THE ASSESSMENT OF STRESS IN CAPTIVE JUVENILE AFRICAN ELEPHANTS (*LOXODONTA AFRICANA*).

By

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ABSTRACT

In 1998, 30 juvenile elephants were captured in Botswana and transported to a holding facility in South Africa to be trained and sold to zoos and safari parks. The welfare of the 'Tuli Elephants' as they became known, became the source of acrimonious dispute between a number of conservation and animal groups. The case highlighted concerns over the welfare of elephants during capture, transport and confinement. Questions asked were: can an objective assessment of the effect on juvenile elephants on the removal from matriarchal group be made. This study was aimed to take an objective approach to assessing the welfare of captive juvenile elephants using behavioural and physiological methods of investigation.

A behavioural study to identify indicators of stress was conducted in five groups of elephants subject to various husbandry systems. Thirteen behavioural indicators of stress were identified. A group of two elephants held in an enclosure 70 m^2 that was devoid of mud and sand baths showed the highest number of behavioural indicators of stress. Elephants in larger enclosures with mud and sand baths showed fewer indicators of stress. The group able to range freely during the day showed the least number of stress-related indicators.

Conventionally the physiological assessment of adrenal responses to stress relies upon blood sample collection and the measurement of

glucocorticoids but this is impossible without immobilisation or restraint that influences results. This study validated a recently established enzyme immunoassay (EIA) measuring faecal glucocorticoid metabolites in elephants. A preliminary investigation into the biological relevance of this non-invasive method was made for use in assessing welfare in elephants.

Four juvenile elephants were injected i.m. with synthetic adrenocorticotrophic hormone (ACTH) (Synacthén, Novartis; 2.15 mg). Blood and faecal samples were collected over 4 h and 7 days respectively. Concentrations of serum cortisol and faecal cortisol metabolites were determined using immunoassay. Variability of basal and peak values in blood and faeces were observed among the elephants. After ACTH injection, serum cortisol concentrations increased by 400-700%. When compared to cortisol and corticosterone EIAs, 11-oxoaetiocholanolone EIA proved best suited to measure cortisol metabolites. Concentrations of faecal 11,17-dioxoandrostanes increased by 570-1070% reaching peak levels after 20-25.5h. Samples left outside could be collected up to 8hrs after defecation without a significant effect on metabolite concentrations. A correlation between enclosure size, presence of stress-related behaviour and faecal 11,17-DOAs was observed. Elephants kept in small enclosures exhibited more stress-related behaviour and had higher levels of glucocorticoid metabolites than those ranging in a larger area. The results of the study suggest that non-invasive faecal monitoring of glucocorticoid metabolites is useful in investigating adrenal activity in African elephants.

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CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

1.1 African Elephants in Captivity

Mankind has harnessed the strength and intelligence of elephants for hundreds of years. One of the earliest references to the domestication of the Asian elephant (*Elephas maximus*) dates back to 1,200 BC in Egypt[20]. Asian elephants were routinely trained for war, and are still used for logging and ceremonial duties today. The first written record of African elephants being caught for training purposes comes from the Red Sea in the Third Century BC. In 218 and 202 BC, Hannibal used African elephants in battles against the Romans, however the use of elephants in battle gradually declined[20]. In the early 1900s an attempt was made to train African elephants for forestry and agriculture in the Belgian Congo and until recently, there were over 100 trained African elephants there. Today, there are approximately 550 African elephants housed in zoos and circuses around the world[22].

1.2 'The Tuli Elephants'

In 1998, 24 female and 6 male elephants aged between 3 and 6 years were captured in Botswana's Tuli Game Reserve (29°S 22°E), also known as the 'Tuli Block', that covers approximately 77 000ha and is inhabited by over 1 000 elephants[22]. They were transported to African Game Services (AGS), Brits (25°47' S 27°48 E), South Africa. Twenty two (6:16) of these animals

were icluded in a training which was conducted by Indonesian mahouts. The intent was that they be subsequently sold to zoos and safari parks.

After their arrival in South Africa, the young 'Tuli Elephants', as they became known, were the focus of local and international media attention and the source of much legal contention. The method of handling the elephants ignited a heated debate between the National Council of the Society for the Prevention of Cruelty to Animals (NSPCA), international animal rights organisations, and non-governmental organisations such as the Rhino and Elephant Foundation, Elephant Management and Owners Association and The World Wildlife Fund for Nature.

Opinions pertaining to the welfare of the elephants gave rise to acrimonious dispute. Opposing sides used different methods to assess the wellbeing of the animals and arrived at completely different conclusions. An objective method giving a reliable indication of the animals' welfare would make a crucial contribution in such debates[19].

Although certain differences of opinion, for example 'Captivity vs Freedom' can never be resolved, it is safely assumed that all parties concerned would like to optimise conditions for behaviour and health thus ensuring the well being of elephants maintained in captivity.

1.3 The Concept of 'Stress'

People in all walks of life, from ethologists to retiring psychiatrists, frequently use the term 'stress' however, when asked for a concise definition of the term, few would be able to provide one. Amongst the scientific community there is no universal agreement on the definition of 'stress'. As Toates write, "It seems as if stress, in addition to being itself and the result of itself, is also the cause of itself" [61].

The term 'stress' is commonly used, particularly in physics to denote the sum of all forces which act against a resistance[56]. Selye, the first person to coin the use of the word 'stress' in a biological sense, defines it as "The sum of all non-specific biologic phenomena (including damage and defence). It may be local or topical, as exemplified by inflammation or systemic responses, as exemplified by the General Adaptation Syndrome" [56]. As described in the review below, an animal's reaction to stress is complex, thus for the purposes of this study, stress shall be defined as:

A physiological and psychological condition that is manifested as a consequence of neuronal and hormonal changes resulting from environmental or psychological influences (stressors).

A stressor is an agent capable of producing stress. They can be qualified and quantified as described in Figure 1.

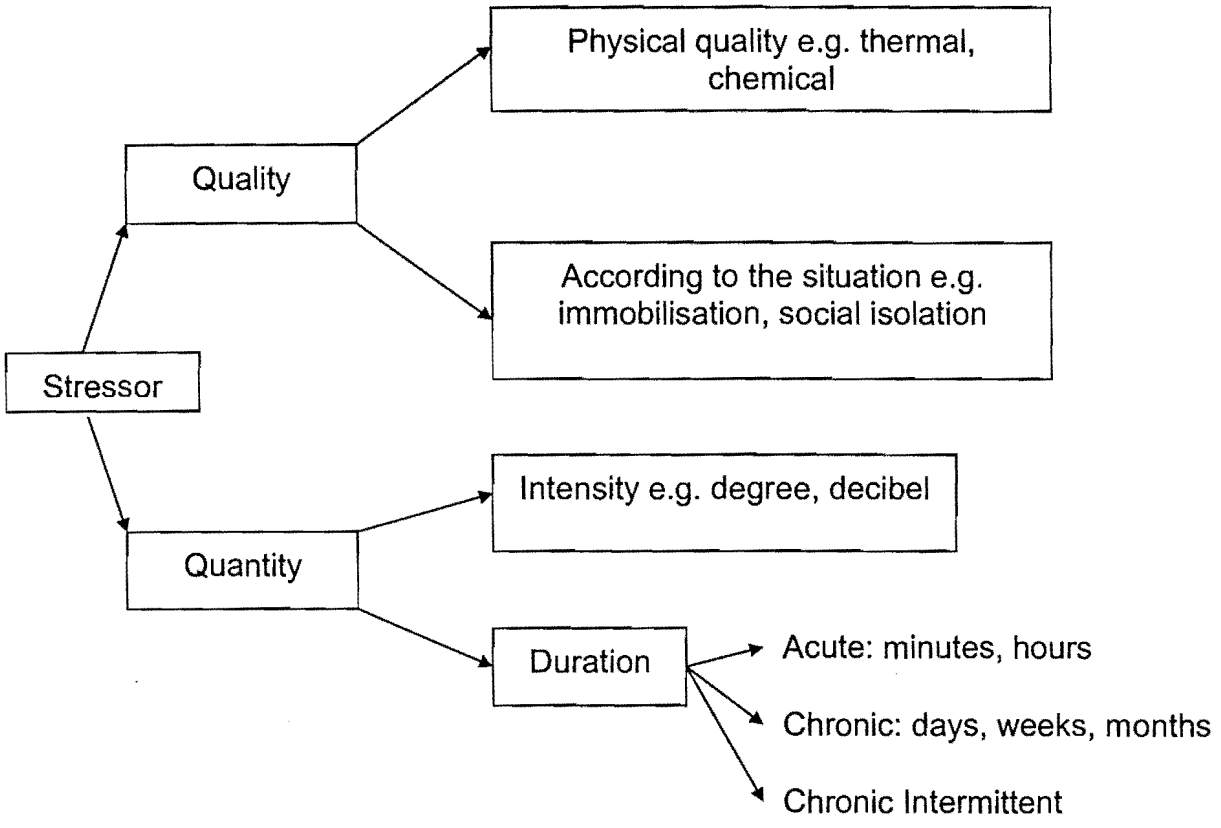


Figure 1. Describing Stressors
Adapted from Ladewig, de Passillé, Rushen, Schouten, Terlouw & van Borell[61]

1.4 The role of glucocorticoids in stress

The determination of corticosteroids in blood is one of the frequently used ways of measuring stress[43].

Physiological and behavioural stress responses are initiated and co-ordinated by the central nervous system. The stress response can be broadly

sub-divided into two effector pathways - the autonomic nervous system and the neuroendocrine system[71].

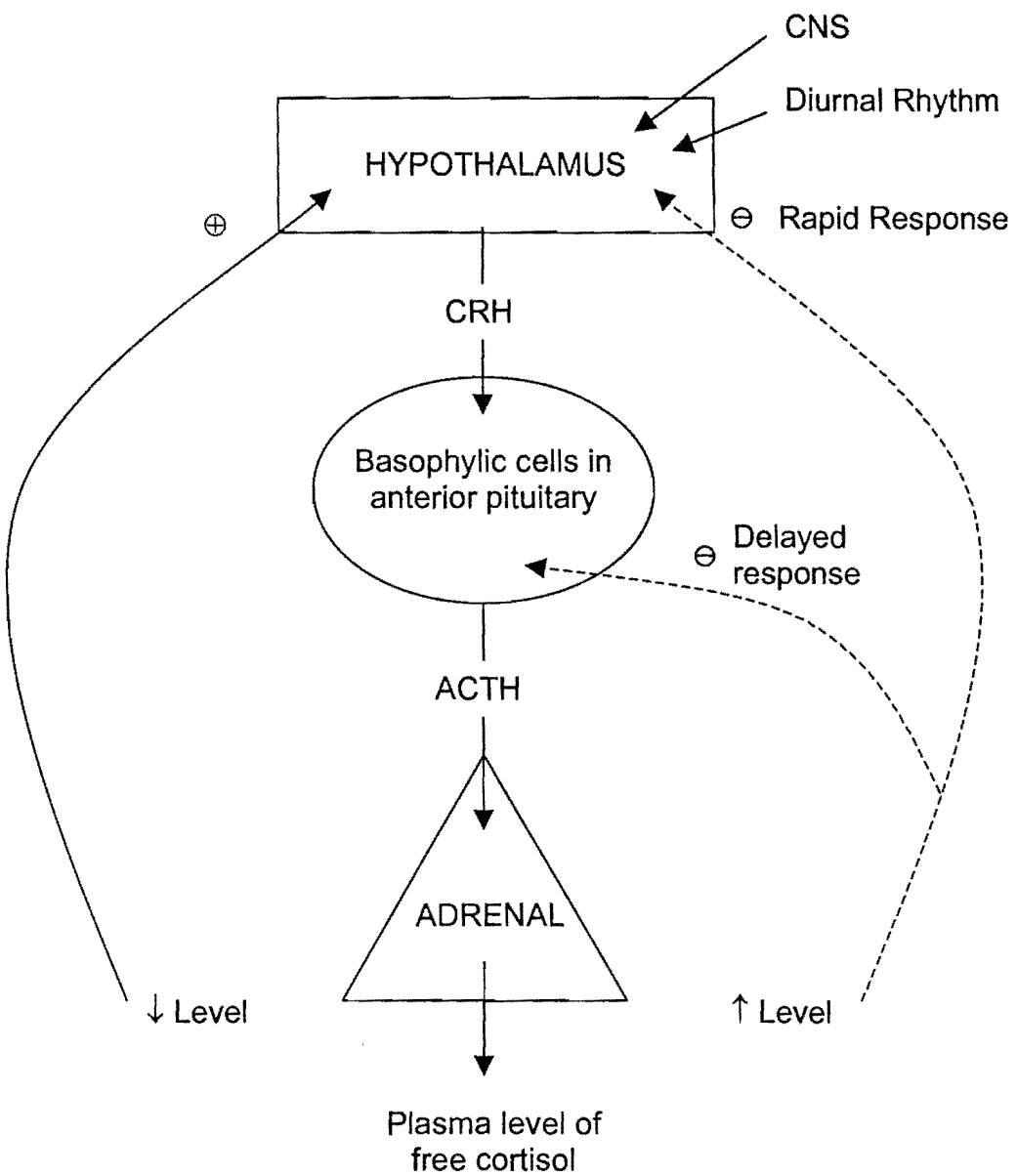
The activation of the sympathetic system results in the release of catecholamines eliciting a 'flight-fight' response and corticosteroid release. The fight-flight response can be described as 'acute stress' if it is of relatively short duration and allows the animal to remove itself from a stressor. However, when circumstances do not allow an appropriate behavioural reaction, the sympathetic tone and as a result the concentration of corticosteroids can be elevated for long periods. This is a state of chronic stress[71].

The hypothalamic-pituitary-adrenocortical axis

Seyle was the first to demonstrate the activation of the hypothalamic-pituitary-adrenocortical axis by stressors[56]. Later studies have also shown that the levels of predictability and controllability of stressors may also activate the system[68].

Impulses activated by stressors are relayed to the hypothalamus via the central and autonomic nervous systems. The hypothalamus then acts as a 'transducer' between neural impulses and hormonal secretion by releasing corticotrophin-releasing hormone (CRH) into the hypothalamic-hypophyseal portal system. The releasing hormone stimulates the production and secretion of adrenocorticotrophic hormone (ACTH) by the adenohypophysis into the blood. ACTH stimulates the adrenal cortex to synthesise and secrete

corticosteroid hormones. A negative feedback loop controls hormone secretion (Figure 2).



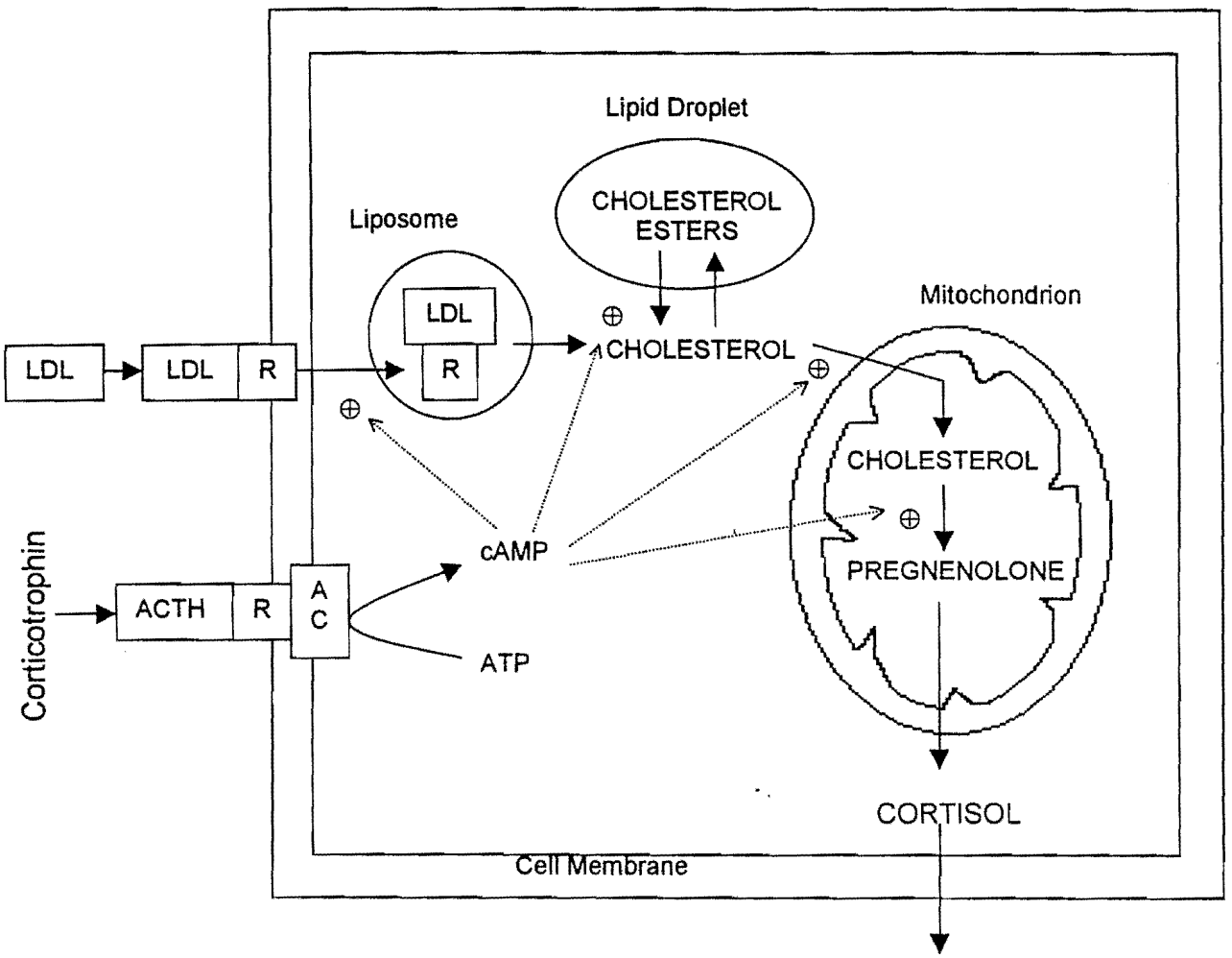
Solid arrows = stimulatory pathways. Dashed arrows = inhibitory pathways

Figure 2. Feedback regulation of glucocorticoid biosynthesis and secretion
Adapted from Cunningham[16]

Synthesis, transport and metabolism of glucocorticoids

Cortisol, the major glucocorticoid is synthesised from cholesterol in the zona fasciculata and reticularis of the adrenal cortex[16] (Figure 3). A number of processes take place upon stimulation by ACTH. The uptake of low-density lipoproteins increases and these are further processed to free cholesterol. Cholesterol is also produced by the hydrolysis of cholesterol esters stored in lipid droplets. The transport of cholesterol into the mitochondria is stimulated wherein cleavage of the cholesterol side chain occurs. Binding of cholesterol to the side chain cleavage enzyme is promoted and a C-21 steroid known as pregnenolone is formed[16]. Pregnenolone is converted to progesterone in the endoplasmic reticulum. Progesterone is hydroxylated by 17α-hydroxylase and 21-hydroxylase to 11-deoxycortisol. This re-enters the mitochondria and is hydroxylated by 11β-hydroxylase to cortisol, the major glucocorticoid[30].

