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Movement of Water within the Body of Sheep fed at Maintenance under Thermoneutral Conditions

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Abstract

Two mature Border Leicester × Merino wethers were maintained with continuous feeding under thermoneutral conditions. Their water balance was recorded for 2 weeks; at the beginning of each week they were given a dose of tritiated water (TOH) into the pulmonary artery or the rumen and samples were taken from both the pulmonary artery and the rumen. A four-compartment model was developed which simultaneously fitted the balance and tracer data.

The half-time of TOH in body water was 6·7 days for one sheep and 7·6 days for the other; TOH space was about 55% of liveweight in both sheep. The bidirectional flux of water between the plasma-accessible compartment and 'bound' intracellular water averaged 45 litres per hour and that between plasma-accessible water and rumen water averaged 3·2 litres per hour. The mean residence times of a water molecule in the rumen were, for the two sheep, 60 and 63 min and the time constants for flow from the rumen were 12·3 and 13·4 h. Consideration of the rumen water balance suggested that there was net movement of water from the rumen to the plasma at about 200 ml/h; diffusion accounted for 86% of the influx to and 92% of the efflux from the rumen.

Introduction

The survival and productivity of ruminants depends in part upon their ability to maintain their body water content and its distribution within relatively narrow limits. Thus the mechanisms by which this is achieved in the face of environmental and physiological stresses are of particular interest. Water labelled with deuterium has been used to study movement of water between blood and tissues (Edelman 1952), and both labelled water and various solutes have been used to study water movement into and out of the rumen (Engelhardt 1970). In addition, labelled water has been widely used to estimate whole-body water content (Searle 1970; Searle *et al.* 1972; Smith and Sykes 1974) and its turnover rate (Richmond *et al.* 1962; MacFarlane and Howard 1972) in many species. However, changes in solute spaces do not necessarily reflect changes in water movements and many of the experimental conditions listed by Engelhardt (1970) may themselves affect the parameters to be measured.

Edelman (1952) resolved curves describing the equilibration of deuterium oxide, given intravenously to dogs and humans, into two exponential components. However, compartmental analysis of such tracer data (Berman and Weiss 1978), which provides a method for the study of processes within the body, does not appear to have been applied to body water. This paper reports the development of a compartmental model to describe concurrently the distribution of tritiated water (TOH) and water balance in sheep maintaining their liveweight under thermoneutral conditions. The model is

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intended to provide a basis for studies of environmental and physiological situations known to cause perturbations of water status.

Methods

Animals and Diet

Two mature Border Leicester × Merino wethers were used; each animal was fitted with a rumen cannula and an abomasal cannula (near the pylorus). They were held in metabolism cages indoors with continuous lighting. The mean daily maximum and minimum temperatures in the sheep room during the experiment were, respectively, 26·3 and 21·8°C.

The diet was a pelleted mixture of three parts lucerne hay and two parts oats (930 g organic matter and 24·1 g nitrogen per kilogram dry matter). It was given in a single meal of 709 g dry matter each day; beginning 8 days before the first injection of TOH, it was given continuously by means of a moving belt. Water was available *ad libitum*.

Experimental

On the day before the first injection, the sheep were weighed and a polyvinyl chloride catheter (1·5 mm o.d., 1·00 mm i.d.; Dural Plastics and Engineering, Dural, N.S.W.) was inserted into the pulmonary artery of each sheep via an external jugular vein, using the characteristic pattern of pulse pressure as an index of location. The catheters were filled with sterile saline containing 200 units heparin per millilitre. Next day (day 1) a dose of TOH (56 MBq in sterile saline) was injected into the pulmonary artery of sheep 92 and the rumen of sheep 28 and samples of blood and rumen fluid were taken; 22 samples of blood and 17 of rumen fluid were taken at increasing intervals on day 1, two samples were taken on days 2 and 3 and one on days 4–8. After sampling on day 8, sheep 92 was given a similar injection into the rumen and sheep 28 an injection into the pulmonary artery and samples were taken at the same intervals as for the first injection. The intake of feed and water and the output of faeces and urine were recorded from day 1 to day 15 and the sheep were reweighed on days 3, 7, 10 and 14.

