Remote Blood Sampling Device
A stress free blood sampling technique for free ranging animals

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ABSTRACT

Changes in various blood parameters can be used to assess the relative stressfulness of farm practices. In order to overcome confounding effects of stress inherent in standard methods of blood collection, a portable remote blood sampling device ("Dracpac") has been developed and tested. Twelve heparinised blood samples can be taken from the jugular vein and stored in insulated packs on the animal. The stability of blood parameters taken from and stored on red deer was determined. Analyses of subsamples taken from a bulk initial sample over 12 hours showed that levels of cortisol, haematocrit and glucose did not significantly differ from initial values. In a second experiment stags were sampled remotely during restraint in a mechanical crush and subsequent recovery at pasture on 2 successive days. On both days cortisol, haematocrit, glucose and lactate levels were elevated during restraint and thereafter decreased significantly to reach levels substantially lower than previously reported for this species using standard methods of blood sampling.

The development of this device permits reliable remote blood sampling of free ranging animals without the stress associated with manual blood sampling.

Keywords: Remote blood sampling; red deer; stress; cortisol.

INTRODUCTION

Increasing concern about animal welfare has lead to attempts to evaluate the relative stressfulness of various farming practices. Changes in the levels of a number of physiological and biochemical parameters in blood plasma are commonly used to quantify the degree of stress an animal experiences. Traditional blood sampling methods involve some form of handling and restraint which, in many cases, itself induces a stress response and confounds interpretation of the measurements obtained (eg. Seal *et al.*, 1972; Hattingh *et al.*, 1988).

Attempts to minimise the stress involved in blood sampling include: shooting undisturbed animals in paddocks (Smith and Dobson, 1990); habituation to handling and the sampling procedure (Bubenik *et al.*, 1983); the use of static remote systems on tame animals in pens (Ladewig and Stribrny, 1988) and remote portable devices outdoors (Farrell *et al.*, 1970; Bubenik and Bubenik, 1979; Hattingh *et al.*, 1988; Mayes *et al.*, 1988; Stephan and Cybik, 1989). These methods have limitations. Shooting does not allow repeat sampling; habituation and taming result in atypical animals; and although current remote systems overcome the confounding effects of handling, they are not commercially available.

Since remote systems appear to provide the optimal sampling procedure, particularly for application with large, flighty, free ranging animals (such as red deer), we undertook to develop such a device that was both versatile and reliable. The present study outlines 2 experiments carried out to this end. Thus, the stability of blood samples stored on animals, and the measurement of levels of stress parameters during handling and subsequent recovery of red deer in the field, were determined.

MATERIALS AND METHODS

Experiment 1

Two rising 2 yr red deer stags were restrained in a pneumatic deer crush and blood (50 ml) sampled from the jugular vein. The blood from each animal was then mixed with 3 ml heparinised saline (5000 IU/ml) and stored on an animal outdoors for 12 h in insulated packs containing ice. The samples were subsampled at 0, 0.5, 1.5, 3, 6, and 12 h. Whole blood was analysed for haematocrit, and plasma was separated by centrifugation and stored at -20°C until analysed for cortisol, lactate, and glucose (see below). Pack temperature was monitored (Delphi Temperature Logger, model 861, Temperature Logger Systems, Wellington N.Z.).

Experiment 2

Twelve rising 2 yr red deer stags were used; 6 were fitted with remote blood sampling devices (experimental animals), the remaining 6 were included to maintain normal group size. Experimental animals were sedated with Fentazin (1.2 mg xylazine/kg) and a double lumen catheter was inserted into the jugular vein (see Ladewig and Stribrny, 1988). At the same time animals were fitted with a canvas backpack. The sedative was reversed with Yohimbine (0.25 mg/kg i.v.)

The following day (Day 1) the experimental animals were restrained in a pneumatic crush and 10 ml of blood was withdrawn manually from the catheter (sample 1). The animals were then fitted with a battery powered remote blood sampling device ("Dracpac").

This device, developed jointly by the Animal Behaviour and Welfare Research Center and the Engineering Develop-

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ment Group, consists of a small peristaltic pump that delivers heparinised saline (5000 IU/ml) down one lumen to the tip of the catheter where it mixes with blood continuously being drawn up the second lumen. The blood is then pumped through a 12-position rotary switching valve (Mayes *et al.*,1988) and collected in one of 12 separate PVC bags. The samples are stored on ice in an insulated pouch within the backpack. The pumping rate and sample duration are independently controlled by a microprocessor, and together determine sample volume. The device measures 150 x 110 x 60 mm, and weighs 1.3 kg.

The blood sampler was programmed to collect 11 continuous samples, each of 20 minutes duration. The animal was then released to pasture (Time 0; approximately 15 min after sample 1 was taken). Following collection of the last sample at pasture, the experimental animals were returned to the yards and the blood sampler and samples removed from the backpack.