Despite being classified as mineralocorticoids, corticosterone and 11-deoxycorticosterone have glucocorticoid effects[16]. They are formed during the synthesis of aldosterone in the zona glomerulosa of the adrenal cortex[16]. Hydroxylation of progesterone at the C21 position forms 11-deoxycorticosterone. The next hydroxylation at C11 produces corticosterone[30]. They have significant adrenocortical activity, although in most species they are less potent than cortisol[16].



Processes stimualted by corticotropin ⊕

R = receptor AC= adenylate cyclase LDL=low density proteins

Figure 3. Glucocorticoid biosynthesis

Adapted from Cunningham [16]

Upon entering the blood, approximately 75% of cortisol is bound corticosteroid-binding globulin (CBG), 15% to albumin, leaving 10% in an unbound active state[16]. The bound hormone is essentially inactive[32]. Due to its tenacious bond with CBG, the half-life of cortisol in plasma is about 1 hour[16]. Smaller amounts of corticosterone, 11-deoxycorticosterone and progesterone also bind with CBG and circulate in the plasma. Progesterone may displace cortisol from CBG to the free active fraction[32]. There are interspecific differences in the transport of glucocorticoids[44,46,47].

A circadian rhythm controlling the release of CRH and therefore ACTH and cortisol has been recorded in most mammalian species studied[25,38,62]. This circadian rhythm normally results in an increase of plasma cortisol shortly after the onset of sleep. Plasma cortisol levels continue to rise during sleep, peak shortly after waking, fall over the next few hours, and reach a low point in late afternoon and early evening. The negative feedback system and diurnal rhythm can be overridden by responses to physical and emotional stress, apprehension, fear, anxiety and pain[16].

Glucocorticoids are not degraded in the process of exerting their physiologic effects[25]. Modification of glucocorticoids occurs primarily in the liver[16] and can differ between species[44,46,47]. Most of the cortisol is reduced to dihydrocortisol and then to tetrahydrocortisol by the reduction of double bonds in the ring and ketone configurations[16]. The resulting tetrahydroderivatives are subsequently bound to glucuronic acid[32].

Some of the cortisol in the liver is converted to cortisone[25]. Cortisone is reduced and conjugated to form tetrahydrocortisone glucuronide. The tetrahydrogluronide derivatives of cortisol and corticosterone are freely soluble and enter the circulation and have no stress related biological activity (R Palme, University of Vienna, pers. comm., 2000).

Some cortisol is also converted to 17-ketosteroid (also known as 17-oxosteroid) derivatives of cortisol and cortisone including 11,17-dioxoandrostanes[11]. The majority of 17-ketosteroids are bound to sulphate[25]. Other metabolites are formed, including 20-hydroxy derivatives[25] and metabolites that have yet to be identified[11]. The metabolism of corticosterone is similar to that of cortisol except that it doesn't form 17-ketosteroid derivatives[25].

Glucocorticoids and their metabolites are excreted into the intestine in the bile[16]. Bacteria in the gut can metabolise cortisol further and deconjugate metabolites[40,47]. There is some reabsorption of glucocorticoids and their metabolites from the intestine and these return to the blood in the enterohepatic circulation[32]. Excretion of free and bound glucocorticoids by the kidney occurs, although there is some tubular reabsorption[32]. The majority (70%) of glucocorticoids and their metabolites are excreted in the urine, approximately 20% leave in faeces, and the rest exit through other routes, such as the skin, milk and saliva[32]. There are interspecific differences in the route of excretion[47].

As shown in Figure 4, the activation of the central nervous system by a stressor results in integrated physiological responses of the autonomic, neuroendocrine and organ systems, which in turn result in behavioural responses[71].

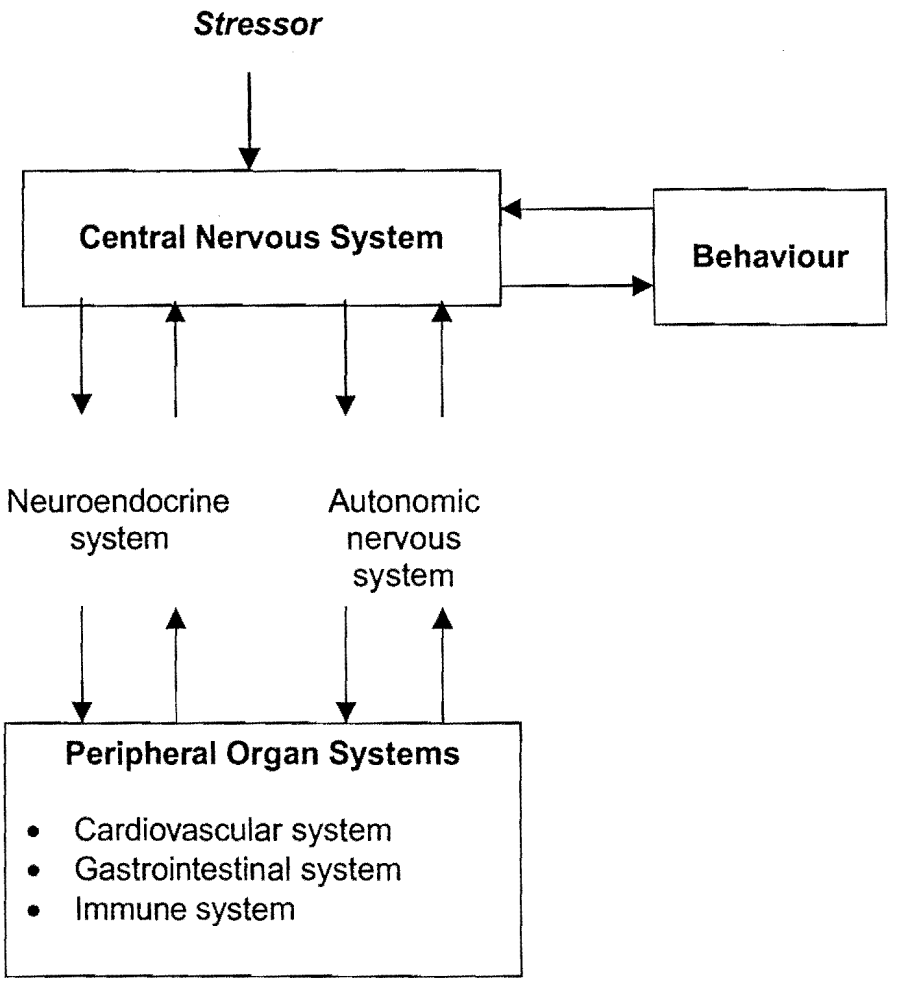


Figure 4. Interconnections between central nervous system, behaviour and organ systems during stress *From Wiepkema & Koolhaas*[71]

Effects of glucocorticoids

Glucocorticoids are important mediators of intermediary metabolism[16]. They stimulate hepatic gluconeogeneisis, involving the formation of carbohydrates from amino acids, thereby increasing blood glucose levels. By promoting the deposition of glycogen in the liver, stimulating lipolysis, inhibiting insulin production and stimulating gastric acid secretion their net effect is the provision of readily metabolisable fuel to the body, thereby preparing the body for action prior to action[61].

However, glucocorticoids also inhibit the uptake and metabolism of glucose in the peripheral tissues, particularly muscle and adipose cells. If glucocorticoids are present in the blood over prolonged periods, a hyperglycemic effect is produced in the liver, and insulin antagonism results in decreased utilisation of glucose in peripheral tissues[16].

Glucocorticoids inhibit protein, RNA and DNA synthesis and enhance protein and RNA catabolism thereby increasing plasma amino acid concentrations[30]. Over long periods, the presence of glucocorticoids results in muscle atrophy and weakening of bone. High levels of amino acids in the blood result in an increase of urinary excretion and a negative nitrogen balance[16]. Glucocorticoids also facilitate water excretion by increasing the glomerular filtration rate and inhibiting antidiuretic hormone activity[16].

Glucocorticoids inhibit fibroblast growth and replication and the formation of collagen and fibronectin. This can lead to weakening of the skin substratum, resulting in thin skin, easy bruising, and poor wound healing[30].

Glucocorticoids have been described as 'mediators of negative feedback', that protect the body against over activity of the body's natural defence system. For example, whereas a stressor induces inflammation, glucocorticoids inhibit the process[45]. This includes interfering with the development of connecting tissue, fibrin deposition, and leukocyte migration. Glucocortiocoids inhibit the synthesis of prostoglandins, thromboxanes and leukotrienes, stabilise lysosomal membranes and prevent the activation of phospholipase A_2[16].

The production of sex hormones can be influenced by glucocorticoids[16]. In some species, cortisol inhibits the production of gonadotrophin releasing hormone, lueteinising hormone, oestradiol and testosterone, whilst in other species they remain unaffected[42].

1.5 Methods of assessing stress

Subjective experiences, such as feelings of pleasure and pain, are notoriously difficult to study because they are essentially private[19]. The assessment of an animal's general health and condition may provide a short term, preliminary assessment of the levels of stress in an animal. However, it

is insufficient to assess welfare as a whole. The fact that physiological and behavioural abnormalities are commonly observed in apparently healthy animals suggests that a more sensitive means of assessing welfare should be used[19].

It is possible to describe behaviours that may be indicative of the presence or absence of stress[27], and compare these observations to other such studies, or between different study groups.

The assessment of adrenocortical activity is confounded by capture or restraint needed to enable samples to be collected; the periodicity of cortisol release and other hormones which interact and effect the adrenocortical system may also influence findings. Physiological findings should therefore be correlated with behavioural observations when interpreting the results[19].

1.6 Aims

As described above, health, physiological studies and behaviour cannot be used in isolation to quantify the welfare of animals - a synthesis of approaches is required. This study aimed to:

- identify and compare behavioural indicators of stress between study groups of elephants
- develop and validate a method allowing non-invasive assessment of adrenal function through the analysis of cortisol metabolites in elephant faeces

- relate glucocorticoid metabolite concentrations to aspects of behaviour and environmental stressors.

CHAPTER 2

GENERAL MATERIALS AND METHODS

2.1 Animals and housing

Twenty-two African elephants (Groups 1-5) aged 4 – 7 years housed at African Game Services (AGS), Brits (25°47' S, 27°48' E) and three African elephants (Group 6) aged between 7 – 10 years housed at Glen Afric Country Lodge, Broederstroom (25°49' S, 27°51' E) were the subject of the study. None of the elephants in the study were pregnant. Details of each study group are presented in Table 1.

Table 1. Details of each study group

Group	Number of elephants	Sex	Boma size (m^2)	Level of human contact/training	Data collected from group
1	2	1:1	140.65	minimal contact untrained	behaviour faecal samples
2	2	0:2	284.21	minimal contact untrained	behaviour faecal samples
3	2	0:2	337.56	minimal contact untrained	behaviour faecal samples
4*	4	2:2	2 500	high level of contact 8mths intensive training	behaviour ACTH challenge faecal samples
5	12	3:11	2 500	high level of contact 8mths intensive training	blood samples
6	3	1:2	7 500 000	high level of contact no formal training caught aged 1-2	behaviour faecal samples blood sample

* Group 4 is a group of 4 animals from group 5 chosen specifically for more intensive study as they were the most habituated to handling and contact with humans. Throughout the study they remained as part of group 5, occupied the same enclosures and were treated in the same way.

Husbandry of all the groups at AGS was the responsibility of AGS under the supervision of the National Council of the Society for the Prevention of Cruelty to Animals. The elephants in Groups 4, and 5 were tethered by a front and a back leg in a barn overnight between 17:00 and 10:00 and provided with fresh fruit, vegetables, *Erogrostis tef, Erogrostis curvular*, oat hay, lucern and bedding. During the day they were released into a 2500sq m enclosure and had free access to a similar variety of feed. They took part in two 30-minute training sessions at the beginning and end of each day. Blood samples were taken from Group 5 animals from time to time during the study when they were tethered and fed in the stable.

At the beginning of the behavioural study, Group 4 had been in captivity for 8 months. At the beginning of the study, Groups 1, 2 and 3 had been held for 6 months in enclosures intended for the housing of rhino at AGS. They were not accustomed to human contact and their diet was as described above. Group 1's enclosure did not have mud or sand baths. Group 2's enclosure had a mud bath and some loose sand. AGS enclosure designs are presented in Figures 5-8 overleaf.

Group 6 at Glen Afric were tethered by one leg in a barn overnight between 16:00 and 07:00 and fed with 2-3 kg horse cubes and varied amounts of lucern. During the day they were released into a 750 ha reserve under the supervision of a ranger.

