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Measurement of Faecal Cortisol Metabolites in Cats and Dogs: A Non-invasive Method for Evaluating Adrenocortical Function

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ABSTRACT

The aim of this comparative study was to gain more information about the metabolism and excretion of glucocorticoids in cats and dogs in order to establish non-invasive methods for evaluating stressful conditions. Therefore, in a first experiment, [^{14}C]cortisol was administered intravenously to 8 animals (two of each sex and species). Over a period of 6 days, faeces and urine were collected immediately after spontaneous defecation and urination. Marked species differences were found, as cats mainly excreted cortisol in the faeces (82% $\pm$ 4% of the total recovered radioactivity), whereas in dogs only a small portion was found there (23% $\pm$ 4%). The highest urinary radioactivity was observed after 9 $\pm$ 3 h in cats and 3 $\pm$ 1 h in dogs. Peak concentrations in the faeces occurred after 22 $\pm$ 6 h in cats and after 24 $\pm$ 4 h in dogs. Most of the radioactivity was not extractable with diethyl ether, indicating that the metabolites excreted in urine and faeces were mainly of the conjugated or polar unconjugated types. This was confirmed by RP-HPLC, which also revealed marked differences between cats and dogs concerning the metabolites formed. In addition, the immunoreactivity of the metabolites was tested in cortisol, corticosterone and 11-oxoaetiocholanolone EIAs. The latter, measuring 11,17-dioxoandrostanes (11,17-DOA) detected the highest quantities of immunoreactive metabolites in cats, but not in dogs. In a second experiment, the adrenal cortex of both species was stimulated by ACTH and, three weeks later, suppressed by dexamethasone. In this study, only faeces were collected over a period of 7 days. In both species, inter-animal variability in the basal and maximal/minimal faecal cortisol metabolite concentrations and the time course was observed. The 11-oxoaetiocholanolone EIA in cats and the cortisol EIA in dogs proved best suited for monitoring changes in adrenocortical activity. ACTH injections resulted in an increase above baseline values of 355% (median) in 11,17-DOA concentrations in cats and of 702% in the concentrations of cortisol equivalents in dogs by about 25 h and 22 h (median) after injection, respectively. Minimal concentrations after dexamethasone administration were about 17% in cats and 31% in dogs (in relation to baseline values) and were reached in 66 h and 72 h, respectively. It was concluded that measuring cortisol metabolites in faeces should be a useful non-invasive tool for monitoring stress in carnivores.

Keywords: adrenocortex, carnivores, cat, corticosteroid, diagnosis, dog, EIA, faeces, glucocorticoids, non-invasive, stress

Abbreviations: ACTH, adrenocorticotrophic hormone; HPA, hypothalamo-pituitary-adrenocortical; EIA, enzyme immunoassay; 11-17-DOA, 11,17-dioxoandrostanes; RP-HPLC, reversed-phase high-performance liquid chromatography; IM, intramuscular(ly)

INTRODUCTION

In recent years there has been a growing interest in and concern about animal welfare. However, the assessment of animal well-being is a complex matter. Misinterpretations are to some extent prevented when a wide range of physiological, endocrinological and behavioural parameters are measured simultaneously (Clark *et al.*, 1997a,b). Stressful stimuli can induce ACTH release which, in turn, increases the synthesis and secretion of cortisol by the adrenal cortex. As a result, plasma cortisol concentrations have been widely used as an indicator of stress (McDonald, 1980). In carnivores, glucocorticoid concentrations increase as a result of poor housing conditions (Carlstead *et al.*, 1993; Clark *et al.*, 1997c; Hennessy *et al.*, 1997), surgery (Church *et al.*, 1994), anaesthesia (Fox *et al.*, 1994; Smith *et al.*, 1999) and restraint (Moe and Bakken, 1997; Ogburn *et al.*, 1998; Beerda *et al.*, 1999). A major problem relating to blood sampling is that it is impractical or, especially in zoo or wild animals, nearly impossible. Also, the sampling of blood can itself induce a stress response. Therefore, a non-invasive approach for monitoring physiological responses to stress has been sought.

Measuring urinary cortisol concentrations has mainly been used to characterize disorders of the HPA axis in dogs (Rijnberk and Mol, 1989; Kaplan *et al.*, 1995) and cats (Goossens *et al.*, 1995). Only a few studies have reported on the value of estimating urinary cortisol concentrations to assess stress responses in these carnivores (Carlstead *et al.*, 1992, 1993; Beerda *et al.*, 1996, 1999; van Vonderen *et al.*, 1998). Unfortunately, many felids void urine by spraying and, even in other carnivores, it is not always easy to collect the urine, thus limiting the practicality of this approach. Another possibility for measuring cortisol levels is the collection of saliva (Vincent and Michell, 1992; Beerda *et al.*, 1996), but this also entails some handling of the animals.