For each sheep, a bulk sample of faeces and urine was prepared for each period (injection–sampling run) by compositing 20% of the daily faecal output and 2% of the daily urine production; a portion of the faecal sample was macerated with water and stored with the bulk samples at −10°C. Blood samples were taken into dry syringes and transferred immediately into pre-cooled, stoppered tubes containing dry heparin. The tubes were held in an ice-bath and plasma was separated by centrifugation as soon as possible; it was then stored at −10°C. Rumen fluid was obtained by straining through Terylene cloth the digesta collected by repeatedly thrusting an open-ended Perspex tube (10 mm i.d.) into the rumen; the fluid was stored at −10°C.

Analyses

The water content of feed, faeces, urine, rumen fluid and blood plasma was determined by drying for 24 h at 105°C. The radioactivity in 0·2 ml samples of rumen fluid and of blood plasma was assayed as described by Searle (1970); counting efficiency was determined by means of an internal standard. Specific radioactivities were expressed as a fraction of the dose per litre of water. Other methods of analysis were those used previously (Faichney 1972).

Calculations

Metabolic water production in the tissues was calculated from the intake of digestible crude protein and digestible non-protein organic matter (assuming that 8% of the energy intake would appear as methane as a consequence of the fermentation of carbohydrate) on the basis that, during metabolism, 396 mg of water per gram of protein and 556 mg water per gram of non-protein organic matter would be produced (Schmidt-Neilsen 1964).

In order to fit a model to the data, the interactive computer modelling program CONSAM* (Boston *et al.* 1981) was used. The operational units, data entry format and computational procedures used in this program are the same as those of the batch version of the program, SAAM27, and details are presented in the SAAM Manual (Berman and Weiss 1978).

There are a number of features of the SAAM system which facilitate the development of a kinetic model such as that presented here. First, the system of equations (in this case linear, first-order differential) defining

*Copies of this program may be obtained from Dr R. C. Boston, School of Agriculture, La Trobe University, Bundoora, Vic. 3083.

the movement of TOH in the sheep, together with the connective pathway for that transport, is established simply by implication (i.e. by reference to fractional turnover rates) rather than by actual specification. Secondly, using the inverse of the errors of adjustable parameters as weights, the program can automatically adjust the model to fit the data. Thirdly, the program facilitates the addition of statistically weighted data at the normal equation level. (These features of the SAAM system are discussed in detail by Foster and Boston 1983.) Thus it was possible to optimize jointly the model for both kinetic and balance (steady state) measures, which latter included (i) rate of water intake (both as drinking, feed and metabolic water), (ii) faecal excretion of water, (iii) urinary excretion and evaporative water loss (iv) total body water, (v) flow of water from the rumen and (vi) rumen water volume, assigning weights to temporal regions of the kinetic data and to the steady-state observations according to the authors' confidence in their reliability.

Results

The mean liveweight, the digestibilities of dry matter and the water balance data for each sheep are presented in Table 1. There were only small variations between sheep and between periods.

Table 1. Mean liveweight, dry matter digestibility and water balance for two sheep during measurements of water movement

Parameter	Sheep 92		Sheep 28		
	Period 1	Period 2	Period 1	Period 2	
Mean liveweight (kg)	49·9	49·2	51·4	50·6	
Dry matter digestibility	0·73	0·72	0·71	0·73	
Water balance (ml/day):					
Metabolic water	206	206	206	206	U(2)[A]
Feed water intake	91	91	91	91	U(3)
Drinking water intake[B]	2371	2123	2709	2531	U(3)
	(304)	(146)	(378)	(270)	U(3)
Faecal output[B]	191	196	209	188	R(0,4)
	(21)	(12)	(15)	(7)	R(0,4)
Urine output[B]	1322	961	1629	1399	R(0,1)
	(208)	(52)	(181)	(196)	R(0,1)
Balance[C]	1155	1263	1168	1241	R(0,1)

[A] See Fig. 2. [B] Seven-day mean; standard error in parentheses. [C] Assumed equivalent to evaporative loss.