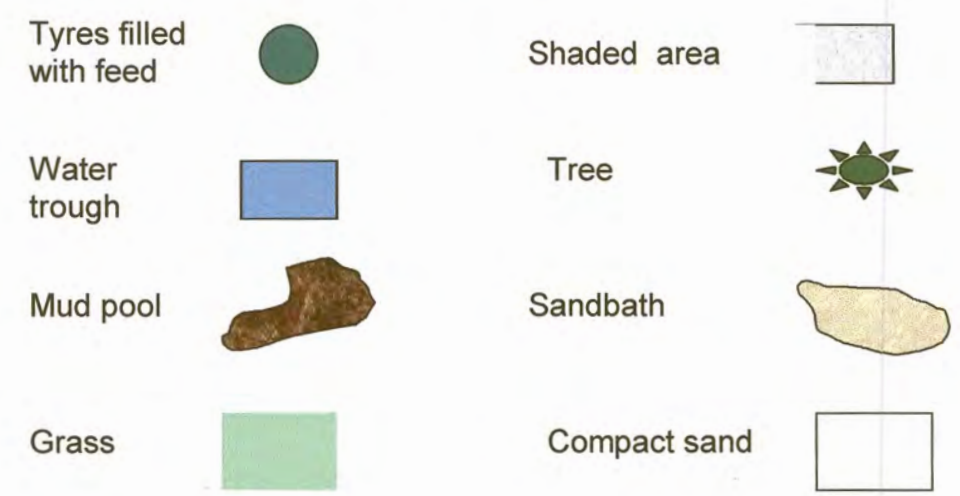


Figure 5. Enclosure housing Group 1

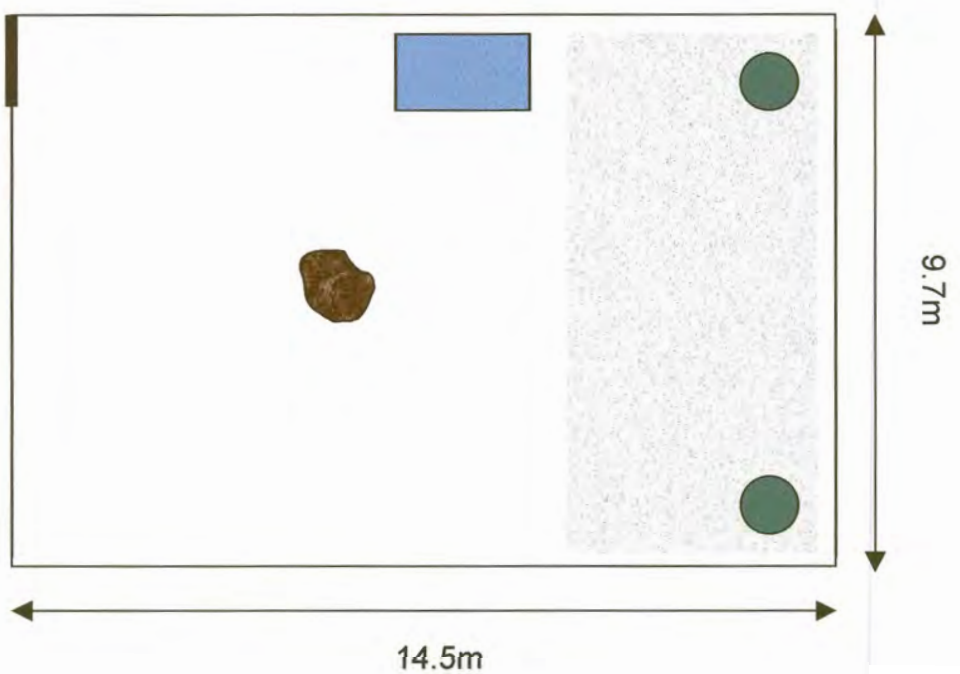


Figure 6. Enclosure housing Group 2

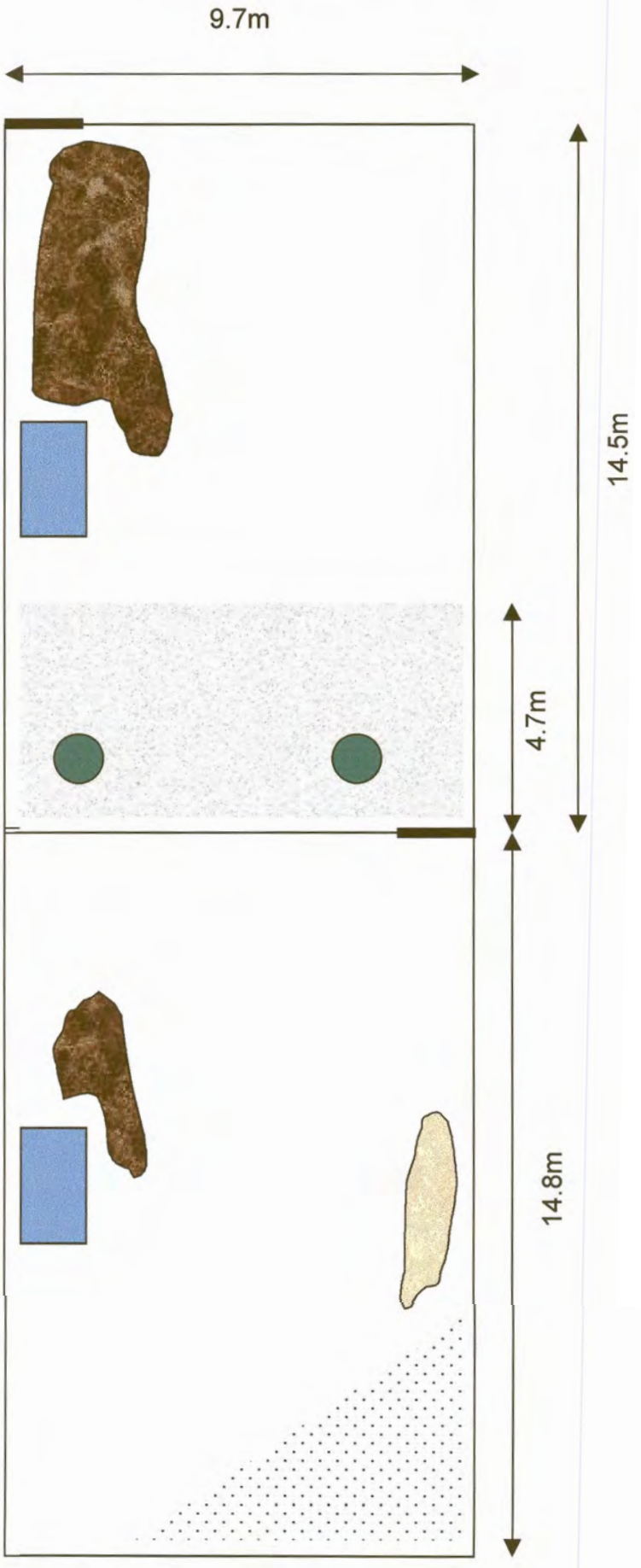


Figure 7. Enc 3

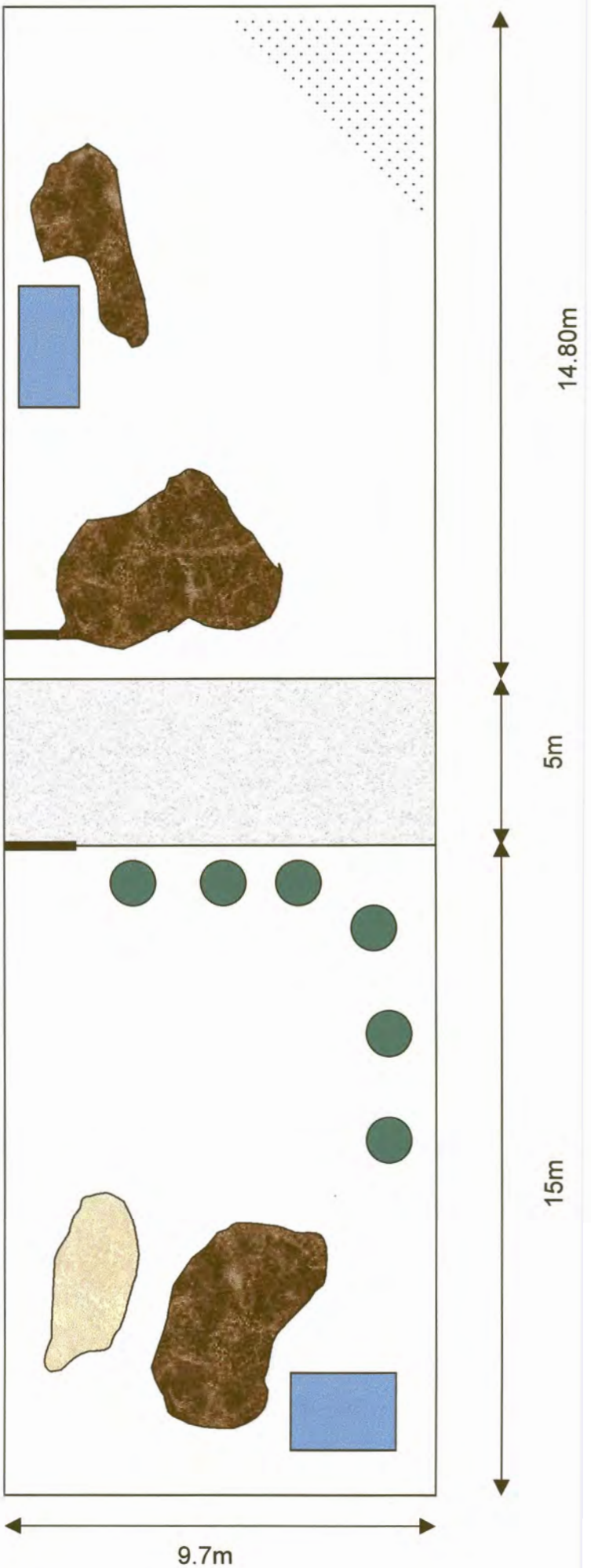
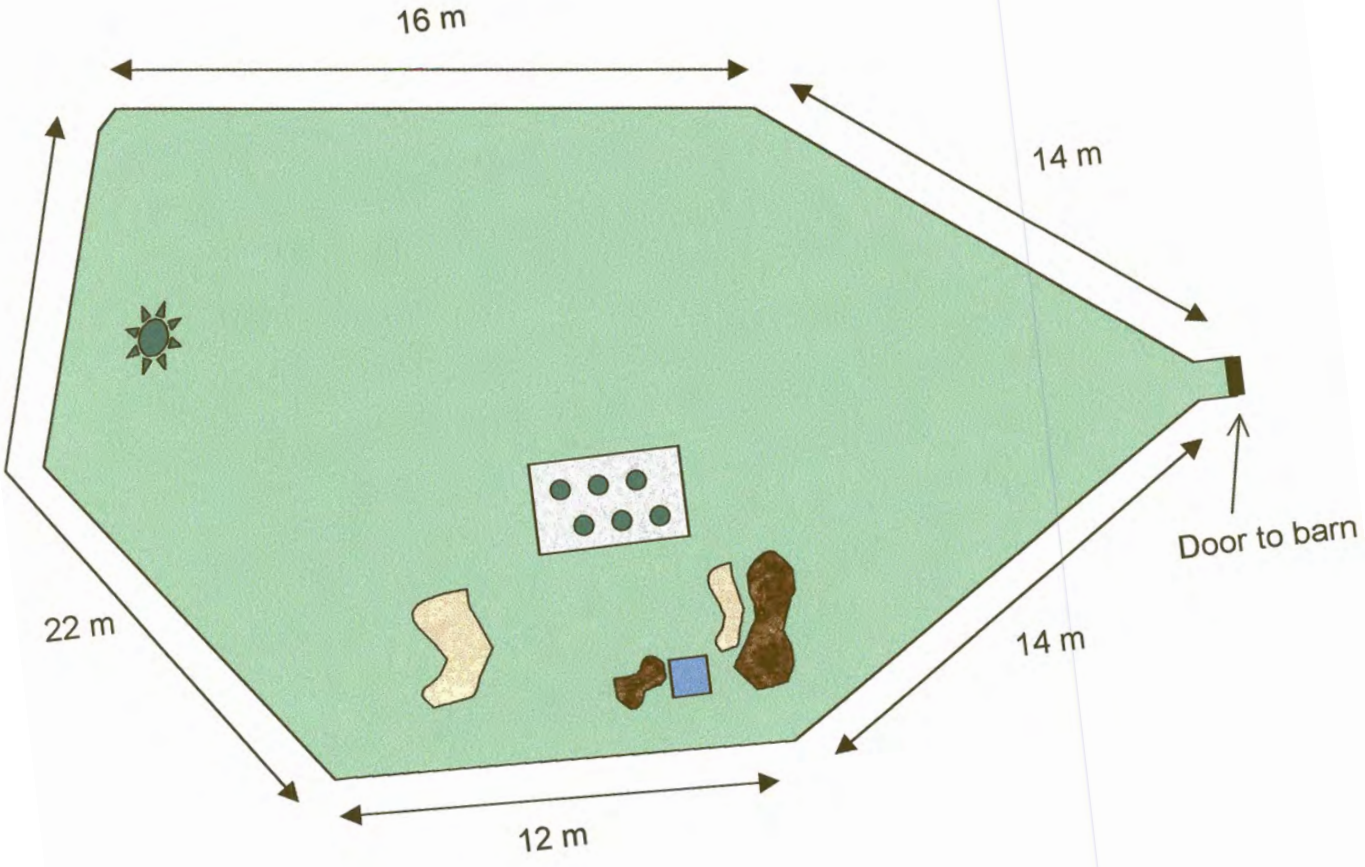


Figure 8. Enclosure housing Group 5



CHAPTER 3

BEHAVIOURAL OBSERVATIONS

3.1 INTRODUCTION

Physiological basis of stress related behaviours

Free-ranging wild animals are faced with a variety of stressors throughout their daily lives. Events such as being faced with danger, a rival or lack of food result in behaviour patterns that are associated with the autonomic nervous system, thus fight-flight responses are common[61]. Such responses should not be of concern because they fall within the natural adaptive range of the animal. However, problems may arise when conditions prevent the animal from expressing behaviours in response to stressors or when behaviours, such as foraging and grooming, cannot be performed[71]. Such conditions are commonly found in captive situations and often result in chronic stress responses, seen as stereotypic or injurious behaviour. These responses are characterised by the fact that the presence of permanent stressors or one or two experiences with a drastic stressor have a long lasting after effect[71]. Chronic stress is often expressed when animals cannot predict and/or control relevant events in their environment[68].

Stereotypic behaviour

A comprehensive review on stereotypic behaviour is provided by Mason[41]. Stereotypies are described as endless repetitive behavioural sequences that appear to be without relevant function and were first described in zoo animals. They are abnormal in the sense that they are not performed in the wild. Stereotypies may be caused by a multitude of factors, but often evolve from early attempts at escape and represent a ritualised form of this behaviour[15]. They may reflect unavoidable lack of control[68], frustration[15], fear[41], the absence of exploratory behaviour[12] or a lack of normal social contacts[6]. They commonly become facilitated by daily routines in captive situations[53]. It has been suggested that in stressful situations, stereotypy is a coping response resulting in a reduction of hormonal response to stress[41].

Identifying stress related behaviour

Assessing the extent to which an animal can perform the behavioural repertoire typical of the species in the wild provides a useful start in the investigation of the welfare of captive animals. The problem with only using wild animals as a comparison is that implies that they are not affected by factors that contribute to stress such as drought, disease or predation[63].

Elephant calves under 5 years old are almost never found by

themselves, and juveniles up to 8 years have been found within 5m of their mothers in 80% of observational scans[39]. It therefore seems reasonable to assume that juvenile elephants separated from their families, captured and translocated to bomas have undergone a highly stressful experience[27]. A number of behavioural indicators that may be stress related in elephants have been described[27]:

- Arousal behaviour including elements such as ears spread and raised; head held high; tail up; running; clustering; loud vocalisation; aggression and other signs related to these behaviours including temporal gland secretion and diarrhoea.
- Reduction in complexity of exploratory behaviour.
- Reduction or absence in play behaviour.
- Increased aggressive or defence behaviour.
- Increased vocalisation.
- Appearance of abnormal behaviours including injurous and stereotypic behaviour.

It has also been suggested that stressed elephants may sleep for shorter periods of time than elephants that feel relaxed in their environment (M Garaï, Elephant Managers and Owners Association, pers. comm., 1999).

This study aimed to identify behavioural patterns indicative of stress and relate their occurrence to environmental stressors.

3.2 MATERIAL AND METHODS

Climate

The study took place during December to February when the weather was hot and rainfall frequent.

Study Groups

Groups 1-4 and 6 were included in the behavioural study (Table 2).

Table 2. Details of study groups in behavioural investigation

Group	Name	Sex	Enclosure size (sqm)	Area per elephant (sqm)	Notes on Husbandry	Level of human contact
1	Legs Lily	m f	140.65	70	no sand/mud-bath unchained at night	minimal
2	Tula Zwana	f f	284.21	142	mudbath only unchained at night	minimal
3	Umseela Makala	f f	337.56	68	3 others in boma unchained at night	minimal
4	Bibi Shirra Sawu Tarzan	f f m m	2 500	625	mud and sand baths chained at night	high
6	Bull Three Mapane	m f f	7 500 000	*	mudbath only chained at night	high

* Group 6 were always within 50m of each other and their keeper.

Behavioural data collection procedures

A pilot study enabled me to identify each elephant and compile a detailed ethogram (see Appendix I). Behavioural 'actions' were recorded

when the behaviour lasted less than 5 seconds. Behaviours were classed as activities if they lasted more than 5 seconds.

I spent 10 minutes 15 m away from the enclosures to habituate the elephants to my presence prior to data collection. Data collection was carried out using the data sheet presented in Appendix 2. Observation times were as follows:

Group	06:00	07:30	09:00	09:30	10:30	12:00	14:00	15:00	15:30	17:00
1, 2, 3	✓	✓	✓				✓		✓	✓
4					✓	✓	✓		✓	✓
6				✓	✓	✓	✓	✓		

Observation times were set to coincide with the elephants' daily routines. In all cases these began when the elephants were given free access to food. Groups 1,2 & 3 were moved into an adjoining open enclosure after being fed and then returned to their enclosure after it had been cleaned. Groups 4 and 6 were observed until they were taken back to their overnight facilities. Each observation period was an hour long. Each elephant in Groups 1, 2 and 3 was observed for 18 hours. Each elephant in Group 4 and 6 was observed for 15 hours.

Temporal gland secretion (TGS) was described as being old if it was present at the beginning of the observations period, and new if it formed during the observation period. The size of TGS was described in relation to the features of the face.

Data Analysis

Values for combined variables ($^{+}$) were calculated by totalling the behaviours described in Table 3.

Table 3. Calculation of combined variables

Combined Variable	**Behaviours summed to form combined variable**
Grooming^{+}	Dust, Mud, Rub activities
Social^{+}	Genitals, Other contact, Trunk touch, Trunk mouth, Trunk over back, Play actions
Dominance^{+}	Moderate push, Hard push, Trunk flick, Trunk hit, Chase, Kick actions
Curiosity^{+}	Reach, Object, Trunk up, Ears open actions
Nervous^{+}	Mock charge, Threat, Head butt bar, Head shake, Tail high, Ears wide, Head high, Eyes wide, Run, Cluster actions
Stereotypy^{+}	Back-forwards, Trunk suck, Head sway, Bar bite actions
Rumbles^{+}	Low rumble, Loud Rumble actions
Trumpets^{+}	Cry, Trumpet actions

A General Linear Model (GLM) was fitted to the data. Fisher's Test was used to test for differences between groups. Since the GLM used cannot

process data that is not normally distributed, the test was unable to identify statistical differences if they had occurred for the following variables:

Back-forwards (action & activity), Head sway, Trunk suck, Stereotypy^{+}, Dust (action), Rub (action), Ears open (activity), Ears wide (activity), Eat mud, Object (action & activity), Trunk over back, Mock charge, Head butt, Cluster, TGS, Low rumble, Loud rumble and Rumbles^{+}.

