

348. Hall, S.J.G., Forsling, M.L. and Broom, D.M. 1998. Stress responses of sheep to routine procedures: changes in plasma concentrations of vasopressin, oxytocin and cortisol. *Vet. Rec.* 142, 91-93.

## Stress responses of sheep to routine procedures: changes in plasma concentrations of vasopressin, oxytocin and cortisol

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*Veterinary Record* (1998) 142, 91-93

IN many studies of the welfare of farm animals, workers have sought a biologically meaningful index of stress and have taken the activation of the hypothalamo-pituitary axis for this purpose (Broom and Johnson 1993). This activation may be inferred from a rise in cortisol concentration, which may be detected in body fluids including blood plasma and saliva (Cook and others 1996). Changes in other physiological variables may be causally linked in some way with elevation of cortisol concentration; for example, in sheep injection of vasopressin may modify secretion of cortisol (Matthews and Parrott 1994, Senn and others 1995). There is also

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**TABLE 1: First experiment (March 1994). Mean (range) concentrations of hormones of sheep during rough and smooth journeys and during confinement in stationary trailer (control) at 0, 40 and 80 minutes**

	Rough journeys			Sample size	Smooth journeys			Sample size	Control			
	Sample size	0	40		80	0	40		80	Sample size	0	40
Plasma cortisol (nmol/litre)	5	6.13 (2.61-9.33)	21.15 (8.69-32.31)	22.18 (5.17-42.17)	6	7.27 (2.45-13.07)	21.13 (3.79-51.67)	17.46 (3.81-49.71)				
Packed cell volume (%)	10	28.9 (23-27)	27.4 (25-30)	26.8 (24-30)	8	23.2 (24-31.5)	26.4 (23-30)	27.3 (23-32)	4	28.25 (23-33)	25.0 (23-27)	25.5 (24-27)
Vasopressin ( $\mu$ U/m)	10	0.51 (0.19-2.31)	0.86 (0.17-2.76)	1.15 (0.17-4.93)	8	1.04 (0.21-2.54)	1.01 (0.15-2.71)	0.86 (0.15-1.89)	4	0.61 (0.32-1.06)	0.47 (0.26-0.83)	0.87 (0.20-2.74)
Oxytocin ( $\mu$ U/m)	10	1.13 (0.19-2.31)	1.33 (0.53-2.18)	0.73 (0.22-1.22)	8	1.03 (0.48-1.93)	0.94 (0.37-1.53)	0.87 (0.39-1.43)	4	0.95 (0.11-1.79)	0.83 (0.14-1.34)	0.86 (0.30-1.37)

evidence that oxytocin, another neurohypophysial hormone, may be released during stress (Forsling 1986). Recent studies on pigs (Bradshaw and others 1996b) are consistent with work on other species and imply that vasopressin may be secreted as a response to vehicular motion as perceived during road transport.

