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Measurement of Extracellular Fluid Volume and Blood Volume in Sheep

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Abstract

Methods are described for the simultaneous measurement of extracellular fluid volume (ECFV) and plasma volume (PV) in sheep using dilution of ^{82}Br (as sodium bromide) and ^{131}I-labelled ovine gamma globulin. Following injection of ^{82}Br (100 μCi), equilibrium in blood was reached after 3 h at which time only 4% of the injected dose was in rumen water. The ECFV was measured as the mean of the 2- and 3-h bromide space after correction for the relative water content of plasma, the Gibbs–Donnan factor and the loss of ^{82}Br into red blood cells. ^{131}I-labelled ovine gamma globulin (20 μCi) was injected after the 3-h ^{82}Br space was obtained and blood samples were taken at 10, 20, 30 and 40 min.

In 16 determinations in 11 sheep (25–47 kg body weight) the mean (± s.e.m.) ECFV was 9112± 289 ml (or 245±9 ml/kg). The mean PV for 16 observations in 11 sheep measured together with ECFV was 1597±62 ml (or 42·8±1·8 ml/kg). Although there was no relationship between body weight and PV there was a significant correlation between ECFV and body weight and also significant negative correlations between body weight and ECFV or PV when these were expressed as a function of body weight. The variation in ECFV measured on four occasions over 7–10 days in four sheep was 3·5% (range 2·6–4·6%). For PV measured in two animals on two consecutive days at the same time as ECFV the coefficient of variation was 1·5 and 2·1%. Acute sodium depletion (250–670 mmol) by parotid duct cannulation in three sheep resulted in a fall in ECFV which would account for only 15–20% of the sodium deficit. The remainder is presumably derived from ruminal sodium stores.

Introduction

The measurement of extracellular fluid volume (ECFV) and plasma volume (PV) are important in physiological studies of body electrolyte and fluid homeostasis. It is not sufficient to measure either PV or ECFV; in many situations there is a close relationship between ECFV and PV but under certain circumstances an abnormal relationship may exist between calculated interstitial fluid volume (IFV) and PV such that the value for either PV or ECFV may appear normal. The dilution of thiocyanate (Hix *et al.* 1959; Macfarlane *et al.* 1959; Panaretto 1965) thiosulphate (English 1966; Ternouth 1968; Homes and English 1969; Kamal *et al.* 1972) and sulphate (Rico *et al.* 1972) have all been used for measurement of ECFV in sheep. Panaretto (1965) concluded that thiocyanate was not suitable for use in sheep and although thiosulphate and sulphate are more satisfactory the techniques are not as convenient as those which use a gamma-emitting isotope such as ^{82}Br. Labelled bromide has been widely used in man (Gamble *et al.* 1953; McMurray *et al.* 1958; Howe and Ekins 1963) and in ruminants other than sheep (Ward *et al.* 1972) for measurement of ECFV.

The studies described in this paper detail the characteristics, reliability and precision of the distribution volume of ^{82}Br as a measure of ECFV in the sheep. Using ^{131}I-labelled ovine gamma globulin to measure PV simultaneously with ECFV, the normal values for PV and ECFV and the relative distribution of ECFV between the interstitial and plasma volumes are established.

Materials and Methods

Experimental Animals

Twenty-three adult crossbred Merino ewes (body weight 25–48 kg) housed in individual metabolism cages were used. All animals were fed an oaten–lucerne chaff mixture (800 g) and offered water *ad libitum* each day.

On the morning of the experiment sheep were weighed and polyethylene cannulae (i.d. 1·19 mm) were introduced 15 cm into the right and left jugular veins. A Foley retention catheter (14 gauge) was inserted into the bladder to facilitate accurate urine collections. Food and water were withheld on the day of measurement until the serial blood and urine samples had been collected. All animals were thoroughly accustomed to all experimental and handling procedures.

Isotopes

^{82}Br as sodium bromide (specific activity *c.* 0·15 mCi/mg Br) was obtained in 2-mCi batches from the Australian Atomic Energy Commission (Lucas Heights, N.S.W.). On arrival it was diluted from a radioactive concentration of 1 mCi ^{82}Br/ml to 400 μCi/ml with sterile 0·9% sodium chloride. ^{131}I as sodium iodide (radioactive concentration *c.* 500–700 mCi/ml) was obtained in 5–25-mCi batches from the Australian Atomic Energy Commission.

Iodination of Ovine Gamma Globulin

The procedure for iodination was that of Hunter and Greenwood (1962) utilizing chloramine-T. The $Na^{131}I$ was used within a few days of arrival. The iodination was carried out in 0·5 M phosphate buffer (pH 7·4) in a plastic radio-iodination pipette (Catt and Culross 1968). A small volume of phosphate buffer (25 μl) was drawn into the pipette and used to transfer 5–25 mCi of $Na^{131}I$ to the pipette, and this was followed by 25 μl of chloramine-T and 10 μl of a 1% solution of ovine gamma globulin (Cohn Fraction II, Commonwealth Serum Laboratories). The reaction was allowed to proceed for 25 s with gentle tapping of the iodination vessel. The reaction was stopped by adding 25 μl of metabisulphite solution (10 mg/ml) and immediately transferring the mixture to a 13 by 1 cm column of Sephadex G100 (Pharmacia) equilibrated with phosphate buffer. Elution of the column with phosphate buffer and collection of 4-drop fractions yielded two distinct radioactive peaks. The fractions appearing near the midpoint of the first radioactive peak were pooled and stored at −20°C until used. The second peak contained the free [^{131}I]iodide and was discarded. After determination of the radioactive concentration, 50-μCi aliquots were removed from the stock solution and diluted in 14 ml of normal saline immediately prior to use.

