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Comparative Aspects of the Metabolism and Excretion of Cortisol in Three Individual Nonhuman Primates[1]

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A radiometabolism study is described to provide the first comparative data on the time course, route, and characteristics of excreted [^{3}H]cortisol metabolites in three nonhuman primates: the common marmoset (*Callithrix jacchus*), the long-tailed macaque (*Macaca fascicularis*), and the chimpanzee (*Pan troglodytes*). A low dose (40–100 μCi) of ^{3}H-labeled cortisol was administered intravenously to one adult male of each species and the excreta collected over a 5-day period postinjection. The major proportion of radioactivity was excreted in the urine (>80%). Peak radioactivity in urine was recovered within 5.5 h following injection in all three species, while in the feces peak levels of radioactivity were recovered within 26 h postinjection. In all three species, urinary metabolites were primarily excreted as conjugates (61–87%), whereas the percentage of conjugated metabolites in feces was 50% or less. The number and relative abundance of urinary and fecal [^{3}H]cortisol metabolites were determined by reverse-phase high-performance liquid chromatography (HPLC) and immunoreactivity of the radioactivity peaks was assessed by screening HPLC fractions with established cortisol, corticosterone, and 11-oxoetiocholanolone enzyme immunoassays (EIA), the latter being a group-specific assay for measuring 11,17-dioxoandrostanes. HPLC separation of urinary and fecal extracts revealed multiple peaks of radioactivity, several of which were common to all three species. The relative proportion of these peaks, however, differed considerably among species and between urine and feces. HPLC indicated that native cortisol was a major urinary excretory product in the marmoset, while comparatively small amounts were present in the urine of the macaque and chimpanzee. In contrast, in feces, cortisol was only detected in low amounts in the marmoset and was virtually absent in the macaque and chimpanzee. In all three species, one of the major radioactivity peaks showed a retention time comparable to 11-oxoetiocholanolone and high immunoreactivity in the 11-oxoetiocholanolone EIA. The measurement of urinary- and/or fecal-immunoreactive 11,17-dioxoandrostanes is therefore implicated for noninvasive assessment of adrenal function in Old World monkeys, New World monkeys, and great apes.

Key Words: nonhuman primates; cortisol metabolism; urinary and fecal excretion; long-tailed macaque; chimpanzee; common marmoset.

It is well established that physiological and psychogenic stress can have a disruptive effect on the reproductive physiology and behavior of mammals, including nonhuman primates. Studies on captive and free-ranging primates have shown that stress may suppress

[1] The sedation and administration of ^{3}H-labeled cortisol to the common marmoset and the long-tailed macaque, as described in the following study, was approved by the Bezirksregierung Braunschweig (Aktenzeichen 604.42502/08-05.97), Germany. The sedation and administration of ^{3}H-labeled cortisol to the chimpanzee was approved by a relevant animal welfare committee in The Netherlands.

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libido, testosterone secretion, and spermatogenesis in males (Goncharov *et al.*, 1979; Sapolsky, 1987; Norman, 1993), while in females ovarian function, embryonic development, and infant survival may be adversely affected (Johnson *et al.*, 1991; Cameron, 1997; Bahr *et al.*, 1998). Apart from these specific effects on reproductive function, other responses to stress may include more generalized disruptions of physiological and physical status, including hypertension, immune deficiencies, and increased disease susceptibility (Cohen and Crnic, 1983; Breazile, 1987). Assessing an animal's stress physiology is therefore essential to the understanding and improvement of animal well-being and reproduction in captive primates, as well as to monitoring impacts of socially mediated and environmental (e.g., ecological) stressors on behavior, health, and reproduction in free-ranging primate populations.

Stress usually results in increased glucocorticoid output and among primates, plasma cortisol measurements have been widely used as a marker of a stress response in various species (e.g., Saltzman *et al.*, 1994; Johnson *et al.*, 1996). However, difficulties in collecting blood and the negative effects of the procedure itself pose serious limitations to this approach, particularly for free-ranging and group-living animals in which repeated capture and restraint are not feasible (e.g., Reinhardt *et al.*, 1991; Sapolsky and Share, 1998). As a noninvasive alternative, analysis of the predominant glucocorticoid metabolites excreted in urine and/or fecal samples would therefore offer a considerable advantage for assessing adrenal function in both captive and free-ranging primates.

Urinary cortisol has been measured in several nonhuman primate species (Crockett *et al.*, 1993; Smith and French, 1997; Bahr *et al.*, 1998), although cortisol itself may be quantitatively of only minor importance, as has been shown for the human (Beisel *et al.*, 1964) and the domestic cat (Graham and Brown, 1996). Glucocorticoids determined in feces have also been used as an index of stress in a number of nonprimates (e.g., Graham and Brown, 1996; Palme and Möstl, 1997; Monfort *et al.*, 1998), although again, native cortisol appears to be present in only trace amounts or completely absent. Among primates, the use of fecal corticosteroid measurements as a marker of stress is limited to a few recent studies (Whitten *et al.*, 1998; Boinski *et al.*, 1999; Cavigelli, 1999; Strier *et al.*, 1999). Furthermore, although Wallner and co-workers (1999) measured two different immunoreactive glucocorticoid metabolites (cortisol and 11-oxoetiocholanolone) in Barbary macaques, there is no information on the relative abundance of native cortisol in relation to its metabolic products for any primate species investigated to date.

