STRESS HORMONE RESPONSES OF SHEEP TO FOOD AND WATER DEPRIVATION AT HIGH AND LOW AMBIENT TEMPERATURES

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The effects of food and/or water deprivation at different ambient temperatures (7 or 35 °C) on stress hormone release in sheep (n = 8), were studied to provide background data for research into the effects of road transport. Blood samples were taken from catheterized animals at the start and, at 6h intervals, during 48h tests in an environmental chamber. Cortisol release was unaffected by temperature or deprivation state but was stimulated by introduction to the chamber. Prolactin secretion showed a similar tendency and levels of this hormone were generally higher in the first test, whichever chamber was used. Heat exposure also had a prolonged stimulatory effect on prolactin release, especially in the first test. Growth hormone concentrations were rather variable but tended to be greatest when the animals were deprived of food. Measurements of plasma osmolality indicated that sheep remained in water balance, even when water was withheld for 48h, unless they had access to food. The results suggest that under laboratory conditions, and over a wide thermal range, withholding food and water for 48h does not induce cortisol or prolactin release in sheep. However, exposure to novel situations seems to have a stimulatory effect.

Keywords: *animal welfare, cortisol, dehydration, food deprivation, prolactin, sheep, temperature*

Introduction

Changes in plasma concentrations of cortisol and prolactin can indicate that animals are stressed and, when used in conjunction with behavioural and physiological measurements, are of considerable value in the assessment of welfare problems.

Although much is known about the thermoregulatory responses of the sheep, changes in endocrine status after food and water deprivation at different ambient temperatures have not been studied. Moreover, such information should provide useful background data for transport research because it is not possible experimentally to partition the effects of transport-related stimuli (eg movement, noise and vibration), environmental factors (temperature and humidity), and deprivation (food and water) state under commercial conditions.

Previous work in this laboratory has shown that when sheep are dehydrated for 48h at normal temperature (= 20 °C), the secretion of the stress hormones cortisol (Matthews &

Parrott 1991) and prolactin (Matthews & Parrott 1992) does not markedly change. Hence, there is no indication from these measurements that the dehydration which is often imposed upon sheep during long-distance transport causes undue distress. Nevertheless, it is important to note that such journeys can also involve prolonged periods of food and water deprivation and exposure to extremes of temperature which may have important additive effects.

If a particular combination of circumstances induces stress hormone release in the laboratory, then such responses are likely to be enhanced if the same conditions apply during transport. On the other hand, conditions which may not be stressful in the laboratory, however, may predispose the animal to display an endocrine response when an aversive stimulus is applied. There is evidence to indicate that this is the case when sheep deprived of water at normal temperatures are subjected to restraint (Matthews & Parrott 1991, 1992).

The present experiment investigated the endocrine responses of sheep to: food; water; or food and water deprivation, during a 48h period of exposure to a low or a high ambient temperature. The animals were provided with indwelling venous catheters so that the results would not be influenced by the effects of venepuncture. Measurements were made of plasma concentrations of cortisol, prolactin and growth hormone. Plasma osmolality, a measure of the osmotic concentration in relation to solvent weight, was also determined as this gives an indication of the state of dehydration and it is affected by physical and social distress in sheep (Parrott *et al* 1988).

Methods

The animals used were eight fleeced Clun Forest wethers (castrated male sheep). They were housed individually on straw litter in pens on opposite sides of a naturally illuminated barn (ambient temperature about 10–15°C). Ventilation fans ensured appropriate air exchange but no heating was provided. When the sheep were not involved in the experiment they were fed twice daily on hay and concentrates, and water was continuously available.