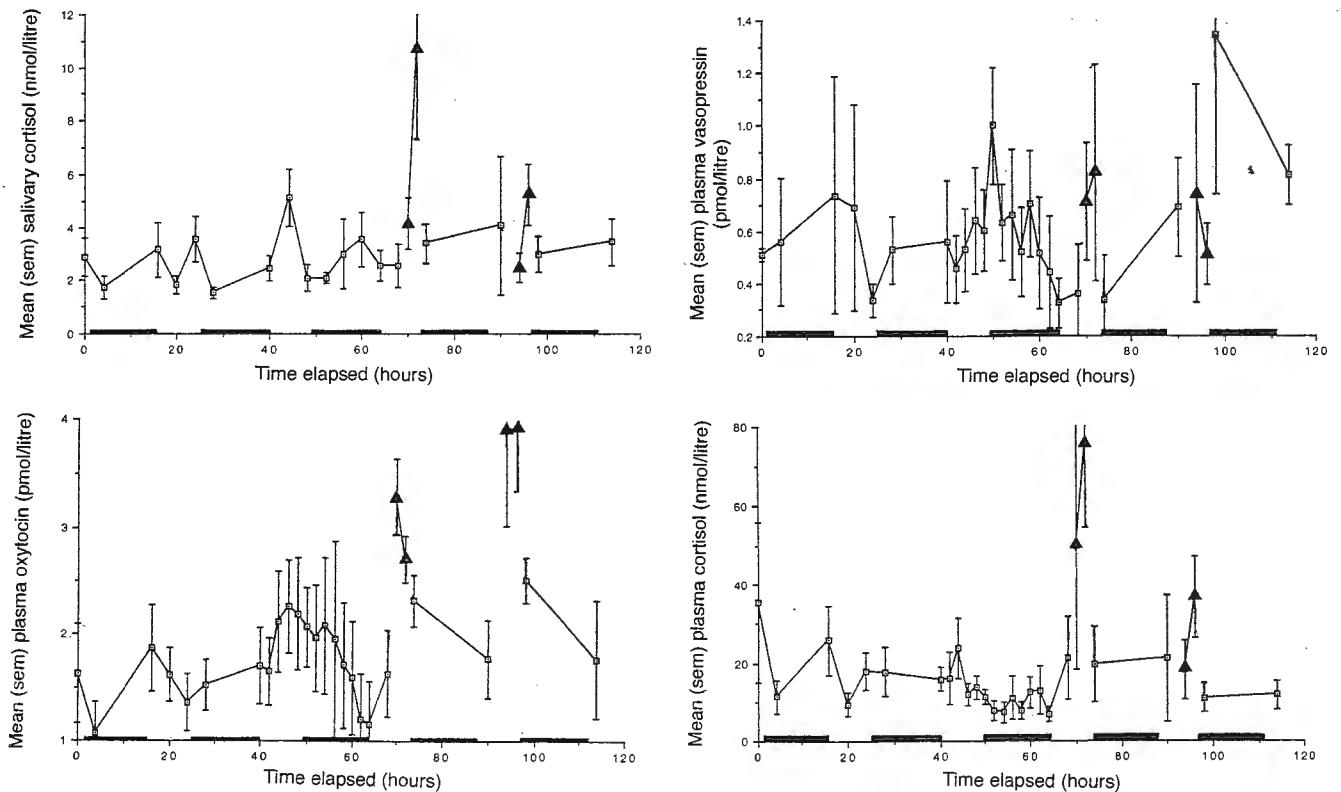
Changes of plasma vasopressin concentrations have been investigated in a welfare context by Cockram and others (1996) who, following a three-hourly sampling protocol, found no relationship with duration and conditions of transport of sheep; by Parrott and others (1988), who reported a decline when sheep were subjected to the stressful procedures of isolation and restraint; and by Ley and others (1991) who found no connection with the experience of chronic pain arising from footrot.

The behavioural neuropharmacologies of vasopressin and oxytocin, and their possible practical applications to the study of welfare of sheep, are therefore obscure. This short communication presents results from two preliminary studies, conducted in March and July 1994, which were designed to contribute to understanding of this subject.

Findings regarding behaviour and salivary cortisol, from the March study, have been reported by Bradshaw and others (1996a).

In this study blood samples were taken into 9 ml Sarstedt Monovette heparinised syringes, by temporary jugular catheter from four adult castrated male sheep before, after, and halfway through journeys (characterised by an accelerometer as 'rough' or 'smooth') and control periods of confinement, of 80 minutes duration, all in a car-towed trailer. In the July study blood samples were obtained in the same way from two groups each of four castrated male sheep, before and after shearing, before and after the two groups (which were previously unacquainted) were placed in the same pen, and over a period of 114 hours during which 12 samples were taken from each sheep at night (18.00-08.00) and 12 during the day. Saliva samples were taken for assay of cortisol (Cooper and others 1989). From each blood sample packed cell volume and plasma concentrations of vasopressin, oxytocin and cortisol were obtained.

Determinations of vasopressin and oxytocin were performed on extracted plasma as described by Forsling and Pevsner (1988) and by Balment and others (1986), respectively. Extraction was by C18 Sep-Pak columns (Waters Associated). The lower limit of detection for the vasopressin assay was 0.12 pmol/litre and for the oxytocin assay 1.6 pmol/litre. The cross-reactivity of oxytocin



**FIG 1: Concentrations of hormones (mean [sem]) during the second experiment (July 1994). Night time (18.00 to 08.00) is indicated by solid bars on the x axis. Means immediately before and after shearing (at time elapsed 70 hours) and mixing (at time elapsed 94 hours) are indicated by triangles**



antisera with vasopressin was less than 0.1 per cent. Cortisol was assayed as described by Parrott and Goode (1992).

