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HORMONAL AND PHYSIOLOGICAL EFFECTS OF A 15 HOUR ROAD JOURNEY IN SHEEP: COMPARISON WITH THE RESPONSES TO LOADING, HANDLING AND PENNING IN THE ABSENCE OF TRANSPORT

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SUMMARY

A controlled study was carried out to investigate the physiological effects of road transport on sheep. Animals ($n=10$; body weight 38.9 ± 1.3 kg), previously with catheters in their jugular veins, were rounded up and loaded onto a vehicle where they were held in a communal pen with eight other lambs. Blood samples were taken at 30 min intervals during the next 15 h while the vehicle remained stationary or was driven a distance of 548 miles (876 km). Measurements were made of plasma concentrations of cortisol, prolactin, creatine phosphokinase and lactase dehydrogenase isozymes and also of plasma osmolality, haematocrit and body weight; heart rate was also recorded in one animal. Loading and the start of driving produced large increases in cortisol and prolactin concentrations. Heart rate also increased whereas osmolality and haematocrit decreased. The major changes in hormone release occurred in the first 3 h period while, during the remaining 12 h, the stimulatory effect of transport was present but small. Body weight loss was similar under both stationary and driven conditions.

KEYWORDS: Transport; sheep; cortisol; prolactin; fluid balance; muscle damage; welfare.

INTRODUCTION

Current concern for the welfare of sheep during long distance road transport is reflected in a series of publications addressing issues such as journey time (Warriss *et al.*, 1990), behaviour during lairage (Jarvis & Cockram, 1995), bruising (Jarvis & Cockram, 1994; Knowles *et al.*, 1994a) and death during transit or lairage (Knowles *et al.*, 1994b). In addition, in order to provide more direct information

on the animals' responses, some studies have examined physiological and hormonal indicators using blood samples collected before and after journeys (Fordham *et al.*, 1989; Knowles *et al.*, 1993, 1994c, 1995). However, in all of these investigations, blood had been collected by jugular venepuncture, which inevitably causes additional distress (Parrott *et al.*, 1988). Furthermore, none of these studies attempted to separate out the effects of transport *per se* from those of rounding up, loading and confinement with unfamiliar animals in pens for the duration of the journey. Hence, the objective of the present study was to carry out an experiment that would enable the effects of transport to be dissociated from those of other variables.

In this investigation, blood was collected at regular intervals from a group of lambs whilst on a vehicle that remained stationary or was driven for the current maximum journey time in the United Kingdom (15 h). The sheep were prepared with catheters in their jugular veins to avoid the adverse effects of blood sampling by venepuncture. In order to assess the degree of distress incurred, measurements were made of plasma concentrations of cortisol and prolactin, both of which increase in sheep subjected to transport simulation (Parrott *et al.*, 1994). Estimations of haematocrit and osmolality were also made to give an indication of changes in fluid balance that might reflect dehydration or distress (Parrott *et al.*, 1994). Similarly, measurements were made of creatine phosphokinase (CPK) and lactate dehydrogenase (LDH) isozyme levels to provide information on the degree of tissue damage (Moss & McMurray, 1979). The sheep were also weighed before and after each test and one animal was provided with a heart rate monitor.

The intention of the study was to provide, for the first time, a detailed physiological profile of sheep subjected to road transport. Although repeated sampling achieves this objective it should be borne in mind that the level of distress experienced is probably greater in this experimental situation than when animals are carried under commercial conditions. This is because there is some disturbance associated with blood sampling *via* catheters, although this is less than with venepuncture. For the same reason, a detailed analysis of behaviour was not carried out in this study.

MATERIALS AND METHODS

Eighteen 10-month-old Clun Forest lambs were delivered from the Institute farm to a holding paddock adjacent to the laboratory. Ten of the animals (four male, six female) were then transferred to indoor pens (two sheep per pen) and, on the next day, temporary jugular vein catheters were inserted under local lignocaine anaesthesia (Willcain, Arnolds Veterinary Products) and protected by an elasticated bandage. Catheter patency was maintained by daily flushing with sterile heparinized saline.