The objective of the present study was to conduct radiometabolism studies to provide basic data on cortisol metabolism and excretion in primates. Specifically, representative species of the three simian primate taxa, the chimpanzee (*Pan troglodytes*), the long-tailed macaque (*Macaca fascicularis*), and the common marmoset (*Callithrix jacchus*), were selected to provide comparative information on: (i) the time course of ^{3}H-labeled cortisol metabolite excretion and the proportion of radiolabeled metabolites excreted into urine and feces; and (ii) the characteristics of the major urinary and fecal cortisol metabolites excreted. A final objective was to assess the suitability of different enzyme immunoassays (EIA) for their determination.

METHODS

Subjects

The study included three healthy adult male nonhuman primates: one chimpanzee (*P. troglodytes*), one long-tailed macaque (*M. fascicularis*), and one common marmoset (*C. jacchus*). The chimpanzee (57 kg) was housed at the Biomedical Primate Research Center (BPRC) in Rijswijk, The Netherlands, and the long-tailed macaque (7 kg) and the common marmoset (0.38 kg) were housed at the German Primate Center in Göttingen, Germany. Male subjects were selected because they were concomitantly used in a study investigating the metabolism of [^{14}C]testosterone (Möhle *et al.*, 1998). During the experimental phase, all animals were kept singly in metabolic cages constructed with a metal waste pan. To avoid cross-contamination of excretory products and for the collection of fecal samples, a wire mesh was inserted between the waste pan and the foot end of the cage. The animals were fed twice daily a diet consisting of monkey pellets, bread, and fresh fruits and vegetables. Water was available *ad libitum*.

Administration of Radiolabeled Cortisol

[^{3}H]Cortisol ([1,2,6,7(N)-^{3}H]hydrocortisone; New England Nuclear, Boston, MA; sp act 84.0 Ci / mmol) was injected into the vena saphena of the common marmoset (40 µCi), the long-tailed macaque (100 µCi), and the chimpanzee (100 µCi). The ^{3}H-labeled cortisol was diluted in either 5 ml (chimpanzee and macaque) or 200 µl (common marmoset) of sterile 0.9% NaCl solution containing 10% (v / v) ethanol. In the macaque and the chimpanzee, the injection solution also contained 1 mg of nonlabeled cortisol as carrier (No. H-4001, Sigma Chemie, Deisenhofen, Germany). Syringes were sonicated for 30 s immediately prior to injection. After isotope administration, the syringe and the tube containing the radiolabeled isotope were rinsed twice with scintillation fluid (Lumasafe, Groningen, The Netherlands), and the residual radioactivity was counted and subtracted from the preinjection total to give the amount of radioactivity administered. Radiolabeled steroids were administered under anesthesia (Ketavet, Pharmacia Upjohn GmbH, Erlangen, Germany).

Following the administration of radiolabeled cortisol, urine and fecal samples were collected individually for 5 days after injection. Samples were stored in polyethylene containers at −20°C until analysis.

Time Course and Route of Steroid Excretion

In all three species, urine samples collected 0–24 h after [^{3}H]cortisol administration were analyzed individually, whereas urine samples collected after 24 h were pooled in 4-h intervals. In the macaque, all fecal samples were analyzed individually, while in the marmoset, fecal samples were pooled in 4-h intervals. In the chimpanzee, fecal samples collected 0–70 h after [^{3}H]cortisol administration were analyzed individually, whereas those collected after 70 h were also pooled in 4-h intervals. Urine (marmoset, 10–20 µl; macaque and chimpanzee, 500 µl) was mixed with 10 ml of scintillation fluid and directly counted in a scintillation counter (Tricarb 2200, Packard Instruments, U.S.A.) for 10 min while running a dual ^{3}H / ^{14}C quench compensation program. Urine samples were indexed by creatinine (Cr; see below) and values of urinary radioactivity expressed as dpm / mg Cr. Radioactivity recovered in fecal samples was determined by catalytic oxygen combustion (Peterson, 1969) of duplicate aliquots (0.2–0.5 g) of freshly thawed and homogenized feces. Following combustion in a liquid scintillation sample oxidizer (Intertechnique Model IN 4101, Plaisir, France), the resulting ^3H_2O and $^{14}CO_2$ were selectively absorbed in 20 ml scintillation fluid (Omni-Szintisol, Merck, Darmstadt, Germany) and counted for 10 min (Heistermann *et al.*, 1998). Average recovered radioactivity ($n = 2$) per fecal sample was expressed as dpm / g wet wt. Total recovered radioactivity (%) was the ratio of the combined sums of the recovered radioactivity in urine and feces divided by the total amount of administered radioactivity. Urine and fecal samples containing peak radiolabel concentration were used for all subsequent analyses.