Faecal samples offer the advantage that they can be collected easily without any need to handle the animal. Graham and Brown (1996) found that the main excretion route for [^{3}H]cortisol metabolites in female domestic cats was via the faeces (about 86%). Following injection of ACTH, they were able to detect an increase in faecal cortisol metabolites using a corticosterone radioimmunoassay. Similar ACTH results were reported for five African wild dogs (Monfort *et al.*, 1998), spotted hyenas (Goymann *et al.*, 1999) and cheetahs (Terio *et al.*, 1999). A longitudinal study in captive female cheetahs demonstrated a correlation between high levels of faecal cortisol metabolites and reproductive failure (Jurke *et al.*, 1997). However, except for the study in female cats by Graham and Brown (1996), glucocorticoid metabolism has not been reported in carnivores at all, or the amount and nature of faecal radioactivity was not determined (e.g. Gold, 1960 in dogs). Based on radiometabolism studies, an 11-oxoaetiocholanolone EIA for measuring faecal 11,17-DOA (a group of cortisol metabolites) was recently developed and successfully applied to measuring adrenocortical activity in domestic livestock (Palme and Möstl, 1997; Möstl *et al.*, 1999; Palme *et al.*, 1999, 2000).

One aim of this study was to gain information about the metabolism and excretion of glucocorticoids in carnivores and to compare these findings with earlier studies in herbivores and omnivores (Taylor, 1971; Palme *et al.*, 1996; Palme and Möstl, 1997; Möstl *et al.*, 1999; Teskey-Gerstl *et al.*, 2000). Observed interspecies differences made it

necessary to differentiate between cats and dogs. The objectives of our radio-infusion experiment were to determine the time course and relative proportion of [^{14}C]cortisol metabolites in the urine and faeces and to tentatively characterize these excreted metabolites, mainly in the latter. In a second experiment, the faecal cortisol metabolites in cats and dogs were measured following stimulation or suppression of the adrenal cortex using ACTH or dexamethasone, respectively, to test whether changes in the HPA activity were reflected in the faecal cortisol metabolite concentrations. The ultimate goal of our study was to establish or improve methods for measuring cortisol metabolites in the faeces as a non-invasive tool for monitoring adrenocortical activity in carnivores.

MATERIALS AND METHODS

Animals and housing

For the whole study, 10 cats and 10 dogs (five of each sex) from the Institute of Nutrition (University of Veterinary Medicine, Vienna) were used. All the animals were born and reared under laboratory conditions and were accustomed to various manipulations, such as blood sampling. During the experiments they were housed singly but had olfactory, vocal and visual contact with each other. The health status of the animals was checked daily by clinical assessment of the pulse, body temperature and respiratory rate. All the animals were healthy throughout the study.

The European Shorthair cats were between 9 and 12 years of age. For this study they were kept in 80 cm long × 40 cm wide × 50 cm high metabolic cages floored with cat litter (Catsan®, Master Foods, Bruck/Leitha, Austria). All the cats were exposed to 12 h of light per day. Water was provided *ad libitum* and they received moist cat food (Whiskas®, Master Foods, Bruck/Leitha, Austria) in the morning. The 10 beagles ranged in age from 1 to 5 years. Each was housed in an individual pen (2.4 × 1.5 m), except on days 0 and +1 of the radiometabolism study, during which they were kept in metabolic cages (120 cm × 75 cm × 125 cm). Their food (Pedigree Pal®, Master Foods, Bruck/Leitha, Austria) was provided once a day, and water was available *ad libitum*. Before the experiments started, the dogs had been familarized with going outside, whereupon they started to urinate and defecating after a few steps.

Experiment 1: Administration of [^{14}C]cortisol

On the first day (–1) of this experiment, a few samples of urine and faeces were collected to determine background levels. On the second day (day 0), about 370 kBq (= 10 μCi) of radiolabelled cortisol, dissolved in 10 ml sterile 0.9% NaCl solution containing 10% (v/v) ethanol, was administered to each of 4 cats and 4 dogs (two of each sex) by a single injection into the *vena cephalica antebrachii*. The [^{14}C]cortisol (NEC-163; 2.2 GBq/mmol) was obtained from New England Nuclear (Dreieich, Germany). Before administration, the purity of the cortisol (over 98%) was checked

by thin-layer chromatography as described by Palme and colleagues (1996). For the dogs, we used a tray on a long stick to collect spontaneously voided urine. This construction was not practicable with cats, which is why the urine samples from cats were collected in the litter (Catsan®, Master Foods, Austria). On days 0 and +1, the animals were monitored continuously for urinating and defecating. Urine and faeces were taken immediately after the cats passed them. To get urine and faecal samples from the dogs, the animals were taken outside at intervals of 1 h on days 0 and +1 and then at intervals of 2–3 h for the next three days (days +2 to +4). The urine samples were collected in glass bottles, the faecal samples in plastic freezing bags. Both urine and faeces were stored at –24°C.

Extraction and determination of metabolites

Duplicates of the canine urinary samples (0.5 ml) were separately mixed with 10 ml scintillation fluid (Quicksafe A, no. 100800, Zinsser Analytic, Maidenhead, UK) and measured in a liquid scintillation counter (Packard Tri-Carb 2100TR, Meriden, CT, USA). Quench correction was automatically performed by the counter. Portions (5 g) of mixed cat litter containing urine were extracted with distilled water–methanol (6 ml+12 ml). Following centrifugation (2500*g*; 15 min), the radioactivity of aliquots of the supernatant (0.5 ml in duplicate) was determined as described above.

