separated by 1 day. Between 0700 and 1100 h, blood samples were taken at 20-min intervals and then at 4-h intervals throughout the remainder of each 24-h period. The data from the two 24-h sample periods were combined. Within 3 days of completion of blood sampling, the 24-h activity pattern of one pig from each treatment was recorded using an 8-mm time-lapse cine-camera which exposed frames at the rate of approximately 50 per hour. The proportions of each hour of the 24-h cycle which each pig spent active (standing) or inactive (lying) were calculated.

A natural winter photoperiod treatment (August), involving seven pigs in individual pens, was subsequently incorporated into experiment 2 as follows:

Treatment 4: natural winter daylength (L:D, $10\frac{1}{2}$:$13\frac{1}{2}$).

Blood samples were taken from the pigs over a 24-h period in mid August 1980.

Samples from experiment 2 were analysed for total corticosteroids and the MCBC, and free corticosteroid concentrations were calculated.

Results

In experiment 1, the pattern of total corticosteroid levels was similar in both animals, with a steep rise in plasma concentrations which began at 0900 h, reached a peak within 1 h and had declined by midday (Fig. 1). The afternoon levels were consistently lower and showed only transient fluctuations of low amplitude.

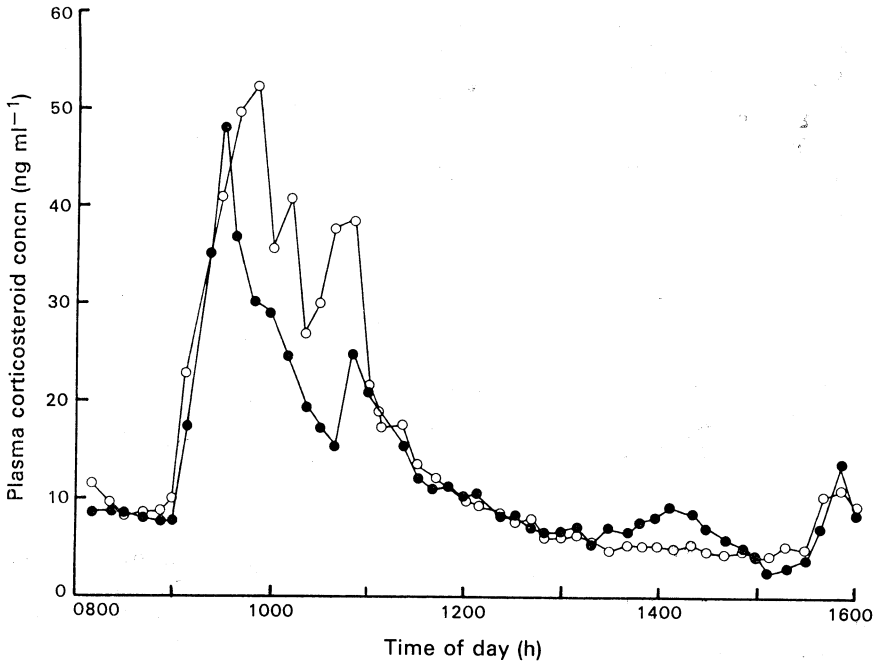

Fig. 1. Changes in plasma total corticosteroid concentration at 10-min intervals in two animals.

Maximum Corticosteroid-binding Capacity

The data for eight animals (one cannula lost patency) in experiment 2 (treatments 1–3) were analysed in two ways. Firstly, the mean MCBC for the blood samples taken at 0700, 1100 and 1500 h were compared with that for blood samples taken at 1900, 2300 and 0300 h. The MCBC's [mean $\pm$ s.e. (n)] were $36{\cdot}19 \pm 2{\cdot}25$ (24) and $31{\cdot}68 \pm 1{\cdot}89$ (24) ng ml^{-1}, respectively, and were not significantly different, and indicate that there was no circadian rhythm of transcortin concentrations. The complete data from four animals (Fig. 2) show the wide variation in MCBC between animals and the lack of circadian rhythm within animals. Secondly, the mean values for the MCBC of all samples from each animal were compared by regression analysis to the mean value of the 1-hourly samples between 0700 and 1100 h for the same animal. The 5-h average (0700–1100 h) was a good predictor ($r = 0{\cdot}99$) of the 24-h mean value of the MCBC (Fig. 3) and was used in subsequent calculations of free hormone concentrations.