Separation of Unconjugated and Conjugated Steroids

The proportion of ether soluble / insoluble metabolites was assessed in urine and feces by ether–water extraction. A total of 250 µl PBS buffer (pH 6.8) was added to 50-µl aliquots ($n = 5$) of urine and was extracted with 7 ml diethyl ether by vortexing for 15 min. The aqueous phase was frozen; the ether was decanted, evaporated to dryness, and reconstituted in 150 µl absolute ethanol; and radioactivity was determined by counting 50-µl aliquots (unconjugated fraction). The aqueous phase was subjected to sequential enzymatic hydrolysis by incubation with 1250 U specific β-glucuronidase (No. G-7396, Sigma Chemie) in 0.5 M PBS buffer (pH 6.8) overnight at 37°C. Deconjugated steroids were then extracted and radioactivity was measured as described above (glucuronide fraction). The residual aqueous phase was adjusted to pH 4.7 with 0.05 N HCl prior to hydrolysis with 1250 U β-glucuronidase / sulfatase (No. G-1512, Sigma Chemie) overnight at 37°C. Deconjugated steroids were determined as described above (sulfate fraction). Combined hydrolysis and extraction efficiency was checked by subjecting duplicate aliquots of 50 µl nonlabeled urine with the addition of 20 µl of [^{3}H]estradiol, [^{3}H]estrone glucuronide, or [^{3}H]estrone sulfate to the above procedure. The extraction recovery of [^{3}H]estradiol was 93.9 ± 12.5% (mean ± SEM, $n = 3$) and the combined hydrolysis and extraction efficiency of [^{3}H]estrone glucuronide and [^{3}H]estrone sulfate was 79.3 ± 9.8% ($n = 3$) and 87.7 ± 1.1% ($n = 3$), respectively.

The proportion of unconjugated and conjugated

steroid metabolites in feces was determined in four aliquots of 0.1 g of lyophilized and pulverized fecal powder. Samples were homogenized in 0.5 ml water and extracted four times with 5 ml of diethyl ether by vortexing for 20 min (Heistermann *et al.*, 1998). Following centrifugation (1800*g*, 1 min), the ether phase (unconjugated steroids) of each extraction step was decanted, pooled, and evaporated to dryness in Lexan combustion capsules. The capsules were subjected to catalytic oxygen combustion as described above and the radioactivity was counted.

High-Performance Liquid Chromatography (HPLC)

HPLC of urinary metabolites (performed at the German Primate Center, Göttingen, Germany) and fecal metabolites (performed at the Institute of Biochemistry, Vienna, Austria) were carried out using a reverse-phase Nova-Pak C18 column (3.9 × 150 mm, Millipore Corporation, Milford, MA) and a solvent system of methanol:water (MeOH:H_2O) at a flow rate of 1 ml/min. Steroids were separated using a linear gradient of 50–75–80% MeOH:H_2O over 0–40–45 min. Prior to HPLC, urine samples (marmoset, 100 µl; macaque and chimpanzee, 500 µl) were ether extracted to remove unconjugated steroids, and the aqueous phase was hydrolyzed with β-glucuronidase/sulfatase and extracted with diethyl ether as described above. Following evaporation, the extract was reconstituted in 350 µl MeOH:H_2O (50:50, v/v), 100 µl was injected onto the HPLC column, and fractions (n = 95) were collected every 20 s. Fecal samples were lyophilized and pulverized and 0.03–0.12 g extracted twice with 5 ml of 80% methanol in water. Extracts were pooled, 30 ml of sodium acetate buffer (0.2 M, pH 4.7) was added, and the total volume was loaded onto a primed Sep-Pak C_{18} cartridge (Millipore Corporation) at a flow rate of 2 ml/min for purification (Palme *et al.*, 1995). The cartridge was washed with 30 ml of water and dried under a stream of nitrogen. The steroids were eluted with 10 ml of dichloromethane, the eluent was evaporated to dryness, the steroids were reconstituted in 350 µl MeOH:H_2O (50:50, v/v), and a 100-µl aliquot separated by HPLC as described above. To determine the presence of radioactivity peaks, 100 µl of each HPLC fraction was counted as described above. In addition, elution positions of cortisol, corticosterone, and 11-oxoetiocholanolone standards (Steraloids, Wilton, NH) were determined separately in the HPLC systems.

Determination of Immunoreactivity

For determination of immunoreactivity in HPLC fractions, 230 µl of each fraction was evaporated to dryness, reconstituted in 350 µl assay buffer, and measured in a cortisol, corticosterone, and 11-oxoetiocholanolone EIA (Palme and Möstl, 1997). Antisera were raised in rabbits against cortisol–3-CMO, corticosterone–3-CMO, and 11-oxoetiocholanolone–3-HS, each coupled with bovine serum albumin. The labels were cortisol–3-CMO, corticosterone–3-CMO, and 11-oxoetiocholanolone–3-glucuronide linked to DADOO biotin (*N*-biotinyl-1,8-diamino-3,6-dioxaoctane (Palme and Möstl, 1994)), and the standards were cortisol (range 80 to 0.3 pg/well) and corticosterone and 11-oxoetiocholanolone (both, range 500 to 2 pg/well). In brief, standards (50 µl) and samples (50 µl) were incubated in duplicate with label (100 µl) and antibody (100 µl) overnight at 4°C. Following incubation, the plates were washed four times with 0.02% Tween 20 (Merck 822184) washing solution and blotted dry before 250 µl streptavidin horseradish peroxidase conjugate (4.2 mU, Boehringer 1089153) was added to each well; plates were then left in the dark on stirring tables for 45 min. The plates were then washed again before tetramethylbenzidine (250 µl/well; Fluka, 87748) was added and the plates were incubated for an additional 45 min at 4°C before the enzymatic reaction was stopped with 50 µl/well of 4 mol/L sulfuric acid. Absorbance was measured at a wavelength of 450 nm (reference filter 620 nm) on an automatic plate reader (Labsystems Multiskan, MCC/340; Szabo). Cross-reactivities and validation of the three EIAs are described by Palme and Möstl (1997).