The following morning, an initial blood sample was taken (06.30 h) and the sheep were weighed. One animal was also provided with a heart rate monitor (Sport Tester, Polar Electro Oy) which was connected to neck and chest electrodes inserted subdermally *via* a 19 G needle. The catheterized sheep were then released into the paddock and all 18 animals were rounded-up and driven up a

ramp into the vehicle. This was a 3.5 tonne standard cattle lorry with ventilation louvres (open) and metal floor and sides. The sheep were confined within a 1.8×2.3 m pen (i.e. 0.23 m² animal⁻¹) the floor of which was covered with wood shavings but neither food nor water was provided.

The vehicle remained stationary for the next 15 h and 10 ml blood samples were collected every 30 min into heparinized syringes (Monovette, Sarstedt). At the end of the sampling period, the sheep were unloaded and the catheterized animals were returned to their pens where they were re-weighed and provided with food and water. The following morning (10.00 h) a final blood sample was collected and the animals were weighed again.

After a 6 day interval, the above procedure was repeated with the difference that the vehicle was driven throughout the 15 h period. A distance of 548 miles was covered (predominantly 'A' class roads and motorways) and there were short breaks (approx 15 min) every 4 h to allow for a change of driver. The experimenters travelled with the sheep, taking blood samples whilst the vehicle was in motion, power for centrifuges and lighting being provided, when required, by a portable generator. Due to unforeseen circumstances, loading took place 30 min later than in the stationary trial with the result that the 31st blood sample was not collected.

On both test days, whole blood packed cell volumes (% PCV) were determined directly after sampling using a haematocrit centrifuge and reader (Hawksley Ltd). The remaining blood was then centrifuged and the resultant plasma divided into aliquots for further analysis. Representative samples were kept at 4°C until the following day for estimations of plasma osmolality using an automatic micro-osmometer (Roebing, Camlab Ltd). Other samples were frozen in dry ice and subsequently stored at -30°C pending radioimmunoassay for cortisol and prolactin, using previously described methods (Parrott & Goode, 1992).

Frozen plasma samples were also used to determine CPK and LDH isozyme concentrations. The CPK assay used an optimized standard method and levels were measured on an automatic analyser (Model CX5CE Beckman Instruments Inc). For LDH, plasma was run on an agarose gel (100 v for 30 min) using a Beckman 'Paragon' electrophoresis system. After staining and fixing, the gels were evaluated with a Beckman densitometer to determine the percentage composition of the five isozymes.

A tri-axial accelerometer reporting to a multi-channel data-logger (Squirrel, Grant) provided a record of physical shocks every 10 min during the course of the driven test. Other sensors measured air temperature, relative humidity and sound pressure levels.

Comparisons between results obtained in the home pen were made using the paired *t* test. However, during the experimental period, differences between stationary and driven treatments were contrasted using an analysis of variance based on the area under the time curve, integrated using the trapezoid rule, for the 0-180, 180-900 and 0-900 min periods. All probability values were two-tailed.

RESULTS

Plasma cortisol concentrations are shown in Fig. 1. Although these were higher in

the home pen (HPD1) before the start of the stationary test ($P<0.001$), loading (time zero) provoked a similar increase in cortisol release in both experimental situations. Concentrations gradually declined during the next 180 min in the stationary condition whereas an additional, but transient, peak in cortisol was associated with the onset of driving (30 min sample). As a consequence, hormone concentrations during the 0–180 min period were significantly ($P<0.02$) greater in the driven condition. By contrast, the pattern of hormone secretion in both tests during the subsequent 12 h was similar and concentrations during the 180–900 min interval were not significantly different. Nevertheless, it should be noted that cortisol concentrations in the driven test were, with one exception (630 min sample), maintained throughout this period above the level seen in the home pen. In the stationary test, however, concentrations tended to be similar or lower than recorded in the home pen. Also, an overall analysis of the results (0–900 min) indicated that cortisol concentrations were generally higher ($P<0.04$) in the driven test.

