accuracy of quantitative collection of urine in carnivores

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EXPANDED ABSTRACT

KEY WORDS:  
- carnivores  
- balance studies  
- inulin  
- mink  
- osmotic pumps  
- urinary markers

In studies of animal nutrition, complete 24-h collections of urine samples are often required for detailed analysis and interpretation of urinary excretion of dietary and metabolic constituents. Most reports include a detailed description of the experimental techniques and the equipment used. However, to our knowledge, there is no information available on the completeness of daily urine collection in animal species commonly used for scientific purposes (Wamberg et al. 1996a and 1996c).

In strictly carnivorous mammals, such as cats, ferrets (Mustela putorius furo) and mink (Mustela vison), accurate collection of urine is extremely difficult because of their habit of squirting and spreading the urine all over the cage and on top of the feces. Furthermore, due to the excretion of a highly concentrated urine with a high nitrogen content, incomplete collection of urine may lead to overestimation of nitrogen balances and, hence, of the true protein requirements of these animals (Elnif 1992, Tauson et al. 1997).

This study was designed to assess the accuracy of quantitative urine collection in conscious female mink by repeated measurements of the recovery, in 24-h urine collections, of two well-documented, radioactively labeled urinary markers, [3H]-p-aminohippuric acid (3H-PAH) and [14C]-labeled inulin (14C-IN), continuously released, for a period of 8 d, by small osmotic pumps implanted intraperitoneally. Details of the experimental procedure and the results obtained on water, electrolyte and nitrogen turnover during six consecutive 24-h balance periods in mink are reported elsewhere (Tauson et al. 1997, Wamberg et al. 1996c).

Materials and methods. In vitro assay. Ten osmotic pumps (Alzet model 2ML1, Alza, Palo Alto, CA), were filled with 2 mL of isotonic (0.154 mol/L) saline containing 3H-PAH (see below) and submerged in sterile isotonic saline in a thermostat-controlled water bath at 39.0 ± 0.1°C (Wamberg et al. 1996a). Each pump was mounted with a 5-cm long PE-60 polyethylene catheter leading to a small collecting vial, which was replaced every 24 h. At the end of d 10, the radioactivity of the vials was determined as described below, and the in vitro pumping rate of the osmotic pumps was calculated from the counts-ratio between the daily output collected in the vials and the stock solution with which the pumps were filled initially. Details of the construction and function of the osmotic pumps (Fig. 1) are given by Theeuwes and Yum (1976).

In vivo study. In this study, the conventional balance technique that uses metabolism cages designed for mink (Elmif 1992, Glem-Hansen 1980) was improved by the application of a new technique for assessing the completeness of urine collection in small animals (Wamberg et al. 1996a) based on daily measurements of the recovery in urine of two urinary markers, 3H-PAH and 14C-IN, which are rapidly and efficiently excreted by the kidneys (Levinsky and Levy 1973).

Animals and surgical procedures. Ten adult female mink, pastel color type, weighing 1100 g, were housed in metabolic cages in a controlled environment (10-h light:14-h dark cycle and temperature 16 ± 1°C) in our laboratory and fed a conventional mink diet, based on industrial fish and slaughterhouse offal, for 1 wk (d –7 to –1) before the experiment. The diet contained (g/kg): dry matter (DM), 310; crude protein, 180; crude fat, 40; and metabolizable energy, 15 MJ/kg DM (Wamberg et al. 1996c). All animals were given free access to drinking water throughout the study.

On d 0, the animals were anesthetized, using ketamine hydrochloride, 40 mg/kg intramuscularly (Ketaminol Vet, 50 mg/mL, Veterinaria AG, Zürich, Switzerland), and midazolam hydrochloride, 2.0 mg/kg (Dormicum, 5 mg/mL, Hoffman La-Roche AG, Basel, Switzerland) as described by Wamberg et al. (1996b); a 2-mL osmotic pump (same as those tested in vitro) was implanted intraperitoneally. In five mink, the osmotic pumps contained an accurately weighed amount of sterile isotonic saline with 1.85 MBq of 3H-PAH; in the remaining five mink, the pumps contained isotonic saline with 1.85 MBq of 3H-PAH and 925 kBq 14C-IN, to be released in the body over the next 8 d. On d 1, the animals were given free access to food...
and water and allowed to recover, and all collected material was discarded. Then, a conventional balance study was conducted for six consecutive 24-h periods, followed by feed restriction (drinking water allowed). Details of the collection procedures, which included separation of urine and feces for the determination of $^3$H and $^{14}$C recoveries in urine and fecal water (bleached with hydrogen peroxide) by liquid scintillation counting, are given elsewhere (Wamberg et al. 1996c).

