Nitrogen balance in adult female mink (*Mustela vison*) in response to normal feeding and short-term fasting

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Ten adult female mink (*Mustela vison*) were studied in a 7 d balance experiment consisting of a 2 d pre-surgery feeding period, followed by surgery, 1 d of recovery, 4 d of *ad libitum* feeding, and a 2 d fasting period. In this experiment (Expt A) the animals had osmotic pumps implanted for continuous release of radioactively-labelled *p*-aminohippuric acid (*p*-aminobenzoyl-2-[^{3}H]glycine; [^{3}H]PAH; *n* 10) and ^{14}C-labelled inulin ([^{14}C]IN; *n* 5). Repeated 24 h collections of urine, corrected to 100 % [^{3}H]PAH or [^{14}C]IN recovery, were used for accurate determination of N balances, 24 h urinary excretion of urea, creatinine, and total N, and calculation of mean 24 h renal clearance rates for endogenous creatinine and inulin. N balances were slightly below zero, but not significantly different between feeding and fasting periods, indicating that correction to 100 % [^{3}H]PAH recovery resulted in slight overestimation of the final balances. During fasting, withdrawal of the dietary water and protein loads resulted in a dramatic decline in 24 h urinary volume, and urea and creatinine excretion. Large individual variations in 24 h urinary creatinine excretion (with relative variation coefficients up to 30 %) confirmed that this is an unreliable index of the completeness of urine collection. In this respect, recovery rates of [^{3}H]PAH proved far more consistent. Renal clearance values obtained in fed mink were in fair agreement with published data from cats, dogs and ferrets (*Mustela putorius furo*). Inulin clearance was about 30 % higher than endogenous creatinine clearance, although its decline in response to fasting was not significant. In a separate study (Expt B) another ten female mink were equipped with osmotic pumps containing [^{3}H]PAH for determination of 24 h excretion rates of purine derivatives. During feeding, allantoin accounted for more than 97 % of the excretion of purine derivatives in urine, uric acid making up less than 2·5 %, xanthine and hypoxanthine less than 1 %. In fasted animals, urinary excretion of each of these purine derivatives declined to less than 50 % of the feeding value. In conclusion, an experimental technique is presented for efficient and accurate measurements of daily urinary excretion of nitrogenous constituents, which allows for correct determination of N balances in adult mink and, presumably, in other mammalian species.

Carnivores: *p*-Amino[^{3}H]hippuric acid: ^{14}C-labelled inulin: N balance: Osmotic pumps

In studies of human and animal nutrition, the balance technique is generally accepted as a useful method for assessing nutritional requirements under well-defined physiological conditions (Fomon & Owen, 1962; Baker, 1986). In the case of N, however, this technique has often led to overestimation of the N balance, mainly due to inaccuracies in quantitative urine collection and, to a lesser extent, to loss of volatile nitrogenous compounds (van Es, 1975; Oddoye & Margen, 1979; Neergaard, 1981; Just *et al.* 1982; Eggum, 1989).

* For reprints.

In carnivores, such as mink (*Mustela vison*), cats and ferrets (*Mustela putorius furo*), this problem is of paramount importance because, owing to the high concentration of nitrogenous substances in the urine, incomplete urine collection will result in considerable experimental errors. Furthermore, these carnivores have the habit of squirting urine and urinating on top of the faeces, which may further limit the accuracy of excreta collection and, hence, balance calculations. Therefore, data derived from N balance studies with carnivores confined in conventional metabolic cages often result in N balances which are substantially higher than justified for the physiological state of the animals studied (mink: Skrede, 1978; Charlet-Lery *et al.* 1980; Glem-Hansen, 1980; Berg *et al.* 1984; ferret: Jarosz & Barabasz, 1988; cat: Miller & Allison, 1958). Taken together with the shortcomings of other experimental methods, this has made determination of the protein requirement of carnivores extremely difficult, and only few reliable data exist, derived mainly from production experiments, for the various life processes (growing cats: Fox *et al.* 1973; growing mink: Skrede, 1978; Työppönen *et al.* 1986, 1987; Børsting & Clausen, 1996; pregnant and lactating cats: Piechota *et al.* 1995; lactating mink: Glem-Hansen, 1979).