Injection of Labelled Tracers

A separate syringe was used for each tracer and 4–5 ml of the appropriate solution (15–20 μCi of ^{131}I-labelled ovine gamma globulin or 100 μCi ^{82}Br) were drawn into a 5-ml Cornwall constant delivery syringe (Becton, Dickenson and Co.) fitted with an 18-gauge needle, care being taken to exclude all air bubbles. After injection of the indicator into the left jugular cannula the syringe was not washed out but with the syringe plunger still depressed the needle was carefully withdrawn from the cannula. The solution was then allowed to run approximately 1 cm along the cannula under gravity to avoid spillage and was flushed into the circulation with 10 ml of heparinized saline (50 i.u./ml). The whole procedure took less than 20 s. For preparation of the standard, the syringe was refilled with the indicator without changing needles. This volume was injected into a 100-ml volumetric flask in the case of ^{131}I-labelled gamma globulin and a 1-litre volumetric flask in the case of ^{82}Br. The flasks were then made up to the mark with saline.

Experimental Protocol

The schedule used for routine determination of ECFV and PV is shown in Table 1. Blood and urine samples were taken at the times indicated in the table. All blood samples were taken from

the contralateral jugular vein to that used for injection. Blood (5 ml) was withdrawn from the venous cannula into a tube containing 0·05 ml (50 i.u.) of heparin. After sampling for microhaematocrit determination, the blood was immediately centrifuged at 4500 rev/min (3400 *g*) for 10 min and the plasma aspirated into a tube. Urine was collected via the bladder catheter into a glass measuring cylinder. At the end of a collection period, the volume was recorded and the bladder washed with two 10-ml volumes of water and one of air. The tube connecting the catheter to the cylinder was drained and the final volume recorded. This was shaken and a 10-ml aliquot was taken into a tube for subsequent analysis.

Table 1. Schedule for injection of ^{82}Br and ^{131}I-labelled ovine gamma globulin and for blood and urine samplings for measurement of ECFV and PV

Time (min)		Procedure
−150		Measure body weight; insert cannulae into left and right jugular veins; catheterize bladder.
−30		Start control urine collection.
−5		Take control blood sample; record and sample urine.
0[A]		Inject 100 μCi ^{82}Br into left jugular vein; commence urine collection.
120		Blood sample; record and sample 2-h urine, start new collection.
180		Blood sample; record and sample 3-h collection.
190		Inject 20 μCi ^{131}I-labelled ovine gamma globulin into left jugular vein.
200	(10 min)[B]	Blood sample.
210	(20 min)	Blood sample.
220	(30 min)	Blood sample.
230	(40 min)	Blood sample.
240		Remove cannulae and catheters; feed animal.

[A] This injection was usually given at 1130 h.
[B] Time after injection of ^{131}I-labelled ovine gamma globulin.

Radioactive Counting

Duplicate 1-ml samples of plasma and urine, together with their respective pre-injection blanks, and duplicate 1-ml samples of the ^{131}I and ^{82}Br dose standards were counted sequentially in a two-channel gamma counter (Packard Instrument Co.). The different energy spectra of the two isotopes allowed simultaneous counting of the samples, without prior separation of the radionuclides, by optimizing each of the channels for one isotope. Count rates were corrected for the contribution of the other isotope into either the ^{131}I or ^{82}Br channel using the simultaneous equations described by Veal and Vetter (1958). These can be simplified as follows:

$$\text{actual } ^{131}\text{I cpm (channel 1)} = N_1 - nN_2,$$
$$\text{actual } ^{82}\text{Br cpm (channel 2)} = N_2 - mN_1,$$

where N_1 is the nett cpm in channel 1, N_2 is the nett cpm in channel 2, $n = N_1/N_2$ for a pure ^{82}Br sample, and $m = N_2/N_1$ for a pure ^{131}I sample. Values for m and n were calculated for each experiment using ^{82}Br and ^{131}I standards. The selected discriminator settings gave values of about 0·052 and 0·170 for m and n respectively. The ^{82} Br counts (channel 2) were corrected for decay ($t_{\frac{1}{2}}$ = 36 h). This was a negligible correction for most routine ECFV measurements as the elapsed counting time between the first and last sample was usually less than half an hour.

The counting procedure (described above) was checked on several occasions by recounting the samples for ^{131}I after a 2-week interval at which time the counts due to ^{82}Br were negligible. The two calculated values for PV never varied by more than 5 ml.