The plasma response of sheep 92 to a plasma dose of TOH is shown in Fig. 1. A curve consisting of the sum of three exponential terms was required to fit these data;

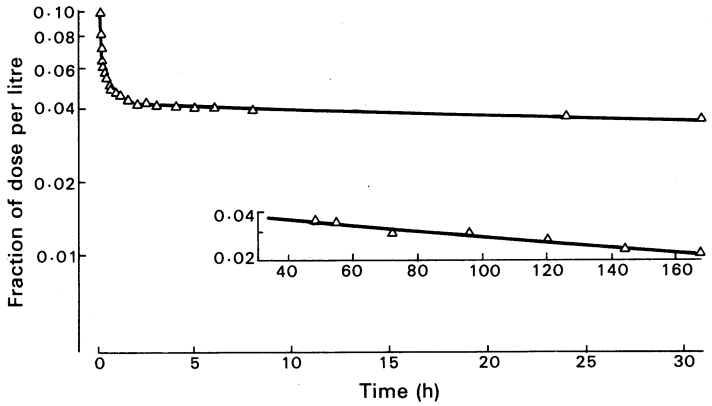

Fig. 1. Specific activity of plasma water following an intra-arterial dose of TOH to sheep 92. The line of the equation described in Table 2 is shown.

the curve is shown in Fig. 1 and the coefficients of the exponential terms are given in Table 2. The time constants of the three components were 3·5 min, 36 min and 10·7 days.

Table 2. Coefficients of the multi-exponential equation fitted to the plasma water specific radioactivity R (as fraction of dose per litre of plasma water) in sheep 92 following an intra-arterial dose of TOH

Values in parentheses are the exponents of 10 by which the value of the coefficients in the multi-exponential equation $R = \sum_{i=1}^{3} A_i \exp(-k_i t)$ are to be multiplied

Term	k (h^{-1})	Standard error	A (l^{-1})	Standard error
i = 1				
=	1·69(1)	2·2	6·07(−2)	4·8(−3)
2	1·66	2·1(−1)	2·33(−2)	2·3(−3)
3	3·88(−3)	9·6(−5)	4·14(−2)	3·0(−3)

The TOH half-times ($T_{½}$) for each sheep were calculated from the decline in plasma radioactivity from day 2 to day 8 (period 1) and from day 9 to day 15 (period 2). The values are given in Table 3 together with those for body water calculated as (water input × $T_{½}$/ln 2). Body water space was similar for both sheep, averaging about 55% of liveweight.

Table 3. Body water space in two sheep calculated from their total water input and the half-time of TOH in their body water

Sheep	Period	Water input[A] (litres/day)	TOH half-time (days)	Body water (litres)
91	1	2·67	7·6	29·3
	2	2·42	7·5	26·2
28	1	3·01	6·6	28·6
	2	2·83	6·7	27·3

[A] Intake in feed and by drinking plus estimate of metabolic water.

When a three-pool model was fitted to the data obtained following the plasma dose of TOH to sheep 92, the rumen response was accommodated best as the slower turnover compartment of a mammillary system with a central plasma-accessible compartment and a fast-turnover compartment that was considered to be a component of tissue water. However, the data describing the rumen disappearance of TOH after the rumen dose was not well described by this three-pool model. When a fourth pool (notionally the post-ruminal gastro-intestinal tract) was included, exchanging with the plasma-accessible pool and providing passage of water out of the system (Fig. 2), good fits to both data sets were obtained.

In addition to fitting the kinetic data, the model was required to be consistent with the balance data obtained during the sampling period (Table 1), i.e. U(2), U(3), R(0,1) and R(0,4), and with rumen water volume, M(3), and outflow, R(4,3), from similar sheep under similar conditions (G. J. Faichney and G. A. White, unpublished data). Direct estimates were also available for the rate of irreversible loss from

compartment 1, R(0,1), and the rate of irreversible loss from the whole system, {[U(2) + U(3)]/[M(1) + M(2) + M(3) + M(4)]}. Initial estimates of the size of the plasma-