In an attempt to quantify the percentage losses of urinary N in balance studies with mink when conventional collection and washing procedures were applied, Elnif (1992) estimated that about 65 % of the total urinary N was retrieved. The losses were mainly due to incompleteness of collection of excreta, while only a minor amount was lost as volatile N, measured in a respiration unit. N retrieval can be further improved by a refined collection and washing technique (A.-H. Tauson, unpublished results). Combining this procedure and the use of osmotic pumps for continuous release and subsequent control of the excretion of labelled urinary markers, Wamberg *et al.* (1996*a*,*b*) found that on average 78 % of the daily excretion of urinary N could be accounted for in the urine collected.

The objectives of the present study were to make use of osmotic pumps, containing radioactively-labelled *p*-aminohippuric acid (*p*-aminobenzoyl-2-[^{3}H]glycine; [^{3}H]PAH) and ^{14}C-labelled inulin ([^{14}C]IN), for accurate determination of quantitative N balances in fed and fasted adult female mink, and to evaluate the effects of feeding and fasting on urinary excretion rates of some N metabolites, including purine derivatives. In addition, preliminary values for mean 24 h renal clearances of inulin (INC) and endogenous creatinine (ENCC) are presented.

MATERIALS AND METHODS

Animals

Expt A. Ten 2-year-old non-pregnant female mink of the pastel colour type, weighing approximately 1100 g were used for measurements of N balances, urinary excretion of N metabolites and changes in nitrogenous plasma constituents during normal feeding and short-term fasting. The females were equipped with implanted osmotic pumps containing [^{3}H]PAH (*n* 10) and [^{14}C]IN (*n* 5; see pp. 85–86).

Expt B. In a separate experiment, blood and urine were collected from another ten adult female pastel mink with implanted osmotic pumps containing [^{3}H]PAH, for the determination of changes in plasma constituents and in 24 h urinary excretion of purine derivatives in response to feeding and fasting.

Housing and feeding

The animals were plasmacytosis-free and appeared healthy when transferred to the laboratory (temperature 14–16°, relative humidity 30–50 %, and a 10 h light–14 h dark cycle) and were confined in individual metabolism cages for a conditioning period of 1 week before the start of the experiment. Once daily they were fed on a conventional wet mink diet with a DM content of 312 g/kg and a crude protein (N × 6·25; CP) content of 177 g/kg (Wamberg *et al.* 1996*d*), and given free access to drinking water throughout the study. The experimental procedure, including feeding, housing and treatment of the animals, blood sampling, and details of the *in vitro* and *in vivo* function of the osmotic pumps, has been described in detail elsewhere (Wamberg *et al.* 1996*a*,*d*).

Balance studies

All animals were studied for two consecutive 24 h pre-experimental collection periods (days −2 and −1; see Fig. 1) after which (day 0) they had a 2 ml osmotic pump (Alzet®, model 2ML1; Alzo Corp., Palo Alto, CA, USA) implanted intraperitoneally during short-term ketamine anaesthesia (Wamberg *et al.* 1996*c*). During the next 24 h the animals were allowed to recover from surgery and, consequently, all collected material was discarded. Another four consecutive 24 h collection periods (days 2–5) were performed during which the animals were given free access to food and water. The feeding period was followed by a 2 d fasting period (drinking water allowed; days 6 and 7). The balance studies, including quantitative collection of excreta, sample preparation etc., were carried out as previously described (Wamberg *et al.* 1996*d*).

Each morning, between 10.00 and 12.00 hours, feed residues and 24 h faecal and urinary excretions were carefully collected, weighed and prepared for analysis or stored at −20° for subsequent analysis. Portions of urine collected on days −2 and −1 were stored separately for the determination of urea, creatinine and purine derivatives.

The experimental procedures used followed Danish National Legislation and the guidelines approved by the member States of the Council of Europe for the protection of vertebrate animals (Anonymous, 1986).

