Faeces is a reliable source of body water for measuring tritium in reindeer in summer and in winter

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Abstract: Rates of equilibration and subsequent wash-out of tritium were measured in parallel samples of blood, rumen fluid and faeces collected from two adult female Norwegian reindeer in summer and in winter. The tritium-concentration was the same in all three body water compartments after no more than 9 h following both intravenous and intraruminal injection of isotope in summer and following intravenous injection of isotope in winter. The biological half-life of the tritium increased from approximately 3 days in summer to approximately 10 days in winter, probably as a consequence of a decrease in water intake. There were no significant differences in disappearance rates of tritium from blood, rumen fluid and faeces within any of the six experiments. Fresh faeces is therefore a reliable source of body water that can be used in place of blood in studies of body water kinetics in reindeer, thus making it potentially possible to conduct such studies on truly free-living and undisturbed animals.

Key words: plasma, rumen fluid, isotope markers, water turnover, seasonal physiology

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Introduction

Isotope dilution methods have been used to measure a variety of physiological parameters in reindeer including total body water and body water turnover rate (r_{H2O}) (e.g. Cameron & Luick, 1972; Holleman *et al.*, 1982; Larsen & Blix, 1985) which can, in turn, be used to estimate body composition (e.g. Searle, 1970; Arnold *et al.*, 1985; Sheng & Huggins, 1979; Rozeboom *et al.*, 1994) and to calculate the rate of CO_2-production by the doubly labelled water method (Lifson & McClintock, 1966). In all cases, a known quantity of one or more water soluble markers is injected into the experimental animal after which body water parameters can be calculated by measuring the subsequent decline in the concentration of the marker(s) in body water resulting from their wash-out. Blood or urine have most commonly been used as the source of body water in which the concentration of marker(s) is measured but this precludes working with truly undisturbed animals because the collection of such samples usually requires restraint of the experimental animal. The aim of this study was to investigate whether water extracted from samples of fresh faeces can reliably be used in place of blood samples in studies of body water parameters in rein-

deer. Faeces is an alternative source of body water which offers the potential advantage that it can be collected from an experimental animal without disturbing it.

Methods

Experimental procedures

Two adult (>4 years old) female Norwegian reindeer *(Rangifer tarandus tarandus)*, reindeer A and B, of approximately 85 kg body mass (BM), were each equipped with a permanent rumen cannula and accustomed to restraint and handling over several years prior to this study. The animals were kept in separate outdoor paddocks (approximately 120 m^2) at the Department of Arctic Biology at the University of Tromsø where they were provided with a commercially available pelleted ration (Bøe and Jacobsen, 1981), snow or water *ad libitum* and a salt lick (98% NaCl). One day before the start of each experiment the animals were put into separate small (4 m^2) roofed outdoor pens where they were kept for up to 12 days while the experiments lasted, still with *ad lib.* access to water or snow, food and a salt lick.

Any potentially deleterious effects of this confinement were evaluated by comparing daily voluntary food intake (VFI, summer and winter, $g \cdot kg^{-1}$ $BM \cdot day^{-1}$) and water intake (summer only, $l \cdot day^{-1}$) during each experiment with values recorded during the preceeding 10 days. Values of VFI or water intake during the experimental period that were ≤ the fifth percentile of VFI or water intake in the preceeding control period were considered significantly different.

Three experiments were conducted in each animal; one winter experiment (March), lasting 10 days, in which tritiated water (^3H_2O) was injected intravenously (I.V.), and two summer experiments (July and August), both lasting 6 days, in which ^3H_2O was injected I.V. and intraruminally (I.R.), respectively.

Prior to injection of ^3H_2O, a sample was collected from each of three body water compartments (blood plasma, rumen fluid and faeces) from which the background levels of tritium (^3H) were determined. Blood samples (10 ml) were collected either via an indwelling catheter in the right jugular vein or by jugular venipuncture using Vacutainer® tubes (Beckton Dickinson Vacutainer Systems Europe, France). Rumen fluid samples (approximately 20 ml) were collected by aspiration via the rumen cannulae. Faeces samples were collected opportunistically from the ground no more than 30 seconds after dropping.