The Kruskal-Wallis test was used to process these variables and those for which Fisher's test found no significant differences. It was also used to detect statistical differences in behaviour between the AGS elephants (Groups 1-4) and those at Glen Afric Country Lodge (Group 6).

Data analysis was carried out by Mike van der Linde, Department of Statistics, with the help of Prof. Groeneveld University of Pretoria.

3.3 RESULTS & DISCUSSION

Behaviours were grouped into general behaviour, grooming behaviour, affiliative interactions, dominance behaviour, investigative behaviour, nervous/alert behaviour, stereotypic behaviour, vocalisations and aggression towards people.

No significant differences were found between groups using Fisher's Test or the Kruskal–Wallis test for the following variables:

Dust (action), Rub (action & activity), Other contact, Genitals, Hard push, Trunk hit, Trunk Flick, Threat, Dominance^{+}, Ears open (activity), Ears wide (activity), Eat mud, Trunk over back, Mock charge, Head butt, Cluster, Backforwards (action & activity), Head sway, Trunk suck, Trunk twiddle, Stereotypy^{+}, Loud rumble and Rumbles^{+}.

3.3.1 Feeding activities, drinking and standing

Results

Mean time (minutes) spent performing each activity per hour per group are shown in Table 4. Statistically significant differences between groups are shown with associated P values in Table 5.

Table 4. Mean time (min) spent performing activity per hour per group.

Group	Feeding	Grazing	Drinking	Standing
1	21.69	0.87	0.47	26.28
2	22.17	1.75	0.81	15.46
3	28.30	3.35	0.69	18.04
4	14.65	11.29	1.91	20.78
6	47.54	Not applicable	2.16	3.08

Table 5. Statistically significant differences between groups

Behaviour	Group	Frequency	Group	P value
Feeding	6	>	1,2,3,4	$\rightarrow 0$
	2	>	4	0.019
	3	>	4	$\rightarrow 0$
Grazing	4	>	1,2,3	$\rightarrow 0$
	3	>	1	0.007
	3	>	2	0.046
Drinking	4,6	>	1,2,3	<0.01
Standing	1	>	2,3,6	<0.017
	1	>	4	0.048
	1,2,3,4	>	6	<0.001

Most feeding activity in Groups 1,2 and 3 occurred in the morning and late afternoon, with periods of standing around midday and in the afternoon. Makala (Group 3) spent 40 minutes of the observed time eating mud and soil. Tula (Group 2) was the only elephant to be observed lying down (27.5 min).

Discussion

The elephants at Group 6 spent 79% of the observed time feeding. At first sight, this appears to correspond with observations of wild elephant that have been reported to spend about 75% of their time feeding[20,72]. However, these observations were carried out over periods of 24hrs whereas Group 6 elephants were only observed during the day. Diurnal observations between 08:00 – 17:00hrs with a mean daily observation time of 7 hr 34min of wild elephants by Guy[31] are more comparable to this study. He recorded a mean feeding time of 36% and 57% during hot and wet weather respectively. The climate during my observations was hot *and* wet, therefore a mean of 46.5% can be used for comparison to the study groups. Unfortunately comparative data from captive situations has not be been published.

Group 6 did not have access to food at night and therefore it is not surprising that the time spent feeding during the day was nearly double that of the elephants observed by Guy[31] and those at AGS. The AGS elephants had access to food day and night but this was restricted by the clumping of

limited food sources. Groups 1, 2, 3 and 4 spent a similar proportion of their time feeding (mean: 43.5%) to those observed by Guy.

Three feeding peaks - during the morning, afternoon and around midnight - have been observed for wild elephants with a general reduction in feeding activity around midday and between 04:00 – 07:00[31,72]. Feeding peaks were observed in all of the study groups but were inextricably linked to times at which food became available.

Group 4 spent less time feeding from the food troughs and more time grazing grass growing in and around the enclosure than the other AGS groups, which did not have access to as much naturally occurring grass. This may be supportive of studies showing that when given the opportunity animals prefer to 'work' for food[21,58]. It has also been suggested that performing functional behaviour related to feeding facilitates adequate endocrine changes in the gastro-intestinal tract (de Passille et al., 1991[71]). Alternatively, all of the AGS elephants may have been hungry, and only Group 4 had ample grass to eat and thus spent significantly more time grazing than the others. This is probably not thè case as Group 1 and 2 did not graze as much as Group 3 despite the fact that long grass was well within reach. On the other hand, the motivation to explore and forage may have been reduced as a result of nervousness[27].

Makala spent almost 14% of her time eating soil and mud. The other elephants spent an insignificant amount of time doing this. Wild and captive elephants have been seen to eat alkaline soil, with an increased content of sodium and clay[52]. It is suggested that this provides essential minerals

lacking in the diet[1]. Makala may well have been suffering from some form of nutritional deficiency, since the lower third of her trunk had been chopped off in an accident. On the other hand, it is possible that eating soil represented a displacement activity that had developed as a result of boredom and lack of normal feeding repertoires.

Coprophagia was observed on a few occasions. This has been reported in captivity[1], but its frequency in the wild has not been reported. It may provide a source of nutrition via undigested material and bacterial proteins. It is unlikely that coprophagia in these elephants resulted from hunger as it was seen very rarely.

Groups 4 and 6 spent significantly more time drinking than the other groups (3.2% and 3.6% of observation period). This was to be expected because they did not have access to water during the night whereas the other groups did. Adult wild elephants usually drink up to 227 litres a day, often in a single session but can also do without water for several days[72]. Guy reported that elephants spend 1.9% of their time drinking during the hot season and that it may occur at any time of day. Commonly drinking precedes mud-bathing[31,72].

Wild elephant are reported to spend 20% and 40% of their time resting in the wet and hot season respectively[31].

The fact that Group 1 spent 44% of observed time standing may be indicative of the effects that the limited space had on them. Group 4 were seen to stand more often in the afternoon just before being led into the barn. It would appear that the presence of people and food preparation distracted them from performing other behaviours. Group 6 were always on the move feeding and as a result had very little time to be inactive (5% of observed time).

There is an inverse relationship between time spent feeding and the time spent standing. The times spent feeding, drinking and standing are reflective of the keeping system and cannot be used as indicators of stress per se.

Prolonged periods of standing reflect that no other behaviours are being performed. They may therefore be indicative of an environment that does not encourage species specific behaviours and one in which stress related behaviours would be expected to develop.

3.3.2 Grooming activities

Results

Groups 1, 2, 3 and 6 were not specifically provided with large sand-baths but used dry dirt on the floor to dust. Group 6 occasionally came upon sand piles intended for construction and would immediately begin excited dusting bouts but were quickly chased away by their keeper.

The mud-baths were generally used during the hottest times of the day. Of the AGS elephants, Tula spent the most time playing in the mudbath. The mean times in minutes spent performing grooming activities per hour per group are shown in Table 6 below. Statistically significant differences are presented in Table 7.

Table 6. Mean time in minutes spent performing grooming activity per hour per group.

Group	Dust	Mud	Rub	Grooming^{+}
1	0.33	0.56	0.042	0.94
2	0.71	3.03	0.56	4.30
3	0.22	0.49	0.38	1.08
4	2.13	0.93	0.24	3.30
6	0.71	4.54	0.06	5.31

Table 7. Statistically significant differences between groups

Behaviour	Group	Frequency	Group	P value
Dust	4	>	1,2,3,6	<0.007
Mud	6	>	1,3,4	<0.02
Grooming[+]	6	>	1,3	<0.03

Discussion

Although the skin of an elephant can be up to 3cm thick, it is well supplied with nerves and blood vessels close to the surface and highly sensitive to touch and temperature variation[5]. It has been suggested that covering the skin with dust and mud provides protective layers, insulating it from the sun and reducing irritation by insect pests[57]. Mud splashed behind the ears and into folds of the skin also has a cooling effect[5].

Comparisons between groups of grooming variables is confounded by the fact that some were provided with grooming facilities whilst others were not. Nevertheless, the absence of grooming repertoires is abnormal in the sense that they do occur in free ranging elephants. The occurrence of grooming and explanations for its frequency in each group is of use in discerning whether or not time spent grooming can be an indicator of stress.

Group 4 were provided with a large sand-bath and spent between 3 and 10 times longer dusting than the other groups which did not have large sand-baths, providing evidence that when available, sand baths are used frequently. It is likely that the incidence of dusting in Group 6 would have increased had they not been prevented from using sand piles by their keeper.

Group 1 were seen to perform dusting action when there was no dust present, suggesting that the motivation to perform the behaviour was present. This may have been a form of stress-related displacement behaviour as described by Adams and Berg[1] who observed that elephants dusted more when browse and food were unavailable and when they were delayed from entering the night-barn. Unlike observations by Adams and Berg, temporal gland secretion was not observed whilst elephants dusted. It is suggested that the inability to dust is a factor that could contribute to stress, and that sand should be made available to captive elephants.

Group 6 spent 7.6% of the observed time using a mud-bath. This exceeds the percentage time observed by Guy[31] in the hot and wet seasons (1 and 4.3% respectively). The infrequent wallow use by wild elephant in the hot season is probably due to lack of shallow pools. Group 1 only used wallows when they were transferred into Group 2's adjacent enclosure during cleaning and food provision. They did not have a wallow in their own enclosure, however, they could have enlarged puddles that were present to construct mud-baths as observed for elephants at San Diego Zoo[1]. Groups 2 and 3 enlarged their mudbaths considerably. The fact that Group 1 did not suggests reduced levels of motivation which were perhaps indicative of stress[27].

Tula, in Group 2, spent the most time playing in the mudbath. This suggests that she felt relatively relaxed in her environment since whilst partially submerged or lying down, vigilance is decreased[27]. The fact that

Zwana, within the same group, did not spend as long playing in mud highlights individual differences in personalities and reactions to the same environmental stressors.

Although Group 4 was provided with a large mud-bath, they were never seen to fully submerge themselves in it, and spent less time mudding than Group 2. This may have been because they did not feel relaxed enough to do so. However, upon closer investigation, the bottom of the bath felt hard and stony. The wallow was subsequently half filled with soft sand, and the use of the bath increased. This suggests that the amount of soft mud in the wallow rather than its depth is important for the elephants.

Use of the mud-baths generally occurred during the hottest times of the day emphasising their role in cooling. In winter months, either the temperature is not high enough to require cooling aid or the mud-baths are too cold to use, even in Southern Africa[31].

In situations where mudbaths are present, lack of use can be attributed to three things. The elephants may be too nervous and alert to use a mud-bath, and therefore low frequency of mudbathing could be indicative of stress[27]. Alternatively the texture of the mudbath may not be attractive to the elephants. It may be too stony, or not contain enough soft mud, and this should be adjusted and the mudbaths use reassessed. Finally, the weather must be considered. During cooler months, grooming activities are likely to be low and therefore unreliable as indicators of stress.

The absence of grooming facilities in a captive environment is a factor that will contribute to the development of stress. The frequency of grooming

cannot be used as an indicator of stress in this study since each group was provided with different grooming facilities.

3.3.3 Affiliative Interactions

Results

Mean numbers of affiliative actions per hour are shown in Table 8. Statistically significant differences between groups are presented in Table 9.

Table 8. Mean number of affiliative actions (n) or time spent playing (min) per hour per group.

Group	Other contact n	Trunk touch n	Trunk mouth n	Play n	Play min	Social+ n
1	5.97	0.53	0.36	0	0	6.94
2	5.97	1.17	0.92	0.92	0.43	8.33
3	11.06	0.17	0.61	0.44	0.11	12.44
4	13.08	0.52	0.50	3.42	3.15	15.33
6	7.62	2.44	1.11	3.67	0.02	11.40

Table 9. Statistically significant differences between groups

Behaviour	Group	Frequency	Group	P value
Other contact	4	>	1,2	0.045
Trunk touch	6	>	1,2,3,4	<0.01
Trunk touch	2	>	3	0.048
Trunk mouth	6	>	1,4	<0.012
Play action	4,6	>	1,3	<0.02
Play action	4,6	>	2	<0.05
Play activity	4	>	1,2,3,6	<0.02

Within Group 4 Tarzan took part in the least number of affiliative actions and spent more of his time away from the group. Within Group 6, Bull was the only elephant to exhibit bouts of play. He only did this with a young white rhino that had been hand-raised with the elephants and often accompanied them during the day.

Discussion

Elephants live in stable family groups, composed of related females and their offspring, in which life long bonds are formed[20]. Tactile communication is an important method of creating and maintaining social bonds[39]. Different calves receive different amounts of friendly attention[39].

The different number of elephants in each group, their familiarity with each other and enclosure size confounds the comparison of social interactions between groups. However, one would expect the group with the most elephants or the least area per elephant to have the highest number of interactions purely due to increased opportunity. This was not the case for all affiliative behaviours. It is therefore useful to examine the frequency of social interactions within each group, investigate the reasons for the differences observed and assess the use of monitoring affiliative interactions to identify stress.

The occurrence of tactile behaviours incorporated under the term 'other contact' is correlated with the number of elephants in each enclosure, with the exception of Group 4. It is suggested that their contact rate was

higher than in Group 3 due to their proximity whilst waiting to enter the night barn in the late afternoon.

Contrary to expectations, the occurrence of trunk touch was not related to group size. It was observed most frequently in Group 6. Both Three and Bull commonly directed tactile behaviour towards the youngest elephant, Mopane. They appear to have adopted an allomothering role defined as comforting, assisting and protective behaviour[39]. Group 2 exhibited the behaviour more than Group 1 despite the fact that group sizes were the same. The reason for this is unclear.

While others have reported that the trunk mouth action was the most frequently recorded affiliative behaviour[1,26], it was the least frequently recorded of tactile actions for groups 1, 2, 4 and 6, and the second most common tactile action in Group 3. The trunk mouth action is an important gesture of recognition and friendship[1]. The number of partners with whom the elephants could interact is below the number that they would have encountered in family groups in the wild, and therefore I did not expect it to be the most frequently recorded affiliative gesture. I expected the frequency of the behaviour to be correlated to group size, however this was not the case. It is likely the action occurred more frequently in Group 6 because they ate a wider variety of food, and thus investigations into what others were eating were more common. Additionally, larger distances were placed between the individuals whilst feeding, so greeting and reassurance gestures would have been more frequent upon regrouping.

Group 1 didn't exhibit playful behaviour throughout the observation period. The absence of play behaviour has been described as reflective of nervousness, insecurity or some other from of emotional stress[27] since it will only occur in relaxed contexts[27,39].

Group 4 spent more time playing than other AGS elephants indicating that they felt relatively relaxed in their environment and had formed strong social bonds. The elephants in group 6 did not spend time playing with each other in sessions. It is likely that the time they spent feeding did not leave time to indulge in long bouts of play. However, Bull was often seen playing with a tame young rhino and tried to mount him frequently. Young males have been observed to spend more time playing with each other than females in the wild[39]. This may explain why Three and Mopane did not engage in playful sessions, but showed playful gestures (actions rather than as an activity).

The lack of affiliative actions between the individuals Group 1 probably indicates that they had not settled into their new captive situation. It appears that there was a lack of bonding between the members of this group, Legs and Lily, and it is likely that they would have benefited from being housed with a larger group within which other affiliative associations could have been sought.

In conclusion, I feel that the frequency of play actions and the combined variable Social^{+} can be used as reliable indicators of how well elephants have settled into their group. If play is absent and/or the frequency

of the combined variable Social^{+} is low, the composition of the group should be reassessed and ways in which social behaviour could be encouraged investigated. The occurrence of other affiliative interactions must be used with caution as indicators of stress. The number of reassuring affiliative gestures could be high when animals are nervous or stressed; or they could be low due to a lack of bonding and unease within the group.

3.3.4 Dominance behaviour

Results

Fisher's test was unable to detect significant differences between groups for Hard push, Trunk flick, Trunk hit and Dominance^{+} because of the frequency of zeros in the data. The low number of elephants per group rendered the Kruskal-Wallis test insensitive to possible differences.

In most cases, dominance actions were associated with the arrival of food or whilst Group 4 were waiting to enter the night-barn. Individuals showed submission towards dominant individuals by presenting their posterior towards them or avoiding them. Mean numbers of dominant actions per hour are shown in Table 10. Statistically significant differences between groups are shown in Table 11. Dominant and submissive elephants are listed in Table 12.

