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Changes in Plasma Volume and Haematocrit in Intact and Splenectomized Sheep during Feeding

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Abstract

Changes in jugular haematocrit during daily 2-h feeding periods in trained sheep with and without spleens were compared with changes in the concentration of the plasma tracer radio-iodinated human serum albumin.

Jugular haematocrit was increased by 16% in intact sheep and 9% in splenectomized sheep 20 min after they started to eat dry rations.

The dilution of tracer in plasma, studied after mixing in the vascular system had been completed, showed four phases. Phase 1 was the rate of removal of tracer in the period before eating began. Phase 2 was the sudden increase in plasma radioactivity that occurred in the first 20 min of eating, indicating a loss of plasma from the circulation. Phase 3 was the decrease in tracer concentration during the remaining feeding period and phase 4 was a post-feeding phase, characterized by a slower rate of decrease of tracer than during phase 3, implying that there was significant recycling of tracer during this phase.

The sudden increase in plasma radioactivity, initiated by the onset of feeding, represented a reduction in plasma volume of 10–12%. Minimum plasma volume coincided with peak haematocrit values. The reduced plasma volume accounted for the increased haematocrit in splenectomized sheep, but only accounted for about half of the increase in intact sheep. The residual increase in haematocrit in intact sheep was most likely the result of splenic contraction.

Introduction

The physiology of sheep is extensively altered when they start to eat dry rations (Blair-West and Brook 1969; Christopherson and Webster 1972; Dooley *et al.* 1972). Several of the observed changes may be related directly to the need of the sheep under these feeding conditions to produce large volumes of saliva rapidly to facilitate swallowing. This need expresses itself in the circulatory system as a reduced plasma volume (Blair-West and Brook 1969) and an ensuing haemoconcentration (Dooley and Williams 1975).

Estimates of haemoconcentration during feeding, based upon changes in haematocrit (or any other measure of red blood cell abundance per unit of volume) are likely to be misleading since the spleen contracts when the sheep starts to eat (Dooley *et al.* 1972) and this elevates jugular haematocrit markedly (Turner and Hodgetts 1959).

The present study attempted to analyse the increase in jugular haematocrit in sheep initiated by feeding in terms of reductions in plasma volume and splenic contraction.

Materials and Methods

Animals and Feeding

Nine mature Merino sheep with spleens (numbers 110, 538, 581, 586, 635, 1162, 1653, 1657, 3521) and three without spleens (numbers 555, 569, 2690) housed in individual metabolism cages were used. During the feeding experiments 800 g of lucerne chaff were offered daily at about 0810 h. The sheep were allowed to eat for 2 h and then the residues were removed and weighed. They were accustomed to this feeding regime during 10 days preceding an experiment.

Cannulation of the Jugular Vein

Sheep were weighed prior to cannulation (see Table 3). Polyethylene cannulae were inserted through 13-gauge needles into the jugular veins of the sheep at least 15 h before an experiment.

Blood Samples

Blood samples of 2·5 ml, taken through the cannulae, were added to stoppered vials containing 500 i.u. dried heparin. The haematocrit of this sample, and the radioactivity in its plasma, were later measured.

Haematocrit

Blood was centrifuged in microhaematocrit tubes (Propper Mfg Co., Long Island City, N.Y.) at a relative centrifugal force of 10 000 *g* for 8 min and within 1 h of obtaining the blood sample. The haematocrit was expressed as a decimal fraction. Haematocrits could be measured in this way in replicate samples with a standard deviation (s.d.) of 0·002 over the haematocrit range 0·12–0·85 (Dooley *et al.* 1974).

Plasma Volume

A sterile solution of human serum albumin labelled with ^{125}I (Radiochemical Centre, Amersham, England) was used as the plasma volume tracer. It was diluted in isotonic NaCl solution and passed through a column (Vogel 1961) of a strongly basic anion exchanger (Dowex AG1X8, 200–400 mesh, Bio-rad Laboratories, Richmond, Calif.) in order to remove unbound ^{125}I. The eluate was stored at 4°C with added resin. Precipitation of protein from aliquots of the eluate showed that less than 1% unbound ^{125}I was present.

For an experiment, tracer solution was centrifuged at 6000 *g* for 4 min to sediment the resin. A small volume of supernatant was removed and diluted with saline to obtain a standard solution with activity similar to that anticipated to be in plasma. A measured volume of the remainder was injected into the sheep through the jugular cannula.