*Calculation of Plasma Volume, Blood Volume and Red Cell Volume**

These parameters were obtained from the ^{131}I-labelled ovine gamma globulin space. Using a Biopipette (Schwarz–Mann) duplicate 1-ml samples of the standard (100 ml) and of the plasma were

* A Fortran IV program for use with an IBM 7044 computer has been written and was used for all calculations and determination of methodological errors.

pipetted into counting tubes. After counting to achieve a relative standard error of less than 1% (usually 2–5 min), the count rate in channel 1 was corrected for background and ^{82}Br contribution as previously described. The ^{131}I count rate was then plotted against time and the line of best fit extrapolated to the time of injection. Then

$$\text{PV (ml)} = (N_s/N_0) \times 100,$$

where N_s is the cpm/ml of ^{131}I standard, and N_0 is the cpm/ml of ^{131}I in plasma at time 0.

Blood volume (BV) was obtained from the following equation:

$$\text{BV (ml)} = \text{PV} \times 100/(100-0{\cdot}95) \times 0{\cdot}96H,$$

where 0·95 is the correction for trapped plasma, 0·96 is the correction for whole body haematocrit, and H is the large vessel haematocrit. Red cell volume (RCV) was calculated as the difference between BV and PV.

Calculation of Extracellular Fluid Volume

The apparent volume of distribution of ^{82}Br was calculated from the injected dose, corrected for urinary losses, and the plasma count rate (corrected for background and ^{131}I contribution if necessary). Then

$$\text{apparent bromide space} = (N_s \times 1000 - \Sigma N_u V)/N_p,$$

where N_s is the cpm/ml of ^{82}Br standard, N_u is the cpm/ml of ^{82}Br in urine and washings at $t = i$, N_p is the cpm/ml of ^{82}Br in plasma at $t = i$, and V is the volume of urine and washings. The concentration of ^{82}Br in plasma is less than its mean concentration in extracellular fluid because of the plasma proteins and the effect of the Gibbs–Donnan equilibrium. Then

$$\text{true bromide space} = \text{apparent bromide space} \times 0{\cdot}930 \times 0{\cdot}977,$$

where 0·930 is the relative water content of plasma, and 0·977 is the Gibbs–Donnan factor for chloride in plasma. A correction must also be made for the proportion of the dose lost into red blood cells (RBC) (see Experimental Procedures below). Then

$$\text{red cell bromide space} = \text{RCV} \times 0{\cdot}930 \times 0{\cdot}977 \times 0{\cdot}65,$$

where

$$0{\cdot}65 = \frac{\text{RBC } ^{82}\text{Br (cpm/ml)}}{\text{plasma } ^{82}\text{Br (cpm/ml)}}.$$

The ECFV can then be calculated as

$$\text{ECFV} = (\text{apparent bromide space} \times 0{\cdot}91) - (\text{RCV} \times 0{\cdot}59).$$

From 16 experiments the average correction of apparent bromide space to obtain ECFV was 0·88 ± 0·002 (range 0·86–0·89). This correction factor has been used in experiments in which PV was not measured. For routine determinations the 2- and 3-h spaces were calculated and the ECFV expressed as the average.

Experimental Procedures

Haematocrit

Calculation of BV, RCV, and ^{82}Br content of red blood cells required measurement of haematocrit. Blood was drawn into heparinized capillary tubes and centrifuged at 10 000 rev/min for 5 min in a microhaematocrit centrifuge (Biodynamics). In four sheep the isotopic haematocrit was determined using ^{131}I-labelled ovine gamma globulin.

To determine the characteristics of ^{82}Br distribution in the sheep the following experiments were carried out:

(1) *Equilibration time.* Blood and urine samples were taken from eight sheep at 2, 4, 6, 12 and 24 h after injection and the volume of distribution calculated.

(2) *Disappearance of ^{82}Br from blood.* In two sheep, blood samples were taken 5, 10, 20, 30, 40, 60, 90, 120, 150, 180, 200, 210, 220, 230, 300, 360 min and 11 and 23 h after injection. Urine samples were obtained at 120, 180, 210, 300, 360 min and 11 and 23 h.

(3) *Distribution of ^{82}Br into rumen.* In two sheep after determination of ECFV at 3 h the animals were killed by thiopentone overdose and the entire reticulum–rumen removed. After weighing and thorough mixing of the rumen contents, two 50-ml samples were centrifuged, the supernatant aspirated and duplicate 1-ml aliquots taken for counting.

(4) *Incorporation of ^{82}Br into red blood cells.* In six sheep ^{82}Br in blood and plasma was determined during routine ECFV measurements. The haematocrit was measured and the radioactivity in plasma-free red blood cells compared with that in plasma. The PV, BV and RCV were measured simultaneously. The total radioactivity in the red cell mass was calculated as the product of the radioactivity in each ml of red blood cells multiplied by the RCV.

(5) *Urinary excretion of ^{82}Br.* All urine produced was collected and sampled 2 and 3 h after injection of ^{82}Br. Urinary losses were expressed as a percentage of the total ^{82}Br administered.