Samples of 0.5 g per 10 g of homogenized wet faeces from each defecation were used to determine the radioactivity, as described by Palme and colleagues (1996). Briefly, the samples were extracted with distilled water–methanol (2 ml+3 ml). After shaking (30 min) and centrifugation (2500*g*; 15 min), the supernatant was removed and duplicate 0.5 ml aliquots were measured in the scintillation counter. To improve the recovery of apolar cortisol metabolites, a further extraction step on the remaining faeces, with 5 ml methanol (100%), was included. To determine the amounts of ether-extractable metabolites, 0.5 ml aliquots of the urinary samples or the combined and concentrated supernatants from the two extraction steps of the faeces were extracted with two portions of 5 ml diethyl ether (Palme *et al.*, 1996). To determine the optimal percentage of methanol for extraction, 0.5 g aliquots ($n = 5$) of a thoroughly homogenized faecal sample from a dog were suspended in increasing percentages (0% to 100%) of methanol, as described by Palme and Möstl (1997).

HPLC

HPLC separations were performed to characterize the faecal ^{14}C-metabolites. The sample of each administration containing the peak radioactivity was subjected to a clean-up procedure with Sep-Pak® C_{18} columns as described by Teskey-Gerstl and colleagues (2000). The dichloromethane fraction (containing unconjugated metabolites) and the ethyl acetate–methanol fraction (mainly conjugated or polar unconjugated metabolites) were separated on a Novapak C_{18} column (3.9 × 150 mm; Waters, Milford, MA, USA) and the eluent was fractionized exactly as described earlier

(Teskey-Gerstl *et al.*, 2000). The elution profiles of the [^{14}C]cortisol metabolites in the fractions were determined by liquid scintillation counting and their immunoreactivity was tested in three established assay systems (11-oxoaetiocholanolane, cortisol and corticosterone EIA; Palme and Möstl, 1997). To evaluate cross-reactions of possible reduced androgen metabolites in the 11-oxoaetiocholanolone EIA, steroids (Steraloids, Wilton, NH, USA) without oxygen present at position C-11 were tested. It was found that 5β-androstanes (5β-androstane-3,17-dione 7.8% and 5β-androstane-3α-ol-17-one, 4.8%) showed some cross-reactivity, but 5α-androstanes did not (5α-androstane-3,17-dione, 5α-androstane-3α-ol-17-one and 5α-androstane-3β-ol-17-one, all less than 0.1%). Some apolar fractions (fractions 65 to 90) were also tested with an epiandrosterone EIA previously established for measuring faecal androgen metabolites possessing a 17-oxo group (Palme and Möstl, 1994). In addition, the amounts of hydrolysable, conjugated ^{14}C metabolites were determined as described by Teskey-Gerstl and colleagues (2000).

Experiment 2: Pharmacological stimulation (ACTH) and suppression (dexamethasone) of adrenocortical activity

To stimulate adrenocotical activity, we injected synthetic ACTH (Synacthen®; Ciba-Geigy, Basel, Switzerland) into 10 cats and 10 dogs (five of each sex). On the third day of the experiment (day 0), we injected (IM) 0.125 mg of ACTH into the cats and 0.25 mg into the dogs. Three weeks later, the adrenocortical activity was suppressed with dexamethasone (Dexa-DAT®, Ogris Pharma, Wels, Austria) in the same animals. The injected dose (IM) was 0.1 mg dexamethasone per kg body weight in both species. All faecal deposits were collected throughout the whole period (days –2 to day +5 of both parts of the experiment), as described for experiment 1.

The amounts of faecal cortisol metabolites were determined by the respective EIAs as described previously (Palme and Möstl, 1997). Briefly, 0.5 g samples of homogenized wet faeces from every defecation were extracted with 5 ml methanol (80%) and aliquots of the supernatant (extracted as described above) were measured in the three EIAs (Palme and Möstl, 1997).

Statistical analysis

Individual baseline cortisol metabolite concentrations were calculated from all samples before treatment. As the values within each animal and species were not normally distributed in all cases (Kolmogorov–Smirnov test), minimum, maximum and median values were calculated. To find the best-suited EIA for reflecting adrenocortical activity, the increase or decrease of faecal cortisol metabolite levels (peak or nadir concentrations after ACTH or dexamethasone injection, respectively) relative to the median basal levels (%) of the EIAs were compared in each species using a *t*-test or a Mann–Whitney rank test, where appropriate. All statistical analysis was performed with Sigma-Stat© (SPSS Inc., Erkrath, Germany).

RESULTS

Experiment 1 ([^{14}C]cortisol)

The percentage excretion of cortisol metabolites via urine and faeces differed in cats and dogs (Table I). Whereas cats preferentially excreted these metabolites via the faeces (81.9% ± 3.8%, mean ± SD), they were eliminated mainly in the urine by dogs (76.6% ± 4.1%). The highest urinary radioactivity was observed after 9 ± 3 h in cats and after 3 ± 1 h in dogs, usually in one of the first two samples following injection, whereas in the faeces the peak concentrations were found after 22 ± 6 h and 24 ± 4 h, respectively (for individual values see Table I). In contrast to the increase, the decline in radioactivity was protracted. During this phase, additional small peaks were present in some animals. Background levels were reached within three days (Figure 1).

