# Analysis of Growth Releasing Factor (GRF) in Red Deer Hypophysial Portal Plasma

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## INTRODUCTION

Numerous methods have been published describing the analysis of hypophysial portal plasma neuropeptides (1,2). Many of these methods involve a non-specific solvent extraction using a large sample, have poor reproducibility and often a high background. All of these factors work against having a sensitive and reliable assay. We have evaluated a specific method using antibody coated polystyrene beads which eliminates most of the problems associated with solvent extraction. This technique has been previously used to analyze rat plasma neuropeptides (3), and we have extended its use to determine GRF in red deer (*Cervus elaphus*) plasma.

## MATERIALS AND METHODS

Specular finish 6.4mm polystyrene beads (Precision Plastics Chicago) were coated with affinity purified sheep anti-rabbit second antibody(Invermay SAR6). After drying they were coated with rabbit anti-GRF primary antibody (Invermay R31). Samples or standards and assay buffer were then added to glass tubes containing the beads and incubated for 3-4 days at 4°C. Beads were then washed four times with 2ml water and 300µl of iodinated GRF was added. Incubation for 24 hours was followed by a further washing and the beads were then placed in a gamma counter.

## RESULTS

Only standard curves and spiked peripheral plasma samples have been analysed to date. A typical standard curve is shown in Fig 1. A sensitivity of 15pg/ml (calculated as two standard deviations from the zero) was obtained. Results from spiked plasma samples are shown in Table 1. The background measured in jugular plasma gave similar values to the non-specific binding of about 3% B/Bo.

## DISCUSSION

Obtaining an assay with a high sensitivity and low background is essential to the successful measurement of GRF in hypophysial portal plasma. The method we have investigated has a very low background, and we believe sensitivity although adequate at 15pg/ml, can be enhanced two or three fold by the use of a method using a chemiluminoassay as the end reading. This could also have the advantage of allowing the use of smaller samples. The next stage of our investigation will be the measurement of a portal plasma profile.

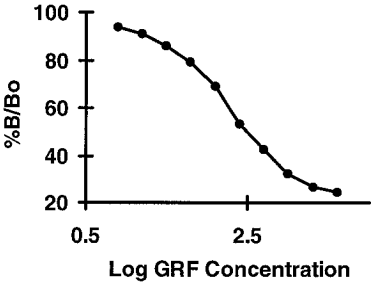


Fig.1: Standard curve GRF using 300µl plasma

| pg/ml expected | pg/ml observed | % recovered | n | CV% |
|---|---|---|---|---|
| 25.0 | 18.9 | 75.6 | 6 | 15.4 |
| 50.0 | 44.5 | 89.0 | 6 | 19.0 |

Table 1: GRF recovered from deer plasma.

## REFERENCES

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