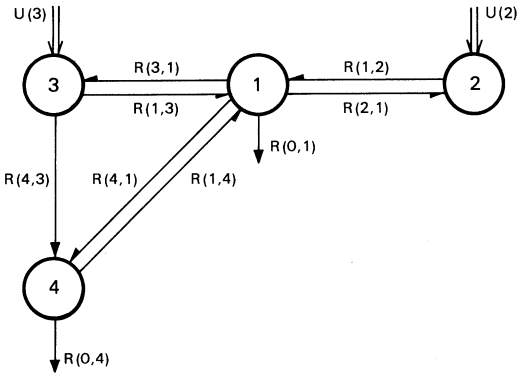

Fig. 2. Model used to describe the flow of water between the 'plasma-accessible' (1), 'intracellular' (2), rumen (3) and post-ruminal gastro-intestinal tract (4) components of body water. The parameters $R(I,J)$ represent the flow of water to compartment I from compartment J; $U(3)$ represents total water intake; $U(2)$ represents metabolic water; $R(0,4)$ represents faecal water output; and $R(0,1)$ represents urine water plus evaporative loss.

accessible pool, M(1), and the rumen pool, M(3), were obtained from the reciprocals of, respectively, the specific activity of plasma and rumen water near time zero. All the available data were weighted, in inverse proportion to their known or assumed

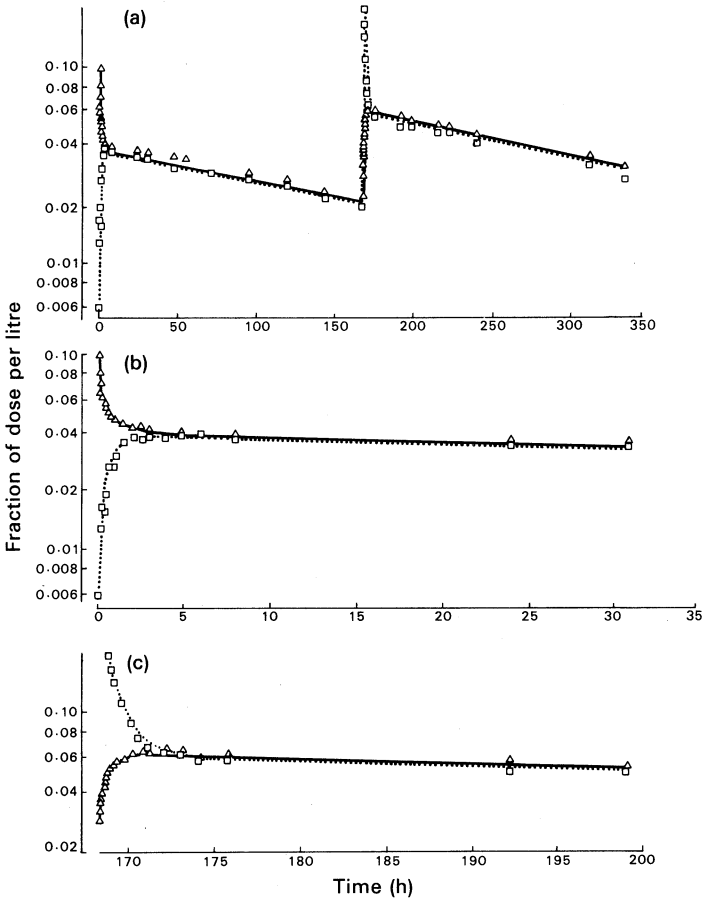

Fig. 3. Specific activities of plasma and rumen water in sheep 92 following intra-arterial (zero hours) and intra-ruminal (168 h) injection of TOH. The lines for plasma water (△___△) and rumen water (□ ····· □) are those fitted by the model described in Fig. 2. (*a*) Periods 1 and 2. (*b*) First 30 h of period 1. (*c*) First 30 h of period 2.

error, and the model was fitted simultaneously to both the kinetic and balance data. The fit of the model is illustrated in Fig. 3.

The data for sheep 28 could not be treated as rigorously as those for sheep 92 because the rumen water specific activities during the first 5 min following the rumen dose were too variable, perhaps because of inadequate mixing, and the rumen water specific activities during the first 3 h following the plasma dose were uncharacteristic, probably because of drinking episodes. Accordingly, because of the very rapid