For I.V. injection of ^3H_2O, a 50 cm long silicone tube leading from a 50 ml syringe containing physiological saline was connected to a catheter inserted into the left jugular vein. ^3H_2O was injected into the lumen of the tube which was then flushed with saline before the needle was withdrawn, thus ensuring complete administration of isotope. For I.R. injection of ^3H_2O, a 50 ml syringe containing physiological saline was connected to a silicone tube which passed through a cork which closed the rumen cannula. ^3H_2O was injected into the lumen of the silicone tube which was then flushed with saline in the manner described above.

The exact amount of ^3H_2O injected (dose: 350–400 μCi) was determined by weighing the syringe and needle containing the dose solution to 0.001 g before and after injection.

Following injection, samples of blood, rumen fluid and faeces were collected as previously described according to the following schedule: blood and rumen fluid samples were collected in parallel every 10 min. for the first 2 h, decreasing to once every 120 min. at 5–25 h post-injection, and then once a day for the remainder of each experiment. Faeces samples were collected approximately every 2 to 3 hours for 25 h after injection of isotope and then once daily. Samples were stored on ice immediately after collection. Blood and rumen fluid samples were centrifuged (1600 g, 15 min.) within 1 h, and the supernatants separated. All samples were stored at -20°C for later processing.

Water was extracted from samples of plasma (2 ml), rumen fluid supernatant (2 ml) and faeces (2 g) by the vacuum sublimation method of Midwood (1990). The extraction efficiency of the method was tested by weighing samples to 0.001g before and again after vacuum sublimation, and again after drying at approximately 60°C for at least 24 h. On average, 99.75% (SEM 0.08, n = 32) of the total amount of water was removed from the samples by vacuum sublimation (Gotaas, 1992). Extracted water was stored in 2 ml ctyo-tubes (Greiner Labortechnik, Germany) at –20°C.

Analyses

Samples of water (approximately 0.5 g) taken from diluted stock solution or recovered from biological material were weighed to 0.0001 g and added to 10

ml of liquid scintillation fluid (InstaGelll®, Hewlett Packard). ^3H activity was measured using a 3375 Tri-Carb Liquid Scintillation Spectrometer (Packard Instrument Company Inc., IL, U.S.A.) set to count for 20 minutes or to a total of 20 000 counts. All measurements were corrected for background level, quenching and counting efficiency and converted from counts per minute (CPM) to disintegrations per minute (DPM).

Calculations

The concentration of ^3H_2O in body water will normally decrease exponentially after injection as the isotope is washed out of the body owing to the animal drinking and excreting water. The ^3H-activity in serial samples is therefore described by the equation $S_t=S_0 \cdot e^{-k_T t}$, where S_t is the specific ^3H-activity in body water ($DPM \cdot g^{-1}$) at a time t after injection of ^3H_2O, S_0 is the specific ^3H-activity in body water ($DPM \cdot g^{-1}$) immediately after injection (assuming equilibration to be instantaneous), and k_T is the fractional turnover of ^3H. From this equation the biological half-life of ^3H, $t_{1/2}$, can be calculated as $t_{1/2}=(1/k_T) \cdot ln\ 2$.

Equilibration times and wash-out rates of isotope were calculated for all three body water compartments using linear regression models (least squares method) after transforming data (^3H-concentrations) to natural logarithms and reformulating the initial equation ($S_t=S_0 \cdot e^{-k_T t}$) as $ln\ (S_t)=ln(S_0) - k_T \cdot t$.

Equilibration time

A variety of methods have been applied to determine the time required for injected isotopes to become equilibrated with body water (e.g. Searle, 1970; Smith & Sykes, 1974; Fancy *et al* , 1986; Midwood, 1990). In this study, the time required for equilibration of injected ^3H_2O was determined by linear regression analysis. A series of analyses was performed in which the concentration of ^3H (independent variate) was related to the time of sample collection (dependent variate) for each body water source. The linear regression models initially included only samples collected 20–24 h after injection but after this each model was expanded by including data from samples collected progressively closer to the time of injection. The first regression model in each series was thus based on just three data points while the last model was based on up to ten data points. Sets of regression coefficients from each series of models derived for each body water compartment were plotted against an arbitrary linear scale in which 1 = the first model, 2 = the second model, etc. The point of inflection of the resulting curve, determined by inspection, was taken as the point of equilibration.