Only small amounts of the radioactivity in canine urine (4% to 9%, median 7%) and a larger proportion of that in the faeces of cats (22% to 69%, median 43%) and dogs (22% to 49%, median 44%) was extractable with diethyl ether. In the homogenized faecal sample from a dog, the percentage of ether-extractable radioactive metabolites in the supernatant steadily increased from 47% to 70% when using increasing (0% to 100%) methanol concentrations for extraction. However, suspending the faeces (0.5 g)

TABLE I
Amounts of radioactivity excreted via the faeces and urine and the delay (h) before the maximal concentrations of radioactive metabolites in the excreta of cats and dogs

		Percentage radioactivity[a]		Time to maximum (h)[b]	
Animal no.	Sex[c]	Faeces	Urine	Faeces	Urine
Cats					
1	M	78.8	21.2	25.2	9.1
2	M	78.3	21.7	23.8	12.7
3	F	85.3	14.7	12.0	9.1
4	F	85.0	15.0	25.2	5.0
Dogs					
1	F	21.5	78.5	22.1	4.1
2	F	25.3	74.7	21.0	3.8
3	M	28.1	71.9	30.0	2.3
4	M	18.7	81.3	22.1	3.6

[a]Portion (%) of recovered radioactivity excreted via faeces/urine

[b]Delay (h) before maximal concentration of radioactivity

[c]F, female; M, male

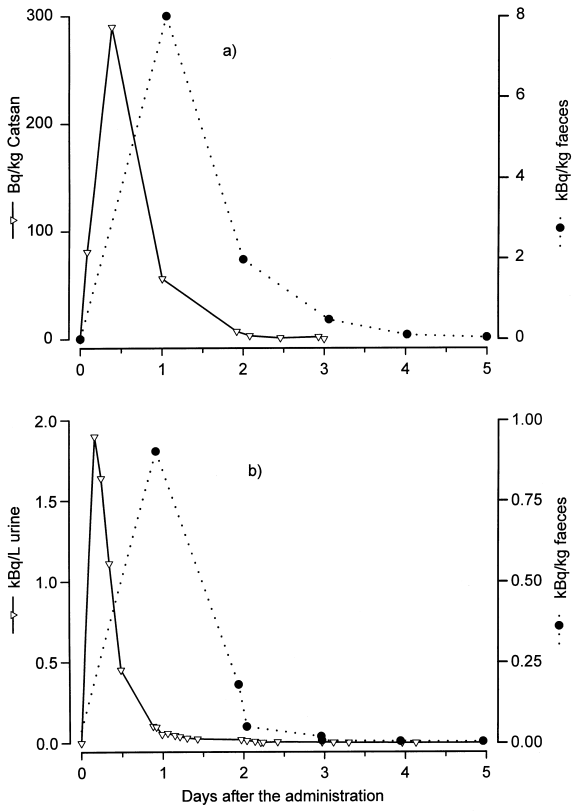

Figure 1. Radioactivity after administration of [^{14}C]cortisol in urine (Bq/kg or kBq/L) and faeces (kBq/kg) in one cat (a) and one dog (b)

with 1 ml water and 4 ml methanol (= 80% methanol) yielded the highest absolute amounts of total and ether-extractable radioactivity. Using water alone, 84% and 61%, respectively, of these amounts could be extracted.

RP-HPLC analysis of both fractions from the clean-up procedure on the faecal samples revealed the presence of a large number (>20) of radioactive metabolites (Figures 2 and 3). Some individual differences concerning the relative amounts of the different metabolites could be observed in both species. The injected [^{14}C]cortisol itself was present only in small amounts. Marked differences were found in the metabolites formed in the two species. Generally, the concentrations of radioactive metabolites were higher in cats. Unconjugated metabolites (Figure 2) showed a chromatographic mobility between cortisol and 20α-dihydroprogesterone in cats, whereas these metabolites were more polar in dogs, eluting around cortisol/corticosterone. In cats, HPLC analysis of the ethyl acetate–methanol fraction (Figure 3) revealed the presence of one