(6) *Exchange of ^{82}Br in various body fluids.* The distribution of ^{82}Br in body fluids relative to the chloride concentration was investigated by comparing the cpm ^{82}Br/mmol Cl in plasma with the cpm ^{82}Br/mmol Cl in urine, saliva and rumen contents.

(7) *Normal values for ECFV and PV.* ECFV and PV were measured on 16 occasions in 11 normal sheep. On a further 22 occasions in 9 animals ECVF alone was measured.

(8) *Reproducibility of ECFV and PV.* ECFV was measured in four sheep on 4 days over either a 7- or 10-day period to obtain day to day variation and method precision. PV was measured simultaneously with ECFV in two animals on two consecutive days.

(9) *Effect of sodium depletion on ECFV and PV.* Three animals were sodium depleted over 48 h by acute parotid duct cannulation (Abraham *et al.* 1976). ECFV was measured on 2 days prior to and then after the 48-h sodium depletion. PV was measured in one animal.

Results

Hematocrit

There was a close correlation ($r = 0{\cdot}945$, $P < 0{\cdot}001$, $n = 8$) between the large vessel haematocrit as determined either by distribution of ^{131}I-labelled gamma globulin or by microcentrifugation. However, the isotopic haematocrit was always less (ratio = 0·95) and this factor was used to correct the large vessel haematocrit determined by microcentrifugation for plasma trapping. As blood flowing through minute vessels has a lower haematocrit than that of blood in larger vessels a correction factor is required to obtain the average distribution of red cells in plasma throughout the vascular system. Based on previous studies in the sheep (Boyd 1967) a factor of 0·96 was used to obtain the whole body haematocrit. Hence

$$\text{whole body haematocrit} = \text{large vessel haematocrit} \times 0{\cdot}95 \times 0{\cdot}96\,.$$

Characteristics of ^{82}Br Distribution

(1) *Equilibration time.* Table 2 shows the individual and mean ^{82}Br volumes of distribution at 2, 4, 6, 12 and 24 h after injection. Urine was collected over each period and the dose was corrected for urinary loss. The data are also expressed as a percentage of the 2-h value. Four hours after injection the apparent bromide space averaged 103·2% of the 2-h value, an average increase of 300 ml ($P < 0{\cdot}05$). The 6-h value (102·8%) was similar to the 4-h value but because of larger variance it was not significantly different from the 2-h value. The 12- and 24-h values were substantially higher than the 2-, 4- and 6-h values, probably reflecting entry of the ^{82}Br into the rumen.

(2) *Disappearance of ^{82}Br from blood.* The results of two experiments in which the disappearance of ^{82}Br from blood was examined are shown in Table 3. In both animals ^{82}Br was removed rapidly from the circulation and had distributed into a volume of 6·6 and 6·0 litres within 5 min for Misty and Bridgell respectively. Samples were not taken prior to 5 min but the minimum volume of distribution would be the PV. PVs measured simultaneously were 1·58 and 1·84 litres for Misty and Bridgell respectively. The apparent volume of distribution appeared to plateau after 120 min although as has previously been shown in equilibration studies the measured space slowly increases with time from 2 to 24 h. The calculated ECFV for

Misty (8673 ml) and Bridgell (2851 ml) is less than the apparent volume of distribution for the average of the 120- and 180-min values since the data in Table 3 have been corrected only for urinary loss and not for loss into red blood cells, the Gibbs-Donnan factor or the relative water content of plasma.

Table 2. Equilibration of ^{82}Br expressed as apparent bromide space and as a percentage of the 2-h space in eight experiments in seven sheep

Statistical comparisons were made using Student's *t* test for paired observations

Animal	2-h space (ml)	4-h space (ml)	% of 2-h space	6-h space (ml)	% of 2-h space	12-h space (ml)	% of 2-h space	24-h space (ml)	% of 2-h space
Yett	9 553	9 940	104·1	9 425	98·7	10 257	107·4	9 747	102·0
Sebo	8 894	9 323	104·8	8 735	98·2	9 771	109·9	9 187	103·3
Rundle	10 897	10 916	100·2	10 827	99·4	11 329	104·0	11 738	107·7
Hancy	9 471	9 461	99·9	10 005	105·6	10 200	107·7	9 521	100·5
Misty 1	9 668	9 883	102·2	10 176	105·3	10 691	110·4	10 298	106·3
Misty 2	9 601	10 301	107·3	10 101	105·2	10 182	106·1	10 565	110·0
Tara 1	9 219	9 468	102·7	9 701	105·2	10 099	109·5	10 257	111·3
Bridgell 1	9 415	9 827	104·4	9 802	104·1	9 287	99·0	9 893	105·1
Mean	9 590	9 890	103·2	9 847	102·8	10 227	106·6	10 151	105·8
s.e.m.	206	185	0·9	215	1·0	213	1·0	276	1·3
P		<0·5		n.s.		<0·001		<0·05	

(3) *Distribution of ^{82}Br into rumen.* In the two animals killed 3 h after injection of ^{82}Br, rumen contents constituted 13% of body weight, giving values for rumen water content (20% dry matter) of 3·2 and 3·7 litres for sheep A74 and A96 respectively. The percentage of the injected ^{82}Br dose in the rumen water was 3·95 and 4·30% for sheep A74 and A96 respectively.