Analytical methods

The DM content of samples of the diet was determined by evaporation at 100° to constant weight. Total N was determined in food, faeces and urine by the micro-Kjeldahl technique using the Tecator-Kjeltec system 1030 (Tecator AB, Höganäs, Sweden). CP was calculated as N × 6·25. In plasma and urine the concentrations of urea were measured by the urease (*EC* 3.5.1.5) method (Hallett & Cook, 1971), those of creatinine by the alkaline picrate method (Chasson *et al.* 1961) and plasma albumin by the bromcresol green method (Doumas *et al.* 1971), using a Technicon® AutoAnalyzer, model RA-1000 (Technicon Instruments Corp., Tarrytown, NY, USA) as previously described (Wamberg *et al.* 1992). Plasma urate was measured by the uricase (*EC* 1.7.3.3) method (Town *et al.* 1985) using the Boehringer Mannheim assay (kit no. MPR 2; Boehringer Mannheim, Mannheim, Germany), and plasma osmolality was determined by means of a vapour pressure osmometer (model 5100B; Wescor, Logan, Utah, USA). The concentrations of the purine derivatives allantoin, uric acid, xanthine and hypoxanthine in urine were measured using the HPLC-technique described by Chen *et al.* (1993). The chemicals used were analytical

grade, purchased from E. Merck, Darmstadt, Germany. All analyses were performed in duplicate and the analytical error was calculated to be less than 4 %.

Radioisotopes

The radioisotopes *p*-aminobenzoyl-2-[^{3}H]glycine (Amersham, code TRA 197, specific activity 520 mCi (19·2 GBq)/mmol) and inulin[^{14}C]carboxylic acid (Amersham, code CFA 399, specific activity 4·92 mCi (182 MBq)/mmol) were obtained from Amersham International Plc, Amersham, Bucks. The radioactivities of [^{3}H]PAH and [^{14}C]IN in plasma and urine were determined by liquid-scintillation spectrometry, using the Mark III Liquid Scintillation System (model 6880; Searle Analytical Inc., Elk Grove Village, IL, USA) as previously described (Wamberg *et al.* 1996*a*,*d*).

Data analysis

Individual samples of urine from each day were analysed separately and the values corrected for inaccuracies inherent in the collecting procedure, using the individual percentage recoveries of [^{3}H]PAH for correction (for details, see Wamberg *et al.* 1996*d*). The 24 h urinary excretion rates of total N, urea, creatinine, uric acid and allantoin were calculated from concentrations in non-acidified urine and in the amount of urine excreted in 24 h corrected to 100 % [^{3}H]PAH recovery.

In the pre-operative period, 24 h urinary excretion rates were corrected to 100 % (mean) post-operative [^{3}H]PAH recovery and, when calculating the renal clearances of inulin, individual percentage recoveries of [^{14}C]IN were used.

Mean 24 h INC and ENCC were calculated according to the conventional definition, $C_x = U_x \times V/P_x$ (Levinsky & Levy, 1973), where U_x is the concentration in urine and P_x the plasma concentration of the substance (X), and V is the (corrected) timed excretion of urine. For the present calculations, it was assumed that 1 g = 1 ml urine (cf. Table 4 and Fig. 1).

Statistics

As indicated by the data presented in a previous study (Wamberg *et al.* 1996*d*) and those given here (Tables 1 and 2, and Fig. 1), the animals recovered to normal behaviour within 24 h after surgery, and no significant adverse effects were observed in any animal during the balance period. Therefore, in the statistical analysis, comparisons were made between the pre- and post-surgery states in fed animals, and between fed and fasted animals post-surgery by means of Student's t test for paired observations (Armitage & Berry, 1994). Throughout the study, statistical significance was set at the 5 % level.

RESULTS

Expt A

Animal performance and N balance. Normal feed consumption was restored when collections started on day 2 (Table 1), and animal live weights remained stable during the feeding period (Table 2). As a response to feed withdrawal, urine excretion decreased dramatically (Fig. 1(a) and Table 1), and the animals began to lose weight (Table 2). The overall mean percentage recovery of [^{3}H]PAH was 77·9 (SE 2.2) % during feeding and 70.3

Table 1. *Expt A. Feed consumption, uncorrected urine excretion, concentrations of urea and creatinine, and some plasma characteristics in fed female mink* (Mustela vison) *before and after implantation of osmotic pumps, and in the same females during fasting*[*]

(Values are period means with their standard errors for ten animals)