To avoid erratic values of tracer concentration in plasma due to incomplete mixing, the first blood sample was not taken until 30 min had elapsed after the injection.

Blood samples were centrifuged at 1500 *g* for 10 min within 1 h of collection. Samples of plasma or of standard solution were transferred to counting tubes with a 500-μl constriction pipette (H. Pedersen, Denmark) and counted in the 25–50 KeV range with a Packard Tricarb Scintillation Spectrometer containing a well-type thallium-activated sodium iodide crystal, until 20 000 counts had accumulated. The s.d. of seven replicates of a standard solution having a mean radioactivity of 24 204 cpm/ml was 223 cpm/ml.

Plots of the concentration of tracer in plasma against time were peeled by a computer method into a series of exponential functions (Dooley *et al.* 1971).

Standard statistical tests were applied to the data (Snedecor and Cochran 1967).

Results

Jugular Haematocrit

(i) *Intact sheep*

Haematocrit usually decreased in the period before feeding to basal values of about 0·28 except in sheep 1653 where the basal value was 0·23 (Fig. 1). Haematocrit

increased rapidly with the onset of eating, and after 17 min it had increased by about 18% to a maximum (Table 1). Values then decreased even though the sheep were still eating, except in sheep 538 in which haematocrit remained elevated. At the end of

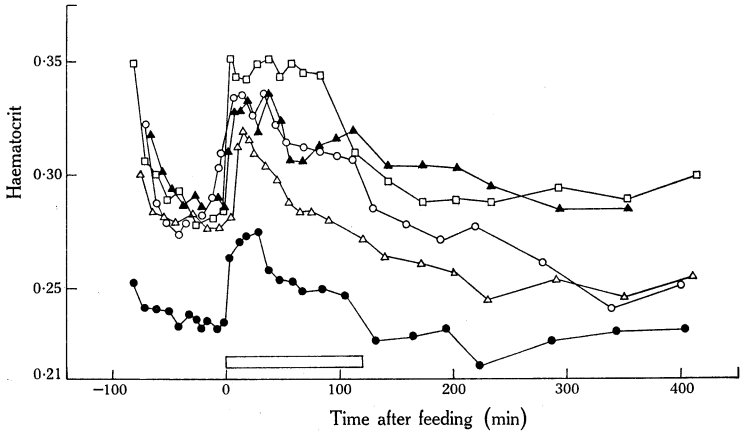

Fig. 1. Changes in jugular haematocrit in intact sheep before and after feeding. The horizontal bar indicates the feeding period. Sheep 1657 (○), 586 (▲), 1653 (●), 581 (△) and 538 (□). Food intakes are given in Table 1.

feeding, haematocrit approximated basal values in two sheep (581, 1653) though in the other three, haematocrit remained elevated.

Table 1. Haematocrit before feeding and maximum haematocrit after feeding

Sheep	Food intake (g)	Prefeeding haematocrit	Maximum post-feeding haematocrit		
			Value	Occurrence after start of feeding (min)	Percentage increase
Intact					
1657	690	0·282	0·335	15	18·8
586	750	0·286	0·333	19	16·4
1653	760	0·235	0·275	29	17·0
581	720	0·277	0·319	16	15·2
538	800	0·286	0·351	4	22·7
Mean	744	0·273	0·323	16·6	18·0
Splenectomized					
2690	800	0·275	0·305	19	10·9
569	800	0·321	0·361	9	12·5
555	720	0·264	0·276	24	4·5
Mean	773	0·287	0·314	17·3	9·3

After feeding, haematocrit returned to values within 5% of basal in three sheep (538, 586, 1653) while in two others (581, 1657) a considerable and persistent reduction

in haematocrit was evident. The last samples of blood taken from these two animals had haematocrits 11 and 18% less than their respective basal values.