To facilitate blood sample collection each animal was provided, at least one day before the start of the experiment, with a vinyl catheter (1.0mm OD, Dural Plastics, Auburn, Australia) in the jugular vein. This was inserted under local anaesthesia ('Willcain', Arnolds, Romford, Essex, UK) using a 12 gauge hypodermic needle (Coopers Needles, Birmingham, UK). The catheters were covered by a protective elasticated bandage and were flushed daily with heparinized sterile physiological saline. The same catheters were used throughout, apart from occasional replacements. It should be noted that the use of catheters enables blood samples to be taken with minimum handling of the animals, thereby reducing the stress of restraint and venepuncture.

The experiment was carried out over an eight-week period in the months of September and October. The animals were divided into groups of four and each week one group was exposed to a low, and the other to a high, ambient temperature, with the treatments alternated in the subsequent week. This was achieved by transferring the sheep to pens inside identical closed, fan ventilated, chambers (1.2x2.7m) thermostatically maintained at approximately 7 or 35°C. The pens were provided with straw bedding and the chambers were illuminated between 0600 and 1800h.

The groups of sheep entered the chambers on the same day each week at 1130h and an initial blood sample was collected 30min later. Further samples were then taken at 6h

intervals over the next 48h. Following this, the sheep were returned to their home pens and given food and water. In the first two weeks of the study, the sheep had water available in the chambers and were fed in the normal manner. In weeks three and four they were just given water and in weeks five and six only food was available. Finally, in weeks seven and eight the sheep were given neither food nor water whilst they were in the chambers.

Blood was collected in 10ml heparinized tubes (Sarstedt, Beaumont-Leys, Leicestershire, UK) which were temporarily stored on ice and subsequently centrifuged. Plasma aliquots for hormone measurements were stored at -30°C and samples for the estimation of osmolality were placed in a refrigerator. The latter were processed as soon as possible using an automated osmometer (Roebling, Camlab, Cambridgeshire, UK). Plasma concentrations of cortisol, prolactin and growth hormone were measured by radioimmunoassay, as previously described (Parrott & Goode 1992).

Statistical comparisons between the responses of the animals to the different experimental treatments were made using analysis of variance and two-tailed paired *t* tests.

Results

The highest plasma cortisol concentrations (Figure 1) were observed at the start of each test. Comparison of initial values with those observed during the next 48h indicated significant differences in the following situations: food only, cold ($P < 0.02$); no food or water, cold ($P < 0.01$); food and water, hot ($P < 0.03$); water only, hot ($P < 0.03$); food only, hot (first 24h, $P < 0.04$); no food or water, hot ($P < 0.01$).

Hormone levels during the subsequent 48h of the first test (food and water) did not differ from the overall concentrations during the same period in later weeks of the experiment, with the exception that lower values were seen in the water only, hot condition ($P < 0.02$). Notably, however, cortisol secretion was not affected by the length of time spent in either chamber, and there were no significant differences between the effects of cold and heat exposure under the various deprivation conditions. There was also no evidence from these data to suggest a rhythmic pattern of cortisol release.

Prolactin concentrations (Figure 2) tended to increase after entry to the chambers and remained high for the following 48h in all tests in the heat. In consequence, overall differences due to temperature condition were statistically significant (food and water $P < 0.001$; water only $P < 0.001$; food only $P < 0.01$; no food or water $P < 0.001$). Moreover, prolactin levels in the subsequent 48h of the first test (food and water) in the cold were higher than when the animals were provided with water only ($P < 0.01$) or nothing ($P < 0.01$). Similarly, in the heat, overall hormone concentrations in the food and water conditions were higher than in the water only ($P < 0.001$), food only ($P < 0.001$) or no food or water ($P < 0.002$) situations. However, notwithstanding the responses of individual sheep that distorted the final means in the food and water (cold) and no food or water (hot) conditions, there was no evidence to suggest that prolactin secretion increased with time.