	Period						Statistical significance of period differences (paired *t* test): *P*	
	Feeding				Fasting			
	Before operation		After operation					
	Mean	SE	Mean	SE	Mean	SE	Feeding; pre- *v.* post-surgery	Feeding *v.* fasting
Feed consumption (g/d)	175	14·8	160	11·2	0		0·15	
Uncorrected urine excretion (g/d)	63	5·7	72	8·0	18	2·4	0·22	< 0·001
Urinary concentration of								
Urea (mmol/l)	1425	33·7	1401	37·2	866	82·7	0·34	< 0·001
Creatinine (mmol/l)	6·3	0·38	5·9	0·56	14·8	1·48	0·62	< 0·001
Plasma characteristics								
Osmolality (mOsm/kg water)	337	3·4	326	2·8	310	1·2	0·04	< 0·001
Urea (mmol/l)	9·8	1·6	13·9	3·1	4·3	0·3	0·17	< 0·001
Creatinine (μmol/l)	67·2	3·1	88·1	3·7	64·2	2·2	< 0·001	< 0·001
Urate (μmol/l)	125·0	14·9	93·3	8·5	81·0	2·8	0·08	0·24
Albumin (g/l)	39·9	0·69	34·8	0·44	35·8	0·33	< 0·001	0·09

* For details of animals and procedures, see pp. 84–86.

Table 2. *Expt A. Animal live weights, recovery of radioactively-labelled* p-*aminohippuric acid* (p-*aminobenzoyl-2-[*^{3}*H]glycine; [*^{3}*H]PAH;* n *10) and* ^{14}*C-labelled inulin ([*^{14}*C]IN;* n *5) in fed and fasted female mink* (Mustela vison) *with implanted osmotic pumps, and data on nitrogen metabolism, corrected to 100 % [*^{3}*H]PAH recovery* (n *10), and renal clearance data corrected to 100 % [*^{3}*H]PAH recovery* (n *10), or [*^{14}*C]IN recovery* (n *5*)*

(Values are period means with their standard errors)

	Period				Statistical significance of period effects (paired *t* test): *P*
	Feeding		Fasting		
	Mean	SE	Mean	SE	
Live wt (g)	1099	37·6	1063	36·6	< 0·001
Wt change (g/d)	1·3	3·4	−39·7	2·8	< 0·001
Recovery (%)					
[^{3}H]PAH, total	77·9	2·2	70·3	2·0	0·01
[^{14}C]IN, total	79·1	2·4	62·7	2·4	< 0·001
Corrected N metabolism					
Urine excretion (g/d)	93	8·3	25	2·7	< 0·001
N intake (g/d)	4·53	0·40	0		
N excretion in faeces (g/d)	0·86	0·10	0		
N excretion in urine (g/d)	4·01	0·46	0·63	0·04	< 0·001
N excretion, total (g/d)	4·87	0·55	0·63	0·04	< 0·001
N balance (g/d)	− 0·34	0·18	− 0·63	0·04	0·08
24 h urinary excretion					
Urea (mmol)	149·4	14·3	21·6	1·4	< 0·001
Creatinine (mmol)	0·60	0·04	0·36	0·02	0·20
Renal clearance (ml/min per kg live wt$^{0.75}$)					
Creatinine	4·4	0·29	3·8	0·27	0·05
Inulin	6·6	0·52	5·5	0·46	0·20

* For details of animals and procedures, see pp. 84–86.

(SE 2·7) % during fasting. In most animals N balances, corrected to 100 % [^{3}H]PAH recovery, were slightly negative during both feeding and fasting (Table 2), the difference between feeding and fasting periods, however, was not significant ($P = 0·08$).

Urinary excretion of N metabolites. During fasting, the concentration of urea in urine decreased significantly ($P < 0·001$), whereas urinary creatinine concentration increased significantly (Table 1). The rapid response to fasting of 24 h urinary excretion of these substances is shown in Fig. 1. Hence, during feeding the mean values (and their relative standard deviation; RSD) for urinary excretion of urea and creatinine were 149·4 mmol/d (RSD 30·2 %) and 0·60 (RSD 18·7 %) mmol/d, respectively; and during fasting the corresponding 24 h excretion rates amounted to 21·6 mmol/d (RSD 17·0 %) and 0·36 mmol/d (RSD 15·1 %) respectively (Table 2). These changes reflected a marked and significant decrease in urinary excretion of urea ($P < 0·001$), while the decrease in creatinine excretion was not significant ($P = 0·20$).