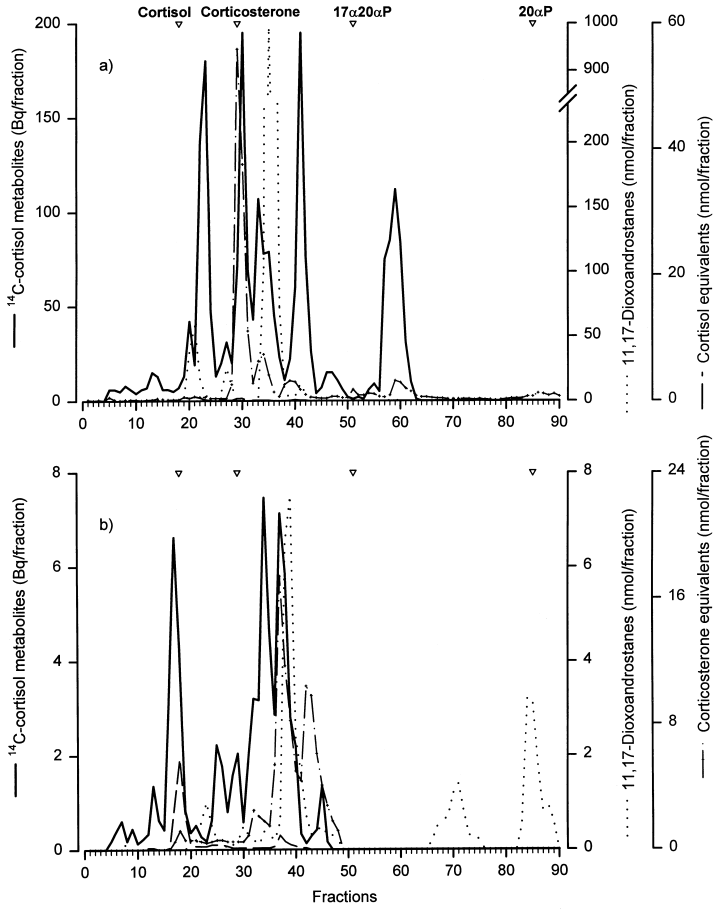

Figure 2. RP-HPLC separations of unconjugated faecal cortisol metabolites after injection of [^{14}C]cortisol in a cat (a) and dog (b). Radioactivity was measured by liquid scintillation counting. Immunoreactivity of each fraction was determined with a cortisol, corticosterone and 11-oxoaetiocholanolone EIA. Fractions marked with ▽ represent the approximate elution time of respective standards (17α20αP_4 = 17α20α-dihydroxyprogesterone, 20αP_4 = 20α-dihydroprogesterone)

main peak, eluting around fraction 10. These conjugated steroids could not be hydrolysed with β-glucuronidase/sulphatase. In contrast, the metabolites in that Sep-Pak fraction were less polar in dogs. The presence of immunoreactive ^{14}C-metabolites in the HPLC fractions was confirmed (Figures 2 and 3). Unconjugated metabolites were more reactive in the EIAs. In cats, significantly higher amounts were measured with the 11-oxoaetiocholanolone EIA, which was not the case in dogs. However, some

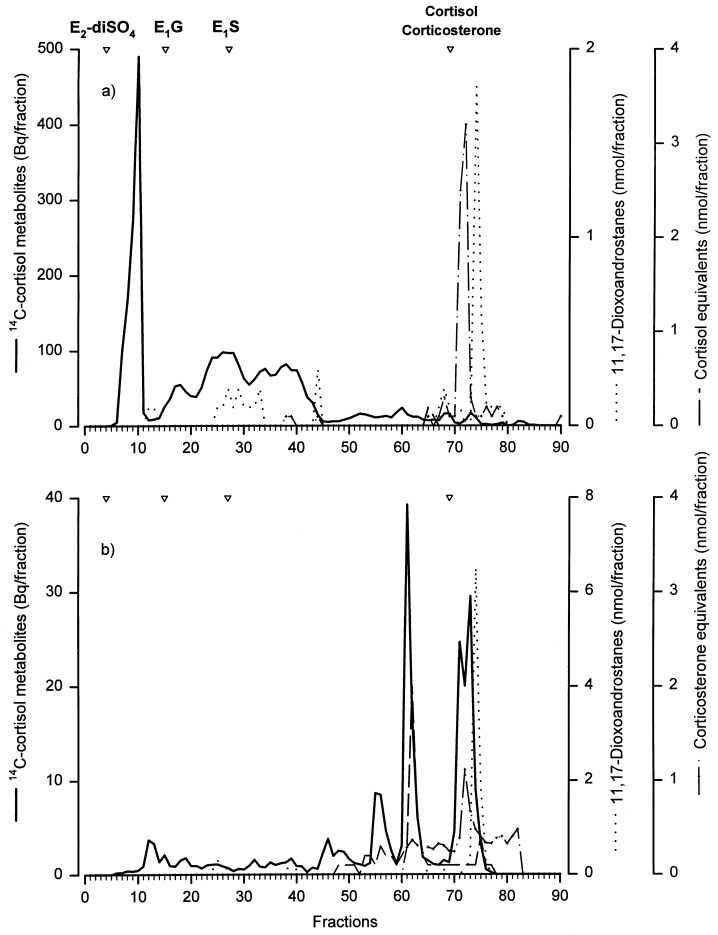

Figure 3. RP-HPLC separations of 'conjugated' faecal cortisol metabolites (ethyl acetate/methanol fraction) after injection of [^{14}C]cortisol in a cat (a) and dog (b). Radioactivity was measured by liquid scintillation counting. Immunoreactivity of each fraction was determined with a cortisol, corticosterone and 11-oxoaetiocholanolone EIA. Fractions marked with $\triangledown$ represent the approximate elution time of respective standards (E_2diSO_4 = oestradiol-disulfate, E_1G = oestrone-glucuronide, E_1S = oestrone-sulfate)

of the main peaks of radioactivity were not accompanied by a peak of immunoreactivity in any of the three EIAs. In addition, some apolar, unlabelled peaks were detected with the 11-oxoaetiocholanolone EIA (eluting between fractions 65 and 90) in the samples from the dogs (especially the males). They also showed high immunoreactivity in an epiandrosterone EIA.