Pattern of wash-out of 3H from different body water compartments

The pattern of wash-out of ^3H from different body water compartments after equilibration was compared by the method of Bland & Altman (1986). The difference in the concentration of ^3H within a pair of parallel samples (D_{ps}) was plotted against the mean concentration of that pair. The significance of each successive D_{ps} value was then determined after calculating what Bland & Altman (1986) called 'the bias', which is given by the mean and the standard deviation (SD) of all the D_{ps} values. D_{ps} values that fell within 2SD were not considered significant. Outliers, detected by this method, were rejected from further analysis.

Rate of wash-out of marker

The disappearance rate of ^3H, k_T, was calculated for each body water compartment within each experiment by regressing the natural logarithm of concentration of ^3H against time after injection. After excluding outliers, all samples collected 23–140 h post-injection in summer experiments and 23–230 h post-injection in winter experiments were included in these regressions. Potential differences in k_T between different body water compartments within each experiment were examined by comparing the slope and intercept of pairs of regression lines (i.e. plasma vs. rumen fluid, plasma vs. faeces and rumen fluid vs. faeces) using t-tests.

Results

Effect of experimental procedures on food intake and water intake

The animals remained calm during all the experiments. In three of six experiments there was a significant reduction in VFI after the animals were put in their small pens. The mean net reductions in VFI were 37.3% over 5 days in the winter I.V. experiment with reindeer A, 41.7% over 2 days in the summer I.V. experiment with reindeer A and 32.4% over 4 days in the winter I.V. experiment with reindeer B. Net water intake was reduced by 28.2% over 3 days in the summer I.V. experiment with reindeer A.

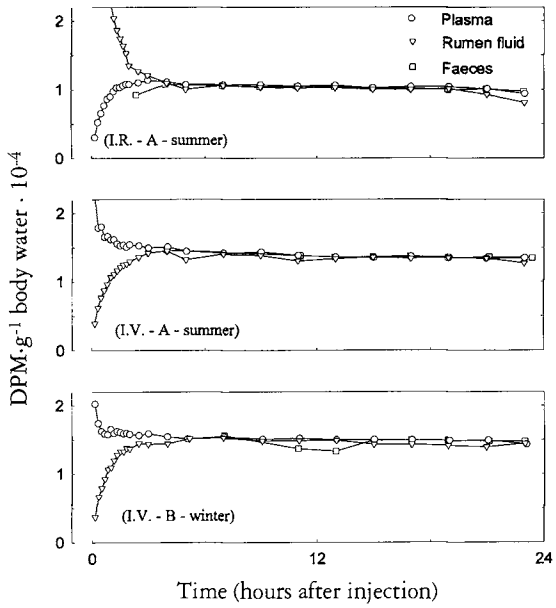

Fig. 1. Concentration of ^3H (DPM·g^{-1} body water) in blood plasma, rumen fluid and faeces over 24 h following intraruminal (I. R., top panel) and intravenous (I. V., middle panel) injection of ^3H_2O in reindeer A in summer and intravenous (I. V., lower panel) injection of ^3H_2O in reindeer B in winter.

Equilibration of ^3H

In all four experiments with I.V. injection of ^3H_2O the plasma concentration of ^3H was highest in the sample collected 10 minutes after injection (Figure 1). Thereafter, the concentration of ^3H in plasma declined rapidly with a concomitant increase in the concentration of ^3H in rumen fluid until the two converged on a common plateau level. A similar pattern was observed following I.R. injection of ^3H_2O (Figure 1). In these two experiments, the concentration of ^3H was initially very high in the rumen fluid but decreased rapidly while the concentration of ^3H in plasma showed a corresponding increase until the concentration of ^3H in these two body water compartments again converged on a common plateau.