Table 3. Calculated percentage dose per litre of plasma and apparent volume of distribution (V) following injection of ^{82}Br (110 μCi) into two sheep

Blood samples were taken between 5 min and 23 h. The percentage dose injected per litre of plasma was corrected for urinary ^{82}Br loss. Weight of sheep: Misty 2, 39·5 kg; Bridgell 1, 34·3 kg

Time (min)	Misty 2 %Dose/l	Misty 2 *V* (l)	Bridgell 1 %Dose/l	Bridgell 1 V (l)	Time (min)	Misty 2 %Dose/l	Misty 2 *V* (l)	Bridgell 1 %Dose/l	Bridgell 1 *V* (l)
5	15·1	6·6	16·6	6·0	180	10·1	9·9	10·8	9·3
10	13·9	7·2	14·8	6·8	200	9·9	10·1	10·4	9·6
20	12·1	8·3	13·2	7·6	210	10·0	10·0	10·4	9·6
30	11·7	8·5	12·4	8·1	220	10·1	9·9	10·2	9·8
40	11·8	8·5	12·2	8·2	230	9·7	10·3	10·2	9·8
60	11·4	8·8	12·1	8·3	300	10·0	10·0	10·1	9·9
90	10·5	9·5	11·2	8·9	360	9·9	10·1	10·2	9·8
120	10·4	9·6	10·6	9·4	11 h	9·9	10·1	10·7	9·3
150	10·1	9·9	11·0	9·1	23 h	9·5	10·5	10·1	9·9

(4) *Incorporation of ^{82}Br into red blood cells.* The ratio of ^{82}Br in red blood cells to ^{82}Br in plasma was 0·65±0·02 (mean±s.e.m.) (range 0·50–0·76) for 18 determinations in six sheep. This was equivalent to 2·7±0·3% of the injected dose at 3 h.

(5) *Urinary excretion of* ^{82}*Br.* Urinary excretion of ^{82}Br in the first 3 h accounted for only $1 \cdot 14 \pm 0 \cdot 18\%$ ($n = 22$) of the injected dose.

(6) *Exchange of* ^{82}*Br in various body fluids.* A comparison of the cpm ^{82}Br/mmol Cl in plasma, urine, saliva and rumen water appears in Table 4. All measurements were made 3 h after administration of ^{82}Br. The ratio of cpm ^{82}Br/mmol Cl in the various fluids to the cpm ^{82}Br/mmol Cl in plasma obtained at the same time is also shown.

Table 4. Comparison of the cpm ^{82}Br/mmol Cl in plasma and various body fluids
Results are also expressed as the ratio of cpm ^{82}Br/mmol Cl in the various fluids to the cpm ^{82}Br/mmol Cl in plasma

Fluid	Sheep	^{82}Br cpm/mmol Cl in: Fluid	Plasma	Fluid : plasma ratio	Mean ± s.e.m.
Urine	Bridgell 2	110	156	0·71	
		405	446	0·91	
		285	310	0·92	
	Nulla 1	78	131	0·60	
		235	273	0·86	
		199	341	0·58	
	Broncia	106	167	0·64	0·73 ± 0·03
		327	492	0·66	
		249	298	0·84	
		252	380	0·66	
	Yago	86	125	0·69	
		328	396	0·83	
		146	272	0·54	
		270	348	0·78	
Saliva	Misty 1	860	113	7·7	4·5
	Tara 1	240	113	2·2	
Rumen water	A74	42	107	0·39	0·56
	A96	64	89	0·72	

Table 5. Measured values of various parameters in normal sheep
The IFV was calculated from the difference between simultaneous measurements of ECFV and PV. Mean body weight (± s.e.m.) was 37·7 ± 1·4 kg. Each value given is the mean ± s.e.m. for 16 determinations in 11 sheep

Units	ECFV	PV	BV	RCV	IFV	PV/IFV
ml	9112 ± 289	1597 ± 62	2091 ± 79	481 ± 30	7519 ± 252	0·212
ml/kg	245 ± 9	42·8 ± 1·8	56·2 ± 2·4	13·3 ± 0·9	202 ± 7	±0·007

Urine: In 14 observations from four sheep, the cpm ^{82}Br/mmol Cl was always less than in plasma. The mean was $0 \cdot 73 \pm 0 \cdot 03$ (range 0·54–0·92).

Saliva: Saliva samples were obtained by parotid duct cannulation. In two sheep the ratio of cpm ^{82}Br/mmol Cl in saliva to that in plasma was 7·7 and 2·2.

Rumen water: Samples of rumen water were obtained from the two sheep described above in (3). The cpm ^{82}Br/mmol Cl was about half of that in plasma.