Determination of Urinary Creatinine

To adjust for variations in water content, urinary radioactivity was indexed against creatinine and expressed as dpm/mg Cr. Creatinine measurement was performed by microtiter plate analysis. Blanks (150 µl water), 50 µl creatinine standard (range 0.075–5.0 µg/50 µl), and 50 µl sample were added to duplicate wells and incubated with 100 µl picrate reagent (mixture of 0.04 M picric acid with 0.75 M sodium hydroxide) in the dark on stirring tables for 15 min. Following

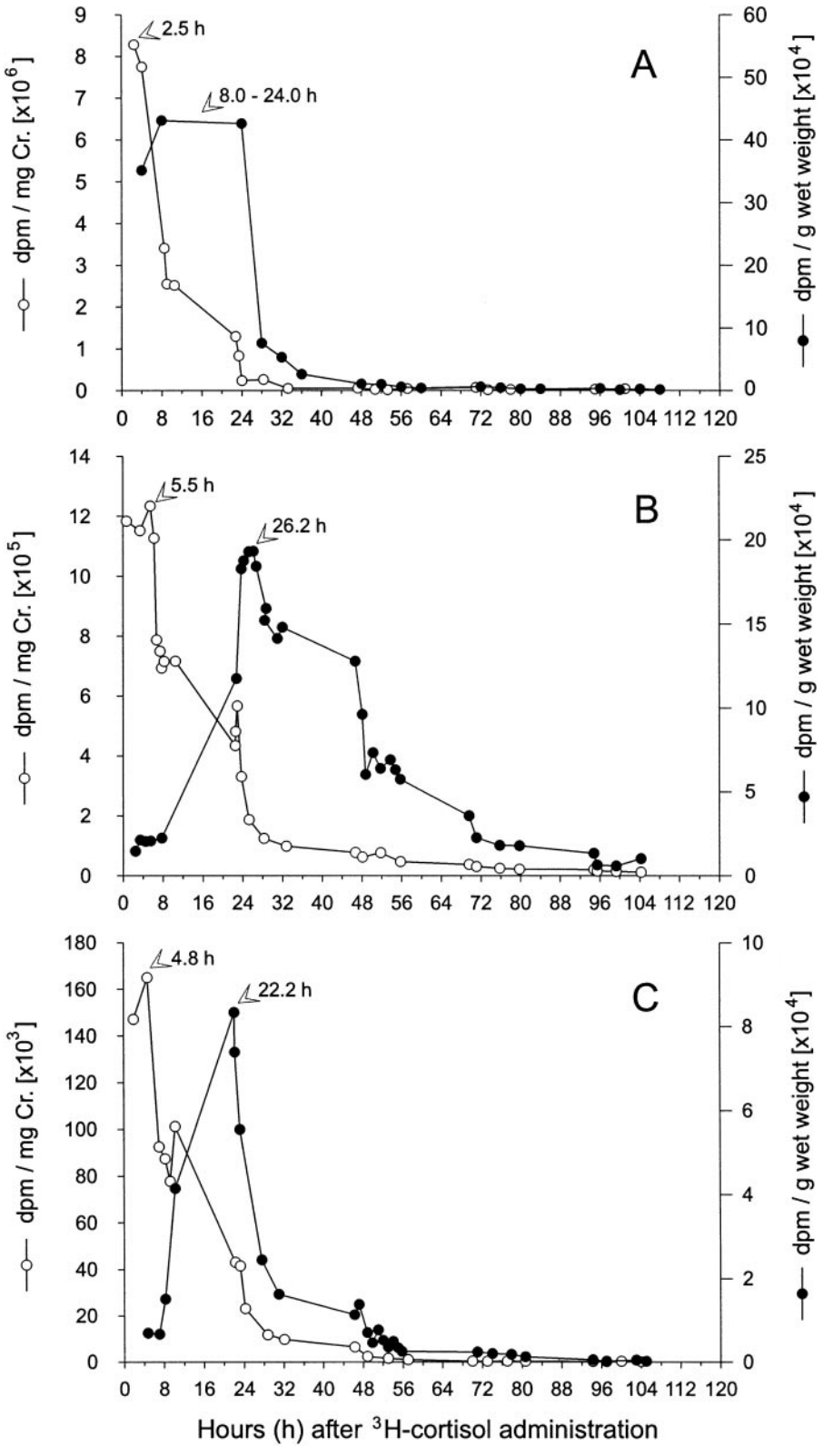


FIG. 1. Time course of [^{3}H]cortisol metabolite excretion into urine (○) and feces (●) of one adult male (A) common marmoset, (B) long-tailed macaque, and (C) chimpanzee following iv [^{3}H]cortisol administration (time 0).