Plasma constituents. The concentrations of urea and creatinine in blood plasma fluctuated in response to feed intake, with significantly lower concentrations being recorded for fasted animals (Table 1). Plasma urate, on the other hand, remained stable when the animals were fasted (Table 1). Plasma osmolality decreased from the pre-

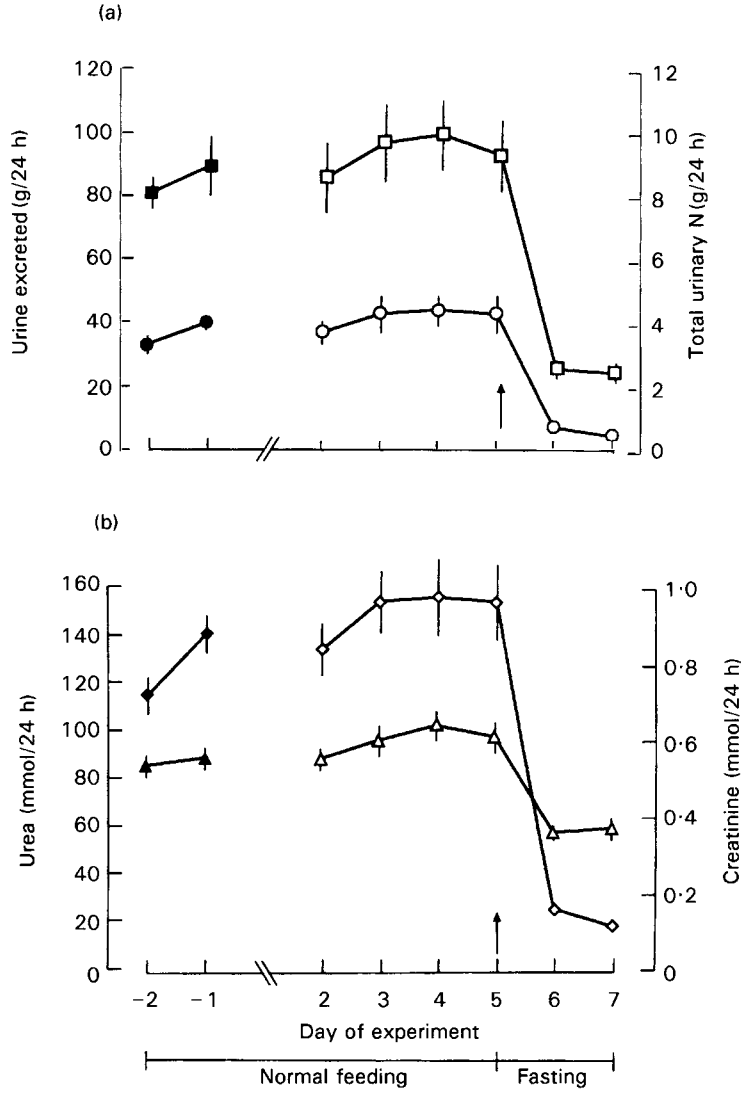

Fig. 1. Expt A. (a) Daily urinary output (□) (g/24 h) and total nitrogen in urine (○) (g/24 h), corrected to 100% radioactively-labelled *p*-aminohippuric acid (*p*-aminobenzoyl-2-[^{3}H]glycine; [^{3}H]PAH) recovery and (b) urinary excretion rates (mmol/24 h) of urea (◇) and creatinine (△), corrected to 100% [^{3}H]PAH recovery, in ten female mink during normal feeding (pre-experimental days −2 and −1 (■, ●, ◆, ▲); and experimental days 2–5) and short-term fasting (days 6–7). ↑, The onset of fasting. Values are means with their standard errors represented by vertical bars. The animals had the osmotic pump implanted on day zero, and no collections were made on day 1. For details of animals and procedures, see pp. 84–86.

operative period to the feeding period, and then further to the fasting period (Table 1), the lowest plasma osmolality being recorded on the second day of fasting. Plasma albumin was higher in the pre-operative period than during the two experimental periods (Table 1).