No differences in plasma prolactin concentrations (Fig. 2) were found between initial samples taken in the home pen. In the first (stationary) test, hormone concentrations increased after loading (time zero) and then rapidly fell. However, in the second (driven) test, concentrations rose transiently with the start of driving (30 and 60 min samples), rather than after loading. Thus, because of these differing patterns of release, no significant difference between treatments was detected during the 0–180 min interval. Hormone concentrations throughout the remainder of the test were, in the main, lower than those observed in the home pen in both tests although some high but variable results were apparent towards the end of the driven test (720, 810 and 840 min samples). However, there was a

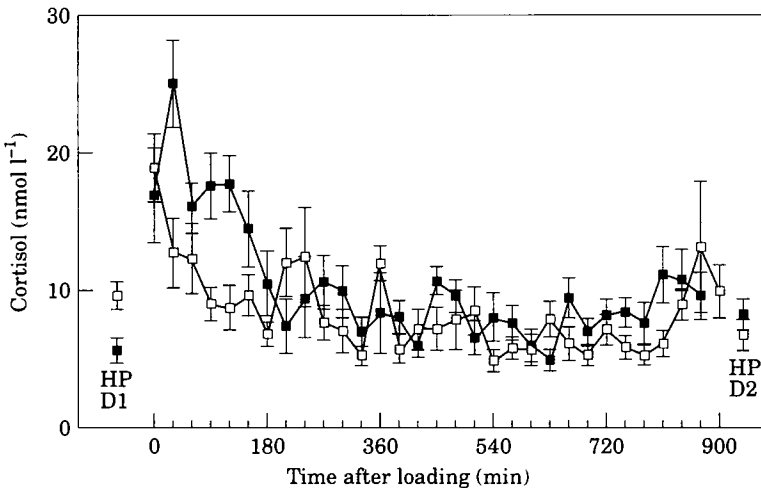


Fig. 1. Plasma cortisol concentrations (nmol l^{-1} ; mean \pm SEM) in sheep ($n=10$) sampled in the home pen on day 1 (HPD1), during the 15 h experimental period under stationary (\square) or driven (\blacksquare) conditions, and 12 h later in the home pen on day 2 (HPD2). In the first 180 min of the experimental period, but not subsequently, hormone concentrations were higher ($P<0.01$) in the driven test; see text for further details.

general tendency for prolactin concentrations to be slightly greater in the driven condition. This is reflected in the statistical analysis which indicates a significant treatment difference both in the 180–900 min period ($P < 0.001$) and also overall (0–900 min, $P < 0.03$).

Estimated plasma osmolality (Fig. 3) was higher initially in the driven test (HPD2, $P < 0.001$). Levels tended to fall on both test occasions during the

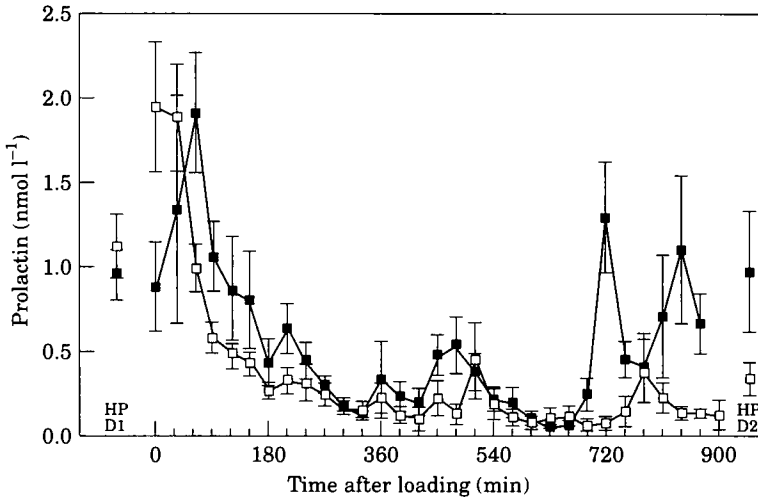


Fig. 2. Plasma prolactin concentrations (nmol l^{-1} ; mean \pm SEM) in the sheep under the conditions described for Fig. 1. Concentrations were greater ($P < 0.001$) in the driven test in the 180–900 min period; see text for further details. (□), stationary; (■), driven.