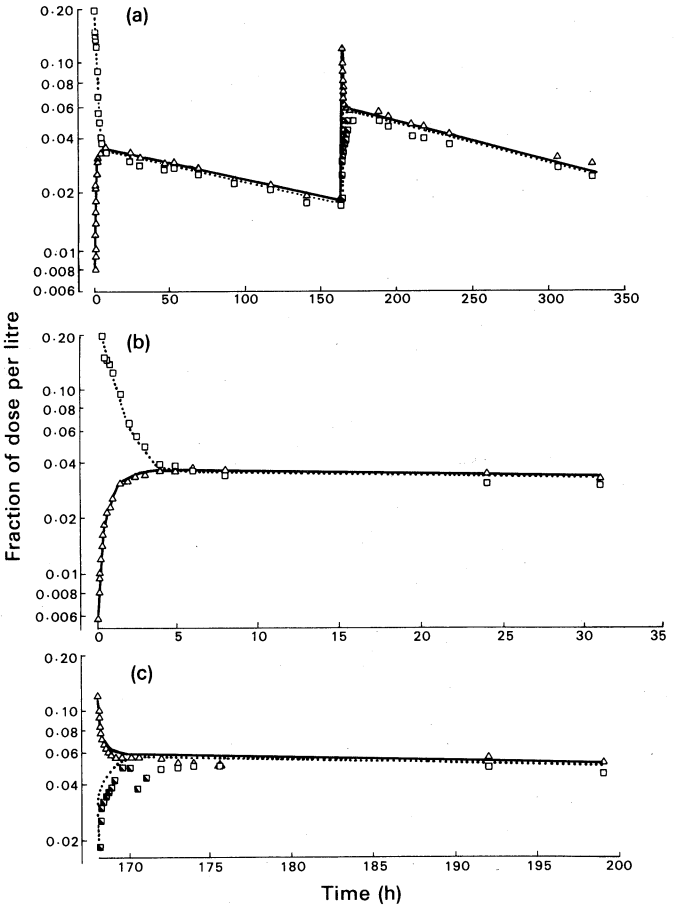

Fig. 4. Specific activities of plasma and rumen water in sheep 28 following intra-ruminal (zero hours) and intra-arterial (168 h) injections of TOH. The lines for plasma water (△___△) and rumen water (□ ····· □) are those fitted by the model described in Fig. 2. The shaded points were excluded during fitting of the model. (*a*) Periods 1 and 2. (*b*) First 30 min of period 1. (*c*) First 30 min of period 2.

equilibration of compartment 2 with compartment 1 and the similarity of the plasma appearance profiles after the rumen doses for each sheep, the model for sheep 28 was fitted using the parameter and error values obtained for the exchange between compartments 1 and 2 in sheep 92 as initial conditions—the first 3 h of the rumen response to the plasma dose was excluded during fitting. The fit of the model is illustrated in Fig. 4 and the estimates obtained for the pool sizes and flows between compartments

for both sheep are shown in Table 4. The high fractional standard errors for R(1,2) and R(2,1) in sheep 28 were associated with the noisy initial part of the plasma response.

Table 4. Distribution of water in two sheep calculated by compartmental analysis of TOH kinetics and water balance

Values in parentheses are fractional standard errors, i.e. $\hat{\sigma}_\theta/\hat{\theta}$

Distribution[A]		Sheep 92		Sheep 28	
		Predicted	Observed	Predicted	Observed
Pools: (litres)	M(1)	8·95 (0·061)	—	9·63 (0·089)	—
	M(2)	8·34 (0·086)	—	8·99 (0·44)	—
	M(3)	3·35 (0·039)	3·05 ± 0·44[B]	3·45 (0·10)	3·05 ± 0·44[B]
	M(4)	5·54 (0·086)	1·69 ± 0·42[B]	5·44 (0·16)	1·69 ± 0·42[B]
	Total	26·2 (0·001)	27·8[C]	27·5 (0·03)	28·0[C]
Flows: (litres/ day)	U(2)	0·206(0·068)	0·206	0·203(0·093)	0·206
	U(3)	2·131(0·049)	2·247 ± 0·620[D]	2·640(0·009)	2·620 ± 0·841[D]
	R(0,1)	2·146(0·049)	2·259 ± 0·635[D]	2·634(0·012)	2·628 ± 0·855[D]
	R(0,4)	0·190(0·083)	0·194 ± 0·043[D]	0·209(0·048)	0·199 ± 0·031[D]
Flows: (litres/ hour)	R(1,2)	43·57 (0·125)	—	46·48 (0·42)	—
	R(2,1)	43·56 (0·125)	—	46·47 (0·42)	—
	R(1,3)	2·96 (0·042)	—	3·18 (0·088)	—
	R(3,1)	3·12 (0·037)	—	3·35 (0·083)	—
	R(1,4)	2·14 (0·27)	—	5·23 (0·41)	—
	R(4,1)	1·89 (0·32)	—	4·96 (0·42)	—
	R(4,3)	0·25 (0·24)	0·26 ± 0·07[B]	0·28 (0·12)	0·26 ± 0·07[B]