Table 10. Mean number of dominant actions per hour per group

Group	Gentle Push	Moderate push	Hard Push	Trunk flick	Trunk Hit	Dominance^{+}
1	0.03	0.06	0.08	0.31	0.01	0.46
2	0.78	0.08	0.08	0.19	0.14	0.49
3	0.67	0.11	0.08	0.03	0	0.22
4	0.17	0.02	0.07	0.10	0.05	0.24
6	0	0	0	0	0	0

Table 11. Statistically significant differences between groups

Behaviour	Group	Frequency	Group	P value
Gentle push	2	>	1,4,6	≤0.0003
Gentle push	3	>	4,6	<0.019
Moderate push	3	>	4,6	<0.019

Table 12. Dominant and submissive elephants in each group

Group	Dominant elephant/s	Submissive elephant/s
1	Legs	Lily
2	Tula	Zwana
3	Umseela	Others in group
4	Bibi	Tarzan
6	No dominance interactions observed	

Discussion

The presence of confounding factors including the length of time that the elephants had spent in captivity, group and enclosure size when comparing social interactions between groups have been discussed previously. One would expect dominance behaviours to be highest in the group with the smallest area per elephant. This was not the case and it is therefore useful to examine the frequency of dominance actions within each group, investigate the reasons for the differences observed and assess the use of monitoring these behaviours to identify stress.

In accordance with other studies[1,26], the majority of dominant incidents in most groups occurred just before the arrival of food. The probability of

aggression in a number of captive species has been shown to increase when eliciting stimuli, or releasers such as the expectation of food are present[17].

Young males have a tendency to exhibit more dominance behaviour than females[20,37,39] and this was reflected in Group 1, all dominant and aggressive behaviour being directed towards Lily from Legs. Dominance behaviour in Group 1 occurred during various times of the day and unlike the other groups, was not always associated with the arrival of or access to food. Lily had little scope to avoid Legs, but appeared to prefer his 'unfriendly' company to solitude, emphasising the need for companionship.

Aggression has been described as being evoked by frustration[17] and lack of behavioural stimulation[71]. It is likely that the opportunity to perform species specific behaviours such as grooming and foraging would have reduced the number of dominant actions directed towards Lily.

Umseela, in Group 3 was seen to direct dominance behaviour to all other elephants in the group and never be the recipient of any, suggesting that she had adopted a matriarchal role. This has also been described in other captive juvenile elephants[1,26,27]. Sikes[57] concluded that elephant leadership is determined by size, strength, age combined with experience. Umseela was the largest within the group, probably the oldest and is the most likely to have had experience as an allomother.

Contrary to expectations, Bibi, who was smaller and younger than Shirra, appeared to have adopted the dominant role in Group 4, leading the others in the enclosure and instigating various activities. This may have resulted from her being the leading elephant during training procedures. The

majority of aggressive and dominance actions were directed towards Tarzan from fellow members of the Group. This is unusual as I expected the youngest and smallest member of a group to receive the most protective and affiliative behaviour as was observed in Group 6.

The frequency of aggression is high in a newly translocated group[27,73]. However since the elephants at AGS had been in captivity for 8 months, I expected the frequency of aggressive behaviour that may have been high during early adjustment to new surroundings to have declined. Unfortunately, the elephants were not monitored when they first arrived at AGS and so this hypothesis cannot be tested.

The lack of aggression within Group 6 was probably due to the length of time that they spent together from a very young age and their long-standing dependence upon one another for positive social interactions. Bull was approaching the age at which he would become independent from the family group and begin to attain status as a breeding male. This may influence the amount of dominant/aggressive actions observed within the group in the future.

I feel that since the number of Dominance^{+} actions in Groups 1 and 2 were double those in Groups 3 and 4, and absent in Group 6 that they can provide a useful indicator of stress within a group. They may be a cause of and/or a product of stress. Hard pushes, trunk flicks and hits would be of particular concern if they occurred regularly and their occurrence was associated with low levels of affiliative behaviour.

3.3.5 Investigative Behaviour

Results

Mean numbers of investigative actions per hour and mean numbers of investigative activities per hour are shown in Tables 13 and 14 respectively. Statistically significant differences between groups are presented in Table 15.

Table 13. Mean numbers of investigative actions per hour per group

Group	Reach	Trunk up	Ears open	Object	Curiosity^{+}
1	2.31	3.42	9.89	0.08	7.16
2	5.00	3.00	12.22	0.28	13.45
3	1.75	3.69	6.25	0.42	4.99
4	0.75	1.32	2.89	1.92	2.21
6	0.20	0.40	4.11	0	0.08

Table 14. Mean time spent performing investigative activities per hour

Group	Reach	Ears open	Object
1	0.04	6.99	0.13
2	1.25	8.17	4.03
3	0.19	4.63	0.17
4	0.07	0.03	2.12
6	0.01	0	0.07

Table 15. Statistically significant differences between groups

Behaviour	Group	Frequency	Group	P value
Reach action	1,3	>	4,6	<0.01
Reach action/activity	2	>	1,3,4,6	0.0001
Trunk up	2	>	6	0.1
Trunk up	1,3	>	4,6	<0.022
Ears open action	2	>	6	0.02
Ears open action	1,2	>	4	<0.03
Object action/activity	4	>	6	<0.05
Curious^{+}	2	>	1,3,4,6	<0.001
Curious^{+}	1,3	>	4,6	<0.035

Discussion

Groups 1, 3 and in particular Group 2 spent the most time 'reaching' out of their enclosure. This usually happened in the presence of their keeper with whom they appeared to have built up a relationship because he hand fed them. Zoo elephants have been observed to spend time reaching ("begging for food") from the public, but quickly stopped if no notice was taken of them[1]. Groups 1, 2 and 3 also reached into adjoining enclosures towards neighbouring elephants, from my observer position it was difficult to see whether or not tactile contact was made with other elephants and thus whether or not 'Reach' was a form of affiliative behaviour. Group 6 tended to ignore people nearby, rarely reaching out towards them and were never seen 'begging for food'.

Elephants use their trunks to gather olfactory information[23], and trunk-raising in addition to ear opening are the first signs that something has

attracted their attention. Groups 1, 2 and 3 performed these behaviours the most suggesting that they were more alert to stimuli outside their enclosure than other groups. Groups 4 and 6 appeared to have habituated to the sights and sounds of people in their vicinity and subsequently raised their trunks the least.

Of all non-primate mammals, elephants have been cited to use tools with the highest frequency and diversity[14,70]. Tula, in Group 2, spent the most time of all the elephants observed investigating and playing with objects. She learned how to turn on the tap within her enclosure, supporting the suggestion that elephants possess high levels of intelligence and memory for adaptive behaviour as reported by a number of authors[1,7,14,20].

High levels of investigative behaviour towards novel, manipulative objects such as stones, pieces of wood, tyres reflect the elephants orientation to their environment and need for stimulus change[1]. This explorative behaviour should be maximised in captive situations and could be used to encourage foraging behaviour by hiding small food items in puzzle feeders, sandbaths, tree trunk and tyres.

Chevalier-Skolnikoff and Liska[14] report that an average of 22.8 acts of tool use by elephants occurred per hour in captivity. Unfortunately my data is not directly comparable to these results due to different methods of data collection. I recorded 6 different types of uses that had obvious functions including cooling, obtaining water, and scratching. No throwing of objects towards humans was observed as others have reported[20,37,70].

It appears that that long bouts of playing with objects will only occur when activities such as feeding and vigilance are not of immediate importance, and if stimuli are present to instigate the behaviour. Tula evidently felt relatively at ease spending upto 13% of her time playing with her food trough (a tyre). By comparison, Legs and Lily spent 0.2% of the observed time playing with objects. I do not feel that this was a result of a lack of objects in the enclosure since all groups were provided with tyres containing food which were easily transformed in to play objects. It is more likely that the absence of play reflects a higher state of vigilance and unease. Group 6s daily activities were consumed by feeding and wallowing, leaving no free time to play with objects. This is to be expected since free-ranging elephants have been reported to manipulate objects including mud and dust less frequently than those in captivity do[14].

It is concluded that the frequency of investigative actions reach, trunk up and ears open reflect the extent to which the animals have habituated to their environment and cannot be used as indicators of stress per se. None of the elephants at AGS could be described as severely apathetic which would have been characterised by a total lack of investigative behaviour[27]. Whether or not elephants play with objects could be used as an indicator of stress for non-free ranging groups. However, the incidence of playing with objects may decrease with age. Tool use should be encouraged by associating objects with particular goals, for example obtaining food.

3.3.6 Nervous/alert behaviour

Results

Nervous/alert actions were shown most frequently when vehicles and/or people came near the enclosures. The elephants' apparent state of alertness was prolonged if the person approaching was unfamiliar to them. Mean numbers of alert actions per hour are shown in Table 16 Statistically significant differences are presented in Table 17.

Aggression towards people was seen in the form of threats, mock charges, head shaking and banging the enclosure bars with the head. The majority of these incidents occurred whilst people unloaded food into neighbouring enclosures. The occurrences of aggressive actions directed at people are presented in Table 18.

Table 16. Mean numbers of nervous/alert actions per hour per group

Group	Tail high	Ears Wide	Head high	Head shake	Nervous^{+}
1	0.28	6.86	1.17	0.61	10.31
2	0.50	4.69	1.17	1	8.44
3	0.39	1.78	0.61	0.97	5.94
4	0.05	0.25	0.05	0.02	0.52
6	0.13	1.42	0.58	0.09	2.56

Table 17. Statistically significant differences between groups

Behaviour	Group	Frequency	Group	P value
Tail high	2	>	4	0.049
Ears wide	1	>	3,4,6	<0.01
Ears wide	2	>	4,6	<0.05
Head high	1,2	>	4	0.05
Nervous+	1	>	4,6	<0.02
Nervous+	2	>	4	0.012

Although Groups 4 and 6 did not perform the action 'Head shake' as often as Groups 1, 2 and 3, Fisher's test could not detect differences between groups.

Table 18. Number of aggressive actions towards humans per elephant.

Elephant	History of human contact	Headshake	Threat	Headbutt	Mock Charge	Total
Legs	minimum	17	8	37	-	62
Lily		5	3	2	-	10
Tula	minimum	10	6	11	3	30
Zwana		26	2	1	2	31
Umseela	minimum	24	4	18	-	46
Makala		11	7	20	-	38
Bibi	8mths training	-	-	-	-	0
Shirra		1	-	-	-	1
Sawu		-	-	-	-	0
Tarzan		-	-	-	-	0
Bull	high contact from young age no formal training	2	-	-	-	2
Mopane		1	10	-	3	14
Three		1	-	-	-	1

Running was seen on 14 occasions in Group 2 and always occurred when food was being delivered. Five incidents of running were observed in Group 4 when the elephants chased birds and on three occasions when they formed a cluster upon hearing loud noises (a helicopter and a motorbike). Similarly Mopane in Group 6 ran twice upon hearing a low flying plane and a helicopter. Elephants in Group 3s enclosure formed a cluster on 15 different occasions upon hearing a low flying aircraft, loud vehicle noises at times other than feeding, and occasionally when a person with whom they were unfamiliar approached. Makala ran on 7 occasions during cluster formations and when food was delivered. Umseela always stood apart from clusters when they formed and was the first to investigate stimuli and the last to retreat from them.

The action of regurgitating water from the stomach was seen in two juveniles outside of the study period when an adult Asian elephant was used to catch them in order that their neck ropes be removed. The procedure also resulted in all elephants in the enclosure running, clustering, bellowing, defaecating and secreting copiously from their temporal glands.

Discussion

Groups 1 and 2 exhibited the most alert actions, usually when the tractor delivering food arrived, and when people with whom they were not familiar approached the enclosure as reported by Young and Oelofse[73]. Group 1 were the most alert, supporting earlier conclusions that they had not

settled well into their captive situation and were not habituated to the sounds and sights of people and vehicles.

Within Group 3, Umseela's reaction to stimuli confirms the earlier suggestion that she had adopted a matriarchal role. Groups 4 and 6 showed the least number of nervous and alert actions reflecting the longer time that they have spent growing accustomed to people. Regular contact with humans has been shown to result in a lowering of heart rate and beta-endorphin blood levels, suggesting that habituation to the presence of people is beneficial to captive animals[28].

The groups that had the most contact with people showed the least aggression towards them. However, as exemplified by Mopane in Group 6, individual variation in reactions to people occurs and this can sometimes be unpredictable. In one instance outside the study period, Tarzan pushed a woman to the ground with his head, breaking three of her ribs despite the fact that he had shown no prior threatening behaviour. This is a reminder that despite their relatively small size, juvenile elephants possess enormous strength, which can only increase as they grow older. In another incident at AGS, an adult Asian female circus elephant who had no track record of aggression towards people, attacked a worker on the farm when he raised a stick to her. The worker admitted that he was extremely lucky to be alive, as she repeatedly tried to chase and stamp on him. In February this year a keeper was killed by a 14 year old Asian elephant at Howletts Wild Animal Park, UK in unknown circumstances.

These statements emphasise the dangers involved in keeping elephants in captivity and warn against complacency when handling them.

Extreme levels of nervous/alert behaviours including running, forming clusters, regurgitating water, bellowing and diarrhoea were not observed frequently. These behaviours can be used as a measure of stress when elephants are first translocated[27]. Their usefulness as indicators of stress declines with the time spent in captivity as habituation occurs. The behavioural stress response may then change to include abnormal social interactions and stereotypies.

It is concluded that after several months in captivity, the frequency of Ears Wide and Head High actions and/or the collective variable Nervous^{+} can be used as indicators of stress.

3.3.7 Stereotypic behaviour

Results

Group 6 did not exhibit any stereotypic behaviours. Group 4 showed minimal amounts of stereotypy. Only 1 elephant showed 'head sway'. Mean numbers of stereotypic actions and mean times spent performing stereotypic activities per hour are shown in Table 19. The high frequency of zeros in the data rendered Fishers Test unable to detect significant differences between groups.

Table 19. Mean numbers of stereotypic actions (n) and mean time spent performing stereotypic activities (min) per hour

Group	Backforward min	Backforward n	Trunk twiddle n	Trunk suck n	Stereotypy^{+} n
1	2.44	1.26	0	0.33	2.78
2	0.86	0.08	0.31	0.31	0.03
3	2	2.36	0.06	0.08	0.06
4	0	0	0.13	0.02	0.003
6	0	0	0	0	0

Stereotypy occurred in groups 1, 2 and 3 in the form of a backward and forward pacing that was always associated with temporal gland secretion. It was performed most frequently by Lily and in most cases always occurred in the late afternoon or early morning before the arrival of food.

Zwana spent 10 minutes standing and twiddling her trunk one afternoon. Trunk sucking was performed most frequently by Lily and was most commonly associated with pacing behaviour, temporal gland secretion and during periods of standing in the afternoon.

Group 4, in particular Bibi, and to a lesser extent Group 6, exhibited head swaying behaviour in the early morning whilst shackled and waiting for their morning feed. The head swaying action comprised of the front part of the body swinging to and fro, with the forelegs lifting in accordance with the rhythm.

Discussion

The stereotypic pacing observed in groups 1, 2 and 3 always occurred before the arrival of food which suggests that it was likely to have been triggered by the sound of the tractor and a general increase of activity around the food store 100m away. It has been stated that hunger is not sufficient to account for the occurrence of stereotypies since they do not occur in the wild[3]. This has given rise to the suggestion that the expectation of food and/or reduction in time spent performing functional behaviour related to feeding trigger stereotypic behaviour[3,41,53].

Lily was the only elephant to exhibit the behaviour in the afternoon up to 2 hours prior to food arrival, suggesting that other triggers, such as the lack of positive social interactions and inability to perform grooming routines were involved in its development. The fact that Legs did not show any

stereotypic behaviour illustrates the individualistic nature of coping with the same environmental stressors[41].

Young elephants have often been seen playing with their own trunks[20]. The bout of trunk twiddling by Zwana in the afternoon occurred during a period where no other specific activity took place and may have been a result of boredom. However, the action was not observed during the remainder of the study, and its' relevance as a form of stereotypic behaviour is questionable.

Trunk sucking by Lily is likely to be a form of comfort behaviour since it was performed most frequently in association with stereotypic pacing when tractors and people were nearby. Before the study had formally begun, a subordinate elephant that was subject to high levels of dominant actions by others in it's enclosure was seen to suck its trunk regularly, for up to 30 minutes at a time. Trunk sucking has been observed in a hand-raised week-old elephant[7]. It is suggested that this behaviour corresponds to thumb-sucking in primates and has a similar function as a displacement activity to satisfy a sucking drive and thereby releasing nervous tension[7]. A casual observation that the majority of elephants liked to suck my hand or have their tongues stroked may support the theory that sucking provides some form of enjoyment for them.

The head swaying behaviour exhibited by Bibi and to a lesser extent Group 6 whilst shackled in the morning before being released is similar to stereotypic weaving described for circus elephants shackled for 12-23hrs a day[54]. The time Groups 4 (up to 15 h) and 6 (up to 17 h) spent shackled falls

within the range reported for 5 European circuses of between 12 and 23 hrs per day[54].

Chaining elephants at night is a traditional method of keeping and is still practised in the majority of zoos today. It has been defended as the best method to provide the elephants with security and an undisturbed sleeping place (Schutz, 1986[54]), and a way in which keepers can demonstrate their dominance (Dittrich, 1988[54]). However, some zoos now see this method of keeping as outdated and unnecessary (H Schwammer, Vice Director, Vienna Zoo, pers. comm., 2000).

It is interesting to note that in other studies both unchained[37] and chained[1] elephants did not show any stereotypic behaviour. Stereotypies either did not develop, or, they may have been present but occurred out side the observation periods and were not recorded.