(7) *Normal values for ECFV and PV.* For 16 determinations in 11 sheep of body weight $37 \cdot 7 \pm 1 \cdot 4$ kg (range 25–47 kg) the ECFV (mean ± s.e.m.) was 9112 ± 289 ml

(or 245±9 ml/kg) (Table 5). There was a significant correlation between body weight and ECFV ($r = 0{\cdot}523$, $P < 0{\cdot}05$) and also a significant negative correlation ($r = -0{\cdot}665$, $P < 0{\cdot}01$) between body weight and ECFV expressed as a function of body weight (Fig. 1). The values for PV, BV and RCV obtained at the same time as the ECFV measurement are shown in Table 5. The mean PV was 1597±62 ml (42·8±1·8 ml/kg). There was no correlation between PV and body weight ($r = 0{\cdot}350$,

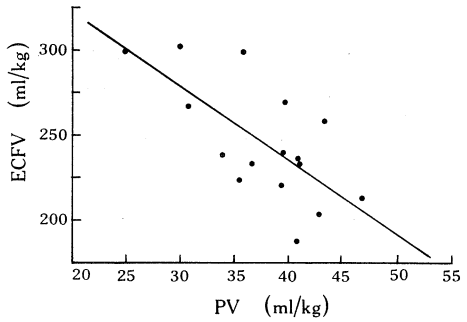

Fig. 1. Relationship between body weight and ECFV on 16 occasions in 11 animals. The negative correlation coefficient ($r = -0{\cdot}665$) was significant ($P < 0{\cdot}01$).

$P > 0{\cdot}01$), but as shown for ECFV the was a significant negative relationship between body weight and PV expressed as a function of body weight ($r = -0{\cdot}558$, $P < 0{\cdot}05$). Calculated interstitial fluid volume (IFV) was 7519±252 ml (or 202±7 ml/kg). The ratio of PV to IFV was 0·195±0·007 (Table 5). The relationship ($r = 0{\cdot}673$, $P < 0{\cdot}01$) between PV and ECFV expressed on a body weight basis is shown in Fig. 2. A significant relationship ($r = 0{\cdot}637$, $P < 0{\cdot}01$) was also found between PV and ECFV expressed as a volume (ml). ECFV was measured on a further 22 occasions in nine animals without simultaneous measurement of PV. The mean ECFV was 8731±189 ml (or 233±5 ml/kg), a value not significantly ($P > 0{\cdot}05$) different from that obtained in the studies in which PV was measured. In these experiments the apparent bromide space was corrected by 0·88 to obtain the ECFV.

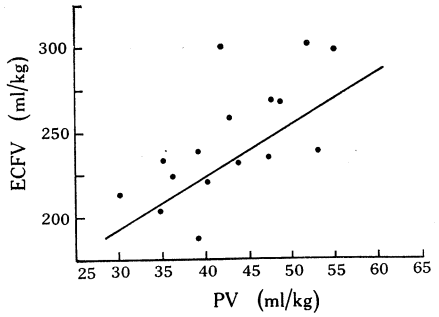

Fig. 2. Relationship between PV and ECFV on 16 occasions in 11 animals ranging in body weight from 25 to 47 kg. The correlation coefficient ($r = 0{\cdot}673$) was significant ($P < 0{\cdot}01$). The data are for identical experiments to that shown in Fig. 1.

The variability of this factor (ratio of ECFV to apparent bromide space) obtained from the 16 experiments outlined above was less than 2% and would only slightly reduce the precision of the estimate of ECFV. The mean ECFV for all 38 observations in 20 sheep (25–47 kg body weight) was 8891±164 ml (or 238±5 ml/kg). The correlation of ECFV and body weight was significant ($r = 0{\cdot}514$, $P < 0{\cdot}01$) as was that for ECFV expressed as a function of body weight ($R = -0{\cdot}560$, $P < 0{\cdot}001$).

(8) *Reproducibility of ECFV and PV.* The between day variability in ECFV expressed as a volume and on the basis of body weight for four animals is shown in Table 6. The coefficient of variation for the four observations for each animal

Table 6. Reproducibility of measurements of ECFV

ECFV was measured over a 10-day period in two sheep and over 7 days in two other sheep. Results are expressed as volume and also as a function of body weight. PV was not measured in these experiments. Apparent bromide space has been corrected (×0·88) to give ECFV

	Nulla 1			Bridgell 2			Misty 3			Tilly 1		
	Day	ECFV (ml)	(ml/kg)	Day	ECFV (ml)	(ml/kg)	Day	ECFV (ml)	(ml/kg)	Day	ECFV (ml)	(ml/kg)
	1	10431	252	1	8466	241	1	8783	226	1	7841	206
	3	10329	246	3	8869	250	2	8730	228	2	7522	207
	8	10167	252	8	8386	244	6	9222	239	6	7430	201
	10	9412	248	10	8040	235	7	9088	238	7	7440	204
Mean		10884	249		8440	242		8956	233		7558	204
s.e.m.		231	1·5		170	3·2		119	3·5		97	1·3
Coefficient of variation (%)		4·6	1·1		4·0	2·7		2·7	2·9		2·6	1·3

ranged from 2·6 to 4·6% (mean 3·5%). On a body weight basis the mean coefficient of variation was 2·0% (range 1·1–2·9%). This represents a mean variation of 300 ml. At another time in two animals PV was measured together with ECFV (Table 7). The variation in PV over 2 days was 2·1% expressed on a volume basis and 1·5% when expressed as a function of body weight.