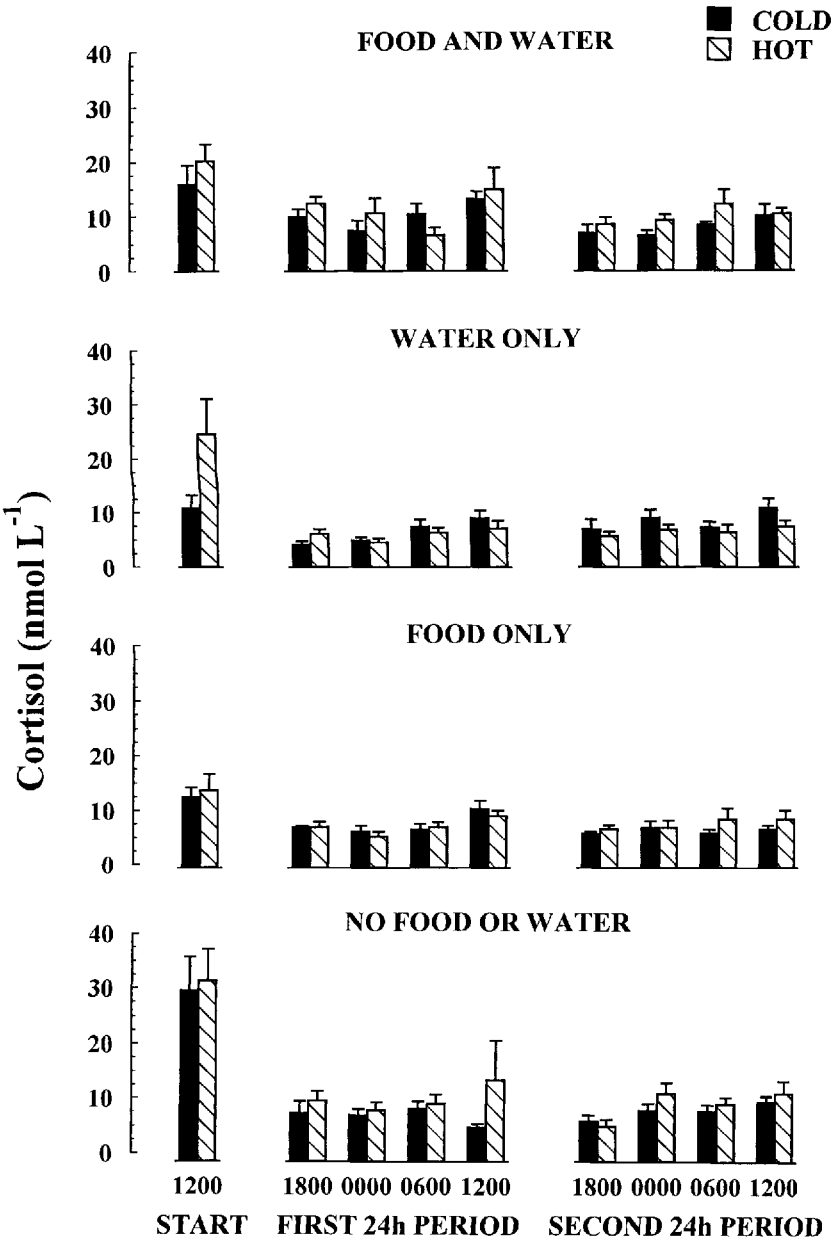

Figure 1 Plasma concentrations (mean ± SEM) of cortisol (nmol l^{-1}) in sheep 30min after entering the chambers (START) and at 6h intervals during two consecutive 24h periods of exposure to cold (+7°C, black columns) or hot (+35°C, shaded columns) ambient temperatures. In the first test, the sheep were provided with food and water; in the second test they received water only; and in the third test, food only. Finally, in the fourth test the animals were deprived of both food and water.