Back-forwards pacing, trunk sucking and head swaying behaviours appear to be important indicators of stress. It appears that Lily was the most stressed of the study elephants since she performed stereotypic behaviour most frequently.

3.3.8 Vocalisations

Results

The infrequent recording of rumbles in Group 4 resulted from their distance from the observer and cannot be included in discussion. Other vocalisations (cry, snort, soft trumpet and squeak) occurred too rarely to be included in the statistical analysis. Mean numbers of vocalisations per hour are shown in Table 20.

Table 20. Mean numbers of vocalisations per hour per group

Group	Low rumble	Loud rumble	Rumbles^{+}	Trumpets^{+}
1	0.89	0.19	1.08	0.06
2	0.83	0.5	1.33	0.72
3	0.28	0.06	0.33	0.08
4	Not applicable	Not applicable	Not applicable	0
6	1.47	0.04	1.51	0.04

Although it appears that more low rumbles were heard from Group 6 than other groups, Fishers and Kruskal-Wallis tests could not detect differences. Group 2 exhibited more Trumpet vocalisations than the other groups ($P \rightarrow 0$).

Trumpeting occurred most frequently in Groups 1, 2 and 3 when the tractor arrived to deliver food.

Discussion

Four major classes of vocalisation have been recorded in elephants. Each one can vary in pitch, duration and volume, thereby resulting in a number of sounds expressing a wide range of emotional states[23]. Many of the vocal communications made by elephants are below our frequency of hearing and can cover large distances[50], therefore interpretation of their meaning can be difficult unless an obvious trigger is present.

The common occurrence of low rumbles within Group 6 may have been a consequence of their inability to maintain visual contact whilst feeding amongst foliage, thus vocal communication was used instead. Quiet rumbles are often heard as a group of wild elephant feeds and when they greet each other[20].

The high incidence of trumpeting by Group 2 when food arrived has been reported in other captive groups[37]. Individual differences in response to the same stimulus are highlighted by this behavioural response.

Trumpeting often accompanies aggressive behaviour in both wild and captive situations however, contrary to expectations, Legs did not perform many such vocalisations. The reason for this is unknown. I do not feel that vocalisations can be used as indicators of stress in this study.

3.3.9 Temporal Gland Secretion (TGS)

Results

Data describing the incidence of temporal gland secretions throughout the observation period is shown in Table 21.

Table 21. Incidence of temporal gland secretions at various times of day

Name	6-12am	12-4pm	4-5pm	Total
Legs	6 old to eye 2 new to eye	3 old past eye 3 new past eye	4 new to mouth	18
Lily	5 old above eye 4 old past eye	2 old past eye 1 old to mouth 1 new to eye 1 new past eye	1 new to eye 1 new past eye	16
Tula	1 new above eye 1 new past eye	2 old above eye	1 new to eye	5
Zwana	3 new above eye	2 new above eye	3 new past eye	8
Umseela	-	-	1 old past eye 2 new above eye	3
Makala	1 new above eye 2 new past eye	1 old above eye	2 new above eye	6
Bibi	1 old to eye	1 old above eye	1 old above eye	3
Shirra	2 old to eye	2 old above eye	1 new above eye	5
Sawu	1 old above eye	1 old above eye	1 new above eye	3
Tarzan	1 old to eye	1 old above eye	2 new above eye	4
Bull	-	-	-	0
Three	2 old above eye	-	-	2
Mapane	2 old above eye	-	-	2

Different amounts of secretion were seen from left and right glands. The majority of new secretions occurred in the early morning or late afternoon.

Discussion

The temporal glands are situated bilaterally on the head between the opening of the ear and the eye[13]. A sticky, long lasting temporal gland secretion is commonly associated with musth in sexually mature males[50]. A second, more watery secretion is reported in elephants of all ages in a variety of different contexts including excitement[1], anticipation[27] and stress[13]. The secretion in this form has been observed more in females than in males[27]. This could not be confirmed in this study due to the low numbers of animals. The rate of secretion from left and right glands differs. Secretions were observed from one or both glands in different amounts as observed by Garai[27]. The individualistic nature of TGS occurrence and degree reflects differences in each elephant's response to the same stimuli, as reported by Garai[27].

Group 1 had the highest incidence of TGS. It has been suggested that elephants showing continuous old TGS, such as Group 1, are secreting from the gland more frequently[27]. Alternatively, the streak may remain for longer periods of time if it has not been removed by rapid evaporation, mud or sand bathing.

The formation of the majority of new TGS in Groups 1, 2 and 3 occurred when the tractor delivering food was nearby, as reported by Adams

and Berg[1]. The anticipation of food and/or the presence of people and a loud vehicle could have triggered temporal gland secretion. It is not possible to determine whether or not it resulted from anticipation or from stress. In Group 4, the formation of TGS in the afternoon did not occur in the presence of a loud vehicle, suggesting an anticipatory reaction to the arrival of trainers, as observed by Adams and Berg[1]. The early morning TGS in groups 4 and 6 may have been a response to overnight shackling, since in Group 6 it formed during the night. However, Adams and Berg found that TGS was significantly correlated with lying down, illustrating the caution with which TGS must be interpreted.

Other behaviours with which Adams and Berg have reported TGS to be significantly correlated include investigative and manipulative behaviour, vocalisations, dusting and when elephants were released from the barn in the morning. Since Group 1 did not perform these behaviours during the day, it is concluded that TGS in this group was caused by conditions that lacked environmental and social enrichment. It is therefore concluded that the occurrence of TGS can be used in conjunction with a description of the context within which it takes place as an indicator of stress.

3.4 CONCLUSIONS

There were many confounding differences between the study groups in terms of their enclosure size and complexity, time spent in captivity, group size, contact with people and husbandry. However, I feel that investigating the behaviours in each group and making comparisons between them was a useful way to identify behaviours indicative of stress in different captive situations (Table 22).

Table 22. The occurrence of behavioural indicators of stress in each group.

Indicator of stress	Occurrence within Group ✓= occurrence x = absence of behavioural indicator per elephant (n)				
	1	2	3	4	6
	n=2	n=2	n=2	n=4	n=3
Standing >40% of observed time	✓✓	xx	xx	xxxx	xxx
Low frequency of grooming	✓✓	x✓	x✓	xxx✓	xxx
Absence of play	✓✓	xx	xx	xxxx	x✓✓
<10 affiliative actions per hr	✓✓	x✓	xx	xxxx	xxx
>0.3 Dominance+ actions per hr	✓✓	✓✓	xx	xxxx	xxx
>2 ears wide actions per hr	✓✓	✓✓	xx	xxxx	xx✓
>1 head high actions per hr	✓✓	✓✓	xx	xxxx	xx✓
>5 Nervous+ actions per hr	✓✓	✓✓	✓✓	xxxx	xx✓
lack of object manipulation	✓✓	x✓	✓✓	xxxx	✓✓✓
Back-forwards pacing	x✓	✓✓	✓✓	xxxx	xxx
Head sway	xx	xx	xx	✓✓✓✓	✓✓✓
Trunk suck	x✓	xx	xx	xxxx	xxx
TGS in over 75% observations	✓✓	xx	xx	xxxx	xxx

Twelve of the 13 behavioural indicators were observed in Group 1. They did not appear to have settled well into their captive environment and subsequently lacked normal affiliative and exploratory behaviour. Legs and Lily adopted different strategies to cope with their situation. Legs exhibited increased levels of aggressive and threatening behaviour, whilst Lily performed stereotypic pacing. Both showed high levels of nervousness/alertness around people and vehicles; and secreted from their temporal glands more than other study groups. Behavioural observations suggest that stress levels were highest in this group.

In Group 2, Zwana showed 8 of the 13 behavioural indicators, whilst Tula showed 5, illustrating how individuals in the same group can react differently to the same environmental stressors. In Group 3, Umseela and Makala showed 4 and 3 of the indicators respectively. Both groups showed signs of alertness and stereotypic pacing prior to feeding times. Eliminating these behaviours is difficult due to their link to daily husbandry routines present in captive situations. It is suggested that over time, the frequency of alert behaviours would decrease as habituation occurred. However, stereotypies have been described as self-reinforcing; their frequency may increase or be displaced; and they may begin to occur in response to other stimuli[41]. Levels of stress in groups 2 and 3 were observed to be lower than in Group 1, and greater than in Groups 4 and 6.

Group 4 exhibited 1 of the 12 indicators of stress, head swaying, in the early morning whilst waiting to be unchained and fed. This was also observed

to a lesser extent in Group 6. The behaviour appears to be a direct result of chaining and it is suggested that overnight housing be designed to exclude the use of shackles. Behavioural observations suggest that of the AGS elephants, Group 4 were less stressed that the others. In Group 6, the absence of play actions between elephants and with objects is reflective of the time spent performing other activities, primarily feeding, rather than stress per se. It is suggested that if food was accessible 24 h a day the time spent playing may increase. Mopane was the only elephant in the group to exhibit alert and threatening behaviour when vehicles and people were present. The reason for this is unknown, but illustrates individual variation within the group. Group 6 appeared to be the least stressed of all the groups, probably due to the fact that they have had the longest time to adjust to their captive environment, and are allowed to range freely during the day.

It is concluded that Groups 1, 2 and 3 were inadequately housed in the enclosures originally designed for rhino, and that a larger, more behaviourally enriched enclosure, would have been more suitable for them. Ideally, elephant keeping facilities should adopt a method of husbandry that does not require overnight shackling and encourages foraging, sociable behaviour, investigative behaviour and the performance of grooming repertoires.

CHAPTER 4

MONITORING GLUCOCORTICOID CONCENTRATION

4.1 INTRODUCTION

Although there is no universal scientific agreement on the definition of stress, stress responses cause an increase in the release of ACTH which stimulates the synthesis of glucocorticoids, primarily cortisol and corticosterone, in the adrenal cortex[35].

Conventionally, the assessment of adrenal responses to stress relies upon the collection of blood samples and the measurement of glucocorticoids[43]. There have been relatively few studies investigating blood glucocorticoid concentrations in African elephants (Table 23). Some studies have measured cortisol concentrations in samples from elephants that had been shot or darted with succinyldicholine (SDC) [10,33,34] whilst others have collected blood samples from immobilised elephants[33,36,43]. The effect of a brain shot on blood cortisol levels cannot to be determined. It is likely that cortisol concentrations in immobilised elephants were the result of capture procedures and are not representative of baseline levels. The process of blood collection from live, free-ranging animals is impossible without the use of capture or restraint and will, in itself, elicit elevated cortisol levels[33,51,69].

A serum sample collected from one captive African elephant[9] is likely to be the most representative of baseline cortisol concentrations in a resting state.

Table 23. Cortisol concentrations in blood taken from African elephants

Ref	Sample taken from	Circumstances of collection	Mean cortisol concentration nmol/l	S.D
Brown et al.(1995)	1 captive adult female	Untranquilised, in lateral recumbancy	49	-
		After ACTH injection (3.75mg)	440	-
Brown & White (1979)	10 males, 23 females, aged 1-60	Shot in early morning, minimum disturbance, serum	315 range: 66-825	182
Hattingh et al. (1984)	5 adult males	Undisturbed, shot, plasma	111	25
	1 young, 5 adult males, 1 female	Herded 3- 20 min, shot, plasma	132	27
	5 adult males, 1 young female	Undisturbed, SDC, plasma	125	105
	7 adults, 8 young, 2 calves	Herded 3-20 min, SDC, plasma	858	283
	2 adult females, 5 young females	Herded 6 – 20 min, shot, SDC, plasma	688	270
Hattingh et al. (1984)	1 young, 8 adult males	Immobilised with M99 induction time 13.7 ± 4.9 minS	106	43
		Undisturbed, peak whilst anaesthetised	332	96
		Herded, peak whilst anaesthetised	493	57
	4 adult males	Darted with SDC Induction time 10.3 ± 9.2 min	146	49.3
Morton et al. (1995)	27	Immobilised with M99/xylazine, plasma	347	26
Kock et al. (1993)	15 adult females	Immobilised with M99/xylazine, 10 min induction, serum	345	113

There have been few studies to investigate the possibility of using non-invasive methods to assess adrenocortical activity in elephants. Cortisol has been measured in the saliva from 2 Asian elephants[18] and in urine from one African and one Asian elephant[9]. The collection of faecal samples is more practical than that of urine and saliva, and provides measurements that are independent of short-term fluctuations[48].

The collection of faeces to measure reproductive steroids has been used extensively in domestic and wild animals[55,66]. However, methods to identify and measure faecal glucocorticoids and their metabolites are relatively new and constantly being improved upon. Palme and Möstl[47] have established an 11-oxoaetiocholanolone enzyme immunoassay (EIA) that measures 11,17-dioxoandrostanes (11,17-DOAs) a group of faecal cortisol metabolites. The biological relevance of this method has been proven in ruminants following administered ACTH stimulation of cortisol release by the adrenal cortex[48] and used to monitor transport stress in cattle[49]. This non-invasive technique has been applied to a number of domestic, zoo and wild animals[4,29,44,60,64]. There are large inter-species differences with respect to the metabolites formed and their route of excretion[4,44,46,47] and therefore interspecfic comparisons cannot be made.

The aim of this study was to validate a method for measuring glucocorticoid metabolites in elephant faeces, and to conduct a preliminary investigation into the method's biological relevance including it's potential use as a tool in assessing welfare.

4.2 MATERIALS AND METHODS

Sampling plan

Due to the sensitive nature of the project, blood samples could only be taken when the opportunity arose - when all parties concerned with the care of the elephants at AGS permitted their collection. Blood samples were collected from Group 5 and one elephant in Group 6.

Only elephants in Group 4 (n = 4) were made available by AGS for the ACTH study. As a result, an experimental design in which control animals would have been given an injection of saline solution instead of ACTH could not be used.

Faecal samples were collected from Groups 1, 4 and 6. They were also collected from Groups 2 and 3, henceforth named '2+3', which had unexpectedly been joined together by AGS half way through the study.

Blood samples

Blood samples from Groups 5 and 6 were collected from an ear vein using a 10 ml syringe. Five ml samples of whole blood were placed in plain vacutainer tubes (Becton Dickenson, USA), 4.5 ml samples of whole blood were placed in lithium heparin tubes (Becton Dickenson, USA) and K_3 -EDTA tubes (Becton Dickenson, USA). Blood in serum tubes was allowed to clot for one hour. All tubes were centrifuged at 3000 rpm, then plasma and serum

samples were placed into cryotubes (Amersham, Johannesburg) and stored at –20 °C until analysis.

ACTH administration and sample collection

Each elephant was injected i.m. with 300 mg azaperone. After 15 minutes, an 18-gauge catheter was inserted into an ear vein and a blood sample collected using a 10 ml syringe. Two further blood samples were collected prior to intramuscular administration of 2.15 mg ACTH (Synacthén, Novartis, Switzerland). Thereafter, a 5 ml venous blood sample was collected every 30 minutes for 4 hours.

Faecal samples from almost all defaecations were collected for 3 days before and 4 days after the ACTH injection. Handful-sized samples were collected within 30 minutes of defaecation and frozen at -20° in plastic freezer bags before the preparation for extraction and EIA analysis.

Analysis of serum/plasma cortisol

Serum/plasma concentrations of cortisol were determined using a Clinical Assays™ GammaCoat™ Cortisol ^{125}I Radioimmunoassay kit (DiaSorin; SA Scientific, Johannesburg). The analysis is based upon the competitive binding principles of radioimmunoassay. Briefly, standards and unknown samples were incubated with cortisol tracer in antibody coated

tubes where the antibody was immobilised onto the lower inner wall of the GammaCoat tube. After incubation the contents of the tube were aspirated or decanted and the tube counted. A standard curve was prepared with 5 serum standards ranging from 1-60µg/dL. Unknown values were interpolated from the standard curve. The entire assay was performed in the coated tube. No separate sample dilution, protein denaturation or extraction steps were required for serum or plasma samples. The calculated sensitivity of the test was 5.79nmol/l; Intra-run coefficient of variation 7%; Inter-run coefficient of variation 9.2%.

Preparation of faecal extract

Frozen faecal samples were placed in an oven and dried at 100°C. Each sample was powdered and mixed thoroughly. A 0.5 g subsample was mixed with 10 ml 80% ethanol, shaken for 30 minutes and centrifuged at 3000rpm for 15 minutes. One ml of the supernatant was drawn off and stored at –20 °C until EIA analysis.

High-Performance Liquid Chromatography (HPLC)

HPLC of the faecal metabolites was performed at the Institute of Biochemistry, Vienna as described by Teskey-Gerstl et al.[60]. Faecal extracts containing peak 11,17-DOAs concentrations were subjected to a clean up procedure (Sep-Pak C18). Separation was performed on a reverse-phase

Nova-Pak C18 column (3.9 x 150 mm, Millipore Corporation, Milford, Massachusetts, U.S.A.) using a linear gradient starting at 50% methanol. Three fractions per minute were collected, dried under a stream of nitrogen, and reconstituted in assay buffer. Immunoreactive glucocorticoid metabolites were quantified with the cortisol, corticosterone and 11-oxoaetiocholanolone EIAs as described below[47].