The procedure of fitting the sampler and obtaining the samples was repeated the next day (Day 2).

Blood Sample Analyses

Haematocrit was determined by centrifugation of whole blood in microcapillary tubes in duplicate. Plasma lactate and glucose were determined using Bohringer Mannheim kits on a Hitachi 717 random access analyzer according to manufacturers instructions. Plasma cortisol concentrations were measured in duplicate, and after extraction in ethyl acetate, by an ^{125}I radioimmunoassay method with PEG separation. All samples were measured in a single assay; intra-assay co-efficient of variation was 10.1%. Assay sensitivity was 0.33 ng/ml.

Statistical Analysis

The data from Experiment 1 was subjected to linear regression analysis (Genstat, 1990) to determine trends. The data from Experiment 2 was analysed by orthogonal polynomial contrasts of the time profiles; least squares ANOVA (Genstat, 1990) was used to test for animal and day effects. Significance levels were set at $P<0.05$. Exponential functions were fitted to individual data on each day to describe their rate of change. Estimates of asymptotes were used to derive apparant basal values of the blood parameters. All values are presented as the mean ± S.E.M..

RESULTS

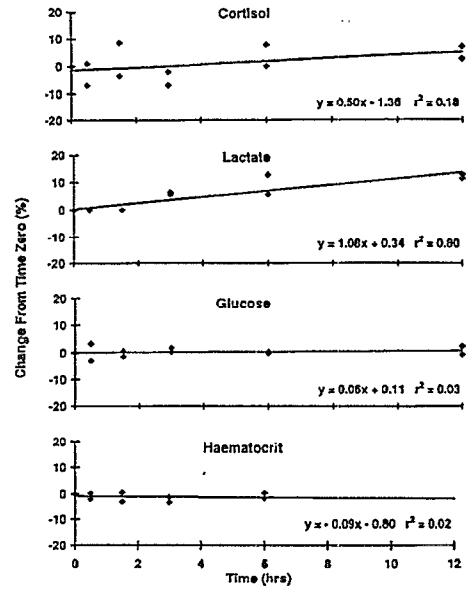
Experiment 1

The temperature inside the insulated blood pack remained below 4°C over the 12 h experimental period. Figure 1 shows the percentage change from time zero for haematocrit and plasma cortisol, lactate and glucose, for both bulk samples. The levels of cortisol, haematocrit and glucose did not change significantly over time, whereas, lactate levels changed significantly with increasing storage time ($P= 0.001$) increasing by 12.8 % over 12 h.

Experiment 2

Jugular catheters were found to be patent in 5 animals on Day 1 and 3 animals on Day 2. Blood samplers were fitted to these animals and 99 % of expected samples were successfully collected.

FIGURE 1: The effect of storage time on the percentage change in levels of cortisol, lactate, haematocrit and glucose relative to initial values (Time 0). All values for the samples from the two different animals are shown. The equations and lines for the least squares linear regression are also shown.



The haematocrit, plasma cortisol, lactate and glucose levels during restraint and successive 20 min sampling intervals after release to pasture on days 1 and 2 are shown in Figure 2. Mean plasma cortisol concentrations were 56.5 ± 5.1 ng/ml during restraint and there was no significant difference between the levels on days 1 and 2. There was also no significant difference between cortisol levels in samples taken manually and the first samples taken with the blood sampler on each day. On both days cortisol levels decreased significantly with time after release to pasture. The rate of decrease was not significantly different (P=0.08) between the 2 days.

Plasma cortisol levels declined exponentially ($r^2 = 0.96$) after release of animals to pasture. Estimated basal levels (the mean asymptotic value for the individual exponential curves) and estimated recovery times (the mean time at which the fitted curve attained a value within 10% of the asymptotic value) are shown in Table 1 for days 1 and 2 combined. The estimated basal level for cortisol was 8.4 ng/ml with the estimated recovery time been 3.75 h after release.

Data on haematocrit, and plasma lactate and glucose levels did not differ significantly between days 1 and 2, therefore the data for both days were combined. The mean values for haematocrit, lactate and glucose from samples taken manually were not significantly different from the first sample taken remotely using the dracpac. Levels declined significantly after release from restraint to estimated basal levels of 30.5 % for haematocrit, 0.94 mmol/l for lactate, and 3.74 mmol/l for glucose (Table 1). The times taken to achieve the estimated basal values were 0.5 h for haematocrit, 2.7 h for lactate, and 5.3 h for glucose.