TABLE 1
Proportion of Radioactivity (A) and Types of [^{3}H]Cortisol Metabolites (B) Recovered in Urine and Feces Following iv [^{3}H]Cortisol Administration

	Marmoset	Macaque	Chimpanzee
(A) Urine[a]	81.7	90.7	85.3
Feces[a]	18.3	9.3	14.7
(B) Urine			
Unconjugated[b]	39.4 ± 1.1	24.3 ± 0.2	12.7 ± 0.3
Glucuronidated[b]	50.6 ± 1.3	43.5 ± 1.5	55.5 ± 1.3
Sulfated[b]	7.0 ± 0.2	5.7 ± 0.1	7.0 ± 0.1
Nonhydrolizable[c]	3.0	26.5	24.8
Feces			
Unconjugated	59.9 ± 1.9	49.6 ± 0.9	61.8 ± 0.6
Conjugated[c]	40.1	50.4	38.2

[a] Values are presented as percentages and are based on the total radioactivity excreted during the first 5 days postinjection.
[b] Values are presented as percentages (mean ± SEM) and refer to extracts of urine ($n = 5$) and feces ($n = 4$) containing peak radiolabel concentration.
[c] Values are based on 100% recovery.

incubation, absorbance was measured at 490 nm, 50 µl/well stopping reagent was added (1.8 ml sulfuric acid and 3.0 ml acetic acid in 44.2 ml water), and the absorbance was remeasured at 490 nm. The differential of both readings was then computed for the creatinine standards and the differential of unknowns was interpolated from the resulting standard curve.

RESULTS

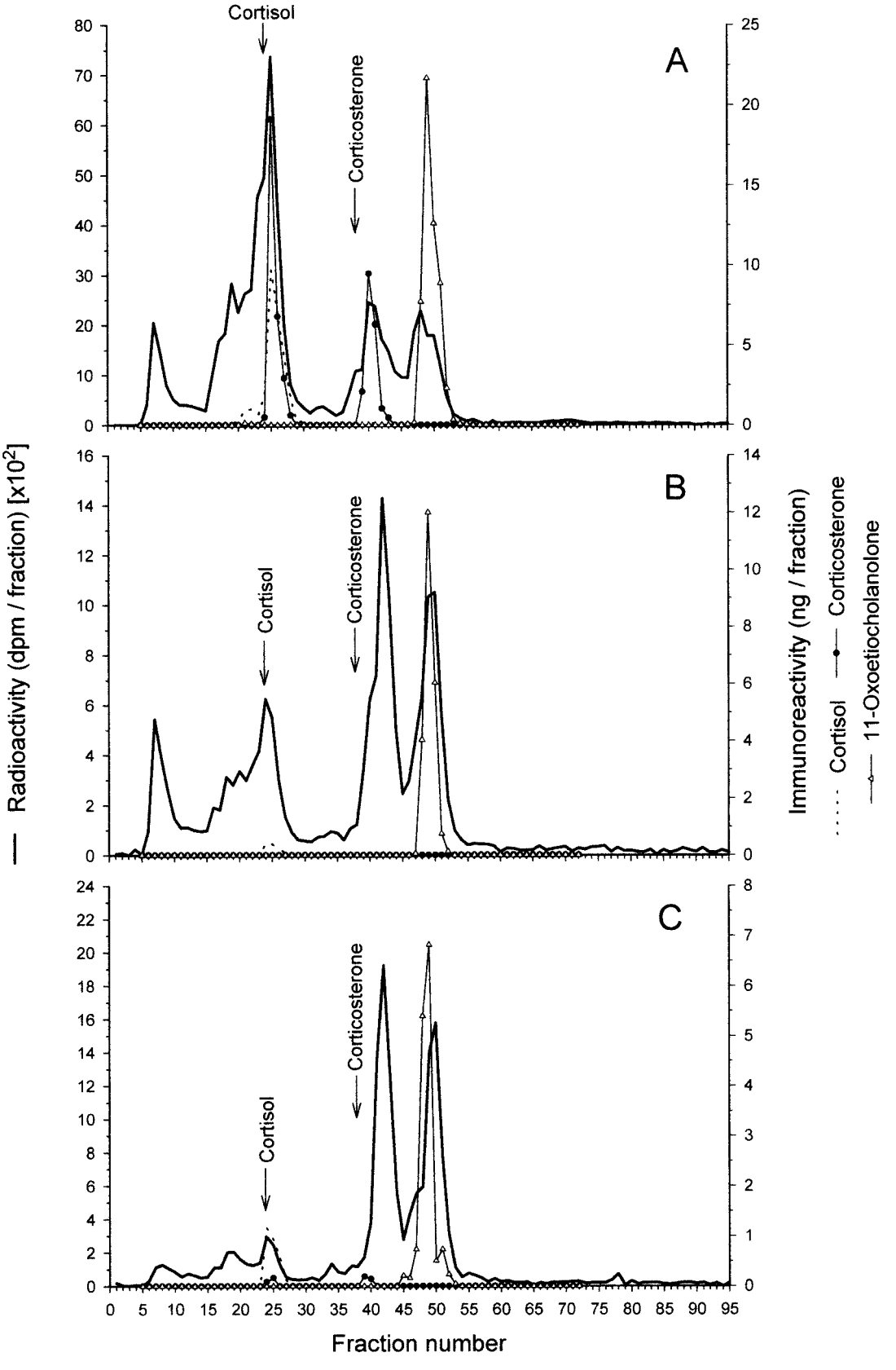
Time Course and Route of Steroid Excretion