Analysis of glucocorticoids and their metabolites in faeces

Aliquots of the extract were analysed using 3 EIA systems (cortisol, corticosterone and 11-oxoaetiocholanolone) as described by Palme and Möstl[47]. Briefly, 50 µl of each sample was pipetted into microtiter plate wells (coated with sheep-anti-rabbit IgG) and 100 µl antibody and enzyme solution was added. After incubation overnight at 4°C, plates were washed four times with ddH_2O/Tween 20 (1:500000); 250 µl enzyme solution (streptavidin-perioxidase conjugated, 500 U, 1:30000; Boehringer, Mannheim, Germany) was pipetted into each well and incubated for 45 min at 4°C. Plates were then washed again and 250 µl tetramethylbenzidine/H_2O_2 substrate solution was added. After incubation for 45 min at 4°C, the reaction was stopped with 50 µl H_2SO_4 (3 M) and absorbance measured at 450 nm/630 nm, using a DIAS reader (Dynatech, Guernsey, Great Britain). Standard curves and sample concentrations were calculated with Immunofit 3.0 (Beckman Inc., Fullerton, CA).

Effect of drying on faecal cortisol metabolites

Two faecal samples were left outside for 30 hours. Subsamples were taken from each sample at 0, 2, 4, 8, 12, 24 and 30 hours, extracted and concentrations of cortisol metabolites analysed as described above.

Preliminary investigation of the effect of stress-related behaviour

Faecal samples were collected from groups 1, 2+3, 4 and 6.

Data analysis

Data obtained from samples collected from groups 5 and 6 (see Appendix 3) was statistically analysed using a General Linear Models Procedure (The Fisher Test). The class variables used were Time (morning vs evening); and Samples Type (Serum vs Plasma vs EDTA) and the interaction between them. Means were adjusted to become directly comparable, therefore least-square (LS) means will be presented in the results and used for discussion.

Baseline concentrations of cortisol metabolites in faeces were determined from Group 4 using median values before and >60 h after ACTH injection. Basal values for assessing the effect of ACTH injection on serum cortisol could not be calculated because concentrations began to rise after

the first sample collection. Changes in serum cortisol concentrations were related to the lowest value of serum cortisol that corresponded to the first sample taken. Descriptive statistics (ranges and medians) were used because the data was not always normally distributed.

4.3 RESULTS

4.3.1 Blood cortisol

Effect of sample time, sample type and individual variation

Cortisol levels in samples taken in the morning were significantly higher than those taken in the evening (P→0) as shown in Table 24.

Table 24. Mean cortisol concentration nmol/l in morning vs evening

Time	n	LS Mean cortisol concentration nmol/l
Morning	16	145.1
Evening	37	81.6

Cortisol levels were significantly higher in serum samples than in EDTA samples (P=0.016) and lithium heparin samples (P=0.01). There was no significant difference between cortisol levels in EDTA and lithium heparin samples, as shown in Table 25 overleaf.

Table 25. Mean cortisol concentration nmol/l in EDTA, lithium heparin and serum samples.

Sample Type	n	LS Mean cortisol concentration nmol/l
EDTA	10	101.9
Lithium heparin	16	104.0
Serum	27	134.1

There was no interaction between sample type and the time at which samples were collected.

Table 26. Cortisol concentration nmol/l in samples from Groups 5 and 6

Group	Number of samples	Collection time	Sample type	Cortisol nmol/l	LS mean
5	19	Morning	serum	46-274	167
5	6	Evening	serum	55-215	101
5	11	Morning	plasma*	41-223	136
5	16	Evening	plasma*	26-148	72
6	1	Morning	serum	35	35

* from lithium heparin samples

Variation between individuals was observed as illustrated in Figure 5. overleaf.

	(38)														
	Bibi	(35)													
Bull		Bull	(81)												
Fiona		••	Fiona	(100)											
Kelly	••	••		Kelly	(145)										
Laura		•••	••	••	Laura	(87)									
Maya		••			•	Maya	(151)								
Nellie	•••	•••	••			•	Nellie	(71)							
Rosy		••			••		•	Rosy	(151)						
Sarah	•••	•••	••	••		••		••	Sarah	(185)					
Shirra	•••	•••	•••	••		••		•••		Shirra	(145)				
Sironga	•••	•••	••								Sironga	(201)			
Sawu	•••	•••	••	••		••		••				Sawu	(42)		
Sally				•	•••		•••		•••	•••	•••	•••	Sally	(189)	
Tina	•••	•••	•••	•••		•••		•••					•••	Tina	(164)
Tuli	•••	•••	••	•		•		••					•••		Tuli (84)
Yoga		•			•				•	•	••	••		••	• Yoga

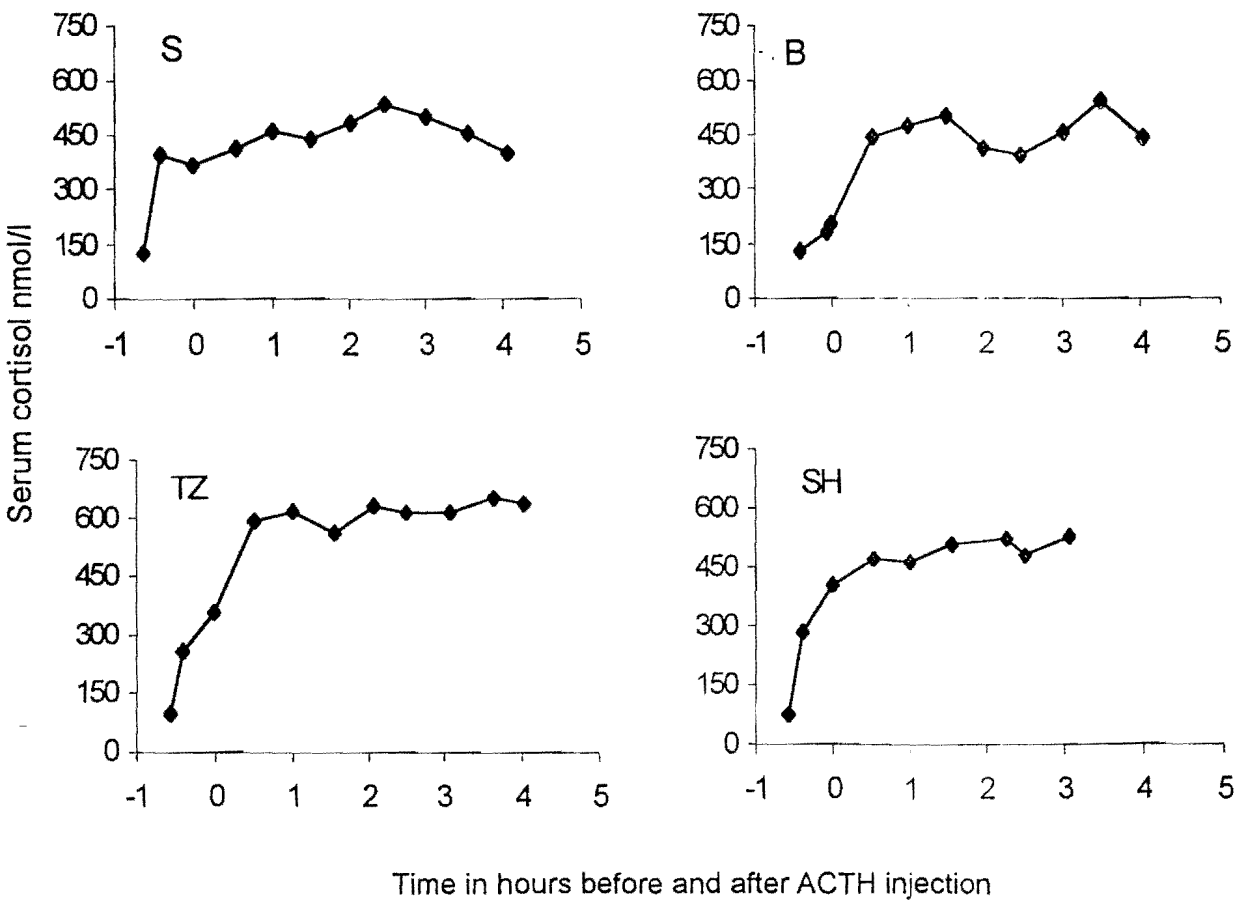
Figure 5. Individual variation in blood cortisol concentrations. LS means for each individual are shown above in brackets

Statistical differences are shown by • where ••• = P≤0.001, •• = P≤0.01and • = P≤0.05

ACTH Challenge

Injection of ACTH resulted in an increase of serum cortisol concentrations as shown in Figure 10. Serum cortisol levels began to rise after injection with azaperone and insertion of catheters. Following ACTH administration serum cortisol increased between 4 and 7 fold, reaching highest recorded values (526 to 652 nmol/l) after 2 hours. No distinct peaks were observed.

Figure 10. Time course of serum cortisol concentrations (nmol/l) in four elephants before and after intramuscular injection ACTH (2.15 mg) at time 0.



4.3.2 Faecal Samples

HPLC Analysis

HPLC separations revealed a number of immunoreactive substances present in elephant faeces. They showed a chromatographic mobility between cortisol and 17,20-dihydroxyprogesterone (Figure 11. overleaf). The main metabolite determined with the 11-oxoaetiocholanolone-EIA eluted around cortisol. Lower amounts of immunoreactive substances were detected by testing the HPLC fractions with the corticosterone-EIA and negligible amounts with the cortisol-EIA (detection limit =2nmol/kg faeces).

ACTH Challenge

Injection of ACTH resulted in an increase of faecal cortisol metabolite concentrations (Figure 12). Individual differences in basal and peak values of faecal cortisol metabolites were observed. Basal values of faecal 11,17-DOAs and corticosterone equivalents ranged from 21 to 168 nmol/kg (median: 48 nmol/kg) and 33 to 133 nmol/kg (median: 50 nmol/kg) respectively. ACTH induced peaks were between 572-1104% (11,17-DOAs) and 160-353% (corticosterone) higher than basal values. These peak concentrations occurred 20 - 25.5 h after the injection. Additional peaks of varied height were observed for both groups of metabolites prior to and after the ACTH induced peaks.

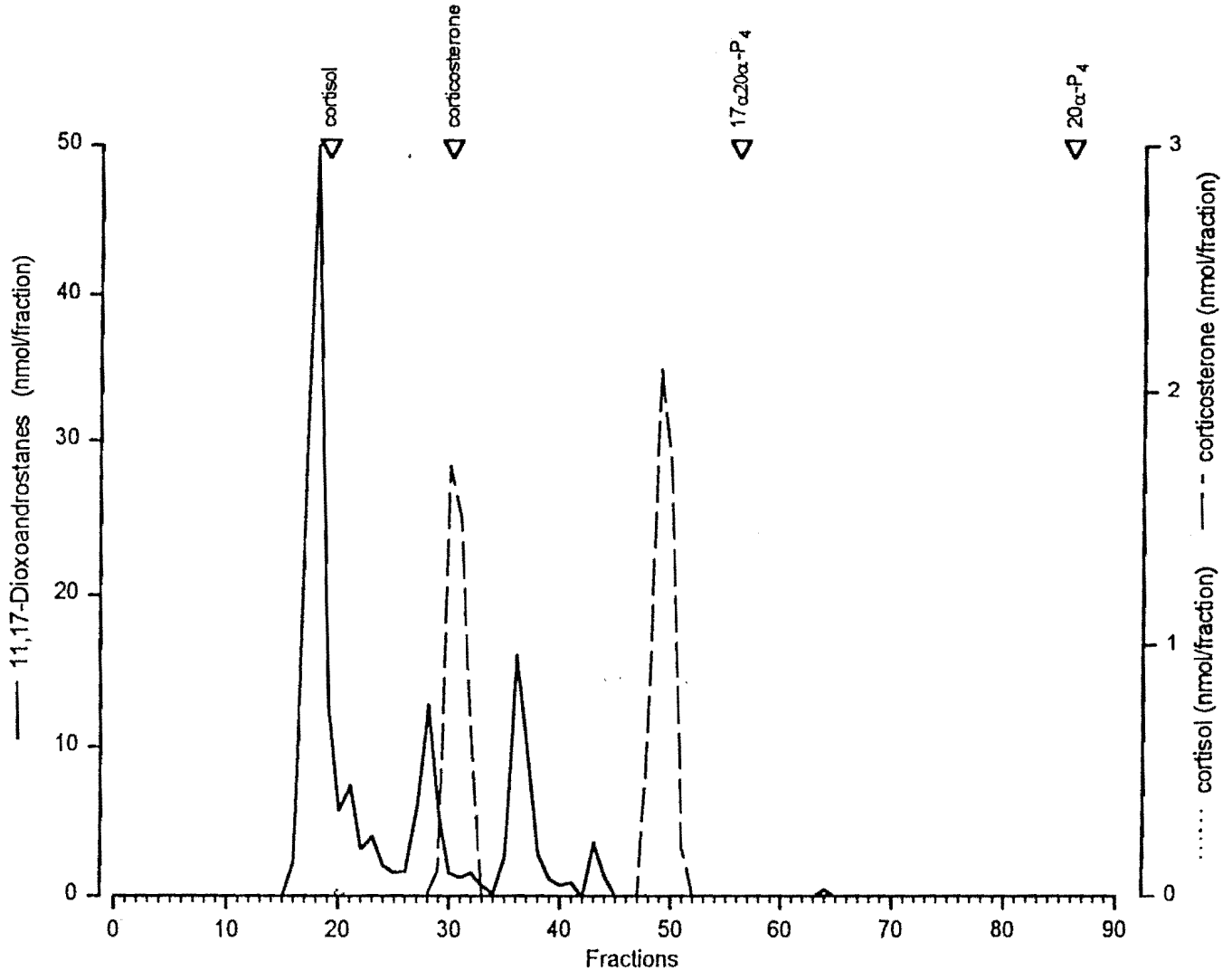


Figure 11. HPLC separation of immunoreactive glucocorticoid metabolites in one faecal sample from an elephant as tested in a cortisol-, corticosterone- and 11-oxoaetiocholanolone-EIA. (P_4=dihydroxy-progesterone)

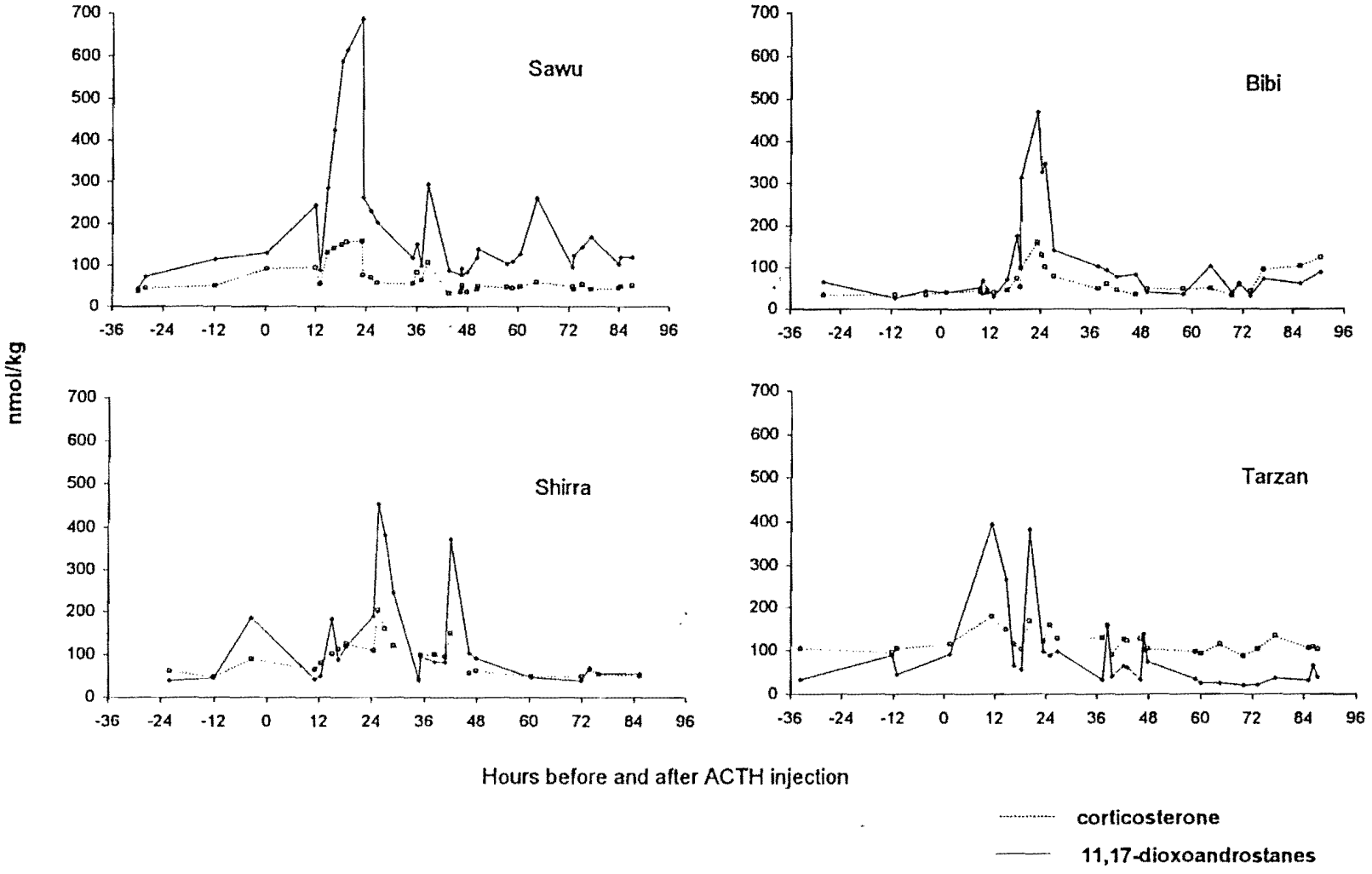


Figure 12. Time course of concentrations of faecal 11,17- dioxoandrostanes and corticosterone equivalents (nmol/kg) in four elephants before and after intramuscular injection ACTH (2.15 mg) at Time 0.