Experiment 2 (ACTH/dexamethasone)

The baseline and peak/nadir faecal cortisol metabolite concentrations varied among individuals, both in cats and dogs. In addition, there was inter-animal variability in the time course. The median basal values of cortisol equivalents (concentration measured with the cortisol EIA) and 11,17-DOA, the absolute and relative peak and nadir concentrations and the delay time until they were reached after ACTH and dexamethasone injection are given for cats and dogs in Table II. In general, activation and suppression of the adrenocortex was well reflected by measuring the faecal cortisol metabolite concentrations in cats and dogs (Figures 4 and 5). In cats the 11-oxoaetiocholanolone EIA, but in dogs the cortisol EIA, revealed higher relative peak or lower nadir concentrations (% of basal values) after ACTH or dexamethasone, respectively. The differences between the results from the two EIAs were statistically significant ($p < 0.05$), with the exception of the ACTH test in dogs. Although the corticosterone EIA reacted with some metabolites (Figures 2 and 3), it did not reflect these changes very well, so not all the samples were analysed with this EIA and the data are not shown. Remarkably, there were higher basal concentrations of 11,17-DOA in male dogs than in females.

DISCUSSION

The aim of this study was to gain more information about the metabolism and excretion of glucocorticoids in cats and dogs. This should help to establish or improve methods for measuring cortisol metabolites in faeces as a non-invasive tool for monitoring stress in carnivores. In the first experiment, we focused on the time course of the excreted [^{14}C]cortisol metabolites in urine and faeces, on the amounts of metabolites in the excreta and on characterization of the faecal metabolites.

The results were in line with previous observations in metabolism studies with radiolabelled cortisol in various species (Graham and Brown, 1996; Palme *et al.*, 1996; Palme and Möstl, 1997; Möstl *et al.*, 1999; Teskey-Gerstl *et al.*, 2000), which had revealed marked species differences. Such differences were also observed between the two carnivore species. Most of the cortisol metabolites in cats were excreted via the faeces, but in dogs they were mainly present in the urine. The percentage of radiolabelled cortisol that was excreted in the faeces was 85% in the female cats. This compares to the 86% reported by Graham and Brown (1996). The percentage in male cats was somewhat lower, at 78.6%. Sex differences in the amounts of excreted steroid metabolites were also observed in ponies and pigs (Palme *et al.*, 1996), but the significance of this observation needs further evaluation owing to the small number of animals used.

As in our study, a rapid appearance of the radioactivity in the urine has been observed in all steroid metabolism studies (e.g. Graham and Brown, 1996; Palme *et al.*, 1996). The fact that urine was sampled after being voided spontaneously accounts for the great variation in the time of appearance of the maximal concentration of radioactive metabolites in the urine. This was also reported in sows and boars by

TABLE II

The median basal values, absolute (cortisol equivalents and 11,17-DOA in nmol/kg faeces) and relative levels (percentage of basal values) of peak and nadir values and the delay (h) prior to these values after injection of ACTH and dexamethasone (Dex.) in cats (n = 10) and dogs (n = 10), respectively

			Median basal values[a]			Peak/nadir (nmol/kg)			Peak/nadir (%)[b]			Delay (h)[c]		
Species	Treatment	EIA	Min	Max	Med	Min	Max	Med	Min	Max	Med	Min	Max	Med
Cats	ACTH	Cortisol	4	18	6	5	22	15	88	395	172	24	49	25
		11,17-DOA	317	1960	644	1488	6345	1947	118	956	355	24	37	25
Dogs	ACTH	Cortisol	4	12	8	10	383	36	129	3482	702	8	71	22
		11,17-DOA	11	392	36	11	709	117	105	1393	240	8	55	22
Cats	Dex.	Cortisol	3	25	10	0	7	4	8	84	45	32	120	60
		11,17-DOA	568	7400	1717	119	673	430	4	49	17	32	96	66
Dogs	Dex.	Cortisol	2	25	7	1	8	2	14	67	31	48	95	72
		11,17-DOA	6	122	23	2	107	11	15	109	54	47	95	71

[a]Minimum, maximum and median concentrations (nmol/kg) of median basal levels of all administrations are given

[b]Peak/nadir values are expressed as a percentage of the respective median basal values