The concentration of ^3H in faeces followed a trajectory similar to the ^3H-concentration in rumen fluid following I.V. injection of marker and a trajectory similar to the ^3H-concentration in plasma following I.R. injection (Figure 1). Thus, the pattern of change in the concentration of ^3H in the two 'non-injected' body water compartments always matched closely.

Examination of the plots based on serial regression models showed that in all six experiments ^3H was equilibrated with the body water pool no more than 9 hours after injection.

Pattern of wash-out of ^3H from different body water compartments

On average, less than one in 20 of the D_{ps} values in each comparison in each experiment was significant. Furthermore, we detected no pattern in the localisation of these outliers; they were neither clustered at the beginning nor at the end of the experiments, nor were they more frequent in the summer experiments than in the winter experiments.

Rate of wash-out of ^3H

Inspection of the plots showing the change in ^3H-concentration throughout each experiment showed that there was no difference in the wash-out rate of ^3H from the three different body water compartments (Figure 2). This was confirmed by subsequent analyses; in no case was there any significant difference between regression coefficients from different

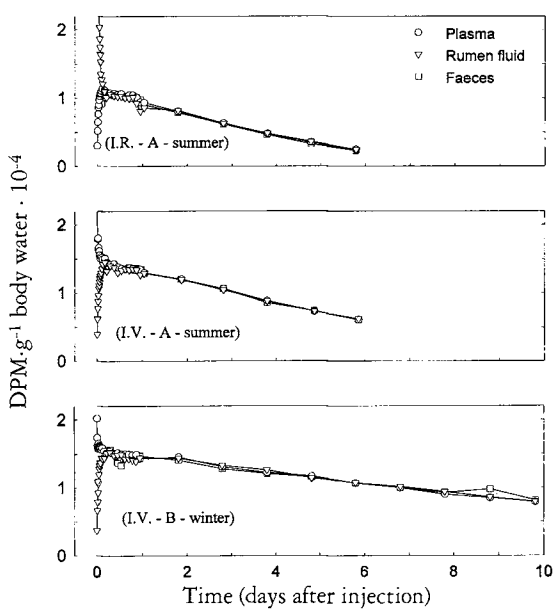

Fig. 2. Concentration of ^3H (DPM·g^{-1} body water) in blood plasma, rumen fluid and faeces throughout the experimental period following intraruminal (I. R., top panel) and intravenous (I. V., middle panel) injection of ^3H_2O in reindeer A in summer and intravenous (I. V., lower panel) injection of ^3H_2O in reindeer B in winter.

Table 1. t-values from comparison of regression models of disappearance rates of ^3H from different body water compartments.

Reindeer	Injection route	Season	Model	Test for parallelism		Test for common intercept		d.f.
A	I.V.	Winter	k_P vs. k_{RF}	0.50	n.s.	1.91	n.s.	14
			k_P vs. k_F	0.72	n.s.	1.26	n.s.	13
			k_{RF} vs. k_F	1.16	n.s.	2.95	*	13
A	I.V.	Summer	k_P vs. k_{RF}	0.93	n.s.	2.00	n.s.	9
			k_P vs. k_F	0.07	n.s.	0.13	n.s.	8
			k_{RF} vs. k_F	0.85	n.s.	1.86	n.s.	9
A	I.R.	Summer	k_P vs. k_{RF}	0.37	n.s.	1.00	n.s.	8
			k_P vs. k_F	0.40	n.s.	0.55	n.s.	9
			k_{RF} vs. k_F	0.70	n.s.	0.62	n.s.	9
B	I.V.	Winter	k_P vs. k_{RF}	0.50	n.s.	0.02	n.s.	16
			k_P vs. k_F	1.41	n.s.	2.47	*	15
			k_{RF} vs. k_F	0.97	n.s.	2.82	*	15
B	I.V.	Summer	k_P vs. k_{RF}	0.81	n.s.	1.88	n.s.	10
			k_P vs. k_F	0.42	n.s.	0.23	n.s.	9
			k_{RF} vs. k_F	0.45	n.s.	2.71	*	9
B	I.R.	Summer	k_P vs. k_{RF}	0.90	n.s.	1.62	n.s.	9
			k_P vs. k_F	1.09	n.s.	0.84	n.s.	10
			k_{RF} vs. k_F	0.05	n.s.	0.96	n.s.	9