Renal clearance. Mean 24 h values for ENCC and INC are recorded in Table 2. INC was about 30 % higher than ENCC in the fed as well as in the fasted state. However, during the fasting period, the decrease in INC was not significant ($P = 0{\cdot}20$), whereas ENCC declined to the borderline of significance ($P = 0{\cdot}05$).

Expt B

Purine derivatives. The 24 excretion rates of purine derivatives in urine as well as urinary excretion of urea and creatinine and some plasma characteristics for animals in Expt B are presented in Table 3. During feeding, allantoin and total purine excretion were more than double the amounts excreted during fasting. Allantoin accounted for more than 97 % of total purine excretion in fed animals, and in fasted animals it made up more than 98 %, which turned out to be significantly ($P = 0{\cdot}05$) higher than that in fed animals.

DISCUSSION

Nitrogen balance

Conventional mink diets are largely based on by-products from the fishing industry and abattoirs and, therefore, are rich in protein and other N-containing constituents; their protein content usually exceeds the animals' requirement. The mink has a very short intestine (Kainer, 1954), and a feed passage time of only 4–6 h (Hansen, 1978; Szymeczko & Skrede, 1990). Thus, ingested dietary protein is rapidly digested and absorbed, the excess being metabolized to C fragments used for energy metabolism and to N-containing endproducts which are excreted in the urine. Therefore, in fed animals, the urinary load of N metabolites is high, with urea accounting for the vast majority of osmotically-active substances (Eriksson *et al.* 1984). Moreover, large diurnal variations in plasma concentrations and urinary excretion rates of nitrogenous waste products can be expected in response to feed intake, and fasting is likely to induce profound changes in metabolism. Since the dietary intake of protein usually is more than sufficient to fulfil the animals' requirements, adult animals kept on maintenance level are assumed to be in zero N balance (Owen, 1967), but data in the literature often indicate that positive N balances have been obtained in adult animals (Charlet-Lery *et al.* 1980; Jarosz & Barabasz, 1988; Elnif, 1992). However, as demonstrated by Elnif (1992) the N balances of adult males approach zero after correction for the estimated retrieval rate of urinary N.

In the present study the animals remained at constant body weight during the feeding period, and N balances were slightly negative, indicating that the correction to 100 % [^{3}H]PAH recovery resulted in a slight overestimation, presumably due to accumulated analytical errors. In the fasting period rapid weight loss was accompanied by an increased negative N balance which was of the same order as that for growing mink on a protein-free diet (Berg *et al.* 1984). Hence, the N balance data achieved here, and previous data on electrolyte balances (Wamberg *et al.* 1996*d*), indicate that the technique used is suitable for accurate determination of nutrient balances in small carnivores, and represents a valuable tool in studies of nutrient requirements. However, owing to its invasive character, it may be emphasized that it is likely to be applied on a limited number of animals only, for example when new experimental procedures or measurements are to be evaluated.

Table 3. *Expt B. Plasma albumin, osmolality and concentrations of urea and creatinine and 24 h urinary excretion rates of urea, creatinine and purine derivatives in ten fed or fasted female mink* (Mustela vison), *with urinary excretion rates corrected to 100 % [^{3}H]PAH recovery**

(Values are period means with their standard errors for ten animals)

	Feeding		Fasting		Statistical significance of difference (paired *t* test): *P*
	Mean	SE	Mean	SE	
Plasma data					
Albumin (g/l)	36·7	0·58	35·6	0·65	0·24
Osmolality (mOsm/kg water)	331	1·7	316	2·1	< 0·001
Urea (mmol/l)	13·0	0·78	5·0	0·62	< 0·001
Creatinine (μmol/l)	65·3	2·05	75·5	2·22	0·003
24 h urinary excretion rates					
Corrected urine (g)	75	11·7	34	5·7	0·006
Urea (mmol)	44·4	10·6	20·7	2·3	0·04
Creatinine (mmol)	0·68	0·12	0·46	0·06	0·12
Allantoin (μmol)	189·5	33·6	90·4	9·9	0·01
Uric acid (μmol)	4·0	0·74	1·2	0·12	0·002
Total purines (μmol)	194·5	34·5	91·8	10·0	0·01
Purine derivatives (% of total purine)					
Allantoin	97·2	0·49	98·3	0·20	0·05
Uric acid	2·3	0·38	1·4	0·16	0·05
Xanthine + hypoxanthine	0·6	0·11	0·4	0·04	0·08

[^{3}H]PAH, *p*-aminobenzoyl-2-[^{3}H]glycine (*p*-aminohippuric acid).
* For details of animals and procedures, see pp. 84–86.