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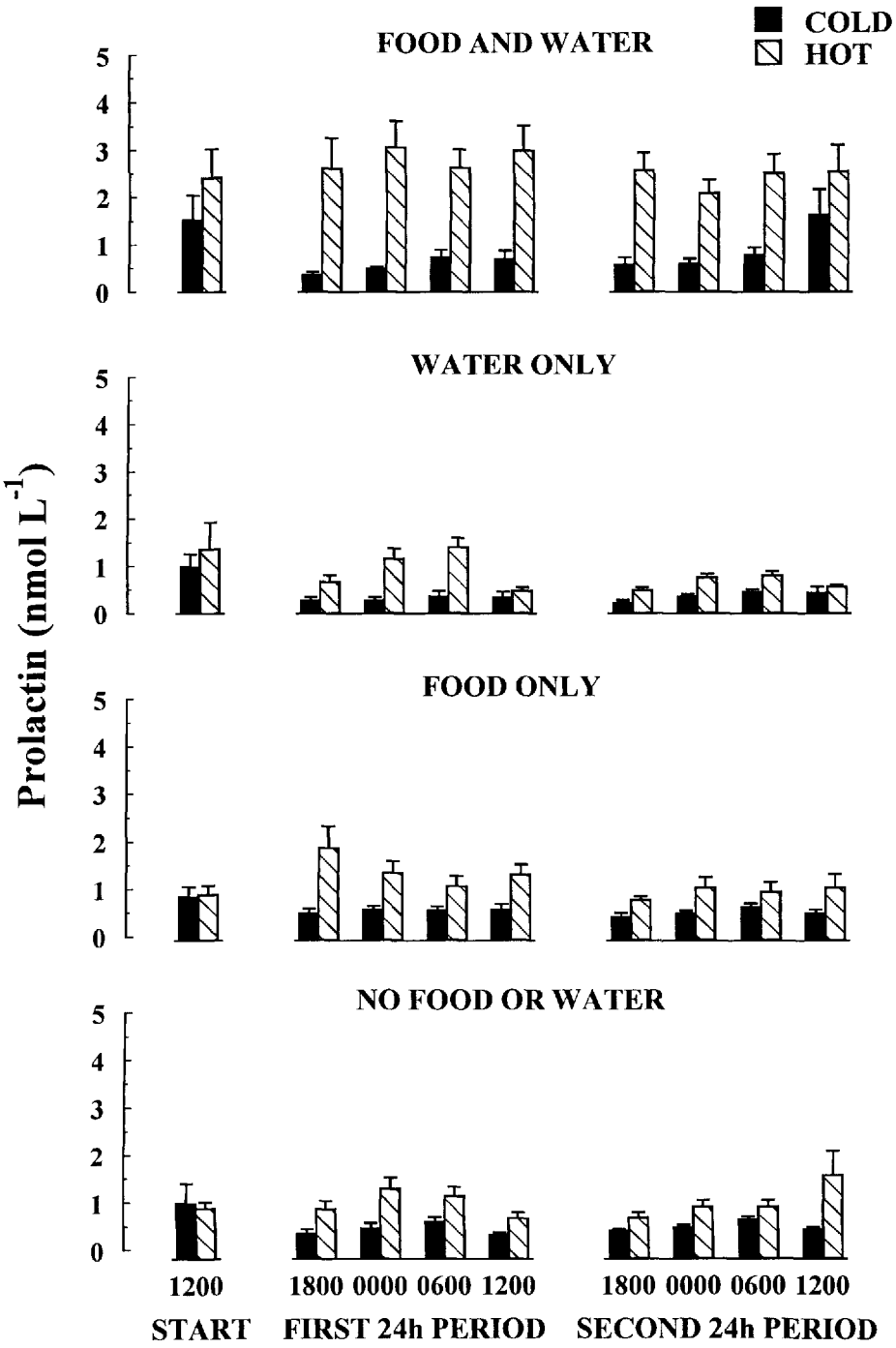

Figure 2 Plasma concentrations (mean ± SEM) of prolactin (nmol l^{-1}) in the sheep tested as described for Figure 1.