[A] See Fig. 2.
[B] Mean ± s.d. for six sheep (G. J. Faichney and G. A. White, unpublished data).
[C] Mean from Table 3.
[D] Standard deviation, n = 14 days; Table 1.

The predicted flows of water into and out of the system were very close to the observed values as was the predicted flow from the rumen (compartment 3) to compartment 4, indicating that the model satisfactorily reconciled the kinetic and balance measurements.

The plasma-accessible compartment (1) was estimated to be 34–35% and the fast-turnover tissue compartment (2) 32–33% of total body water. The predicted rumen water content (3) was about 13% of total body water and was within 1 standard deviation of the mean value observed in similar sheep under similar conditions but the predicted size of compartment 4 was considerably greater than the observed value for the post-ruminal gastro-intestinal tract (G. J. Faichney and G. A. White, unpublished data).

The movement of water between compartments 1 and 2 averaged 45 litres per hour and it was calculated that the mean residence time of a water molecule in compartment 1 was 11·0 min for sheep 92 and 10·5 min for sheep 28; in compartment 2 the values were, respectively, 11·5 and 11·6 min.

Water moved across the rumen epithelium between rumen fluid and the plasma at 3 to 3·4 litres per hour. The mean residence time of a water molecule in the rumen was calculated to be, respectively, 63 and 60 min for sheep 92 and 28. The time constants for flow to the omasum from the rumen [M(3)/R(4,3)] were 13·4 and 12·3 h, average

12·9 h. This parameter is the equivalent of the mean retention time for solutes, which was found to be 12·9 ± s.d.2·9 h when determined using ^{51}Cr-EDTA in six similar sheep (G. J. Faichney and G. A. White, unpublished data).

Discussion

The four-pool model developed in this study provided a very good description of both TOH distribution with time and water balance measurements. The model was developed using data obtained in two consecutive experiments with one sheep. When it was applied to a more variable data set from a second sheep studied at the same time the model was conserved, confirming that the concepts embodied in it were reasonable.

Within the model, compartment 3 was clearly identified as the rumen because of its size and turnover rate but compartments 1 and 2 did not resemble the commonly determined extracellular and intracellular fluid spaces. However, these spaces are defined in terms of the volumes within which a specific solute is distributed or from which it is excluded (e.g. Na^+ distributes in extracellular fluid, being excluded by the cell membrane from the intracellular fluid in which K^+ is concentrated) but the cell membrane is not the rate-limiting barrier to the movement of water between cells and their environment (Ling *et al.* 1967). The various solutes used to estimate extracellular fluid volume (Elkington and Danowski 1955) enter gut water to varying degrees leading to further uncertainties of interpretation (Hix *et al.* 1959; Panaretto 1965). The very rapid equilibration between pool 1, which was accessible via the plasma, and pool 2 suggests that pool 1 may be free water and pool 2 intracellular 'bound' water, i.e. water whose freedom of movement is restricted by association with macromolecules such as proteins (Ling *et al.* 1967; Cope 1969). Pool 4 was clearly larger than the post-ruminal GI tract and its flux rates were quite different for the two sheep. This pool was not well resolved because of its distance from the sampling sites and further studies in which samples are taken at sites distal to the rumen are required to define it more clearly.