Photoperiod had a significant effect on the MCBC. The means (± s.e.) for the 5-h average for treatments 1, 2, 3 and 4 were 35·06 ± 2·17, 45·51 ± 2·78, 23·6±0·84 and 66·9 ± 3·29 ng ml^{-1}, respectively. When the pigs were kept on natural daylengths, the mean MCBC value was lower ($P < 0·01$) in summer (treatment 1) than in winter (treatment 4), and on artificial short days the value was lower ($P < 0·01$) on reverse daylight (treatment 3) than in treatment 2.

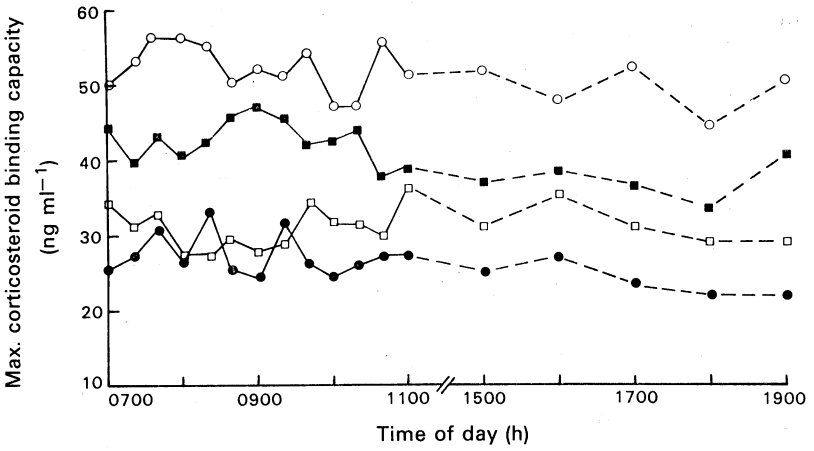

Fig. 2. Changes in the maximum corticosteroid-binding capacity in four individual pigs.

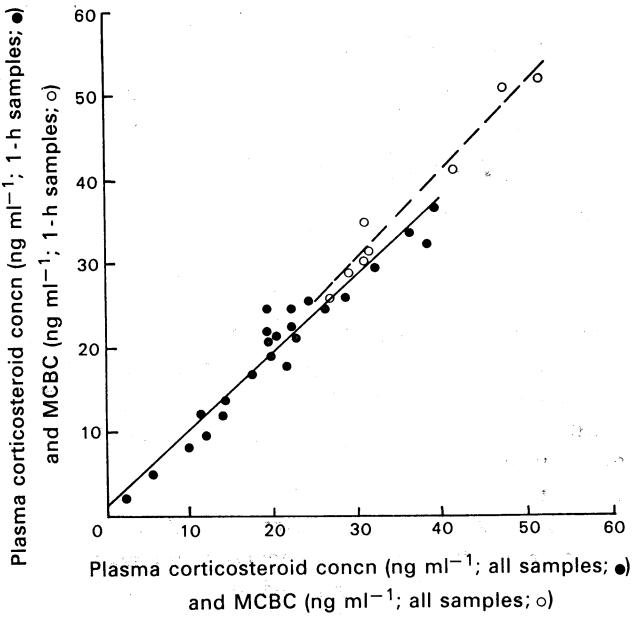

Fig. 3. Plot of mean plasma corticosteroid concentration of all samples from experiment 2 between 0700 and 1100 h (ordinate) against mean value of 1-h samples between 0700 and 1100 h (abcissa); $n = 24$; and of mean maximum corticosteroid-binding capacity of all samples from eight individuals from experiment 2 (ordinate) against mean value of 1-h samples between 0700 and 1100 h (abcissa); $n = 8$.
○ $y = 0·25 + 1·02x$.
● $y = 1·70 + 0·90x$.