Urinary urea and creatinine excretion

As might be expected because of the high dietary N intake, urinary excretion of urea and creatinine was substantial during the feeding period, and the dramatic decrease during fasting reflected the withdrawal of the normal dietary load of nitrogenous constituents. This response is in good agreement with recent observations by Wamberg & Tauson (1996).

As previously discussed, under-collection of urine may be one of the most important sources of error in nutritional studies, and in order to minimize this kind of error, determination of the reproducibility of excretion of suitable endogenous or exogenous substances has been applied. The 24 h urinary excretion of creatinine has, for instance, been used for several years, and in many species, as an internal standard in the evaluation of quantitative urine collection. Unfortunately, urinary excretion of endogenous creatinine has turned out to be highly variable (rats: Kumar *et al.* 1959; dogs: Bartges *et al.* 1994; human subjects: Jackson, 1966; Scott & Hurley, 1968), depending not only on the rate of glomerular filtration (GFR) but also on the amount of dietary protein intake (mink: Wamberg & Tauson, 1996; Wamberg *et al.* 1996*b*) and on the cooking (Jacobsen *et al.* 1979; Watson *et al.* 1981) of dietary meat. The wide range in creatinine excretion (expressed as a percentage of the group mean) found in the present investigation confirms the views of Edwards *et al.* (1969) and Bingham & Cummings (1983) that urinary creatinine excretion is unreliable in control of the completeness of urine collection. From our own data it is obvious that recovery rates of continuously delivered [^{3}H]PAH are far

more reliable. In human studies, oral administration of a suitable urinary marker may be used to solve the problem of incomplete collection of urine (Bingham & Cummings, 1983).

Finally, the plasma concentrations of urate obtained here were rather high when compared with the levels found in cats (42 (SE 8) μmol/l; Zhang *et al.* 1994) and in rats (49 (SE 5) μmol/l; Brulé *et al.* 1988), which may be due to different sources of dietary protein. In our study there were no apparent differences between plasma values obtained in post-operative animals and animals fasted for 1 or 2 d.

Response to fasting

The response to withdrawal of the feed was rapid, and reflected by a dramatic fall in the renal excretion of water, total urinary N, urea and creatinine. A similar response in urinary water and electrolyte excretion was demonstrated by Wamberg *et al.* (1995, 1996*d*). The slight increase in the negative N balance, as demonstrated for the fasting period, could hardly be explained by extensive breakdown of muscular tissue or liver protein, since the fasting period lasted for only 2 d. This interpretation is supported by the decreased excretion of urea and creatinine in urine.

The diurnal fluctuations in plasma osmolality and plasma concentrations of N-containing metabolites are caused by several factors. The most important of these is the rate of urinary excretion, which depends on the functional status of the kidney. Other influences are dietary composition, episodes of feeding and fasting, gastrointestinal absorption, storage, synthesis and/or metabolic degradation. Thus, the decrease in plasma protein in the post-operative and the fasting periods results from a lower rate of net protein synthesis in the liver. In carnivores, plasma nitrogenous constituents are likely to fluctuate considerably in response to feeding, as demonstrated for dogs (Watson *et al.* 1981). For mink, Wamberg & Tauson (1996) demonstrated that both plasma urea and creatinine were markedly influenced by feeding and the time elapsing from the last meal to blood sampling. The present results concur with this concept by demonstrating a profound decrease in plasma urea and creatinine in fasted animals, which was reflected by a significant decrease in plasma osmolality on the second day of fasting.