DISCUSSION

During this study the Dracpac remote blood sampler functioned reliably and resulted in a high percentage return of expected samples. Non-patency of catheters was the reason

FIGURE 2: Levels of cortisol, lactate, haematocrit and glucose during restraint and following release to pasture (Time 0) on the two experimental days. The two values to the left of Time 0 on each plot are for the samples taken manually in the crush. Day 1 n = 5; Day 2 n = 3.

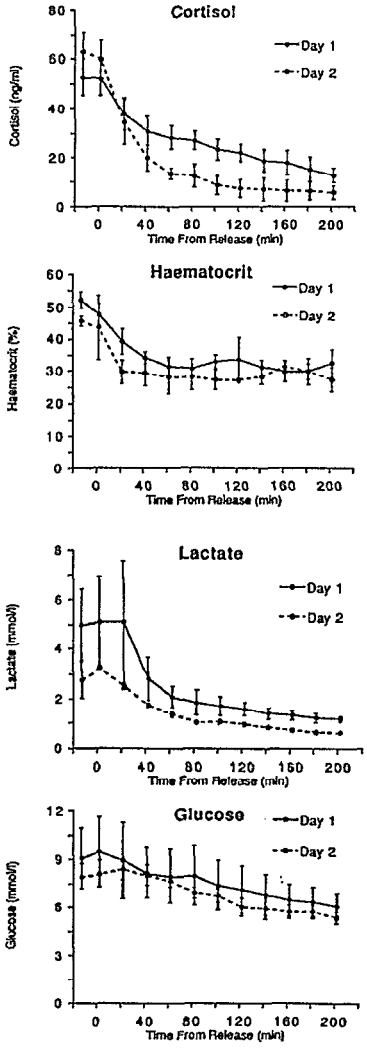


TABLE 1: Estimated basal levels* (± S.E.M.)and estimated recovery times**(± S.E.M.) at pasture for cortisol, glucose, lactate and haematocrit derived from fitting exponential functions to individual data (Days 1 and 2 combined). Goodness of fit is shown by r^2 values.

Parameter	Estimated basal level	Estimated recovery time	r^2
Cortisol (ng/ml)	8.41 ± 2.36	3.75 ± 1.04	0.96
Glucose (mmol/l)	3.74 ± 0.75	5.30 ± 2.69	0.96
Lactate (mmol/l)	0.94 ± 0.12	2.69 ± 0.38	0.98
Haematocrit (%)	30.54± 1.67	0.54 ± 0.12	0.47†

* The mean asymptotic value for the individual exponential curves.
** The mean time at which the fitted curve attained a value within 10% of the asymptotic value.
† Indicates a poor fit to the exponential function hence estimated values may be unreliable.

for reducing animal numbers during the study. This problem has since been rectified through the use of an improved method for fixing catheters in place.

We were concerned that the storage of whole blood on the animal for several hours could have affected the levels of some of the important stress indices in the samples. The results of Experiment 1 revealed that levels of cortisol, glucose and haematocrit were unaffected by storage periods of up to 12 h. Lactate levels, however, showed a significant increase over the storage period, though the actual percentage increase was relatively small. These findings are similar to published results with other species (Reimers *et al.*, 1983, 1991; Wittwer *et al.*, 1986). Hence, the levels of these stress indices in blood collected and stored on the animal would be similar to those in samples collected and processed immediately.

The elevated levels of the four parameters measured in the first samples in Experiment 2 suggest that the procedure for attaching the blood sampler which involved yarding, drafting, and restraint is stressful. Similar high values were reported by Seal *et al.*, (1972) for manually restrained white tailed deer (*Odocoileus virginianus*), and by Matthews *et al.*, (1990, 1991) in red deer stags restrained in a pneumatic deer crush.

Soon after release to pasture the levels of each of the parameters began to decline. The estimate of the time taken to reach basal values varied from 0.5 h for haematocrit to 5.3 h for glucose. This indicates that a substantial period of time must be allowed to elapse following fitting of the sampler, and before the effects of subsequent stressful events can be assessed.

The estimated mean basal concentration of cortisol was 8.4 ng/ml. This value is similar to that reported for undisturbed red deer stags shot in the field (5.7 ± 3.7 ng/ml, Smith and Dobson, 1990) and tame Eld's deer (*Cervus eldi thamin*) sampled by remote catheter (range 5.5 - 14.5 ng/ml, Monfort *et al.*,1993). Similarly, the predicted basal values for lactate, glucose and haematocrit are close to those reported for other unstressed ruminants (Hattingh *et al.*,1988, 1989). It would seem that the levels of stress parameters measured in this study are approaching those found in unstressed deer. Thus, it appears that red deer adapt readily to the Dracpac remote blood sampling system and that there is little or no stress associated with its use.

It is envisaged that the Dracpac remote sampler will find application in studies defining variations in a wide range of physiological, haematological and biochemical parameters.

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