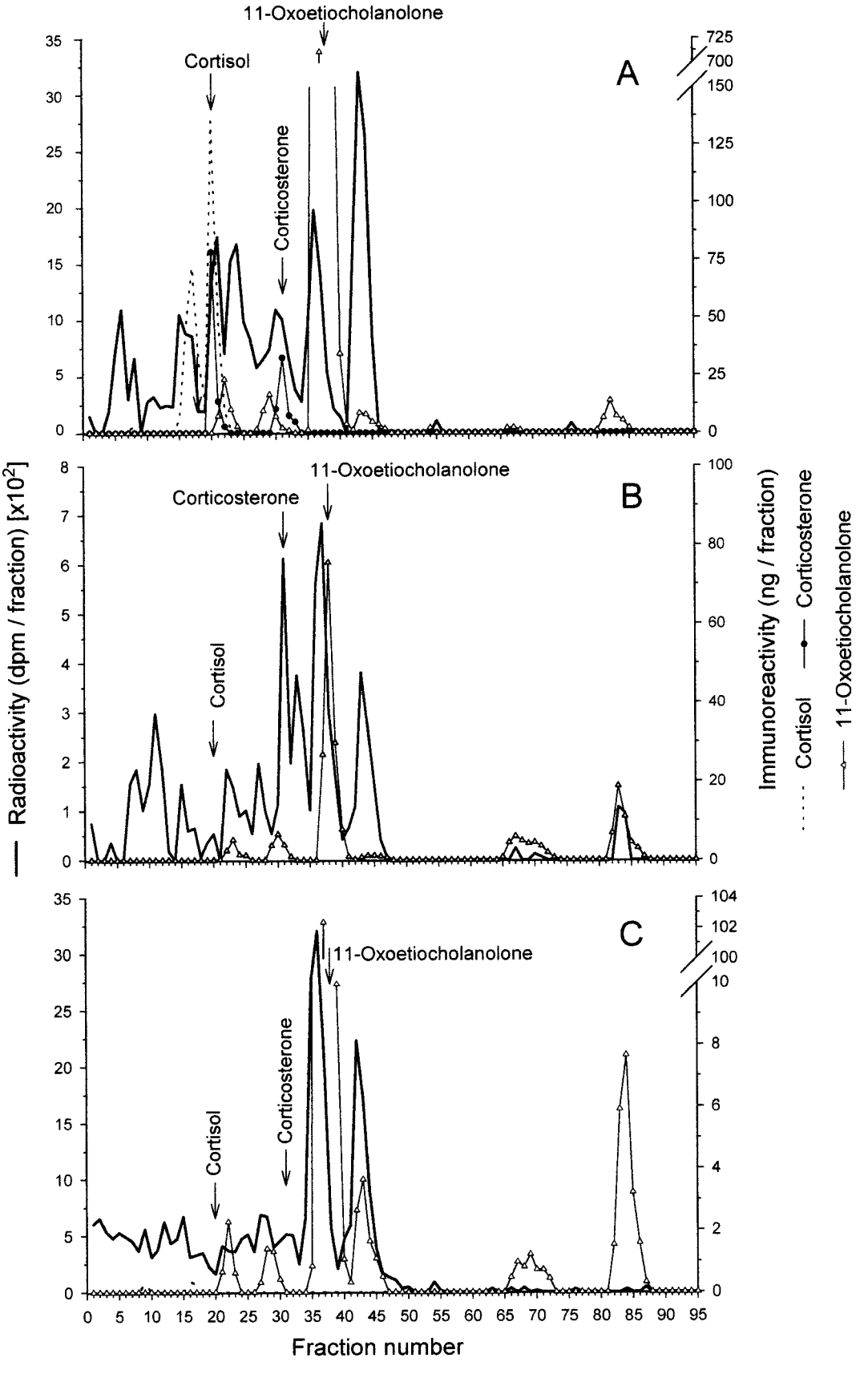
The time course of excretion of radioactivity in urine and feces is shown in Figs. 1A–1C. In all three species, peak radioactivity in the urine was recovered within 5.5 h, with the majority (marmoset, 91.4%; macaque, 69.7%; chimpanzee, 89.9%) having been eliminated by 24 h. Peak radioactivity in feces was detected between 8 and 24 h in the marmoset and after 22 and 26 h in the macaque and chimpanzee, respectively. Nearly all radioactivity in feces was excreted within the first 72 h. Total recovery of administered radioactivity (urine and feces combined) was 99.1% in the macaque, 87.2% in the chimpanzee, and 39.4% in the common marmoset. More than 80% of radioactivity was excreted in the urine in all species (Table 1), most of which was present in the form of glucuronides (Table 1). A higher proportion of unconjugated metabolites was measured in the urine of the marmoset compared with the other two species, while in the macaque and chimpanzee roughly 25% of the metabolites were nonhydrolizable. In feces, unconjugated metabolites predominated in the marmoset and chimpanzee, while in the macaque unconjugated and conjugated forms were excreted in similar amounts (Table 1).