The influx of water to the rumen from the plasma-accessible pool, R(3,1), includes water entering in the saliva. If saliva flow is estimated from its relationship to dry matter intake (Black *et al.* 1980–81), the rumen water balance can be calculated (Fig. 5). The values fall within the range noted in a review by Engelhardt (1970). Diffusion accounted for 86% of the influx to and 92% of the efflux from the rumen. The calculations suggest that net water absorption occurred at about 200 ml/h. This absorption probably occurred against an osmotic gradient because similar sheep under similar conditions were found to have rumen osmolalities of 310–362 mosmol/kg (G. J. Faichney and G. A. White, unpublished results). Warner and Stacy (1968) concluded that daily net water absorption, calculated as the difference between intake plus saliva and outflow, could be similar to the amount imbibed; they reported a range from 50 ml/h in resting periods to 100–300 ml/h for a few hours after drinking. The present results fall within this range, although amounting to about twice the daily water intake, but differ in that Warner and Stacy (1968) found rumen fluid to be hypotonic to plasma at the higher net absorption rates. However, Dobson *et al.* (1970) reported uptake of water against an osmotic gradient when solutions in the temporarily isolated ventral sac of the rumen of the cow were gassed with CO_2, a treatment that increases blood flow in the rumen epithelium; increases in blood flow were associated with increased absorption of TOH from the rumen (Dobson 1979). The present results are consistent with these findings.

Smith and Sykes (1974) reported that TOH concentrations in rumen fluid of sheep took 8 h to equilibrate with plasma concentrations following intravenous TOH injection; Searle (1970) reported equilibrium in about 6 h. In both experiments, food and water were withheld during equilibration. It can be seen from Figs 3 and 4 that equilibrium in the present study occurred in from 3 to 6 h. This finding does not support the suggestion that ingestion of food or water delays equilibration (Robertshaw 1982). It should be noted that equilibration of rumen and plasma TOH concentrations does not necessarily mean that these concentrations must be equal. After equilibration, rumen TOH concentrations were always less than plasma concentrations (Figs 3 and 4) because entry of unlabelled water occurred primarily via the rumen.

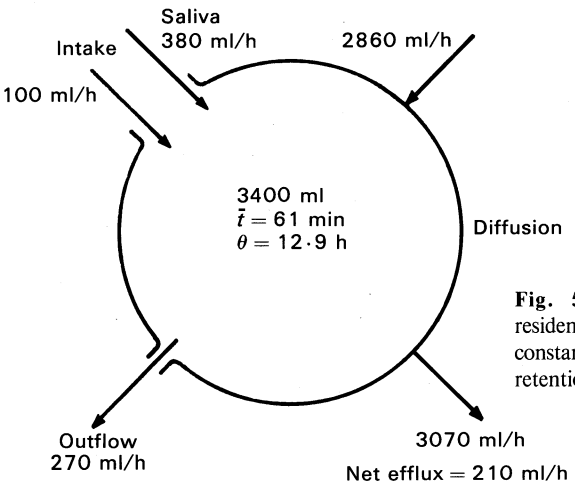

Fig. 5. Rumen water balance; $\bar{t}$ = mean residence time of a water molecule; θ = time constant for water outflow (equivalent to the mean retention time for unabsorbed solutes).

The value of about 55% for body water space determined in this experiment (Table 3) is close to the interspecies mean of 60% (Richmond *et al.* 1962) and agrees well with values for body water in mature sheep determined using 4-iodoantipyrene (Hansard and Lyke 1956), antipyrene (Hix *et al.* 1959) and TOH confirmed by slaughter and desiccation (Searle 1970; Smith and Sykes 1974). Searle *et al.* (1972) reported an equation for body water which gave a value of 26 litres for a 50 kg sheep fed at half *ad libitum*; the equation describes data from sheep fasted for 30 h, so would be expected to give values a little lower than the average of 27·7 found here (Table 3). The method used to calculate body water space provided a mean value for a 6-day period and is less prone than the methods used by these workers to the methodological errors discussed by Nagy and Costa (1980).

Acknowledgments

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References

Berman, M., and Weiss, M. F. (1978). SAAM 27 Manual, U.S.D.H.E.W. Publ. No. (NIH) 78-180.

Black, J. L., Beever, D. E., Faichney, G. J., Howarth, B. R., and Graham, N. McC. (1980-81). Simulation of the effects of rumen function on the flow of nutrients from the stomach of sheep. I. Description of a computer program. *Agric. Systems* **6**, 195-219.

Boston, R. C., Greif, P. C., and Berman, M. (1981). Conversational SAAM—an interactive program for kinetic analysis of biological systems. *Comp. Prog. Biomed.* **13**, 111–19.