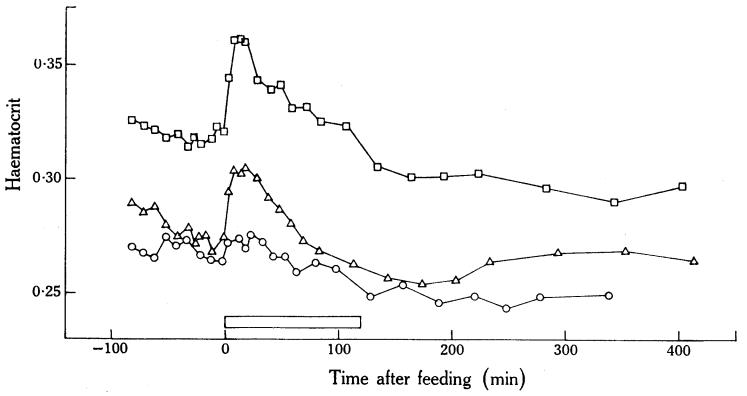

Fig. 2. Changes in jugular haematocrit of splenectomized sheep during feeding. The horizontal bar indicates the feeding period. Sheep 2690 (△), 569 (□) and 555 (○). Food intakes are given in Table 1.

(ii) *Splenectomized sheep*

The general pattern of haematocrit changes in splenectomized sheep (Fig. 2) was similar to that described for intact sheep. The major difference was that the rise in haematocrit was only half that of intact sheep (Table 1).

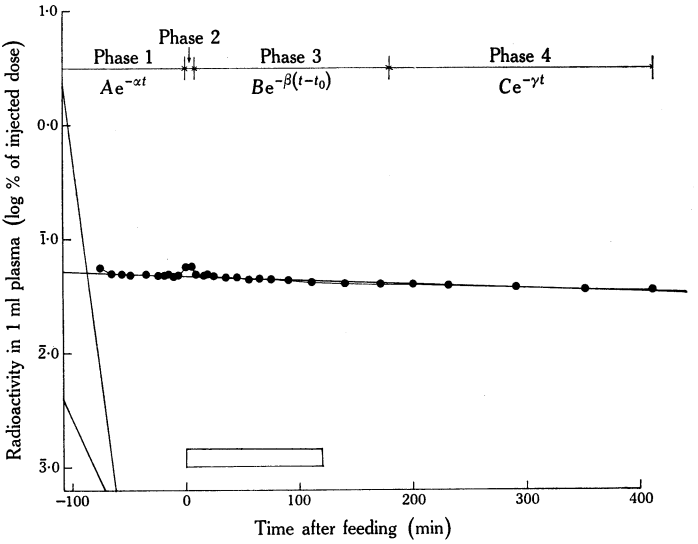

Fig. 3. Tracer loss from sheep 581 during feeding. The horizontal bar indicates the feeding period. Tracer was injected 108 min before feeding commenced. Visual appraisal of the data revealed that the disappearance of tracer followed four phases. Three phases (1, 3 and 4) were analysed into equation (1) of the text. The exponent associated with each phase is shown at the top of the figure.

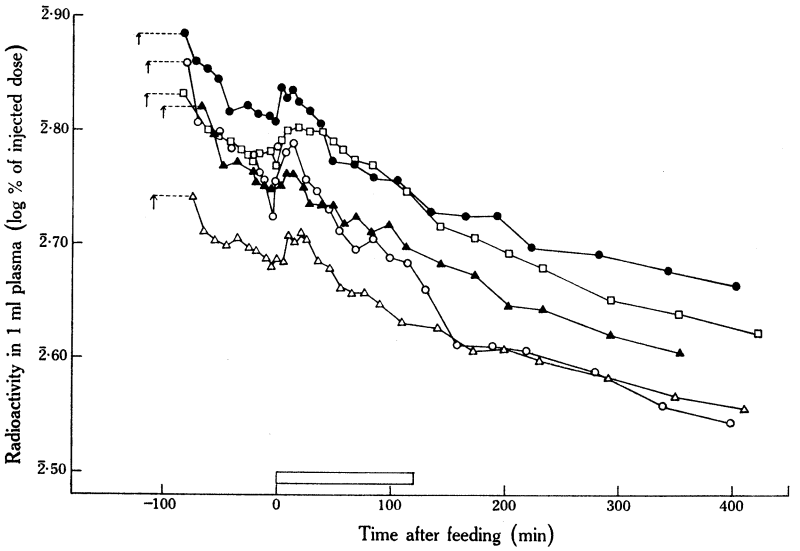

Fig. 4. Disappearance of ^{125}I-labelled serum albumin from plasma of intact sheep during feeding. The horizontal bar indicates the feeding period. The arrows represent the time of injection of the labelled albumin for the series indicated by dotted lines. Sheep 1657 (○), 586 (▲), 1653 (●), 581 (△) and 538 (□).