Table 7. Reproducibility of measurements of ECFV and PV in two sheep on consecutive days

Sheep	Day	Body weight (kg)	ECFV		PV	
			(ml)	(ml/kg)	(ml)	(ml/kg)
Nulla 2	1	40·8	9962	244	2025	49·6
	2	39·5	9980	253	1950	49·4
Misty 4	1	37·5	8451	225	1720	45·9
	2	37·0	8765	237	1760	47·6

(9) *Effect of sodium depletion on ECFV and PV.* Sodium depletion for 48 h by withdrawal of parotid saliva produced sodium deficits of 248–667 mmol (Table 8). This was accompanied by a fall in body weight and ECFV. Plasma sodium concentration fell in all animals. On the basis of the measured change in ECFV and the plasma sodium concentration, only 15–20% of the sodium deficit can be accounted for from the ECFV.

Table 8. Effect of acute sodium depletion over 48 h on body weight, ECFV, PV and plasma sodium concentration

PV was not measured in Tilly 1 or Misty 3. The apparent bromide space has been corrected (×0·88) to give ECFV

Sheep	Time (h)	Na loss (mmol)	Body wt (kg)	ECFV (ml)	PV (ml)	Plasma [Na] (mmol/l)
Tilly 1	−24	0	37·0	7430[A]	—	—
	0	0	36·5	7440[A]	—	140
	48	−250	34·8	7084	—	138
Misty 3	−24	0	38·5	9222[A]	—	—
	0	0	38·0	9088[A]	—	147
	48	−670	33·5	7975	—	140
Nulla 2	−24	0	40·8	9962[B]	2025[B]	—
	0	0	39·5	9980[B]	1950[B]	143
	48	−650	37·0	9657	1764	137

[A] Data also appear in Table 6.
[B] Data also appear in Table 7.

Discussion

These studies describe the use of ^{82}Br (as sodium bromide) for the measurement of ECFV in the sheep and show that if the necessary corrections for entry into red blood cells, the Gibbs–Donnan equilibrium and the relative water content of plasma are made to the apparent bromide space, then ECFV can be determined with acceptable reproducibility. The described technique also enables PV and BV to be determined simultaneously with ECFV using ^{131}I-labelled ovine gamma globulin.

A tracer whose volume of distribution corresponds to the true or anatomical ECFV has not yet been found but this is not surprising when the heterogeneous composition of the ECFV is considered. Large molecules such as inulin although excluded from cells, probably underestimate the ECFV because of very slow diffusion into transcellular water (Nichols *et al.* 1953). The other classes of tracer used such as chloride, bromide, sulphate and thiocynate avoid the problems of slow equilibration but may overestimate ECFV because they do not remain extracellular (Edelman and Leibman 1959). However, it has become accepted that the distribution volume of chloride when corrected for the chloride content of red blood cells is a good estimate of functional ECFV (Nichols *et al.* 1953)—functional in that a change in the anatomical ECFV will be represented by a proportional change in the chloride space. Sodium bromide and, more recently, radioactive ^{82}Br (as sodium bromide) have been used to measure the chloride space because of the disadvantages of using ^{36}Cl or ^{38}Cl, and in measuring plasma chloride. Although there are differences in the handling of chloride and bromide, they have similar volumes of distribution in man (Hellerstein *et al.* 1960).

Although ^{82}Br has been used in cattle and goats (Ward *et al.* 1972), estimates of ECFV in sheep have previously been with thiosulphate (English 1966; Ternouth 1968; Holmes and English 1969; Kamal *et al.* 1972), sulphate (Rico *et al.* 1972) or thiocyanate (Hix *et al.* 1959; Macfarlane *et al.* 1959; Panaretto 1965).

Thiosulphate has been used with satisfactory results but requires that an extrapolative method be used for calculation because of removal of the tracer from plasma during the 60–90 min of equilibration. It does not penetrate the rumen or red blood cells to any significant extent (Holmes and English 1969). Reported values for ECFV using thiosulphate in adult sheep show considerable variation, ranging from 268 ml/kg (Ternouth 1968) to 205 ml/kg (Kamal *et al.* 1972). English (1966) reported a value of 234 ml/kg in Suffolk sheep and one of 200 ml/kg for Merinos (Holmes and English 1969).

Using thiocyanate, values of 25–35% of body weight have been obtained for ECFV in sheep (Hix *et al.* 1959; Macfarlane *et al.* 1959; Panaretto 1965). Although thiocyanate reaches equilibrium within a few hours, its rapid urinary excretion also makes the use of an extrapolative method mandatory. Furthermore, in the sheep thiocyanate is not uniformly mixed (as in man) or excluded (as in the heifer) from the rumen compartment. For this reason Panaretto (1965) concluded it was an unsuitable tracer for ECFV measurement in the sheep.