[c]Time after which the peak or nadir concentrations were observed

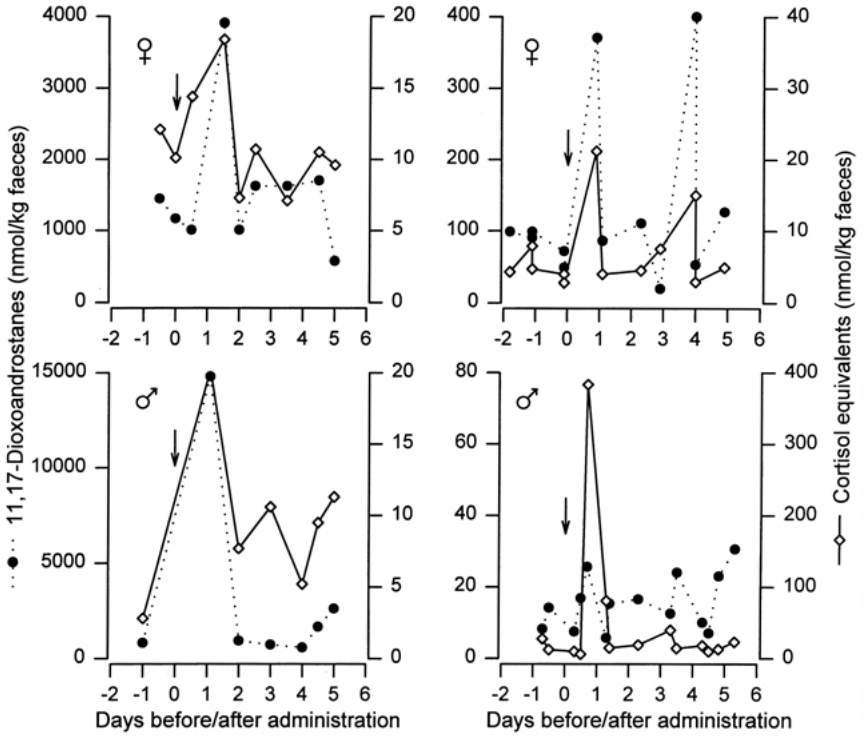

Figure 4. Concentrations of cortisol equivalents and 11,17-DOA in faecal samples (nmol/kg) before and after IM ACTH injection (0) in two cats (left panels) and two dogs (right panels). The time of the administration is marked with an arrow

Palme and colleagues (1996). In our study, special attention was given to the faecal samples, as these can be easily collected. The delay prior to the peak faecal concentration reflects the intestinal passage time (Palme *et al.*, 1996) and was much the same in cats as that reported by Graham and Brown (1996). In this respect, dogs showed a similar excretion pattern. The presence of small peaks of radioactivity and the protracted decrease after maximal concentrations could be due to mixing in the intestine and/or to an enterohepatic recirculation, as was found in other species (Palme *et al.*, 1996; Teskey-Gerstl *et al.*, 2000).

The highest amounts of total and ether-extractable radioactivity were recovered when dog faeces, containing the naturally occuring [^{14}C]cortisol metabolites, were extracted with 80% methanol. This has been observed in all other species (sheep, Palme and Möstl, 1997; ponies, Möstl *et al.*, 1999) in which such studies have been performed. Therefore, it seems advisable to extract mammalian faeces with this percentage of methanol for measuring glucocorticoid metabolites.

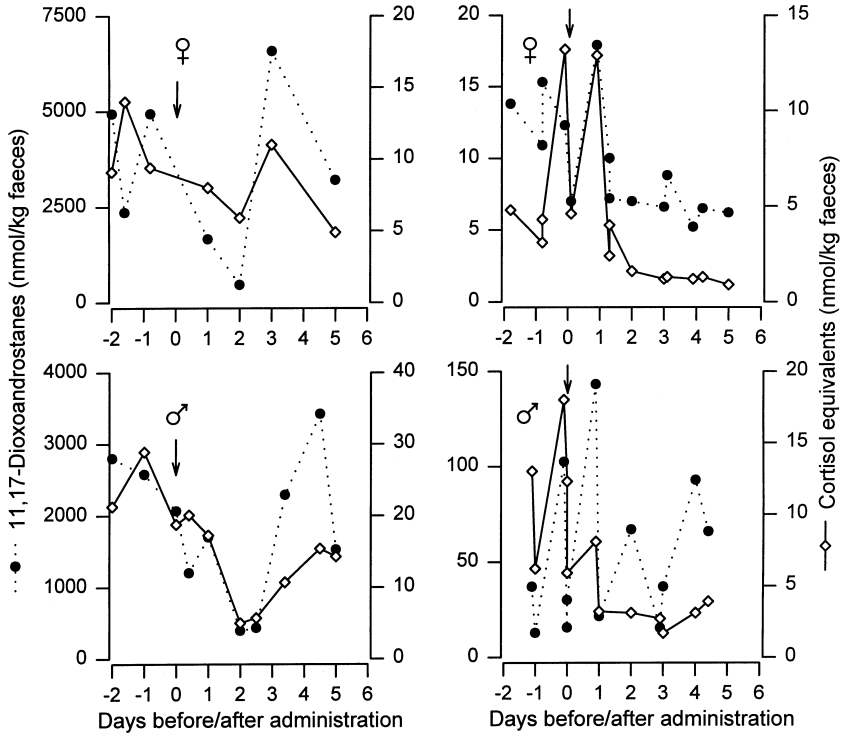

Figure 5. Concentrations of cortisol equivalents and 11,17-DOA in faecal samples (nmol/kg) before and after IM dexamethasone injection (0) in two cats (left panels) and two dogs (right panels). The time of the administration is marked with an arrow

Glucocorticoids are heavily metabolized (Brownie, 1992). In contrast to domestic livestock (sheep, ponies and pig, Palme *et al.*, 1996; Palme and Möstl, 1997; Möstl *et al.*, 1999), which excrete mainly unconjugated (ether-extractable) metabolites, most of the faecal metabolites in the present study were not extractable with diethyl ether, indicating a predominance of conjugated or polar unconjugated metabolites, which was confirmed by RP-HPLC separation of the faeces. However, there were marked differences between the two carnivore species in the metabolites formed. In cats (Figure 3a), there was one main peak of conjugated metabolites, which could not be hydrolysed. The same situation occured in hares (Teskey-Gerstl, 2000), in contrast to domestic livestock (Palme *et al.*, 1996). As in sheep (Palme and Möstl, 1997), significant amounts of a number of metabolites eluting between cortisol and 20α-dihydroprogesterone were present. In contrast, most of the faecal metabolites in dogs eluted around cortisol (partly not ether extractable). These differences in carnivores with regard to the formation of faecal metabolites may be due to a different