Characterization of Steroid Metabolites by HPLC and Antibody Screening

Multiple peaks of radioactivity were found after HPLC of both urine and feces extracts. Although several peaks were common to all three species (Figs. 2 and 3), their relative proportions differed considerably among species and between extracts of urine and feces in the same species. HPLC of marmoset and macaque urinary extracts revealed four substantial peaks of radioactivity (Figs. 2A and 2B, fractions 6–9, 22–27, 38–44, and 47–52), two of which were also seen as prominent peaks in chimpanzee urine (Fig. 2C, fractions 38–44 and 47–52). In the macaque and chimpanzee the majority of radioactivity was associated with the metabolites eluting in fractions 38–44 and 47–52, whereas the major peak of radioactivity in the marmoset was more polar, eluting in fractions 22–27. In all three species, the radioactivity peak measured in fractions 22–27 coeluted with the cortisol standard, and cortisol immunoreactivity in these fractions was confirmed by EIA. Corticosterone immunoreactivity was detected in the urine of the marmoset, where it was associated with two peaks of radioactivity in

FIG. 2. High-performance liquid chromatographic (reverse-phase) separation of [^{3}H]cortisol metabolites in hydrolyzed urine of a male (A) common marmoset, (B) long-tailed macaque, and (C) chimpanzee. Radioactivity per fraction was determined by liquid scintillation counting and immunoreactivity was measured by EIA (cortisol, corticosterone, and 11-oxoetiocholanolone). Retention times of radioactive and immunoreactive peaks are compared to those of the cortisol and corticosterone standard.





fractions 26 and 40. In each of the three species, the radioactivity peak in fractions 47–52 corresponded with a prominent peak in immunoreactivity detected by the 11-oxoetiocholanolone EIA. The elution position of the 11-oxoetiocholanolone standard was not tested, but based on the relative retention times of the standards used in the HPLC separation of fecal cortisol metabolites (see below), it was predicted to elute in fractions 47–49.

Multiple peaks of radioactivity were obtained after HPLC of fecal extracts (Figs. 3A–3C), although in all species a relatively high proportion of radioactivity was found in fractions 35–39 and 41–45. No radioactivity peaks were found at the elution position of cortisol and corticosterone in the macaque and chimpanzee, and cortisol and corticosterone immunoreactivity was not measurable in any HPLC fraction in these two species (Figs. 3B and 3C). In contrast, prominent peaks of radioactivity were found at the elution positions of the cortisol and corticosterone standard in the marmoset and the presence of both metabolites was confirmed by EIA (Fig. 3A). The highest levels of immunoreactivity, however, were detected with the 11-oxoetiocholanolone EIA in all three species, with the majority being found in fractions 36–38, corresponding to one of the major radioactivity peaks and the elution position of the 11-oxoetiocholanolone standard.

DISCUSSION

This study provides comparative information on the excretion pattern of exogenously administered ^{3}H-labeled cortisol in a common marmoset, a long-tailed macaque, and a chimpanzee. The time course of urinary cortisol excretion in the chimpanzee concurs well with findings of an earlier study (Layne *et al.*, 1964) which reported that the majority of administered [^{14}C]cortisol was excreted into the urine within the first 24 h. Although information on the time course of urinary cortisol excretion is lacking for other primates, urinary excretion profiles obtained in the present study are in general agreement with those reported for nonprimate species (Graham and Brown, 1996; Palme *et al.*, 1996) and with data on the time course of urinary excretion of ovarian steroids in primates (Ziegler *et al.*, 1989; Shideler *et al.*, 1993; Wasser *et al.*, 1994). As expected, radioactivity in feces appeared later than in urine, but in all three species peak values were obtained within 26 h of injection, which is considerably shorter than the 36–48 h usually reported for peak excretion of ovarian steroids in primates (Ziegler *et al.*, 1989; Shideler *et al.*, 1993; Wasser *et al.*, 1994). With respect to the chimpanzee, this interval is also shorter than would have been expected from the reported gut transit time of 48 h in this species (Milton and Demment, 1988) and the 2-day lag to maximum fecal-immunoreactive cortisol excretion following an anesthesia-induced stress response (Whitten *et al.*, 1998). The reason for this difference is unclear, but the lower sampling frequency in the latter study may have resulted in an overestimation of the interval to peak excretion. Moreover, differences in fecal excretion rates within a species may also be related to differences in diet and/or individual adaptations in hepatic and gastrointestinal function (Goldin *et al.*, 1982; Wasser *et al.*, 1993). The faster elimination of the bulk of radiolabel in the urine and feces of the marmoset suggests an overall increased rate of cortisol metabolism and excretion compared with the other two species. With respect to fecal excretion this finding agrees well with a report on gut passage time in Callitrichidae (Price, 1993).