k_P, k_{RF} and k_F are regression coefficients based on samples of plasma, rumen fluid and faeces respectively. I.V. and I.R. denote intravenous and intraruminal injection, respectively.
* $p<0.05$

body water compartments within experiments (Test for parallelism, Table 1). Likewise, there was no significant difference in the intercepts of the regression lines within experiments in 14 of 18 comparisons (Test for common intercept, Table 1).

We found substantial seasonal variation in k_T values, illustrated by the increase in biological half-life ($t_{1/2}$) of ^3H from 3.4 ± 0.8 (SD) days, n = 12 body water compartments/experiments in summer, to 10.4 ± 0.6 (SD) days, n = 6 body water compartments/experiments in winter (Table 2).

Discussion

In both animals (BM approximately 85 kg), ^3H equilibrated between blood plasma, rumen fluid and faeces no more than 9 h after injection of ^3H_2O in both summer and winter. Fancy *et al.* (1986) reported equilibration time of ^3H between blood and rumen fluid in excess of 9 h in reindeer in winter. In contrast, equilibration times for ^2H and ^3H have been reported to be 4-6 h in sheep (body mass 20–80 kg) (e.g. Searle, 1970; Midwood, 1990) and 3–5 h in cattle (body mass 219–717 kg) (e.g. Arnold *et al.*, 1985; Martin & Ehle, 1986). The rate of equilibration of these isotopes in the body water pool may thus be species specific and not generally related to body mass in ungulates.

We found no difference in the rate of equilibration following intravenous or intraruminal injection of isotope in our summer experiments. In sheep, by contrast, the rate of equilibration has been reported to vary substantially with route of injection. Smith & Sykes (1974) reported equilibration after approximately 5 h following combined intraruminal and intraperitoneal injection (25% of dose intraruminally and 75% intraperitoneally), approximately 8 h following intravenous injection, and more than 8 h following intraruminal injection of ^3H_2O in sheep. These authors suggested that the slow rate of equilibration following intraruminal injection was due to slow diffusion of water across the wall of the rumen.

Table 2. Biological half-life ($t_{1/2}$) of ^3H in reindeer calculated from regression models based on recovery on isotope from different body water compartments ≥23 h after injection of ^3H_2O.

Reindeer	Injection route	Season	Body water compartment	regression coefficient	SE	r^2	d.f.	$t_{1/2}$ (days)
A	I.V.	Winter	Plasma	–0.06	<0.01	0.99	7	10.75
			Rumen fluid	–0.07	<0.01	0.99	7	10.47
			Faeces	–0.06	<0.01	0.98	6	11.36
A	I.V.	Summer	Plasma	–0.16	0.01	0.99	4	4.32
			Rumen fluid	–0.15	0.01	0.99	5	4.63
			Faeces	–0.16	0.01	0.99	4	4.34
A	I.R.	Summer	Plasma	–0.28	0.01	0.99	4	2.48
			Rumen fluid	–0.27	0.02	0.97	4	2.58
			Faeces	–0.29	0.01	0.99	5	2.41
B	I.V.	Winter	Plasma	–0.07	<0.01	0.99	8	9.96
			Rumen fluid	–0.07	<0.01	0.99	8	9.91
			Faeces	–0.07	<0.01	1.00	7	10.34
B	I.V.	Summer	Plasma	–0.19	0.01	0.98	5	3.62
			Rumen fluid	–0.18	0.01	0.99	5	3.89
			Faeces	–0.18	0.01	0.99	4	3.76
B	I.R.	Summer	Plasma	–0.25	0.02	0.98	5	2.77
			Rumen fluid	–0.23	0.02	0.98	4	3.05
			Faeces	–0.23	0.01	0.98	5	3.07

I.V. and I.R. denote intravenous and intraruminal injection, respectively.