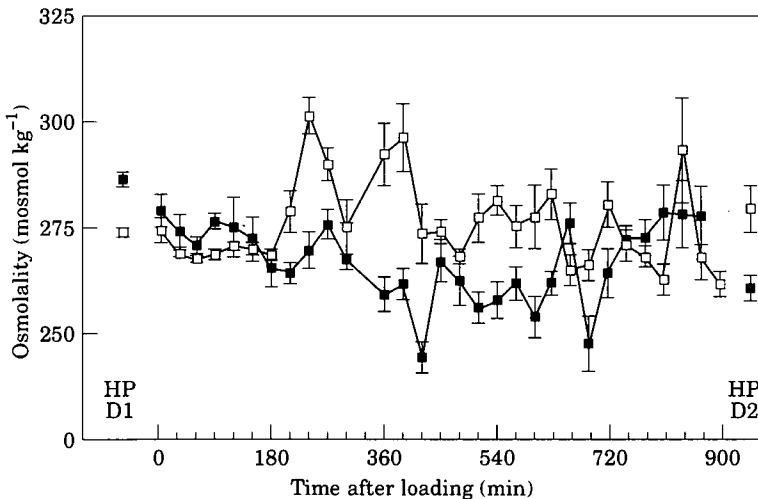


Fig. 3. Plasma osmolality (mosmol kg^{-1} ; mean \pm SEM) in sheep under the conditions described for Fig. 1. Values were greater ($P < 0.04$) in the driven test in the first 180 min but lower ($P < 0.002$) in the 180–900 min period; see text for further details. (□), stationary; (■), driven.

0–180 min period but still remained higher in the driven test ($P<0.04$). Subsequently, however, osmolality rose in the stationary condition and values during the 180–900 min interval were higher ($P<0.002$) than those in the driven situation. Also, it should perhaps be noted, that all osmolalities measured in the driven test were lower than those initially seen in the home pen whereas this was not so for the stationary test.

Haematocrit results are displayed in Fig. 4. Readings were higher ($P<0.001$) before the stationary trial. On both test occasions, haematocrit decreased abruptly after loading (time zero) and more gradually subsequently. In all three parts of the experimental period, the haematocrit was greater ($P<0.001$) in the stationary condition.

Concentrations of CPK and LDH isozymes 1 and 5, expressed as the difference (mean \pm SEM) from initial values in the home pen on day 1 are shown in Fig. 5. Each of these indices provided highly variable results, as indicated by the size of the error bars. Moreover, there was considerable overlap between the data obtained for each measure in both the stationary and driven trials. Although CPK concentrations appeared to be higher in the first 180 min of the driven study, this difference was not statistically significant. There were no obvious effects of either loading or driving on LDH1 and LDH5 isozyme concentrations.

Heart rates recorded in an individual sheep during both tests are illustrated in Fig. 6. After high values recorded at the start, the smoothed frequency (median no. beats per 10 min) decreased in a similar fashion under stationary and driven conditions. Also, behavioural observations indicated that the majority of sheep were standing on each sampling occasion in both stationary (9.3 \pm 0.2, mean \pm SEM) and driven (9.4 \pm 0.2) tests. None was seen to ruminate while lying and only a few did so while standing (0.4 \pm 0.2 stationary, 0.01 \pm 0.1 driven). Other behaviours