Corticosteroid Concentrations

There was a marked photoperiod effect on the concentrations of total and free plasma corticosteroids (Fig. 4). Blood samples from pigs on long natural daylength

(treatment 1) showed a steeper increase ($P < 0{\cdot}001$) in mean hormone concentrations between 0800 and 0900 h, higher ($P < 0{\cdot}001$) overall peak values and higher ($P < 0{\cdot}001$) mean values between 0700–1100 h (mean values were 5·06, 3·45, 1·81 and 2·58 ng ml^{-1} for treatments 1, 2, 3 and 4, respectively) than animals on other daylengths (Fig. 4). The level of the morning increase was variable between animals.

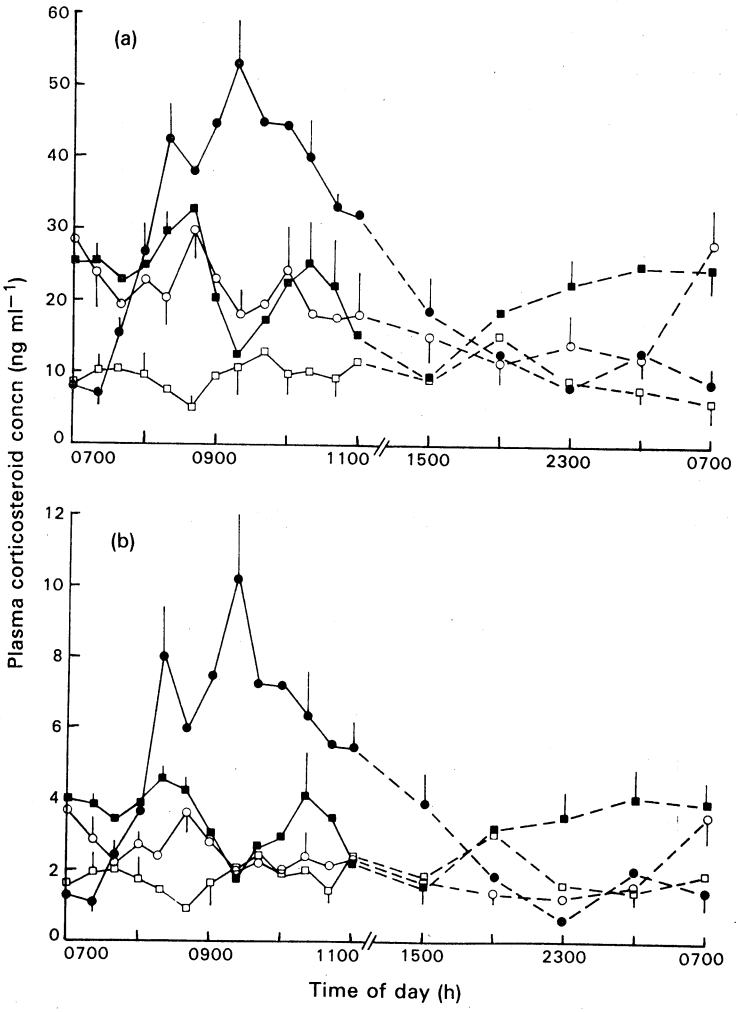

Fig. 4. Changes in plasma total (*a*) and free (*b*) corticosteroid concentrations. ● Pigs on natural long daylength. ○ Pigs on natural short daylength. ■ Pigs on artificial short daylength. □ Pigs on reverse short daylength. Standard errors are represented by vertical lines. Sample size is 5, 6 or 7. All animals were fed between 0720 and 0740 h.

For example, in treatment 1 (L:D, 14:10) between 0720 and 0820 h there were 2–10-fold increases (mean, 6) in mean total corticosteroid concentration and 5–11-fold increases (mean, 8) in mean free corticosteroid levels. By 1500 h, corticosteroid levels in all treatments had generally returned to early morning levels, and this lower level appeared to be maintained throughout the rest of the day.