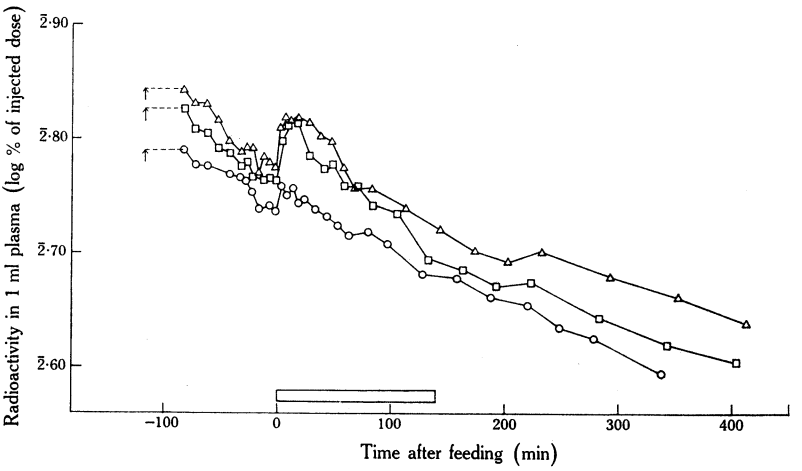

Fig. 5. Disappearance of ^{125}I-labelled serum albumin from plasma of splenectomized sheep during feeding. The horizontal bar indicates the feeding period. The arrows represent the time of injection of labelled albumin for the series indicated by dotted lines. Sheep 2690 (△), 569 (□) and 555 (○).

Disappearance of Iodinated Albumin

(i) *Fed sheep, intact and splenectomized*

The disappearance of tracer from the plasma of intact and splenectomized sheep in each feeding experiment, plotted semilogarithmically, appeared to consist of four sequential phases (Figs 3, 4 and 5):

(1) Phase 1 represented the loss of tracer in the interval before the sheep were fed but after the tracer had mixed in the circulation.

Table 2. Values for parameters in the general equation describing disappearance of tracer from the circulation

For the feeding experiments the disappearance of the tracer is described by equation (1) of the text. For the fasting experiments on intact sheep the disappearance of the tracer is described by equation (2) of the text

Sheep	A	α	B	β	C	γ
			(*a*) *Feeding experiments*			
Intact						
1657	939	−0·0089	174	−0·0137	1319	−0·00054
586	2309	−0·0834	47	−0·0154	1586	−0·00116
1653	1881	−0·0429	256	−0·0645	2678	−0·00087
581	69810	−0·1807	126	−0·0517	1611	−0·00079
538	16932	−0·1369	202	−0·0092	2172	−0·00088
Mean	18374	−0·0906	161	−0·0309	1873	−0·00085
Splenectomized						
2690	835	−0·0192	516	−0·0310	2497	−0·00064
569	979	−0·0374	423	−0·0181	3652	−0·00091
555	317	−0·0161	80	−0·0098	3001	−0·00081
Mean	710	−0·0242	340	−0·0196	3050	−0·00079
			(*b*) *Fasting experiments on intact sheep*			
3521	705	−0·0397	4338	−0·00066		
110	1037	−0·0085	4443	−0·00048		
1162	1390	−0·0102	3798	−0·00052		
635	957	−0·0142	3733	−0·00048		
Mean	1022	−0·0182	4078	−0·00054		

(2) Phase 2 occupied the first 20 min after feeding during which time plasma radioactivity abruptly increased by 12·5%.

(3) Phase 3, which followed phase 2, represented a steadily falling plasma radioactivity during the remainder of the feeding period which usually persisted into the early part of the post-feeding period. Computer analysis (see below) indicated that phase 3 dominated the disappearance of tracer from 20 min after eating commenced (i.e. the end of phase 2) to between 20 and 125 min after the end of the feeding period. Sheep 586 was the exception since phase 3 finished during the eating period in this animal.

(4) Phase 4 followed phase 3 and defined the loss of tracer up until the end of the sampling period. The rate of loss was less than during phase 3.

It was possible to fit an approximate mathematical model to the disappearance of tracer in each sheep. The equation had the form

$$Y = Ae^{-\alpha t} + Be^{-\beta(t-t_0)} + Ce^{-\gamma t}, \quad (1)$$

where t is the time (min) after injection of tracer and t_0 is the time (min) elapsing between injection and the commencement of feeding.