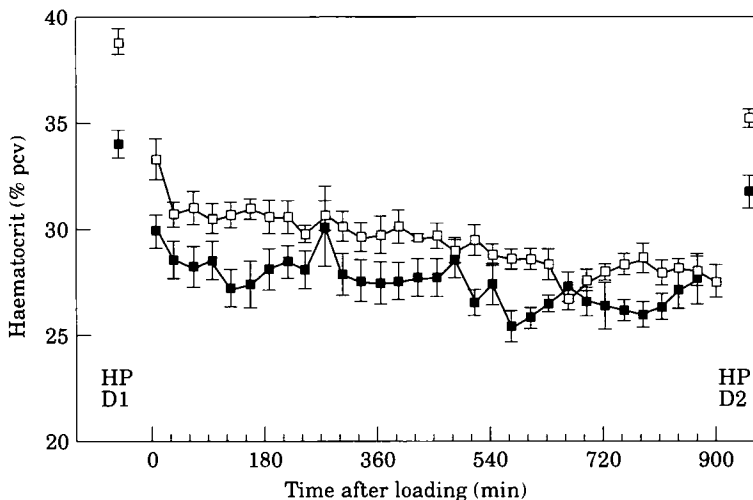


Fig. 4. Whole blood haematocrit (% PCV; mean \pm SEM) in sheep under the conditions described for Fig. 1. Measurements were significantly ($P<0.001$) higher in the stationary than the driven test in both the 0–180 and 180–900 min periods; see text for further details. (□), stationary; (■), driven.

observed sporadically, but not systematically, were tooth grinding and wool nibbling.

The sheep lost weight on both test occasions. In the stationary phase, the animals weighed 38.9 ± 1.3 kg initially, 37.5 ± 1.2 kg at the end of the test period and 37.4 ± 1.1 kg the next day. Weight loss in the driven test was not significantly greater, i.e. 38.1 ± 1.5 kg before and 36.0 ± 1.5 kg after testing and 36.3 ± 1.4 kg the next day. Notably though, the weights recorded the day following each test were still significantly ($P < 0.001$) below initial values.

On both test days, the air temperature 1 m above the floor was about 10°C and the relative humidity varied between 70 and 85%. There was no marked variation