In the three primate species studied here, cortisol metabolites were predominantly (>80%) excreted in the urine, a finding comparable with reports on cortisol excretion in domestic livestock (Palme *et al.*, 1996), but not in the domestic cat, in which cortisol (as other steroids) is almost exclusively excreted via the feces (Graham and Brown, 1996). Data for the relative proportion of renal versus gastrointestinal cortisol excretion in other nonhuman primates are not avail-

FIG. 3. High-performance liquid chromatographic (reverse-phase) separation of unconjugated [^{3}H]cortisol metabolites in the feces of a male (A) common marmoset, (B) long-tailed macaque, and (C) chimpanzee. Radioactivity per fraction was determined by liquid scintillation counting and immunoreactivity was measured by EIA (cortisol, corticosterone, and 11-oxoetiocholanolone). Retention times of radioactive and immunoreactive peaks are compared to those of the cortisol, corticosterone, and 11-oxoetiocholanolone standard.

able, but ovarian steroids are usually preferentially excreted in the urine (Ziegler *et al.*, 1989; Shideler *et al.*, 1993; Wasser *et al.*, 1994; but see Perez *et al.*, 1988), although this can vary according to the hormone (e.g., Ziegler *et al.*, 1989). The finding that urinary cortisol metabolites were primarily eliminated as conjugates, whereas the bulk of radioactivity excreted into the feces was associated with steroids in an unconjugated form, is in general agreement with previous findings in both primate (Ziegler *et al.*, 1989; Shideler *et al.*, 1993; Wasser *et al.*, 1994) and nonprimate species (Heistermann *et al.*, 1998; Schwarzenberger *et al.*, 1996).

While the number and relative retention times of radioactivity peaks following HPLC of urine were similar among all three primates, the relative proportions of these peaks differed. In the macaque and chimpanzee, relatively apolar metabolites were more abundant, whereas more polar forms predominated in the marmoset. Despite these species specificities, the present data suggest similarities in cortisol metabolism and urinary excretion between the three species and suggest that urinary metabolites eluting in fractions 38–44 and 47–52 may also be abundant cortisol excretory products in other primates. The data for the marmoset also suggest that the metabolites excreted in fractions 22–27 might be quantitatively more important in callitrichid primates. While the nature of these substances cannot be confirmed without further analysis, the immunoreactivity profiles and comparison with the elution position of standards suggest that the peak eluting around fraction 50 contains 5-reduced 3α-hydroxylated 11,17-dioxoandrostanes. Small amounts of cortisol were also present in urine from all three species, although in quantitative terms, excretion of urinary cortisol was lower in the Old World monkey and great ape compared to the New World monkey. Corticosterone immunoreactivity was measured only in the marmoset and in very small amounts, and it is therefore unlikely that corticosterone represents an abundant urinary glucocorticoid metabolite in primates.

HPLC of fecal extracts revealed prominent peaks of radioactivity around fractions 37 and 44 in all of the three species studied, suggesting that measurement of either of these relatively apolar substances may provide a useful fecal marker of adrenocortical activity suitable for broad application. In particular, radioactivity eluting at the position of authentic cortisol was either low (marmoset) or virtually absent (long-tailed macaque, chimpanzee), and this, together with the presence of only trace amounts of immunoreactivity, suggests that cortisol is not excreted in significant amounts into the feces of primates as previously shown in cats (Graham and Brown, 1996) and sheep (Palme and Möstl, 1997). With respect to the chimpanzee, the present results contrast with a recent finding by Whitten and co-workers (1998), reporting the measurement of immunoreactive cortisol in chimpanzee feces which, according to HPLC analysis, suggested substantial amounts of authentic cortisol. Since sensitivities of the procedures used were comparable between studies, the reason for the discrepancy is unclear, although it may be that other immunoreactive glucocorticoids were not detected by Whitten and co-workers, who analyzed only a relatively small number of HPLC fractions using a single highly specific antibody.

Together, the present data indicate that cortisol is of only minor quantitative importance in the feces of representative species of two of the three simian primate taxa and that measurements of other fecal metabolites are better for assessing cortisol secretion (see also Graham and Brown, 1996; Palme and Möstl, 1997). In this respect, a group-specific determination of 11,17-dioxoandrostanes may be more useful as a fecal measure of cortisol excretion in a range of nonhuman primates, and this would agree with recent results for the Barbary macaque (Wallner *et al.*, 1999). Given that 11,17-dioxoandrostanes appear to represent major urinary and fecal metabolites of cortisol in species of various primate taxa, as well as in nonprimate species (Palme and Möstl, 1997), their group-specific measurement offers the potential for multispecies application for the noninvasive assessment of adrenal function. Use of such an assay may in turn help to elucidate the impact of environmental and/or social stressors on reproductive function in both captive and free-ranging primates and thus may help to better understand the role of stress in regulating reproduction and reproductive success in this group of mammals.

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