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Growth hormone concentrations (Figure 3) were highly variable and, therefore, any statistical analysis of these data would not be appropriate. Hormone levels were not consistently higher at the start and there did not appear to be any obvious effects of temperature. However, there was a tendency for overall concentrations to be raised in the two tests when the sheep were deprived of food.

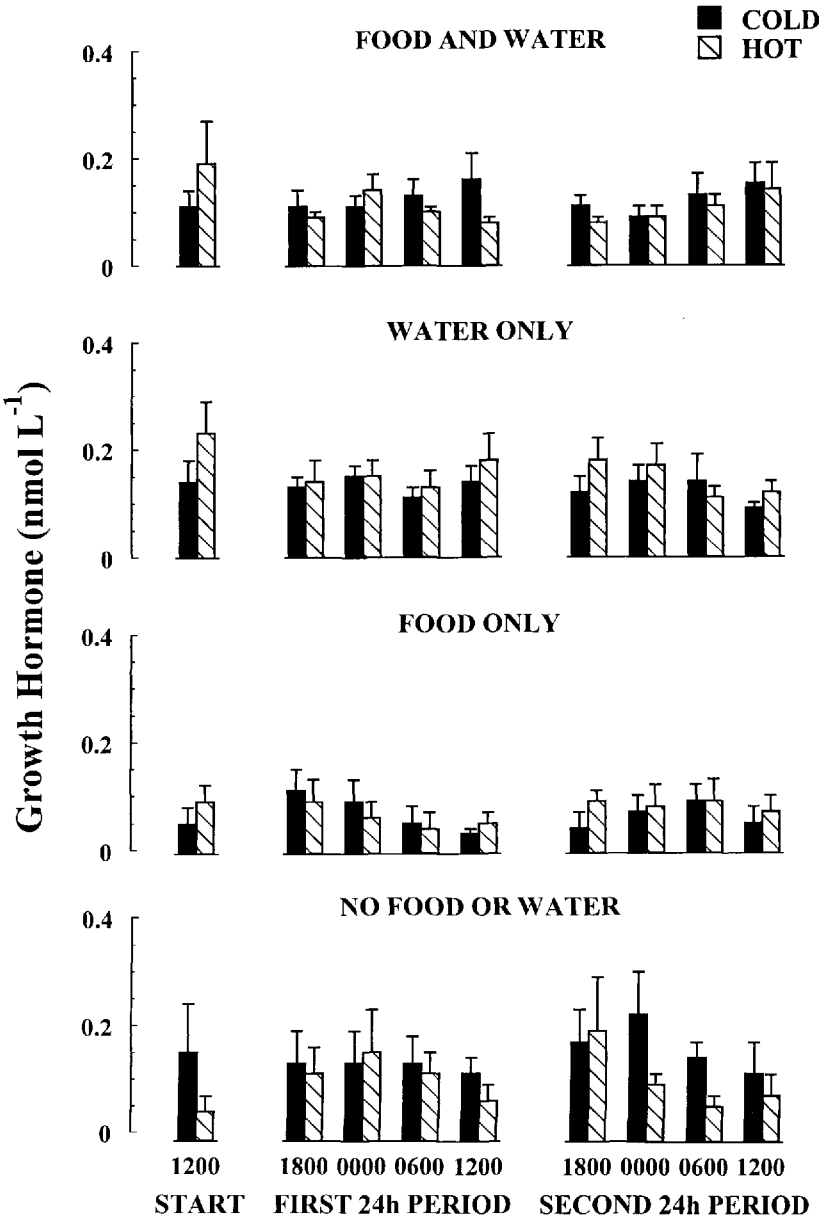

Figure 3 Plasma concentrations (mean ± SEM) of growth hormone (nmol l^{-1}) in the sheep tested as described for Figure 1.