There were no significant differences ($P > 0{\cdot}05$) in mean values of free hormone concentrations calculated from the samples obtained at 4-h intervals between natural short daylength (treatment 4: mean, $1{\cdot}99$ ng ml^{-1}) and reverse short daylength (treatment 3: mean, $1{\cdot}99$ ng ml^{-1}) (cf. total corticosteroid concentrations: means $\pm$ s.e. $16{\cdot}36 \pm 1{\cdot}73$ and $10{\cdot}42 \pm 1{\cdot}17$ ng ml^{-1}, respectively; $P < 0{\cdot}05$). The mean values ($\pm$ s.e.) for free hormone concentrations calculated from samples obtained at 4-h intervals for treatments 1 and 2 were $2{\cdot}59 \pm 0{\cdot}35$ and $3{\cdot}17 \pm 0{\cdot}30$ ng ml^{-1}, respectively; these values were higher ($P < 0{\cdot}05$) than those from treatments 3 and 4. The night-time values (1900–0700 h) of free and total corticosteroid concentrations of the artificial short daylength treatment (treatment 2) were higher ($P < 0{\cdot}05$) than in other treatments (Fig. 4).

Effects of Feeding and Activity Patterns

There was no evidence of a rise in plasma corticosteroid levels either in anticipation of or immediately after feeding (Fig. 4).

The pigs in treatments 1, 2 and 3 spent $14{\cdot}9$, $13{\cdot}9$ and $12{\cdot}2\%$, respectively, of the day standing. Most of this activity (79, 46 and 59%, respectively) occurred 2 h either side of feeding (Fig. 5). None of the three pigs was very active during the dark period.

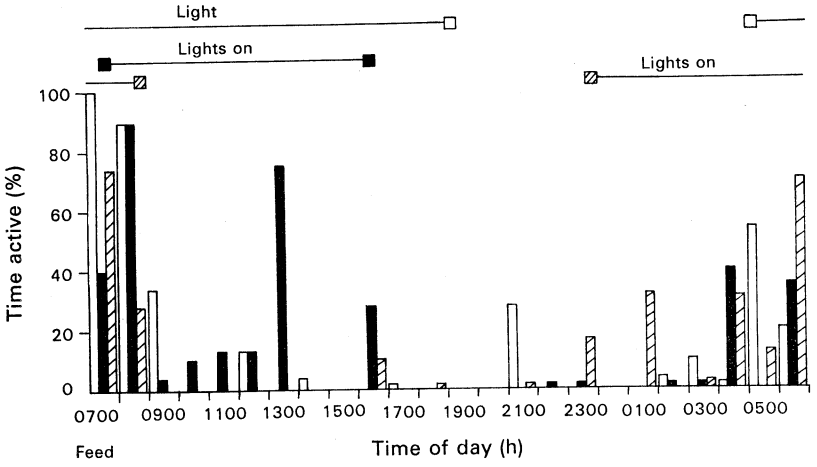

Fig. 5. Changes in activity patterns of one pig from each treatment during the day. Open histogram, treatment 1 (natural daylength). Solid histogram, treatment 2 (short daylength). Cross-hatched histogram, treatment 3 (reverse short daylength).

Simplified Blood Sampling Regime for Plasma Corticosteroids

To determine a simpler blood-sampling regime to estimate the degree of elevation in corticosteroid levels between 0700 and 1100 h, the mean corticosteroid value of all blood samples from individual animals (experiment 2) was compared with the mean value of samples taken at 1-h intervals between these times. The mean of five blood samples taken at 1-h intervals between 0700 and 1100 h was a good predictor ($r = 0{\cdot}97$) of the mean value from samples taken at 20-min intervals during this period (Fig. 3).

There was a marked photoperiod effect on the concentrations of total and free

Discussion

In pigs kept on natural daylengths, the level of both total and free plasma corticosteroids was higher in the morning (experiment 1, 0900–1100 h; experiment 2, 0800–1100 h) than at other times of the day. This was most marked on long or near equinoctial daylengths where mean levels rose sharply between 0900–1000 h in experiment 1 and between 0720–0820 h in experiment 2, but there was considerable variation between animals of all treatments in the peak values. The average levels and pattern of change in plasma corticosteroid levels on natural daylight are consistent with published information on the diurnal rhythm of corticosteroid levels in pigs with sampling at 1-, 2-, 4- or 8-h intervals (Whipp *et al.* 1970; Bottoms *et al.* 1972; Baldwin and Stephens 1973; Edqvist *et al.* 1980).