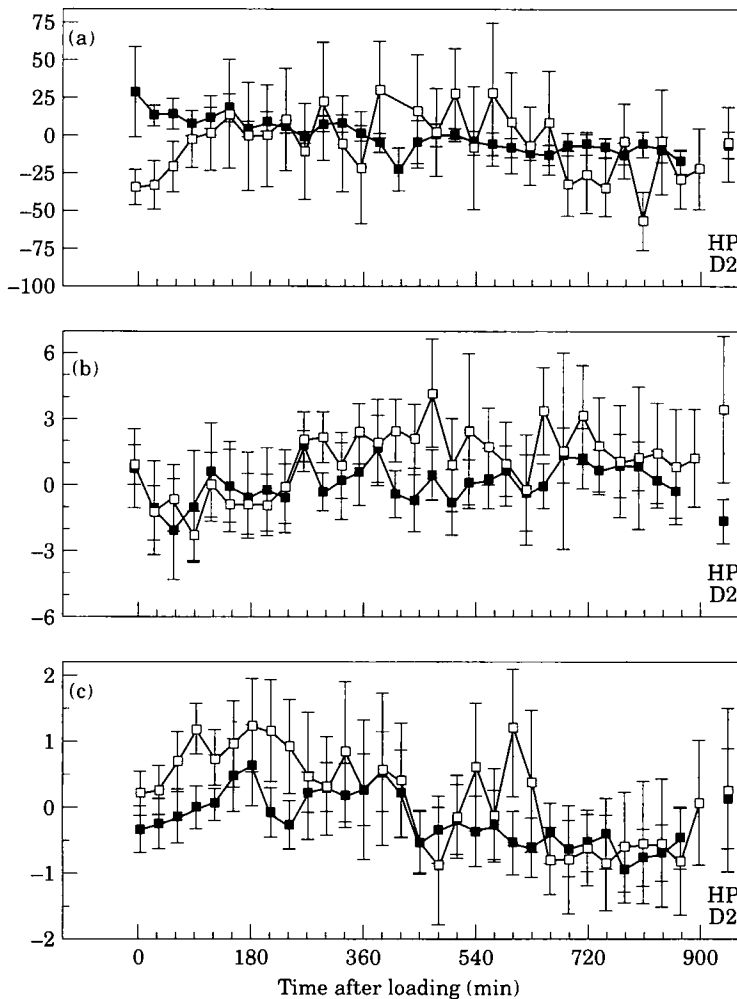


Fig. 5. Plasma concentrations (difference from HPD1; mean \pm SEM) of (a) CPK (IU l^{-1}) and (b) LDH1 and (c) DH5 isozymes (% total LDH) in sheep under the conditions described for Fig. 1. No significant treatment effects were detected.

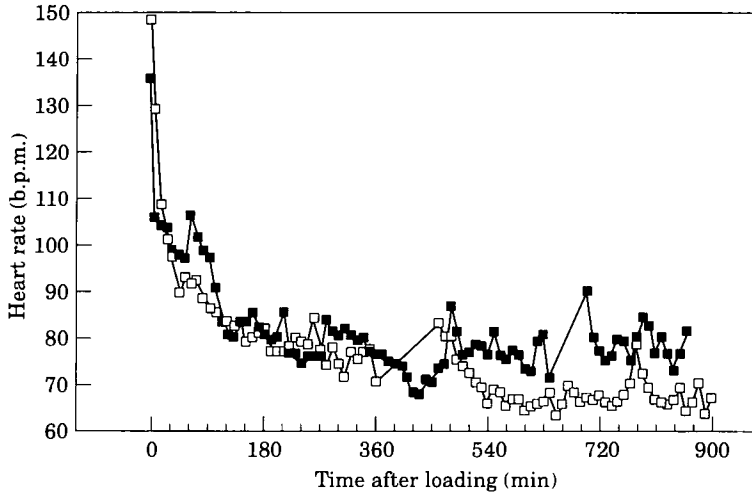


Fig. 6. Heart rate measurements (median beats per 10 min) in an individual sheep under the conditions described for Fig. 1. The recording interval was set to 1 min. (□), stationary; (■), driven.

in sound pressure level (87.9 ± 1.1 dB), or physical shocks (3.3 ± 0.5), based on analysis of 10 min time bins, during the course of the driven test.

DISCUSSION

In the present study, large increases in plasma concentrations of adrenocortical (cortisol) and pituitary (prolactin) hormones during the first 180 min of the test period were induced by loading, penning and the start of transport. However, the long-term effects of transport on hormone release were small. Osmolality and haematocrit decreased in response to loading and remained depressed, except in the stationary test where osmolality subsequently rose. Also, the weight loss induced on both test occasions was not fully restored by 12 h access to food and water.

There are no other published studies in which sheep have been sampled at regular intervals in moving vehicles and where the effects of transport can be separated from those of loading and penning. In this experiment, the greatest stimulation of cortisol release was brought about by transferring the sheep from their individual holding pens to the communal pen on the vehicle. The start of driving produced an additional cortisol peak (350% above the initial value; 5.4 to 24.3 nmol l⁻¹) followed by a slow decline. Moreover, although the effects of loading and the start of driving had largely disappeared after 180 min, there was evidence to indicate that transport had a stimulatory effect on cortisol release throughout the 15 h period. Nevertheless, caution is needed in the interpretation of these data as there is a possibility that the experimental design may have influenced the results. Because the same animals were used for both tests, adaptation could have occurred. However, this is unlikely since repeated handling of sheep at

2 week intervals has no effect on peak cortisol concentrations (Hargreaves & Hutson, 1990). Also, minimal adaptation to repeated confinement in an environmental chamber was found in another study (Parrott *et al.* in press). Furthermore, even if the sheep had adapted to loading and penning as a result of the stationary test, they would not have had any opportunity to become accustomed to the noise, vibration and movement stimuli associated with transport in the driven test. Thus, as the response to loading had disappeared by 180 min, hormonal and physiological changes during the remaining 720 min of the driven test would appear to be a specific response to transport.