Table 3. Plasma volume (PV) in fed and fasted sheep

Sheep	Body wt (kg)	PV before feeding		PV after feeding			% Decrease in PV predicted from haematocrit data[A]
		(ml)	(ml/kg)	(ml)	(ml/kg)	% Decrease	
				(a) Feeding experiments			
Intact							
1657	27·2	1334	49·1	1177	43·4	11·8	28·3
586	30·6	1482	48·4	1395	45·6	5·9	24·6
1653	29·0	1234	42·5	1108	38·2	10·2	23·2
581	33·4	1782	53·4	1600	47·9	10·2	22·5
538	38·3	1446	37·8	1252	32·7	13·4	34·9
Mean	31·7	1456	46·2	1306	41·2	10·3	26·7
Splenectomized							
2690	33·9	1339	39·5	1131	33·4	15·5	15·9
569	38·2	1414	37·0	1223	32·0	13·5	19·4
555	32·7	1488	45·5	1376	42·1	7·5	6·3
Mean	34·9	1414	40·7	1243	35·8	12·2	13·9
				(b) Fasting experiments on intact sheep			
3521	40·8	1551	38·0				
110	37·0	1485	40·1				
1162	33·6	1280	38·1				
635	45·5	1931	42·4				
Mean	39·2	1562	39·7				

[A] By the method of van Beaumont (1972).

The first exponent represents phase 1, the second phase 3, and the third phase 4. Phase 2, representing the increase in plasma radioactivity during the first 20 min of feeding, was omitted from the model because of the few observations made during this time interval. Values for each parameter in the above equation are shown for each sheep in Table 2.

To clarify the procedure the occurrence of each phase, and the exponential functions in the general equation which defined them, are shown for one animal in Fig. 3.

The computer analysis was carried out for two main reasons. Firstly, the analysis defined the time intervals of phase 3 and phase 4. Secondly, it provided the best possible estimates of the rate of loss of tracer from the circulation upon which the calculation of plasma volume depended.

Plasma volume of sheep before they started eating, and the reduction in plasma volume during feeding, were estimated from the tracer disappearance curves as follows.

(1) *Before feeding*. The slope of the curve before feeding (phase 1) was extrapolated to the time of injection. The antilog of this value, divided into 100, gave the plasma volume in millilitres. The difference between means for intact sheep (46·2 ml/kg body weight) and splenectomized sheep (40·7 ml/kg) was not significant (Table 3).

(2) *During feeding*. The slope of phase 3 was extrapolated to the time of injection and the new plasma volume was calculated in the manner described above. Plasma volume was reduced by about 10% to 41·4 ml/kg in the intact sheep and by about 12% to 35·7 ml/kg in the splenectomized sheep (Table 3). The means did not differ significantly.

Our estimates of the reduction in plasma volume for each sheep during feeding, however, are valid only if the loss of tracer from the plasma was the same during phases 1 and 3; major differences between these rates would restrict the inferences that could be derived from the extrapolated data. Therefore, disappearance rates of tracer during phases 1 and 3 were calculated. Values for rate constants shown in

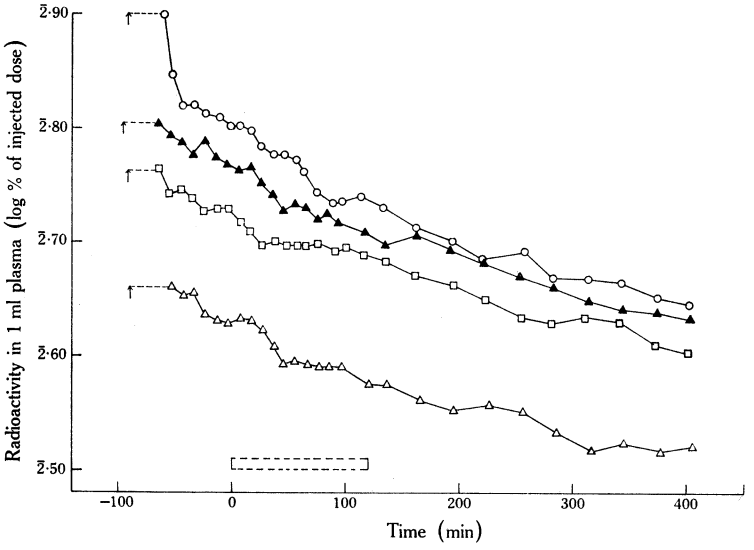

Fig. 6. Disappearance of ^{125}I-labelled serum albumin from plasma of intact, fasted sheep. The horizontal bar, shown in broken lines, indicates the period in which feeding usually occurred. The arrows represent the time of injection of labelled albumin for the series indicated by dotted lines. Sheep 1162 (○), 110 (▲), 3521 (□) and 635 (△).