Renal clearances

As a response to dietary protein loading a temporary increase in renal blood flow and GFR can be expected (Watson *et al.* 1981). Moreover, the rate of formation of metabolites will increase, and together with the change in GFR, this will lead to increased rates of urinary solute and water excretion. In the present study, this was reflected in relatively large variations in the plasma concentrations of urea and creatinine during feeding. Since ENCC and INC were based on a single blood sample obtained for each 24 h period, the calculated mean values for ENCC and INC presented here should only be taken as rough estimates of the mean 24 h value for GFR. Despite this fact, the mean 24 h clearance values obtained in the present study are in accordance with the clearance data reported in the literature for endogenous creatinine in fed (awake) animals as well as for exogenous creatinine, inulin or radioactively-labelled substances in fasted, anaesthetized cats, ferrets and mink (Table 4). Comparable values for 24 h ENCC are found in awake dogs (mean 3·7 (SE 0·13) ml/min per kg; Bovée & Joyce, 1979) and in dogs fed on diets containing varied amounts of protein (range 2·2–3·3 ml/min per kg; Bartges *et al.* 1996). The unexpectedly small decrease in the

Table 4. *Literature data on estimated glomerular filtration rates (GFR) in cats, ferrets* (Mustela putorius furo) *and mink* (Mustela vison)

Author(s)	Animal species	*n*	Estimated GFR (ml/min per kg) Mean	SE	Clearance technique	Anaesthesia and comments
Ross & Finco (1981)	Cats	11	2·94	0·10	EXCC	Pentobarbital
	Cats	8	3·51	0·21	INC	Pentobarbital
Russo *et al.* (1986)	Cats	12	2·31	0·14	ENCC	Awake, 24 h urine collection
Rogers *et al.* (1991)	Cats	6	2·56	0·25	EXCC	Halothane
			3·07	0·31	INC	Halothane
			2·60	0·29	^{99m}TcDTPA	Halothane
Adams *et al.* (1991)	Cats	6	2·15	0·12	INC	Halothane
Kim *et al.* (1992)	Cats	29	2·96	0·15	INC	Thiopentotal
Esteves *et al.* (1994)	Ferrets	26	2·55	0·21	ENCC	Awake, 24 h urine collection
	Ferrets	12	3·32	0·46	EXCC	Isoflurane (50 ml/l)
	Ferrets	12	3·02	0·27	INC	Isoflurane (50 ml/l)
Müller-Peddinghaus *et al.* (1979)	Mink, female	3	6·5	1·0	^{51}CrEDTA	Xylazin + ketamin
Present study	Mink, female	10	4·37	0·25	ENCC	Awake, fed animals
			3·80	0·35	ENCC	Awake, fasted animals
	Mink, female	5	6·64	0·43	INC	Awake, fed animals
			5·51	0·61	INC	Awake, fasted animals

EXCC, Exogenous creatinine clearance; INC, inulin clearance; ENCC, endogenous creatinine clearance; DTPA, diethylene-triaminepenta-acetic acid.

mean 24 h INC in response to fasting observed in our study can be explained mainly by the calculations being based on a single blood sample for each 24 h period.

Purine derivatives

Data on excretion of purine derivatives have, to our knowledge, not previously been reported for mink. The present results indicate that allantoin is the major route of excretion, that uric acid makes up only a minor part, and that fasting resulted in a marked reduction in urinary excretion of total purine derivatives to less than half the level observed during feeding, and that the relative importance of allantoin increased.

Conclusions

The results of the present study show that the use of implanted osmotic pumps for continuous release of a urinary marker permits reproducible and accurate determination of total urine excretion, and calculation of correct N balances in experimental animals. Moreover, they demonstrate profound differences in urinary excretion of N metabolites between fed and fasted animals, and that the response to fasting is rapid. Allantoin was shown to be the major route for excretion of purine derivatives. INC and ENCC were of the same order of magnitude as those for other carnivores. In both cases, however, the decrease in response to short-term fasting was less than expected.

Finally, the use of implanted osmotic pumps has proved a valuable tool for the accurate determination of nutrient balances in small carnivores kept under well-defined experimental conditions. In a broader perspective, the results of the present investigation

emphasize the importance of adequate control of quantitative urine collection in nutritional, pharmacological or toxicological studies in all mammalian species, including human subjects.

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