The results of this experiment can also be compared with those from uncontrolled studies in which blood samples were collected by venepuncture before and after journeys of different durations. For example, cortisol concentrations prior to transport in 4–5-month-old lambs (Fordham *et al.*, 1989) were much higher than in the present investigations (133 nmol l^{-1}) and increased by 67% (to 221 nmol l^{-1}) after a journey lasting 1 h. By contrast, cortisol levels increased by 187% (from 5.4 to 15.5 nmol l^{-1}) after 60 min transport in this study. Furthermore, in sheep samples before and after a 9 or 14 h journey carried out under commercial conditions (Knowles *et al.*, 1993), cortisol increased by 19% in August (107 to 127 nmol l^{-1}) and 65% in November (105 to 173 nmol l^{-1}). The greater hormonal response in winter, however, was probably not due to the change in ambient temperature as catheterized sheep deprived of food and water for periods up to 48 h at high or low temperatures do not differ in their cortisol concentrations (Parrott *et al.* in press). Also, in a similar study (Knowles *et al.*, 1994c), a significant increase (18%) in cortisol occurred after an 18 h journey (130 to 154 nmol l^{-1}) whereas a decrease (7%) was noted following 24 h transport (113 to 105 nmol l^{-1}). Finally, in a recent report (Knowles *et al.*, 1995), sheep transported for 3 h showed a greater increase in cortisol (90%) than others transported for 9, 15, 18 or 24 h.

The above comparisons indicate that the degree to which plasma cortisol increases during travelling depends on the initial concentration. When this is low, as in the present study, the hormone release due to transport is proportionally greater. Clearly, rounding-up and venepuncture in the field induces cortisol release whereas catheter sampling in the home pen results in a lower baseline. Thus, in the latter situation it is possible to generate a large (percentage change) adrenocortical response to loading and driving. This suggests that the present results may be more meaningful in terms of transport-related stress responses. Nevertheless, both the venepuncture (Knowles *et al.*, 1995) and the present catheter study suggest that considerable adaptation of the cortisol response occurs within a few hours of the start of driving.

Physically taxing situations which are also stressful, for example transport simulation, stimulate prolactin release in sheep, although the hormonal response to psychologically disturbing stimuli is often less consistent (Parrott *et al.*, 1994). In the present experiment, loading induced prolactin release in the first (stationary) but not in the second (driven) test. This may represent adaptation to handling as there is some evidence to suggest that this can occur in sheep (Parrott *et al.* in press). On the other hand, this hormone is rather labile and variability is often encountered which cannot easily be explained, as for example, at the end of the

driven test. However, when driving commenced, prolactin release was transiently stimulated but, as with loading, the response had faded within 180 min. Also, although concentrations in both tests during the 180–900 min periods were generally lower than initial values, driving significantly increased prolactin secretion during this period and also overall. These changes, therefore, parallel those found for cortisol both in terms of the effects of loading and driving and in the rapidity with which adaptation occurred.

One reason for measuring osmolality in this experiment was to determine whether sheep transported and deprived of food and water for 15 h would remain in fluid balance. In agreement with previous studies involving journey times ranging between 3 and 24 h (Knowles *et al.*, 1993, 1994c, 1995), no consistent increases in osmolality were detected. Similarly, sheep deprived of water at high temperatures for periods of up to 48 h also do not become dehydrated (Parrott *et al.* in press).

