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RESEARCH NOTE

FIELD COLLECTION AND PRESERVATION OF URINE IN ORANGUTANS AND CHIMPANZEES

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ABSTRACT

Urine allows for the measurement of physiological correlates of reproduction, behavior, and health. However, field collection of urine from free-ranging animals has been limited due to the difficulties of urine storage and transport. I report here on techniques which I have found effective for collection of urine from wild orangutans (*Pongo pygmaeus*) and chimpanzees (*Pan troglodytes*) and a field method for urine preservation on filter paper in the absence of refrigeration. Use of urinalysis strips and hormonal test kits for field evaluation of reproductive functioning and general health are described. This method's applicability to other primate species and importance for conservation is also discussed.

Keywords: urine, *Pongo pygmaeus*, *Pan troglodytes*, primates, hormone, radioimmunoassay

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INTRODUCTION

Over the past 15 years analysis of urine has become accepted as one of the standard methods of studying primate reproductive physiology (Czekala *et al.*, 1988). Such studies have allowed for the characterization of hormonal changes during the menstrual cycle (e.g. Dahl, 1991), pregnancy (e.g. Bonney and Kingsley, 1982), lactation (e.g. Hearn, 1984), and development (e.g. Kingsley, 1988) of many primate species. However, the great majority of these studies, and thus what is currently known about the hormonal control of primate reproduction, comes primarily from research on captive animals (e.g. Graham, 1981; Faiman *et al.*, 1981; Shideler and Lasley, 1982; Czekala *et al.*, 1988; Masters and Markham, 1991). Studies of captive animals have the advantage of being in a controlled environment, however, they may not adequately represent the physiology of free-ranging animals. Nutrition, social behavior and developmental timetables may vary significantly between captive and wild populations.

Reproductive functioning of wild primates has largely been studied through behavioral observations alone. However, in animals such as orangutans who do not exhibit visual signs of ovulation, very little can be learned about their reproductive cycles in this manner. In chimpanzees, knowledge of the relationship between estrus swelling

and ovulation could greatly aid our understanding of reproductive behavior and paternity in the wild. The collection and analysis of urine samples from wild primates can thus unite the more precise reproductive measures obtained from laboratory analysis with the natural behavior of free-ranging animals.

Until very recently logistical considerations have limited the analysis of primate urine samples to the captive setting. This is largely due to the lack of adequate storage facilities in the field. The few primate field studies that have analyzed urine from free-ranging animals have preserved and transported samples using a freezer, liquid nitrogen and/or dry ice (Andelman *et al.*, 1985; Van Schaik *et al.*, 1991; Czekala *et al.*, 1994). At many remote field sites, however, the ability to keep samples under these conditions is either difficult or impossible. I thus report here a method for collecting urine from free-ranging animals and preserving it on filter paper that I have successfully used with orangutans and chimpanzees. It is hoped that this paper will help facilitate application of this technology to other primate field studies. I further describe tests of primate physiology and reproductive functioning that can be conducted using urine under field conditions.

METHODS

Study Sites

The methods reported here were first developed in 1992 for orangutans at the Cabang Panti research site in Gunung Palung National Park, West Kalimantan (Indonesian Borneo), and for chimpanzees in the Kibale forest of Uganda (Knott, 1993). They have subsequently been used in an intensive study of orangutan reproductive ecology beginning in 1994 (Knott, unpublished). Urine collection following these methods is ongoing at each site.

Urine Collection

Orangutans and chimpanzees, as well as most other primates, urinate upon awakening. This is the ideal time to collect a urine sample. Individuals characteristically urinate over the side of their nest. Thus, to collect urine, a large (1.5 x 1.5 m), clean plastic sheet can be placed on the ground beneath the sleeping nest before the animal awakens. The side of the nest from which the animal will leave can often be anticipated and the sheet placed accordingly, adjusting if necessary. The urine is collected from the sheet using disposable plastic pipettes and then transferred into plastic collection tubes. Often volumes over 15 ml can be collected in this way. Plastic disposable gloves should be worn for protection and to avoid contamination of the sample. Care should be taken not to collect urine that has intermixed with feces on the plastic sheet. Plastic sheets can be reused by washing them with water and a small amount of soap.

Urine can also be opportunistically collected during focal follows. The largest volumes of urine are obtained by keeping a plastic sheet handy which can be quickly tossed beneath the urinating animal. Alternatively, urine can be collected directly from

vegetation, although this method usually yields smaller volumes. A short-stemmed pipette works best for small volume collection. Suspended drops on leaves can be captured directly into plastic bottles. I found 15 ml screw top centrifuge tubes convenient for collecting large samples and 1.5 ml snap tubes worked well for small samples (available from Fisher Scientific). All collection vials should be labeled in the field with an indelible marker to avoid later sample confusion and can be transported in a plastic bag.