Cope, F. W. (1969). Nuclear magnetic resonance evidence using D_2O for structured water in muscle and brain. *Biophys. J.* **9**, 303–19.

Dobson, A. (1979). The choice of models relating tritiated water absorption to subepithelial blood flow in the rumen of sheep. *J. Physiol.* **297**, 111–21.

Dobson, A., Sellers, A. F., and Shaw, G. T. (1970). Absorption of water from isolated ventral sac of rumen of the cow. *J. Appl. Physiol.* **28**, 100–4.

Edelman, I. S. (1952). Exchange of water between blood and tissues; characteristics of deuterium oxide equilibration in body water. *Am. J. Physiol.* **171**, 279–96.

Elkington, J. R., and Danowski, T. S. (1955). 'The Body Fluids.' (Williams & Wilkins Co.: Baltimore.)

Engelhardt, W. v. (1970). Movement of water across the rumen epithelium. In 'Physiology of Digestion and Metabolism in the Ruminant'. (Eds A. T. Phillipson *et al.*) pp. 132–46. (Oriel Press: Newcastle-upon-Tyne.)

Faichney, G. J. (1972). Digestion by sheep of concentrate diets containing formaldehyde-treated peanut meal. *Aust. J. Agric. Res.* **23**, 859–69.

Foster, D. M., and Boston, R. C. (1983). The use of computers in compartmental analysis: the SAAM and CONSAM programs. In 'Compartmental Distribution of Radiotracers'. (Ed. J. S. Robertson.) pp. 73–142. (CRC Press: Florida.)

Hansard, S. L., and Lyke, W. A. (1956). Measurement of total body water in sheep using ^{131}I-labelled 4-iodoantipyrene. *Proc. Soc. Exp. Biol. Med.* **93**, 263–6.

Hix, E. L., Underbjerg, G. K. L., and Hughes, J. S. (1959). The body fluids of ruminants and their simultaneous determination. *Am. J. Vet. Res.* **20**, 184–91.

Ling, G. N., Ochsenfeld, M. M., and Karreman, G. (1967). Is the cell membrane the rate-limiting barrier to the movement of water between the living cell and its surrounding medium? *J. Gen. Physiol.* **50**, 1807–20.

MacFarlane, W. V., and Howard, B. (1972). Comparative water and energy economy of wild and domestic mammals. *Symp. Zool. Soc., Lond.* No. 31. pp. 261–96.

Nagy, K. A., and Costa, D. P. (1980). Water flux in animals: analysis of potential errors in the tritiated water method. *Am. J. Physiol.* **238**, R454–R465.

Panaretto, B. A. (1965). Body composition *in vivo*. VIII. Some physiological implications with respect to extracellular fluid volume arising from the distribution of thiocyanate in sheep. *Aust. J. Agric. Res.* **16**, 667–73.

Richmond, C. R., Langham, W. H., and Trujillo, T. T. (1962). Comparative metabolism of tritiated water by mammals. *J. Cell. Physiol.* **59**, 45–53.

Robertshaw, D. (1982). Potential errors in the technique for estimating total body water and water turnover using tritiated water. In 'Use of Tritiated Water in Studies of Production and Adaptation in Ruminants'. pp. 33–42. (International Atomic Energy Agency: Vienna.)

Schmidt-Nielsen, K. (1964). 'Desert Animals: Physiological Problems of Heat and Water.' (Oxford University Press: London.)

Searle, T. W. (1970). Body composition in lambs and young sheep and its prediction *in vivo* from tritiated water space and body weight. *J. Agric. Sci., Camb.* **74**, 357–62.

Searle, T. W., Graham, N. McC., and O'Callaghan, M. (1972). Growth in sheep I. The chemical composition of the body. *J. Agric. Sci., Camb.* **79**, 371–82.

Smith, B. S. W., and Sykes, A. R. (1974). The effect of route of dosing and method of estimation of tritiated water space on the determination of total body water and the prediction of body fat in sheep. *J. Agric. Sci., Camb.* **82**, 105–12.

Warner, A. C. I., and Stacy, B. D. (1968). The fate of water in the rumen. 2. Water balances throughout the feeding cycle in sheep. *Br. J. Nutr.* **22**, 389–410.

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