The value for ECFV in the present study was $24{\cdot}5 \pm 0{\cdot}9\%$ (mean $\pm$ s.e.m.) of body weight. This value is slightly higher than that determined by thiosulphate (English 1966) but less than the thiocyanate space (Hix *et al.* 1959; Macfarlane *et al.* 1959; Panaretto 1965), and reflects the different distribution characteristics of these substances. The expressing of ECFV as a percentage of body weight is probably of limited value for comparison purposes between animals. Although there was a positive correlation between ECFV and body weight, there was also a significant negative correlation between body weight and ECFV when expressed as a function of body weight (Fig. 1), the lighter animals tending to have a higher proportion of ECFV. The wide variation in values for ECFV between different studies is to be expected when the known influences of age, sex, temperature, latitude, food quantity and quality, water and salt status, shearing, lactation and disease on body fluid distribution are taken into account (Macfarlane 1975).

In the past the main criticism of the use of bromide has centred on its entry into cells, especially the red blood cells, and its selective concentration in the gastro-intestinal tract. In the present study PV and RCV were measured simultaneously with the bromine space and, based on the ratio (0·65) of ^{82}Br in red blood cells to plasma, an exact correction could be made. On average 3% of the ^{82}Br dose injected entered the red blood cells. At 3 h after injection the relative specific activity (cpm ^{82}Br/mmol Cl) in the rumen was only half that of plasma, and the fraction of the dose in the rumen was 4–5%. Thus at the equilibration times used, the rumen volume is essentially excluded from the calculated ECFV. Loss of tracer into the rumen was not corrected for since it could not be accurately measured in every experiment and thus would not increase the precision of the ECFV measurement. Equilibration in plasma was probably achieved within 2 h, as the 2-, 4- and 6-h ^{82}Br spaces differed by only 3%. Only 1·1% of the injected dose was excreted by the kidney in the 3 h after injection of ^{82}Br.

The mean value (±s.e.m.) for PV in 11 sheep from the present investigation was 42·8±1·8 ml/kg. Using a similar procedure, Boyd (1967) reported a value of 47·1±1·8 ml/kg in five sheep. Using Evans Blue, Hodgetts (1961) obtained a value of 44·6 ml/kg. The use of Evans Blue is questionable as its disappearance rate from plasma is approximately 20–30% per hour during the immediate post-mixing period (Hodgetts 1961; Boyd 1967) and the reproducibility of the procedure is poor (Boyd 1967). In addition, the volume of distribution of Evans Blue or ^{131}I-labelled albumin has been shown to be consistently more than the volume of distribution, measured simultaneously, of higher-molecular-weight materials such as ^{131}I-labelled gamma globulin (Anderson 1962; Boyd 1967) and ^{131}I-labelled fibrinogen (Baker 1963). Details of the precision and reproducibility of the method using ^{131}I-labelled ovine gamma globulin for the measurement of PV in sheep have been detailed by Boyd (1967). There was no correlation between absolute PV and body weight. When PV was expressed on a body weight basis there was a significant negative correlation—again, the lighter animals tending to have a higher proportion of PV.

The ratio of PV to IFV (calculated as ECFV−PV) was 0·212±0·007. This is slightly less than the values of 0·223 and 0·214 reported for man using ^{82}Br and iodinated human serum albumin for ECFV and PV measurements respectively (McMurrey *et al.* 1958; Tarazi *et al.* 1969). The importance of this ratio is not its actual value, since an isolated measurement has little significance, but the fact that it reveals changes in the partition of ECFV under various physiological situations (Scoggins *et al.* 1975). Further, the strong correlation between PV and ECFV is as expected and defines the distribution characteristics of ECFV in the normal animal (Tarazi *et al.* 1969).

Although the process by which sodium from the rumen becomes available for maintenance of the integrity of the ECFV and the circulatory system during sodium depletion has been investigated (Denton 1957; Blair-West *et al.* 1965), the magnitude and proportion of the fall in ECFV in relation to sodium deficit has not been defined. In these preliminary studies of sodium depletion, the changes in ECFV and plasma sodium indicate that only 15–20% of the sodium is obtained from the ECFV. Although a small proportion of the sodium would be mobilized from bone, the majority would probably come from the rumen. The fall in body weight during the 2-day depletion period was 4–10 times greater than the fall in ECFV and this suggests that there was a reduction in rumen volume. The greater percentage decrease in PV

than in ECFV has been observed previously in dogs by electrolyte depletion (Elkington *et al.* 1946).

The techniques for the measurement of ECFV and PV described in this paper use the two readily available gamma-emitting isotopes ^{131}I and ^{82}Br, are relatively simple and reproducible, and have been established as routine procedures in our laboratory for the determination of these two body fluid compartments in sheep.

The methods have been described and developed for use in sheep held under laboratory conditions. However, they could be readily applied to animals maintained under less intensive management and since only about 1% of injected ^{82}Br is lost during equilibration it would be possible to obtain accurate results without bladder catheterization.

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