Urine Preservation

The method I utilized for processing urine samples was modified from a similar method employed for human and captive primate urine samples (Campbell, 1994; Shideler *et al.*, 1995). Highly absorbent filter paper (Schlicker & Schloom, #16110) is cut into 2.5 x 2.5 cm pieces. In the high humidity of the rain forest, cut pieces of filter paper should be stored in an air-tight container with a desiccant such as silica gel to prevent moisture absorption. When urine is ready to be processed these pieces of filter paper are placed on a non-absorbent surface such as aluminum foil or parafilm for sample application. On each piece of filter paper the animal's name, collection date, sample letter and aliquot amount are written in pencil. A micropipette is then used to aliquot sample replicates onto each piece of filter paper. Urine can also be applied with calibrated capillary tubes, although I have found a micropipette to be easier. I applied urine samples to filter paper within 24 hours of collection to avoid contamination from bacterial growth.

The number of replicates made depends on the number of tests to be performed. I found 5 replicates, containing 200 μl each, to be adequate for the measurement of creatinine and several hormonal radioimmunoassays, while still providing backup samples. Many hormonal analyses using radioimmunoassay only require small volumes of urine, on the order of 10 μl (Czekala *et al.*, 1981; Czekala, 1987), thus even small volumes of urine can be useful. Aliquot amounts of less than 200 μl can be used if only a small sample was obtained.

After the urine is transferred to filter paper, the piece of aluminum foil with the samples is placed in an air-tight container partially filled with silica gel. Using a container of approximately 5 liters in volume, containing 1/2 liter of silica gel, samples dry within 12 hours of urine application. The drying time may depend on the number of samples in the container. After drying, samples should be stored in plastic. I have found transparent slide sheets to be convenient for storing individual pieces of filter paper, as recommended by Shideler *et al.* (1995). Slide sheets containing samples should also be kept in a plastic container with silica gel to avoid moisture absorption and mold growth under tropical rainforest conditions. The silica gel used for these procedures should have a color indicator and needs to be frequently "cooked" to maintain its effectiveness. Samples should be kept away from light and heat (Campbell, 1994).

I have found urine samples kept in this way to be well preserved, exhibiting no mold growth. However, if a further anti-fungal agent is desired, sodium azide can be used. This method has proved reliable for the stabilization of salivary samples (Ellison *et al.*, 1986) and was used by Van Schaik (1991) for urine samples. The procedure of Ellison *et al.* (1986), recommends a sodium azide application resulting in concentrations of approximately 0.1%.

Urinalysis Strips

Urinalysis strips made for human clinical testing can be used to test samples for the presence of disease and physiological status. These are plastic strips with various reagents on pads that produce a color reaction when urine is applied. Tests available include blood (hemoglobin), specific gravity, leukocytes, bilirubin, ketones, glucose, protein, urobilinogen, nitrite and pH. Specific gravity tests provide a measure of concentration of urine which can be used in addition to or as a substitute for creatinine measures (Campbell, 1994). Leukocytes provide an indication of infection and ketones reflect the presence of fat catabolism. These measures have proven valuable in assessing orangutan physiological status in the field (Knott, 1996). The presence of blood in urine is useful in assessing injury, disease, and menstruation. This method has commonly been used in zoos to detect menstruation in orangutans (Rogers 1989; Smith 1989; Masters and Markham, 1991). Urinalysis strips with various combinations of tests are available from Boehringer Mannheim and Microstix. I have found Boehringer Mannheim Chemstrip 10 with SG to provide the most complete test coverage.

Hormonal Test Kits

The use of test kits for the assessment of pregnancy (HCG) and ovulation (LH) in humans may be appropriately applied to some primate species, particularly great apes. I have found kits made by Quidel to be reliable in orangutans and Czekala (pers. comm.) has applied these to the study of mountain gorillas. The kits are simple to use in the field, requiring the addition of a few drops of urine and, in some kits, reagents to a test pad. These kits have been particularly useful in obtaining feedback on orangutan menstrual cycles since visual indicators of ovulation are not present in these animals.

RESULTS AND DISCUSSION

Precise hormonal analyses can be done of urine preserved on filter paper by reconstituting it in buffer and analyzing it using standard methods of radioimmunoassay (Campbell, 1994) or enzyme immunoassay (Shideler *et al.*, 1995). I have found hormonal levels measured in urine preserved on filter paper to exhibit a high correspondence to hormonal levels in urine preserved by the conventional method of freezing. In a validation study of this method, estrone conjugates recovered from human urine exhibited a correlation of over .91 between samples preserved on filter paper and matched frozen urine samples (Figure 1). All hormonal values were determined using standard methods of radioimmunoassay (Czekala *et al.*, 1987) and all values were indexed by mg of creatinine (Taussky, 1954).