Engelhardt (1970), however, showed that water diffuses very rapidly across the rumen wall. Midwood (1990), likewise, found no difference in the rate of equilibration of ^2H and ^{18}O following oral and intravenous administration in experiments with sheep; in both cases equilibrium was established after approximately 6 h. In contrast both to the present study and the study by Midwood (1990), Smith & Sykes (1974) denied their animals access to food and water for 16 h prior to their experiments. Slow rate of equilibration of isotope between blood and rumen liquor may, therefore, have been partly due to reduced rate of salivation caused by fasting (Church, 1983; Robertshaw, 1982). Similarly, fasting is known to reduce the rate of exchange of water across the wall of the reticulo-rumen (Willes *et al.*, 1970).

The rate of equilibration appeared independent of season, as shown by the fact that there was no difference in rates of equilibration of ^3H between the summer and winter experiments in which ^3H_2O had been injected intravenously. This indicates that the diffusion and osmotic and hydrostatic gradients which drive the exchange of water between different body water compartments are of equal magnitude both summer and winter.

The water contained in the lumen of the gastrointestinal tract (i.e. water in faeces) appears to participate in the rapid exchange of water between body water compartments. This is shown by the fact that the change in the concentration of ^3H in water extracted from both faeces and rumen fluid followed virtually identical trajectories during the equilibration process following I.V. injection of ^3H_2O and, likewise, the pattern of equilibration of ^3H was similar in both faeces and blood plasma following I.R. injection of ^3H_2O.

There was no significant difference between rate constants (k_T) based on analyses of the concentration of ^3H in water extracted from blood plasma, rumen fluid and faeces within experiments. Likewise, Martin & Ehle (1986) found no difference in the slopes of ^2H_2O dilution curves based on parallel samples of blood, milk, urine and faeces taken from cattle. These authors reported that ruminal samples showed no consistent pattern, a fact they ascribed to

difficulties in obtaining representative samples of rumen fluid. Similarly, we found that rumen fluid was involved in three of the four instances in which the intercepts of two regression models differed significantly. The irregularity of the k_T-values calculated from rumen fluid samples compared to other body water compartments suggests that errors are probably caused by the difficulty of withdrawing representative samples from the rumen, rather than slow equilibration of water across the rumen wall.

Values of k_T more than doubled from summer to winter, probably reflecting the increased water intake in summer. Consequently, the biological half-life of ^3H was shorter in summer (3.4 days) compared to winter (10.4 days). These values correspond well with other studies on reindeer in which $t_{1/2}$ is reported as approximately 3 days in summer and approximately 11 days in winter (Fancy *et al.*, 1986), and approximately 4 days in summer and approximately 14 days in winter (Larsen & Blix, 1985).

VFI was significantly reduced for a short period in three of the six experiments and it is probable therefore, that the animals' water intake, too, was reduced because water flux is linearly related to food intake in reindeer (Larsen & Blix, 1985). Even though such a reduction in water flux may have influenced the calculated disappearance rates of ^3H, it is unlikely to have had any significant effect on the primary findings presented here because water exchanges rapidly between different body water compartments, and a reduced water flux would be reflected equally well in all of them.

While it is obviously advisable to collect faeces samples as quickly as possible after dropping, even this is apparently not critical. In a series of subsequent experiments using ^2H and ^{18}O there was no significant difference in the concentration of these isotopes between samples collected immediately after dropping and those left exposed outdoors for 30 minutes (G. Gotaas & N.J.C. Tyler, unpublished data).

Our results show that faeces is a reliable source of body water for measuring tritium in reindeer in both summer and winter, and the method described here can potentially be used to study body water kinetics on truly free-living and undisturbed reindeer. The number of samples that it is practicable to collect after the initial equilibration will obviously vary from situation to situation in field studies. We advise, however, that studies using this technique should be based on no less than four post-equilibrium samples collected over a period equivalent to two to three biological half-lives of the isotope, and that the first post-equilibrium sample should be collected not earlier than 9 h after injection of tritium.

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