The period of high corticosteroid secretory activity during the early part of the day in pigs may be associated with motor activity patterns. In man and sheep, where motor activity is spread throughout daylight and some hours of darkness, periods of corticosteroid secretion occur throughout the 24-h period (Krieger 1975; Fulkerson and Tang 1979). Also, in man, periods of wakefulness (during sleep) are correlated with increased cortisol levels (Alford *et al.* 1973), and in rats there is a phase relationship between the circadian rhythm of plasma corticosteroids and motor activity (Halberg *et al.* 1959; Morimoto *et al.* 1977). In the present study, motor activity coincided with the beginning of the light period (independently of photoperiod treatment). This is similar to the situation in the wild pig (Gundlach 1968). The peak of motor activity coincided with the peak in corticosteroid levels in pigs on natural daylength treatments; the restricted period of corticosteroid activity is possibly a consequence of management and concurrent restriction of motor activity.

When daylength was artificially shortened during summer, the pattern of corticosteroid secretion was similar to that found on the natural short daylength treatments, except that values were higher between 1900 and 0300 h when lights were on from 0700 to 1600 h and were lower between 0800 and 1100 h when lights were on from 2300 to 0800 h. The reasons for the discrepancies between the patterns of corticosteroid secretion between treatments is obscure, but the lower levels of corticosteroids on short daylengths compared with those on longer daylengths suggest that seasonal patterns are controlled more by daylength than by temperature in pigs kept in a mediterranean climate. This contrasts with observations that adrenal function is activated by low temperatures in rodents (Sobel *et al.* 1960; Eleftheriou 1964) and in man (Stein *et al.* 1949), but Haus and Halberg (1970) present evidence that the circannual variation of adrenocortical function may not be primarily temperature dependent. The influence of season on adrenocortical function in the pig remains to be fully examined.

In contrast to studies in man (Angeli *et al.* 1979) and hens (Siegel *et al.* 1976), we found no circadian rhythm in the maximum corticosteroid-binding capacity.

In the present study, feeding was not accompanied by an anticipatory increase in corticosteroid levels. In rats such an increase is associated with feeding and motor activity that is independent of photoperiod (Morberg *et al.* 1975; Morimoto *et al.* 1977).

From the data obtained in these studies, a suitable blood-sampling regime for determination of valid estimates of corticosteroid secretion may be suggested. Firstly, treatments to examine the effects of short-term stressors should be carried out in the

afternoon rather than in the morning because the between-animal variation in hormone levels is less and the hormone levels are more stable. Secondly, to define the period of high corticosteroid activity and changes that occur in corticosteroid levels during other times of the day, blood samples taken at 1-h intervals are sufficient, and thirdly, because of the lack of a circadian rhythm in the MCBC, the average MCBC of five samples gives a value which can be used to determine free corticosteroid levels at any time of the day.

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References

Alford, F. P., Baker, H. W. G., Burger, H. G., de Krester, D. M., Hudson, B., Johns, M. W., Masterton, J. P., Patel, Y. C., and Rennie, G. C. (1973). Temporal patterns of integrated plasma hormone levels during sleep and wakefulness. I. Thyroid-stimulating hormone, growth hormone and cortisol. *J. Clin. Endocrinol. Metabol.* **37**, 841–7.

Angeli, A., Frajria, R., Dogliotti, L., Crosazzo, L., Rizoli, F., and Ceresa, F. (1978). Differences between temporal patterns of plasma cortisol and corticosteroid-binding globulin binding capacity throughout the twenty-four hour day and the menstrual cycle. *J. Endocrinol. Invest.* **1**, 31–8.