Whereas increases in osmolality signal haemoconcentration, decreases occur when sheep are stressed. For example, in isolated animals, plasma osmolality declines when cortisol concentrations increase (Parrott *et al.*, 1987, 1988). Because vasopressin secretion is inhibited in isolation-stressed sheep (Parrott *et al.*, 1987, 1988), and in dogs infused with cortisol (Papanek & Raff, 1994), stress-induced reductions in antidiuresis may be compensated for by an action of cortisol in defence of plasma volume. Interestingly, in the present study, osmolality decreased in the first 180 min of both tests, corresponding with the time of maximal cortisol release. Subsequently, as cortisol concentrations fell, there appeared to be a temporary rebound increase in osmolality in the stationary test. However, in the driven situation, where cortisol concentrations were maintained above initial levels, osmolality remained depressed.

Other workers (Knowles *et al.*, 1993) have reported decreases in haematocrit following 9 or 14 h journeys, although this effect was considered to be due to recovery after an initial rise induced by rounding-up and handling. A more recent study by this group, however, has observed increases in haematocrit with different journey times (Knowles *et al.*, 1995). Splenic contraction due to excitement is known to raise haematocrit in sheep (Turner & Hodgetts, 1959) and high initial values followed by a decrease have been described in association with handling (Fenwick & Green, 1986). However, it should be noted that even when initial values are similar, restraint or isolation stress produce a greater fall in haematocrit than handling alone (Parrott *et al.*, 1988). Hence, it seems that stressors can produce an actual rather than an apparent decrease in haematocrit; this might be expected if cortisol was responsible for moving water, perhaps from the rumen, into the plasma. Similarly, on both days of the present experiment, loading induced an abrupt fall in haematocrit with levels remaining depressed thereafter. Although these results suggest that a real decrease in haematocrit can occur when cortisol release is stimulated, it was unfortunate that large differences in home pen measurements of haematocrit and osmolality were seen in the two tests. It is probable that this was due to the change in diet; in the stationary test the sheep had recently been on pasture whereas in the driven test they had received hay and concentrates for the previous 6 days.

Increases in plasma concentrations of CPK and LDH isozymes are due to leak-

age from muscle, particularly when damaged. Although CPK has been found to increase in pig plasma during transport (Moss & McMurray, 1979), previous studies have failed to find increases in CPK in sheep blood after journeys of differing durations (Knowles *et al.*, 1993, 1994c, 1995). Therefore, the present results, which provide a much more detailed CPK profile, support the earlier observations. Similarly, LDH1 and LDH5, which are the predominant forms in cardiac and skeletal muscle, respectively (Jones & Price, 1992), also did not show any clear changes in either the stationary or driven condition. The conclusion from these data is that minimal tissue damage was engendered by the procedures adopted in this experiment.

As found in other studies (Knowles *et al.*, 1993, 1994c, 1995) the sheep lost weight during transport. Although the losses (3.6% stationary, 5.5% driven) were similar to those previously reported, differences due to treatment were not significant. This suggests that deprivation, metabolism and elimination, rather than stress-related effects, are responsible for weight loss during journeys. Nevertheless, as reported by others (Knowles *et al.*, 1995) these effects were rather long-lasting because the animals had not regained their weight by the following day, despite the overnight provision of ample food and water.

In conclusion, the objective of the present experiment, the dissociation of the effects of transport from those of loading and penning, can be regarded as having been only partially achieved. In retrospect, it would have been better if the sheep had been fully adapted to dry food before both tests and if two separate groups of animals had been used. Alternatively, the specific effects of transport might have been more readily discernible if the animals had fully habituated to the loading procedure. With these caveats in mind, the findings can be summarized as follows. Firstly, the sheep showed clear physiological signs of poor welfare during loading, penning and driving, which lasted for about 3 h. Secondly, the pituitary/adrenocortical and prolactin responses to transport over the subsequent 12 h, although present, were not great, there was no apparent tissue damage and heart rate returned to normal, as previously reported (Knowles *et al.*, 1995). Thirdly, the contemporaneous decreases observed in osmolality and haematocrit may have been related to changes in cortisol concentration whereas the loss in body weight appeared to be due to metabolic rather than stress-related factors. Finally, because of the limitations of the present study, it is desirable in future research concerned with this important welfare issue that experimental protocols are adopted which avoid the re-use of animals.

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