The results for the March study are given in Table 1. Here, temporary or permanent occlusion of catheters and the loss of certain samples in laboratories account for variation in sample size. Most importantly, there was no control for the effects of transport on cortisol concentration, but comparison is still possible between rough and smooth journeys. Had each animal been fully sampled for each journey, the sample size would have been 32 (four animals  $\times$  eight journeys) for the rough journeys and the same for the smooth journeys, and 16 for the control period.

Paired-sample *t*-tests were employed to compare the variates at the start of each journey and after 40 minutes. Rough journeys evoked a response of plasma cortisol ( $t=4.24$ ,  $n=5$ ,  $P<0.05$ ) but smooth journeys did not ( $t=1.77$ ,  $n=6$ ,  $P$  not significant). The responses of the other blood variables were not significant, but that of vasopressin to rough journeys was suggestive ( $t=1.85$ ,  $n=10$ ,  $P=0.098$ ).

In the July study, shearing evoked a response of salivary cortisol ( $t=2.79$ ,  $n=8$ ,  $P<0.05$ ) and social mixing, a response of plasma cortisol ( $t=2.62$ ,  $n=8$ ,  $P<0.05$ ). There was no response of packed cell volume, vasopressin or oxytocin. Differences in blood variables between day and night were evaluated by two-factor anovar (factors: time of day, with two levels, day and night; and identity of animal, with eight levels). Sampling sessions before and after shearing and mixing were analysed separately. In all cases the anovar was significant, due to a strong effect of identity of animal but only for plasma cortisol and oxytocin was there a significant effect of time of day (respectively  $F=4.92$ ,  $df=1136$ ,  $P<0.05$ ;  $F=4.40$ ,  $df=1129$ ,  $P<0.05$ ) and there was no time of day  $\times$  identity interaction. The course of changes over the experimental period is summarised in Fig 1. Mean (n, sem) day and night concentrations were, respectively, for plasma cortisol 17.35 (81, 2.33) and 12.17 (71, 1.36) nmol/litre, for oxytocin 0.97 (77, 0.06) and 0.83 (68, 0.07)  $\mu$ U/ml, for vasopressin 0.32 (77, 0.04) and 0.29 (73, 0.03)  $\mu$ U/ml, and for salivary cortisol 3.26 (60, 0.39) and 2.65 (62, 0.27) nmol/litre. For packed cell volume means were 31.15 per cent (55, 0.81) and 31.70 per cent (55, 0.75) or, as transformed to arcsine, 0.32 (55, 0.008) and 0.32 (55, 0.008).

The authors are not aware of any studies on diurnal variation of plasma vasopressin in sheep, or of any previous reports that oxytocin may exhibit diurnal variation in male or castrated sheep, though in female sheep oxytocin peaks at 09.00 on days six to nine of the oestrous cycle (Flint and Sheldrick 1983). Regarding cortisol, some studies (Brinklow and Forbes 1984, Sillence and others 1987) have shown no consistent diurnal pattern while others (Fulkerson and Tang 1979, Salem and others 1991, Atkinson and others 1995) have implied a peak in the middle of the night and a nadir in late afternoon.

Sample sizes were very small in these studies which must therefore be seen as preliminary. Nevertheless it is evident that events which would be predicted to be stressful did in fact evoke a cortisol response, but not a response of packed cell volume, vasopressin or oxytocin. As it fails to achieve statistical significance ( $P=0.098$ ), the increase of plasma concentrations of vasopressin in response to rough journeys is not proven. It is at least possible that elevation of this hormone could, as has been suggested for pigs (Bradshaw and others 1996b), signal a stress response by at least some sheep to the conditions of transport.

**Acknowledgements.** – The authors acknowledge financial support from the EC (AIR contract PL 920528, held by Professor D. M. Broom). Plasma cortisol assays were performed by J. A. Goode of the Babraham Institute.

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