### P051 – Friday 13 September 2002

**Non-invasively Assessing Stress in the Rat**

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Two non-invasive stress assessment techniques are described: corticosterone (B) assay from single micturitions, and chromodacryorrhoea scores. Single micturitions can be collected by placing rats in a bare/mesh-floored cage. Rats typically urinate within 10-60 min, producing 0.1 - 0.8 ml. Urine is stored at -20°C, and B assayed using an ICN \(^{125}\)I RIA kit (creatinine is assayed to correct for urine concentration). No prior extraction is needed, and small samples can be bulked with distilled water. Individual urinary B concentrations correlate between Lights-off and Lights-on (F1,13 = 110.97, p < 0.0001), but in males are higher at Lights-off (sex*time of day: F1,13 = 122.04, p < 0.0001), tracking circadian changes in plasma B. In rats restrained for 25 min, peak plasma B, and increases over baseline, both correlate with B concentrations in micturitions collected 1.5 - 3.5 h later (F1,23 = 14.89, p = 0.001 and F1,23 = 7.86, p = 0.01, respectively). Severe chromodacryorrhoea (‘bloody tears’: porphyrin-containing Harderian secretions) is a qualitative sign of stress (eg, in rats subjected to trauma). However, slight secretions can appear around eyes/nostrils even in unmanipulated animals. In other species, these are involved in social signalling (eg, appeasement), and rats seem similar: animals subordinate in ‘Tit-bit Tests’ had higher chromodacryorrhoea scores, assessed on a 5-point scale (F1,6 = 7.86, p = 0.03). Scores also increased with disturbance (eg, unfamiliar visitors to the unit; F1,9 = 31.84, p < 0.0001; maintenance work involving drilling; F1,9 = 42.25, p < 0.0001). These secretions may thus signal fear, and were more sensitive to disturbance than urinary B.

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**Keywords:** chromodacryorrhoea, Harderian gland, rat, restraint stress, urinary corticosteroids

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**Non-invasive Monitoring of Stress Hormones by Measuring Their Faecal Metabolites**

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Adverse situations trigger responses that result in an increase in glucocorticoid and/or catecholamine secretion. These hormones are inactivated by various enzymatic steps and are excreted via urine and faeces. Radiometabolism studies were performed in various species (ponies, dogs, cats, hares, pigs and sheep; at least 2 of each sex) by injecting cortisol (all species) and adrenaline/noradrenaline (only the latter two) in order to gain information about their metabolism and excretion. This should help to establish enzyme-immunoassays (EIAs) for the determination of some of the relevant faecal metabolites to allow a non-invasive evaluation of disturbances. Pronounced species differences were found regarding the route of excretion and the delay time of maximum concentrations of faecal radioactivity (reflecting gut passage time). As only a few percentage of the catecholamines (in contrast to cortisol) were excreted via the faeces, further analysis was restricted to the cortisol infusions. High performance liquid chromatography of the faecal metabolites revealed a large number of cortisol metabolites formed in all animals, while cortisol itself was almost absent. However the pattern of the metabolites varied significantly between species. Several group specific EIAs (eg, 11-oxoetiocholanolone-EIA) were developed for measuring some of the relevant metabolites. Results of experiments, testing the biological relevance (eg, ACTH-stimulation, transport) of such determinations, proved them suited for evaluating adrenocortical activity. Unlike blood, faecal samples offer the advantage that they can be collected easily without stressing the animals. Thus the established methods may help to elucidate endocrinological changes during stressful situations and may be applied as a non-invasive tool in a variety of research fields.

**Keywords:** adrenocortical activity, enzyme-immunoassay, faeces, mammals, non-invasive