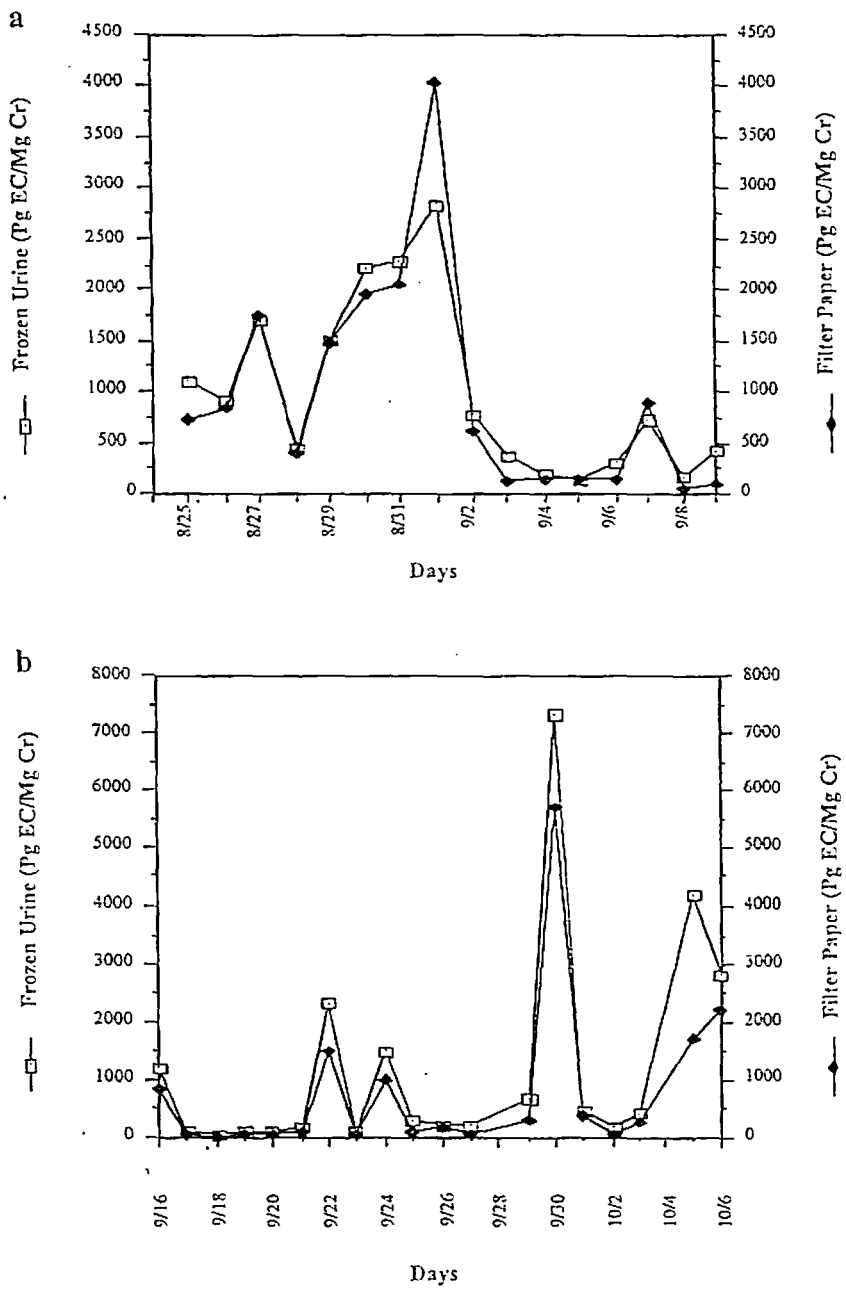

Figure 1. Graphs a and b each show one menstrual cycle of a single human subject. The levels of estrone conjugates measured from matched samples of frozen urine (open squares) and dried urine eluted from filter paper (closed squares) are compared.

Conclusions

The non-invasive collection of urine from free-ranging primates provides an effective way to monitor reproductive physiology in the wild. These methods can be applied to any species in which urine can be readily obtained from individual animals. Collection protocols may need to be modified depending on local conditions and the species involved.

Data collected from the wild, in conjunction with what has already been learned from captive studies, can greatly augment our understanding of primate reproductive functioning. Collection of urine and analysis of hormones from the wild provide the opportunity to study the ecological context of reproduction through questions addressing the interaction between nutrition, energetics, and ultimately food availability on reproductive hormones. Further, as demonstrated by Sapolsky (1982, 1986) and van Schaik *et al.* (1991), evaluation of the hormonal correlates of social behavior in the wild can be effectively used to study how rank, aggression, and stress affect physiology and reproductive success. Hormonal analysis of urine also provides a way to investigate the effect of both male and female hormones on sexual behavior, mate choice, and the timing of mating in the wild.

Analysis of urine in free ranging animals has important conservation applications, as knowledge of reproductive parameters is essential for population viability analysis. Animal populations are limited by female fecundity, thus understanding reproductive functioning in the wild is essential for determining whether viable populations can be maintained in shrinking reserves and increasingly degraded habitats. Comparative data on the relative health status of animal populations can also assist in the conservation of endangered species.

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