Effect of drying

The effect of leaving samples outside on concentrations of 11,17-DOAs is presented below in Table 27.

Table 27. Percentage increase of concentrations of 11,17-DOAs in faecal samples left outside after defaecation.

	Hours after defaecation	**0**	**1**	**2**	**4**	**8**	**12**	**24**	**30**
% Increase	**Sample 1**	0	3	38	37	23	23	10	55
	Sample 2	0	16	25	32	31	169	278	70

Effect of stress-related behaviour

The effect of stress-related behaviour on concentrations of 11,17-DOAs is presented below in Table 28.

Table 28. Effect of stress-related behaviour on 11,17-DOA concentration

Group	**Number of Elephants**	**Occurrence of Stress-related behaviour**	**Range of 11,17-DOA nmol/kg**	**Median**	**n =**
1	2	High	62-1000	176	6
2+3	4	Present	22-152	52	6
4	4	Present	21-168	48	42
6	3	Very low	15-47	39	6

4.4 DISCUSSION

4.4.1 Blood cortisol

The effect of sample time, sample type and individual variation

Blood samples taken in the morning were 150% higher than those taken in the evening. These findings fit with the general trend of cortisol concentrations being highest in the morning and reaching a nadir in the early evening[16,38,62]. The influence of the of time collection on these concentrations highlights the fact that only samples taken at approximately the same time of day can be used in comparative studies.

Serum cortisol concentrations were higher than in plasma collected at the same time. It is suggested that serum samples be taken for the analysis of cortisol concentrations because they are easier to handle in the field than samples collected in heparin or EDTA.

Individual differences in blood cortisol concentrations were observed reflecting different animals' adrenal activity with respect to a number of possible factors including responses to the blood collection procedure, past experiences, innate blood chemistry and metabolism. The presence of individual variation does not support findings by Brown and White[10] who reported that no significant variation due to age, sex, location or season were observed.

The lowest serum cortisol value of 35 nmol/l was recorded in the morning sample taken from a tame elephant in Group 6. The elephant was naive to blood collection, was not restrained in any way and remained calm throughout the procedure. This sample probably represents the true baseline cortisol levels at that time of day, and is similar to that found by Brown et al.[10] in an unanaesthetised captive adult elephant. No further samples could be collected. Loss of naivety to the procedure would have rendered subsequent collections for use in this study undesirable and been disturbing for the elephant.

All samples collected from Group 5 required restraint for approximately 5 minutes. The morning serum cortisol concentrations for the Group were approximately 5 and 8 times higher (for mean and highest values respectively) than 35 nmol/l. An assessment of whether these high levels are due to a chronic elevated state or a response to blood collection procedures is not possible.

A mean plasma cortisol concentration of 104 nmol/l in Group 5 is similar to that found by Hattingh et al. in undisturbed elephants that had been shot[34] and also those that were immobilised with M99[33]. However, a true comparison cannot be made because the effect of a brain shot on blood cortisol has not been discerned and chemical immobilisation is known to influence cortisol concentrations[33,51].

The sampling problems discussed above highlight the difficulty of using blood cortisol to assess adrenocortical activity.

ACTH challenge

Serum cortisol levels began to rise before ACTH was injected suggesting that handling or the injection of azaperone affected levels of serum cortisol within 30 minutes. These findings are in accordance with other studies that report that physical restraint and blood sampling can produce an increase of blood cortisol levels within 15 minutes of handling in sheep[24], deer[59] and elephants[33].

The lowest values recorded in Group 4 prior to ACTH injection (73 to 131 nmol/l) were higher than a baseline sample taken by Brown et al. of 48 nmol/l[10]. This may reflect the effect of azaperone and/or insertion of the catheter on blood cortisol concentrations. After ACTH injection, serum cortisol levels continued to rise and in 3 out of 4 cases reached a plateau. The level of increase was less than double after 30 minutes. This was not as much as that recorded by Brown et al. who observed an eight-fold increase 30 minutes after ACTH injection (1.25 mg)[10].

Brown et al. administered three ACTH injections (1.25 mg) at 2 h intervals to enable the detection of urinary cortisol. At 3 h, after the second ACTH injection, serum cortisol concentration had increased twelve-fold. In my study, cortisol concentrations had increased 4 to 7-fold by 4 h. In the study by Brown et al. concentrations began to decline 2-3 h after the last ACTH injection, but were still elevated approximately fourfold 8 hours afterwards. They had returned to baseline levels by the morning.

In the present study, it is likely that had further blood samples been taken, a similar pattern would have been recorded. Blood samples could not be taken after 4 hours, because the tranquillising effect of azaperone began to wear off and it became distressing for the elephants. In addition, it became increasingly difficult to prevent the elephants from pulling out the indwelling catheters from the ear veins. Sampling from other sites of the body, such as the tail, had been attempted previously and proved unsuccessful.

The highest recorded cortisol concentrations were within the range of those found in 5 elephants that had been herded for 6 - 20 minutes and darted with succinyldicholine before sample collection[34]. This gives some indication of the type of stressor that may produce such elevated levels.

4.4.2 Faecal samples

HPLC analysis

A number of glucocorticoid metabolites were detected using HPLC analysis in elephant faeces. Although the exact identity of the metabolites was not determined due to the cross-reactions of the EIA, a group of them may be collectively described as 11,17-dioxoandrostanes (11,17-DOAs)[47]. As reported in domestic livestock[44,47] negligible amounts of cortisol and low amounts of corticosterone were found in elephant faeces. These findings suggest that the recently developed 11-oxoaetiocholanolone EIA, which

measures 11,17-DOAs, is the most suitable EIA to use for the non-invasive monitoring of adrenocortical activity in elephants.

ACTH Challenge

As found in other species, the time course of faecal cortisol metabolite concentrations reflected the ACTH induced stimulation of glucocorticoid production[44,48]. Concentrations peaked 20 - 25.5 h after the ACTH injection. Similar times were found by Möstl et al. in ponies[44]. It has been suggested that the delay in faecal glucocorticoid excretion is correlated with the transit time of digesta from the duodenum to the rectum[46]. Our findings fit well with the total passage time from mouth to rectum of 33 h reported for Indian elephants[65]. Differences in diet and individual adaptations in hepatic or gastrointestinal function may explain differences in excretion rates[66].

The large increase of 11,17 DOA (572-1104 % above basal levels) after ACTH injection was higher than that observed in ponies (200-660 %) by Möstl et al.[44], but within the range of reported increases in cattle during transport (400-1100 %)[49]. Lower percentage increases in corticosterone were measured, supporting the earlier suggestion that 11,17-DOAs are a more suitable group of metabolites to measure. Additional peaks after the one induced by ACTH could be due to an enterohepatic recirculation of the metabolites[46]. Alternatively, additional peaks may have been caused by stressful events approximately 24 hours before the peaks were recorded. More prolonged periods of behavioural observations conducted before,

during and after the trial may have made it possible to identify events that had caused these responses.

As reported in other species[44,48,60] individual variation in basal and peak values was observed. This fact may be due to differences in previous experiences, body mass, metabolism, age, diet or gender. Further investigations with a greater number of animals are necessary to identify the influence of these confounding factors.

Effect of drying outdoors

Concentrations of 11,17-DOAs in faecal samples that were left outside appear to be relatively stable for up to 8 hours after defaecation. An increase of 23-31% occurred during this time. Thereafter increases of up to 280% were measured. This increase is lower than that found by Möstl et al. who investigated stability of 11,17-DOAs at room temperature and found increases of up to 375-962% in cattle, ponies and pigs[44]. It is suggested that drying in the sun minimised the modification of metabolites by anaerobic faecal bacteria described by Winter et al. (1982)[44]. Aerating the samples by opening or squashing them would minimise modification of metabolites further by encouraging the evaporation of water required for bacterial metabolism. This may be particularly useful in the field application of the technique depending upon weather conditions. More research is required into the effect of collection and storage on the stability of faecal glucocorticoid metabolites.

Effect of stress related behaviour

Preliminary investigations into the application of the technique to assess welfare showed good correlations between the behavioural observations made in Chapter 3 and faecal glucocorticoid metabolite concentrations. As predicted, concentrations of 11,17-DOAs from Group 6 were the lowest, falling within the lower end of the range of basal values measured during the ACTH challenge. Group 6 were exposed to fewer stressors than elephants housed at AGS and had more opportunity to perform species specific behaviours such as foraging in a larger area.

The highest 11,17-DOAs concentrations were measured in Group 1 - 450% higher than Group 6. Some values in Group 1 even exceeded peaks induced in Group 4 during the ACTH stimulation test and the median value for the group lies outside of the range for basal values in Group 6. This suggests high levels of stress, which confirms the conclusions drawn in Chapter 3.

4.5 CONCLUSIONS

It is clear that blood collection procedures can influence the results obtained when determining baseline cortisol concentrations in captive elephants, unless the animal is at ease around people and naïve to the procedure. The study successfully validated a recently established non-invasive method for measuring glucocorticoid metabolites in elephant faeces.

The primary advantages of faecal sample collection are that the collector does not require special skills and there is no need to handle the animals. Secondly, concentrations of faecal glucocorticoid metabolites probably reflect the amounts of cortisol produced and excreted more closely than cortisol measured in blood, which only reflects a point in time during a dynamic process of absorption, metabolism and excretion.

It is concluded that the measurement of faecal 11,17-DOAs is a valuable tool for the non-invasive monitoring of adrenocortical activity in African elephants. This could help to optimise the capture, transport and husbandry of elephants and be useful in investigating stress in free-ranging situations.

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APPENDIX 1 : ETHOGRAM

Feed/forage	Investigation of provided food item and subsequent consumption
Grass Bar	Eating naturally growing grass not specifically provided
Eat Mud	Eating soil, mud or stones
Drinking	Consumption of water
Stand/Walking	Standing (>1minute) or locomotion with no other activity
Resting	Standing with eyes lowered, relaxed posture, sleeping
Dusting	Throwing/blowing sand onto any part of body
Mudding	Throwing mud onto any part of body or lying in mud
Rubbing	Moving body against object
Trunk Touch	Any part of body excluding mouth or genitals with trunk tip
Trunk Mouth	trunk to mouth
Genitals	smelling/touching genitals
Trunk over back	placing trunk over length of spine
Play	Trunks intertwining, heads pushing together
Other contact	Other forms of body contact excluding those described above
Trunk flick	trunk flicked towards elephant, no contact made
Trunk hit	contact made after flick with trunk to another elephant
Threat	lifting head so tusks horizontal or lowering head towards elephant or person (stipulate)
Gentle push	Contact with head causing partner to move (no steps taken)
Moderate push	Medium force used (>2 steps taken), partner displaced
Hard push	Strong force used (>2 steps, vocalisation elicited), partner displaced
Chase	Chasing partner – 2 individuals running
Kick	Kick partner
Mock charge	Ears spread wide, trunk up, short run ends in stopping before 'target'.
Reach	Stretch out trunk to a person or elephant
Object	Manipulating an object with foot, mouth or trunk, object is not eaten.
Trunk up	Lifting trunk up above head towards a stimulus
Ears open	Ears spread outwards upto 45° from body
Ears wide	Ears spread outwards 90° from body

Tail high	Tail held horizontally
Eyes wide	Whites of eyes visible
Head shake	Vigorous shaking of the head – ears flap against body
Heat butt bar	Hard contact made between head and bars of enclosure
Head high	Head held high so that tusks are horizontal to ground
Run	
Cluster	Elephants run in to a tight group
Low rumble	Low frequency quiet sound
Loud rumble	Low frequency loud sound
Trumpet	High pitched loud sound through trunk
Soft trumpet	Medium pitched sound through trunk
Cry	Loud high pitched sound through mouth
Snort	Brief push of air through trunk
Squeak	Short high pitched sound through trunk
TGS	Temporal gland secretion – seen as a moist patch on side of face
Backforwards	Pacing forwards and pacing backwards repetitively
Head sway	Head rocking from side to side repetitively
Bar bite	Mouth open and placed on bar of enclosure
Trunk curl	Curling of the trunk tightly inwards
Trunk twiddle	Twirling trunk around in circles, no object held
Trunk suck	Trunk placed in mouth with no food >5 secs
Stomachwater	Regurgitation of water from stomach and sprayed over body
Diarrhoea	Liquid-like faeces

APPENDIX 2 : DATA COLLECTION SHEET

Date 15-3 | Time 10-30 | Location 4 | Weather sunny | Name Bibi

Time	eat	stwk	grsbar	groom	affiliative	who?	dom	sub	aggress	obj	reach	trkup	eopen	ewide	tail	hh	hdshk	threat	vocal	stimulus	m	sec	other
0	|																			truck	50	20	no food
1	|																					
2	|							,														Ø TGS
3	|																					
4	|																					
5	|																					
6	|																					
7	|																					
8		I																					
9			|											!				lowO!					
10			|											!									
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13		I	|																				
14	|												!										
15	|												!										
16	|																						
17	|			mud																				
18	|			|																			
19	|			|							:												
20	|			|																			
21		I		I																			
22				dust																			
23				I																			
24		|			C	SW				'	stone									pegde	20	|	
25		|			C	SW							!									|	
26		|			trktk	t							!									|	
27		|																				|	
28		|			trkmth	t																|	
29	I																						
30	|																						

APPENDIX 3 : BLOOD CORTISOL CONCENTRATIONS IN GROUP 5

Name	Date	Time	Cortisol nmol/l	Sample type
BB	04-11-98	06:00	79.38	Lithium heparin
BB	25-11-98	06:10	46.81	Serum
BB	27-11-98	05:45	117.18	Serum
FI	19-11-98	07:15	93.68	EDTA
FI	19-11-98	07:15	100.3	Lithium heparin
FI	19-11-98	07:15	128.54	Serum
FI	27-11-98	06:15	152.46	Serum
KY	06-11-98	17:05	68.48	Serum
KY	06-11-98	17:05	55.4	Serum
KY	06-11-98	17:05	37.86	Lithium heparin
KY	06-11-98	17:05	41.65	EDTA
KY	19-11-98	07:35	123.07	EDTA
KY	19-11-98	07:35	130.7	Lithium heparin
KY	19-11-98	07:35	174	Serum
KY	19-11-98	07:40	149.75	EDTA
KY	19-11-98	07:40	174.9	Serum
LA	06-11-98	16:45	127.53	Serum
LA	06-11-98	16:45	97.43	Lithium heparin
LA	06-11-98	16:45	98.32	EDTA
LA	19-11-98	06:10	181.62	EDTA
LA	19-11-98	06:10	169.96	Lithium heparin
LA	25-11-98	05:35	193.09	Serum
MY	06-11-98	17:00	68.08	Serum
MY	06-11-98	17:00	25.93	Lithium heparin
MY	25-11-98	05:55	167.38	Serum

Table continued.....

Table continued.....

Name	Date	Time	Cortisol nmol/l	Sample type
NL	06-11-98	06:00	212.74	Serum
NL	06-11-98	06:00	165.16	Lithium heparin
RY	03-11-98	06:25	118.47	Serum
RY	03-11-98	06:25	103.78	Lithium heparin
RY	25-11-98	06:00	120.55	Serum
SA	06-11-98	16:50	215.15	Serum
SA	06-11-98	16:50	148.47	Lithium heparin
SA	06-11-98	16:50	154.44	EDTA
SA	19-11-98	06:40	121.83	EDTA
SA	19-11-98	06:40	170.06	Serum
SA	19-11-98	06:40	192.54	Serum
SA	25-11-98	05:50	140.24	Serum
SH	05-11-98	06:00	222.91	Lithium heparin
SH	27-11-98	06:00	224	Serum
SI	03-11-98	06:35	204.5	Serum
SI	03-11-98	06:35	163.04	Lithium heparin
SW	03-11-98	05:55	223.27	Lithium heparin
SY	03-11-98	06:45	120.51	Serum
SY	19-11-98	07:30	59.05	EDTA
SY	19-11-98	07:30	41.25	Lithium heparin
TI	06-11-98	16:48	168.08	Serum
TI	06-11-98	16:48	124.42	EDTA
TI	25-11-98	05:54	274.4	Serum
TU	06-11-98	06:30	233.6	Serum
TU	06-11-98	06:30	170.39	Lithium heparin
YG	06-11-98	17:10	64.81	Serum
YG	06-11-98	17:10	50.1	Lithium heparin