metabolism or to a different intestinal flora, as the metabolites undergo further metabolism by bacteria (Macdonald *et al.*, 1983). In cats, extremely high concentrations of 11,17-DOA, a group of cortisol metabolites formed by enzymic side-chain cleavage, were present. Bokkenheuser and colleagues (1986) found high activities of such enzymes (C_{17}–C_{20} desmolase) in the faecal flora of cats, indicating that these metabolites are, at least in part, formed by bacteria. As previously reported in cats (Graham and Brown, 1996) and other species (Palme and Möstl, 1997; Möstl *et al.*, 1999; Teskey-Gerstl *et al.*, 2000) cortisol itself was only found in small amounts, if at all, in the faeces of carnivores.

Interestingly, in dogs (and more pronouncedly in males) but not in cats, some smaller peaks were found with the 11-oxoaetiocholanolone EIA between fractions 60 to 90, without coeluting radioactivity. As large amounts of metabolites were measured in these fractions with an epiandrosterone EIA (detecting 17-oxoandrostanes), they are probably metabolites of androgens. This probably accounts for the higher 11,17-DOA basal levels in the male dogs.

In order to test whether changes in the HPA activity were reflected in the faecal cortisol metabolite concentrations, stimulation and later suppression of the adrenal cortex were performed, using first ACTH and then dexamethasone. Adrenocortical activity was best reflected by the 11,17-DOA concentrations in cats, whereas in dogs our sensitive cortisol EIA proved better suited, even though it measured only small amounts of the metabolites. The group-specific 11-oxoaetiocholanolone EIA (measuring 11,17-DOA) has proved suitable for monitoring adrenocortical activity in other species, including horses (Möstl *et al.*, 1999), ruminants (Palme and Möstl, 1997; Palme *et al.*, 1999, 2000) and hares (Teskey-Gerstl *et al.*, 2000). However, it was not suited for measuring faecal glucocorticoid metabolites in dogs. As in ponies and pigs (Möstl *et al.*, 1999), the 11,17-DOA are not dominant excretory products in the faeces of dogs, as the main peaks of radioactivity in the fractions of the HPLC did not show such immunoreactivity. Therefore, developing EIAs that react with the main faecal cortisol metabolites should significantly improve non-invasive methods in dogs.

Our corticosterone EIA, which also detected some [^{14}C]cortisol metabolites, did not reflect HPA changes very well and therefore could not be applied to measuring faecal cortisol metabolites in cats or dogs. In contrast, others (Graham and Brown, 1996; Goymann *et al.*, 1999; Terio *et al.*, 1999) have found the highest responses in their ACTH studies with immunoassays utilizing a corticosterone antibody. These differences might be due to different cross-reactions by the antibodies used. Especially with faeces, as a large number of steroid metabolites and only small amounts, if any, of the respective blood hormones are present (Palme *et al.*, 1997; Palme and Möstl, 1997; Möstl *et al.*, 1999), the selection of the antibody and label of an immunoassay for measuring the steroids is a serious concern.

After ACTH administration, the highest faecal cortisol and 11,17-DOA concentrations, at around 25 h (median) in cats and 22 h in dogs, were in agreement with the results of our radiometabolism study. Substantial variations were due to constipation in some animals, as was also described by Graham and Brown (1996). In cats, the percentage increase in measured faecal cortisol metabolites above individual baseline values was also in the range reported by Graham and Brown (1996). As they used a

larger total amount of ACTH, faecal 11,17-DOA measurements in cats might be even better suited for evaluating adrenocortical activity. One day after dexamethasone injection, prior to the decrease, a brief peak in faecal cortisol equivalents was observed in some dogs (Figure 5b). This is in agreement with findings in ruminants (Palme *et al.*, 1999) and probably reflects the effects of the injection itself or of the handling, which disturbed the animals. Such an effect in dogs was also described by Hennessy and colleagues (1998), who found an increase in blood cortisol levels 20 min after venepuncture in dogs. Additional peaks, appearing in some animals after the second day, may have been caused by stressful events approximately 24 h before they were recorded. As the animals were not observed continuously during that period, it was not possible to identify the events that may have caused these responses.

In accordance with similar studies reported by others (Graham and Brown, 1996; Palme *et al.*, 1999) and the first experiment in this study, inter-animal variation in faecal cortisol metabolite concentrations was high. To overcome this problem, baseline periods must be included in the experimental design so that each animal can serve as its own control (Palme *et al.*, 1999).

In conclusion, substantial differences were observed in the excretion of cortisol between the two carnivore species studied. However, measured faecal glucocorticoid metabolites (11,17-DOA in cats and cortisol equivalents in dogs) reflected the adrenocortical activity quite well. Therefore, our study underlines the feasibility of measuring faecal cortisol metabolites as a non-invasive indicator of the amounts of cortisol released. A broad approach, including such non-invasive methods for elucidating endocrine changes, to questions such as animal welfare (housing, handling and human–animal interaction) or various stress reactions in domestic and also in wild or captive carnivores should be possible.

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