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Plasma osmolality (Figure 4) was unaffected by temperature in this experiment. The only treatment condition in which a change in osmolality was detected was in the food only test. In this situation, osmolality increased in both the heat and the cold at the end of the first 24h period and remained high throughout the next 24h; statistical analysis indicated that differences between the two 24h periods in both cold and heat exposed animals were highly significant ($P < 0.001$). It is also apparent that osmolality tended generally to decrease throughout the course of the experiment.

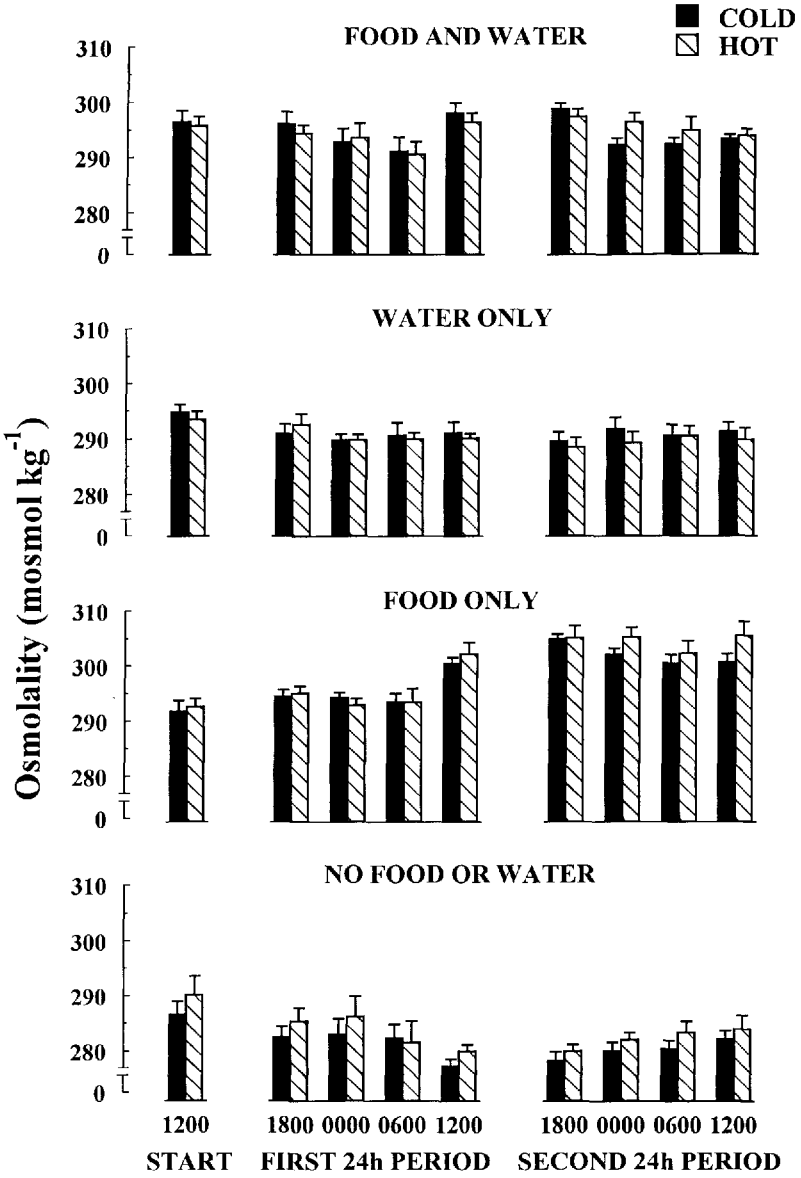

Figure 4 Plasma osmolality (mean ± SEM; mosmol kg^{-1}) in the sheep tested as described for Figure 1.