All experimental procedures involving animal treatment followed the guidelines approved by the Member States of the Council of Europe (Anonymous 1986). The radiopharmaceuticals, $^3$H-PAH (code TRA.197) and $^{14}$C-IN (code CFA.399) were obtained from Amersham, Buckinghamshire, England.

**Results and discussion.** The mean (± SEM) pumping rate of the osmotic pumps, when fully operating in vitro, was 9.96 ± 0.12 μL/h (Wamberg et al. 1996a), which was in agreement with the nominal value, 10.00 ± 0.15 μL/h, given by the manufacturer. This result indicated that the osmotic pumps could be used for accurate and constant delivery of the test substances in vivo for at least 7 d.

In the balance study, all animals recovered quickly from anesthesia, and normal rates of feed and water intake were recorded on d 2–5 after surgery. During the experimental feeding period (Fig. 2, d 2–5), the amount of urine collected in each 24-h period was similar to that obtained on day 2, before surgery, indicating that the experimental procedures did not adversely affect the mink.

The mean 24-h excretion rates of nitrogenous constituents in urine (corrected to 100% $^3$H-PAH recovery), are presented in Table 1; they showed a dramatic decline in response to feed restriction, i.e., to withdrawal of the normal dietary water and protein supplies but free access to drinking water.

The mean daily recoveries of $^3$H-PAH and $^{14}$C-IN in urine (Fig. 3) followed a similar pattern, both in the feeding period and during feed restriction. This observation documents the high reproducibility of our present collection technique when used in balance studies with mink. It may also be taken to indicate that any possible metabolic decomposition of the labeled urinary markers did not play any significant role in this experiment. The lower percentage recoveries obtained during feed restriction can be explained by the dramatic reduction in urinary volume in response to cessation of the normal dietary water supplies.

Considering the individual recoveries of the two urinary markers, we observed distinct differences in behavior among individual animals in respect to the deposition of urine and feces in their cage. Therefore, the amount of (labeled) urine and feces collected via the sump during feed restriction was not considered in the present experiments.

**TABLE 1**

<table>
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<tr>
<th>Urinary volume and nitrogen excretion (per 24 h) in fed and feed-restricted female mink (Mustela vison)</th>
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<tr>
<td>Urinary excretion rates</td>
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<tr>
<td>Volume, g/d</td>
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<tr>
<td>Total nitrogen, g/d</td>
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<tr>
<td>Urea, mmol/d</td>
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<tr>
<td>Creatinine, mmol/d</td>
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<td>Total purines, μmol/d</td>
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1 Values are mean ± SEM, $n = 10$, corrected to 100% $^3$H-labeled p-aminohippuric acid ($^3$H-PAH) recovery.
recovered in the daily feces collections turned out to be highly variable and may explain a considerable part of the scatter observed in the daily percentage recoveries of $^3$H-PAH and $^{14}$C-IN among the mink. Furthermore, the missing number of counts, and hence the amount of urine unaccounted for, may be explained by squirting and evaporation of urine and, to some extent, by the quenching effect on liquid scintillation counting of urine label masked by the dark-colored fecal water (Wamberg et al. 1996a and 1996c).

**Conclusions.** The experimental method described in this paper, using implanted osmotic pumps for continuous release of specific urinary markers, to assess the accuracy of quantitative collection of urine in small, strictly carnivorous mammals, was shown to be feasible and highly reproducible. The technique may also be used in experimental studies on renal clearances and water turnover rates in animal species in which permanent catheterization is not easily performed. Finally, 24-h urinary excretion of endogenous creatinine is a poor index of the accuracy of daily urine collection in mink.

**FIGURE 3** Mean ± SEM daily recoveries of $[3]$H-labeled $\alpha$-amino-hippuric acid ($^3$H-PAH ; □) and $[14]$C-labeled inulin ($^{14}$C-IN; ○) in urine (including screen washings and fecal water) of female mink during normal feeding (experimental d 2–5) and during feed restriction (experimental d 6–7). The animals had the osmotic pump implanted on d 0, and no collections were made on d 1. The arrow indicates the onset of feed-restriction. Figure reprinted with permission, Wamberg et al. (1996a).

**LITERATURE CITED**