Baldwin, B. A., and Stephens, D. B. (1973). The effects of conditioned behaviour and environmental factors on plasma corticosteroid levels in pigs. *Physiol. Behav.* **10**, 267–74.

Barnett, J. L., Cronin, G. M., and Winfield, C. G. (1981). The effects of individual and group penning of pigs on total and free plasma corticosteroids and the maximum corticosteroid binding capacity. *Gen. Comp. Endocrinol.* **44**, 219–25.

Bottoms, G. D., Roesel, O. F., Rauch, F. D., and Akins, E. L. (1972). Circadian variation in plasma cortisol and corticosterone in pigs and mares. *Am. J. Vet. Res.* **33**, 785–90.

Christison, G. I., and Curtin, T. M. (1969). A simple venous catheter for sequential blood sampling from unrestrained pigs. *Lab. Anim. Care* **19**, 259–62.

Edqvist, L. E., Einarisson, S., Larsson, K., and Lundstrom, K. (1980). Diurnal variation in peripheral plasma of testosterone, androsterone and cortisol in boars. *Acta Vet. Scand.* **21**, 451–3.

Eleftheriou, B. E. (1964). Bound and free corticosteroid in the plasma of two subspecies of deer mice (*Peromyscus maniculatus*) after exposure to a low ambient temperature. *J. Endocrinol.* **31**, 75–80.

Fulkerson, W. J. (1978). Synchronous episodic release of cortisol in the sheep. *J. Endocrinol.* **79**, 131–2.

Fulkerson, W. J., and Tang, B. Y. (1979). Ultradian and circadian rhythms in the plasma concentration of cortisol in sheep. *J. Endocrinol.* **81**, 135–41.

Gundlach, H. von (1968). Brutfürsorge, Brutpflege, Verhaltensontogenese und Tagesperiodik beim Europaischen Wildschwein (*Sus scrofa* L.). *Z. Tierpsychol.* **25**, 955–95.

Halberg, F., Peterson, R. E., and Silber, R. H. (1959). Phase relation of 24-hour periodicities in blood corticosterone, mitoses in cortical adrenal parenchyma and total body activity. *Endocrinology* **64**, 222–30.

Haus, E., and Halberg, F. (1970). Circannual rhythm in level and timing of serum corticosterone in standardized inbred mature C-mice. *Environ. Res.* **3**, 81–106.

Kreiger, D. T. (1975). Circadian pituitary adrenal rhythms. In 'Biological Rhythms and Endocrine Function'. (Eds L. W. Hedlund, J. M. Franz, and A. D. Kenny.) pp. 169–89. (Plenum Press: New York.)

Morberg, G. P., Bellinger, L. L., and Mendel, V. E. (1975). Effect of meal feeding on daily rhythms of plasma corticosterone and growth hormone in the rat. *Neuroendocrinology* **19**, 160–9.

Morimoto, Y., Arisue, K., and Yamamura, Y. (1977). Relationship between circadian rhythm of food intake and that of plasma corticosterone and effect of food restriction on circadian and adrenocortical rhythm in the rat. *Neuroendocrinology* **23**, 212–22.

Siegel, H. S., Mitchell, B. W., Gould, N. R., Latimer, J. W., and Wilson, R. L. (1976). Circadian rhythms for corticosterone, corticosteroid binding capacity, plasma glucose, heart rate, respiration rate, and body temperature in white rock males. European Poultry Conference Proceedings, Pt 5. pp. 1050–61. (British Poultry Science Association.)

Sobel, H., Sideman, M., and Arce, R. (1960). Steroid excretion by guinea pigs exposed to cold for prolonged periods. *Am. J. Physiol.* **198**, 1107–10.

Stein, H. J., Bader, R. A., Eliot, J. W., and Bass, D. E. (1949). Hormonal alterations in men exposed to heat and cold stress. *J. Clin. Endocrinol.* **9**, 529–47.

Whipp, S. C., Wood, R. L., and Lyon, N. G. (1970). Diurnal variation in concentrations of hydrocortisone in plasma of swine. *Am. J. Vet. Res.* **31**, 2105–7.

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