Discussion

Previous studies in this laboratory with Clun Forest sheep have shown that there is an initial cortisol response to handling when blood is collected by venepuncture, after which hormone levels in animals receiving no additional stress are in the range of 5–10 nmol l^{-1} (Parrott *et al* 1988; Parrott & Thornton 1989). Similarly, the higher concentrations (10–30 nmol l^{-1}) seen in the present study directly after the sheep were transferred to the environmental chambers probably reflect a response to disturbance and grouping, and the novelty of the situation. Thereafter, however, cortisol levels in each treatment condition decreased and were mainly within the normal range for unstressed animals (> 10 nmol l^{-1}), although they tended to be somewhat higher than those found in very well-adapted catheterized sheep (Matthews & Parrott 1991).

The lack of effect on cortisol release of 48h dehydration at normal temperatures has been reported before (Matthews & Parrott 1992). The present data show that this manipulation does also not induce cortisol secretion when sheep are housed at extreme temperatures. Further, withholding food provides no additional stimulus to cortisol release under these conditions. These findings are consistent with the evolutionary origin of the sheep in the Middle East (Ratner & Boice 1975), their ambivalence towards shade in the summer heat of the Australian subcontinent (Johnson 1991), and their remarkable ability to withstand dehydration (Silanikove 1994) and heat stress (Degen & Shkolnik 1978). For example, it has been reported that restricting water intake to two days per week has little effect on the health or reproductive performance of ewes living in the Indian desert (Mittal & Ghosh 1986).

With regard to the effects of cold exposure, cortisol concentrations are increased in shorn, periodically wetted sheep kept at 2–3°C (Panaretto 1974). By contrast, subjecting lambs each day to 3h at -5°C was calculated to increase blood cortisol levels only on the first occasion (Berman *et al* 1980). Therefore, it seems that larger, fleeced sheep are unlikely to be distressed by temperatures between 7 and 35°C, even when they have no access to food or water. However, it should also be noted that sheep, paradoxically, seem to have lower cortisol concentrations at high temperatures under humid conditions (Guerrini & Bertchinger 1982). A final point relating to the cortisol results concerns the absence of any obvious secretory rhythm. An investigation in sheep sampled at 10min intervals indicated that highest cortisol concentrations occur around 0100h and lowest after 1200h (Fulkerson & Tang 1979). Clearly, therefore, the sampling interval in the present study was too infrequent to detect such a pattern. Moreover, the latter may also have been disrupted by the experimental procedure, as a period of adaptation appears to be necessary before the rhythm becomes apparent (McNatty & Young 1973).

Prolactin is a pituitary hormone well known for its role in reproduction, although there is evidence to indicate that it may also be involved in a number of other physiological and metabolic processes. In the sheep, for example, plasma concentrations of the hormone in animals sampled by venepuncture or using a catheter are rather variable (De Silva *et al* 1981) and, as in other species, have often been reported to increase in response to stress (eg Wolinska-Witort *et al* 1986; Parrott & Thornton 1989). Similarly, in the present experiment, prolactin concentrations in the cold tended to be greatest directly after introduction to the chamber. This result parallels that for cortisol and probably represents a stress response to movement and grouping. However, prolactin secretion under cold conditions was unaffected

by the time of exposure, or the deprivation state, suggesting that these chronic situations were less stressful than the acute effects of transfer to the chamber.

When the sheep were placed in the hot chamber, the initial high concentrations of prolactin were maintained. There was, therefore, a temperature-dependent stimulation of prolactin release that was exaggerated the first time the animals were tested in the heat. The secretion of prolactin by sheep is known to be higher in summer due to an effect of increased photoperiod (Pelletier 1973), but an increase associated with environmental temperature does not appear to have been reported before in this species. However, plasma prolactin has been shown to be positively correlated with ambient temperature in pigs (Dauncey & Buttle 1990) and cattle (Wetterman *et al* 1982) and, therefore, this response may be typical of ungulates generally. Moreover, although drinking following dehydration increases prolactin concentrations in cattle (Doris & Bell 1984), comparison of the response to food deprivation with that of water deprivation indicates that the increased prolactin secretion observed in the hot chamber was not a consequence of drinking. This investigation also confirms previous findings showing that neither dehydration (Bell *et al* 1991; Matthews & Parrott 1992), nor food restriction (Thomas *et al* 1990), affects prolactin release in sheep. However, the possibility that dehydrated (Matthews & Parrott 1992) or heat-exposed (this study) sheep may show a greater prolactin response to stress needs to be borne in mind.

