

ERRATUM (Author's Correction)

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Enzyme immunoassays for water-soluble steroid metabolites in the urine and feces of Japanese macaques (*Macaca fuscata*) using a simple elution method

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Vol. 80, No. 7 (2018), p. 1139, the number of the Plate preparation section should have been as follows:

Error

Plate preparation. Anti-rabbit IgG (H+L) goat serum (Rockland Immunochemicals Inc., Limerick, PA, U.S.A.) was diluted to 0.15 $\mu\text{g/ml}$ in pH 9.6, 0.05 M carbonate buffer, and 50 μl was added to microtiter plates (Maxisorp flat-bottom, Thermo Fisher Scientific, Waltham, MA, U.S.A.) for the E1C, PdG and E3G assays.



Correction

Plate preparation. Anti-rabbit IgG (H+L) goat serum (Rockland Immunochemicals Inc., Limerick, PA, U.S.A.) was diluted to 15 $\mu\text{g/ml}$ in pH 9.6, 0.05 M carbonate buffer, and 50 μl was added to microtiter plates (Maxisorp flat-bottom, Thermo Fisher Scientific, Waltham, MA, U.S.A.) for the E1C, PdG and E3G assays.

commonly with high concentrated alcohol or volatile solvents [15, 16, 18, 25, 29]. Though these solvents allow for a high extraction rate, highly concentrated alcohol or volatile solvents can interfere with the enzymatic and/or immunometric reaction. Therefore, it is necessary to remove the blockage from the extraction. This complicated extraction process in fecal samples requires special equipment and can lead to some errors, which will then bring incorrect results. Consequently, a simpler method of obtaining accurate results from fecal extraction is desirable.

The present study described the establishment of 1) a simple and rapid method for fecal extraction without a large quantity of alcohol or volatile solvent, and 2) EIAs using appropriate polyclonal E₁C, PdG, and E₃G antibodies with cross-reactivity to urinary and fecal conjugated and unconjugated steroid metabolites in Japanese macaques. 3) Then, we measured these steroid metabolites using this method in both the urine and fecal samples of female Japanese macaques.

MATERIALS AND METHODS

Experimental subjects

Eight sexually mature female Japanese macaques (*Macaca fuscata*); with body weights ranging from 7.2–11.5 kg (mean=9.2 kg, SD ± 1.4) were used in this study. All females were housed individually at the Primate Research Institute (PRI), Kyoto University, Inuyama, Japan. They were provisioned commercial monkey chow daily, and supplemented with sweet potatoes. Water was available *ad libitum*. They were maintained in a natural lighting conditioned room at a temperature of approximately 20°C. All females were multiparous, and daily vaginal bleeding was monitored. After confirmation of the onset of menstruation, all females were mated with males between approximately 10–15 days from the beginning of menstruation. The use of laboratory subjects adhered to the Guide for Care and Use of Laboratory Primates (1986) of the Primate Research Institute, Kyoto University.

Sample collection

Urine and fecal samples were collected two times per week during the entire breeding season. Large collection trays were placed beneath the individual cages, and a stainless steel mesh was used to separate the feces from urine to minimize contamination. Morning urine samples were aspirated from the tray using a disposable syringe. Feces were collected and placed into plastic collection bags. All samples were immediately frozen at –30°C and stored until the assay.

At the end of the breeding season, six of eight females conceived and five pregnancies resulted in the birth of healthy offspring. However, one offspring died five days after birth by negligence. One other pregnant female had a premature stillbirth. The urine and fecal samples of pregnant females were collected over the entire gestation period. The menstrual cycles of the two remaining females that had not conceived were monitored until the end of July.

Sample preparation

Urinary samples. Urinary samples were unprocessed, and diluted with deionized water between 100–5,000, 1–50, 1–20, 50–100 and 1–20 times for the E₁C, PdG, and E₃G assays of pregnant females; and the E₁C, and PdG assays of non-pregnant females, respectively. For the E₃G assay, urinary samples were used without dilution.

Fecal samples. Fecal samples were thawed and dried using a vacuum drier at 50°C for approximately 12 hr. Dried samples were pulverized, and foreign substances such as rough fiber and seeds from the dried fecal powder were removed. A fecal sample of the powder representing 0.25 g was placed into a 14 ml polypropylene test tube (Stockwell Scientific Inc., Scottsdale, AZ, U.S.A.), and 2.5 ml of extraction buffer which contained 0.1 M phosphate buffer (pH 7.0) 0.1% BSA, with 0.05% Tween 20 and no alcohol were added, and then rotated on a test-tube rotator (Labinco, B.V., Breda, Netherlands) for 24 hr at room temperature. Following centrifugation at 4°C, 1,500 × g for 10 min, the supernatant was decanted into microtubes and stored at –30°C until the assays.

Enzyme Immunoassays

Antibodies. Previous studies have described that major estradiol (E₂) metabolites were two types of estrone-monoconjugates (E₁C) in urine and unconjugated estrone (E₁) in feces. Progesterone (P₄) metabolites were various 20 α -hydroxy C21 compound-monoconjugates in urine and unconjugated pregnanediol in feces [6, 15, 21]. Moreover, during pregnancy, it has been reported that large quantities of E₂ are produced from the placenta of humans and great apes [7, 17]. Therefore, we employed group-specific polyclonal antibodies against estrone-3-glucuronide-BSA, pregnanediol-3-glucuronide-BSA, and estriol-6-carboxymethylxime-BSA raised in rabbits by Dr. A. Kambegawa.

Plate preparation. Anti-rabbit IgG (H+L) goat serum (Rockland Immunochemicals Inc., Limerick, PA, U.S.A.) was diluted to 15 μ g/ml in pH 9.6, 0.05 M carbonate buffer, and 50 μ l was added to microtiter plates (Maxisorp flat-bottom, Thermo Fisher Scientific, Waltham, MA, U.S.A.) for the E₁C, PdG and E₃G assays. Then plates were left for one or two nights at room temperature (<24°C). The plates were washed of the unbound anti-rabbit IgG with wash buffer (0.15 M NaCl, 0.05% Tween20), and blocked with blocking buffer (0.05 M borate buffer, pH 7.8, 0.1% BSA, 3% sucrose) overnight. The following day, the blocking buffer was discarded and the plates were dried, sealed, and stored in 4°C until the assays.

E₁C EIA. The E₁C polyclonal antibody was diluted with assay buffer (0.05 M borate buffer, pH 7.8, 0.2% BSA) at an appropriate dilution rate (1:8,000,000), and 50 μ l of the antibody was added to IgG-coated plates and left overnight at room temperature. The following day, the plate was washed to remove the unbound first antibody, and all emptied wells were filled with 50 μ l of assay buffer immediately. Afterwards, serially diluted 50 μ l of standards (range 0.001–10 ng/ml), internal controls, and unknown samples were added to each well. Correspondingly, 50 μ l of estrone 3-carboxymethyl ether (CME)-HRP diluted with assay buffer