Growth hormone release is induced by stressors in man and inhibited in rodents (Reichlin 1988) but no stress-related changes have been observed in sheep (Davis 1972; Cronin *et al* 1981; Parrott & Goode 1992). In the present study growth hormone concentrations were highly variable, making interpretation of the data difficult. Nevertheless, there was not an obvious increase in hormone levels in the first test (food and water) nor in response to introduction to the chambers, as was found for cortisol and prolactin. Also, there did not seem to be an increase in secretion at high ambient temperatures, as has been suggested in the pig (Dauncey & Buttle 1990). However, there was a tendency towards higher concentrations in both tests when the animals were deprived of food. This is consistent with previous findings in sheep maintained on a restricted diet (Thomas *et al* 1990).

When sheep are subjected to isolation, a psychologically disturbing situation which increases heart rate (Baldock & Sibly 1990), there is a decrease in osmolality that parallels the increase in plasma cortisol (Parrott *et al* 1988). However, there was no evidence for an effect of this kind in the present study, perhaps because the treatments had only transient effects. When only food was available, osmolality significantly increased at the same time in both the heat and the cold and then remained high for the next 24h. Since sheep normally drink after feeding (Ternouth 1968), this suggests that the animals continued to eat in the absence of water and, thereby, disturbed their water balance. Although it was not possible to quantify individual food intakes because the animals were housed communally, this phenomenon has been noted before in sheep maintained at normal temperatures (Parrott *et al* 1987). However, it does not occur when animals are stressed and have consistently high cortisol levels because, under these conditions, sheep starve themselves and their osmolality remains constant (Parrott *et al* 1987). Similarly, starvation was reported as a cause of death in some sheep during a 13 day sea voyage (Bailey & Fortune 1992) whereas the majority of animals, even though they were kept at high temperatures (approximately 30°C), showed little change in body weight. Nevertheless, exposure to heat tended to produce a small (non-significant) increase in osmolality when sheep were deprived of both food and water in the

present study. As some animals were observed to pant in the hot chamber, it is likely that this reflects water lost through evaporative cooling. Finally, the decrease in osmolality during the course of this study was probably caused by lack of condition resulting from repeated episodes of food restriction.

Taken together, the cortisol, prolactin and osmolality data presented here provide no evidence that fleeced sheep are markedly stressed by prolonged exposure to temperatures of 7 or 35°C and concomitant deprivation of food and/or water. In fact, the only part of the experiment that was distressing to the sheep seems to have been the initial movement and grouping and the overall circumstances of the first test. Psychological factors may have had a contributory effect here because, in subsequent treatment conditions the animals would have been familiar not only with one another, but also with the experimental procedure. These results suggest that extremes of temperature and periods of deprivation may not be major components of the stress associated with long-distance road transport.

Animal welfare implications

There is much concern for the welfare of sheep during long-distance road transport throughout the continent of Europe. Such journeys are likely to involve prolonged periods without access to food or water and extremes of temperature. In order that the response to transport *per se* can be properly evaluated, it is necessary to obtain background information on the effects that some of the conditions might have when sheep are not in transit. Measurement of cortisol and prolactin secretion in catheterized animals provides a useful means by which to address this question. The present findings suggest that neither exposure to high or low temperatures, nor the withholding of food and/or water for 48h is stressful to sheep held under laboratory conditions. Moreover, sheep are able to maintain normal plasma osmolality if deprived of food or both food and water, but not if provided with food alone. This suggests that if access to water is limited during lairage, it might be advisable also to restrict the availability of dry food.

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