Table 2 could not be used for this purpose since in accordance with established curve-peeling procedures the slope of phase 3 is given relative to phase 4, rather than to the x-axis (see Fig. 3). Similarly the slope of phase 1 shown in Table 2 is given relative to the slopes of phases 3 and 4. Calculating the slopes relative to the x-axis revealed that the mean slope of phase 1 for fed sheep ($-0{\cdot}0019\ \text{min}^{-1}$) was not significantly different from the mean slope during phase 3 ($-0{\cdot}0018\ \text{min}^{-1}$), supporting the contention that tracer disappeared at a uniform rate during these phases, and validating our methods for estimating the percentage decrease in plasma volume.

(ii) *Fasted sheep*

Disappearance of iodinated albumin from plasma of intact fasted sheep was described by a two-exponential model (Fig. 6 and Table 2) of the form

$$Y = Ae^{-\alpha t} + Be^{-\beta t}. \quad (2)$$

The first term describes tracer loss during the first 3 h after injection and the second term describes tracer loss in the remaining time interval. Mean plasma volume calculated from the extrapolated value of the first exponential was about 40 ml/kg which was less than, though not significantly different from, plasma volume of intact sheep in the feeding experiment before they were fed.

Comparison of tracer loss from plasma of fasted sheep with that of fed sheep was also used to decide whether or not phase 4 of tracer loss in fed sheep was a consequence of having consumed the earlier meal. The technique adopted was to peel by computer the curves of tracer loss during phases 1 and 4 in the fed sheep into two exponential functions and to compare the rate constants of each exponential function with the rate constants for tracer loss in fasted sheep. The reason behind this procedure was that phases 2 and 3 of tracer loss in fed sheep were obviously associated with feeding; whether phase 4 was an after-effect of feeding remained in doubt.

The result of this analysis was that tracer disappeared in the interval following the injection at a slower rate in the fasted sheep ($-0{\cdot}0182\ \text{min}^{-1}$) than in the sheep that were due to be fed ($-0{\cdot}0657\ \text{min}^{-1}$) but the difference was not significant, due to the wide scatter among individual values. However, subsequent loss of tracer at comparable times after injection of tracer was significantly greater during the final phase in the fed sheep ($-0{\cdot}00083\ \text{min}^{-1}$; $P < 0{\cdot}025$) than in the final phase in starved sheep ($0{\cdot}00054\ \text{min}^{-1}$). Loss of tracer during phase 4 of fed sheep would therefore appear to be a consequence of the earlier meal.

Discussion

The most conspicuous events observed in the present study were the increases in haematocrit in intact and splenectomized sheep after they started to eat their daily ration of dry food, and the simultaneous decreases in plasma volume. The 11% reduction in plasma volume was similar to values for reductions in plasma volume and thiosulphate space reported by others (Ternouth 1968; Blair-West and Brook 1969; Christopherson and Webster 1972). Maximum haematocrit in the present study coincided in each case with the occurrence of minimum plasma volume.

Splenectomized Sheep

It is reasonable to assume that the 14% increase in haematocrit in splenectomized sheep was due principally to the reduction in plasma volume. This argument is strengthened by the fact that the fall in plasma volume can be predicted fairly accurately from the haematocrit data alone (Table 3) using the equations derived by van Beaumont (1972). The possibility that the increase in haematocrit was due to the addition of red blood cells into the circulation is discounted because splenectomized sheep do not have any pools of sequestered red blood cells (Turner and Hodgetts 1959; Dooley *et al.* 1972). Therefore, with the exception of the shrinking of red blood cells in the mildly hypertonic plasma after feeding (Dooley and Williams 1975), the changes in haematocrit in splenectomized sheep indicate changes in plasma volume.

The sudden reductions in plasma volume at the start of feeding are probably related to the need for copious volumes of saliva to lubricate the dry feed and to facilitate swallowing. About 3 ml of saliva are secreted for each gram of dry food consumed and the water for the saliva is abstracted from the plasma (Stacy and Warner 1966). Saliva secretion is continuous throughout ingestion (Bailey 1961). The return of the haematocrit to basal values following the earlier peak, which takes place during the remaining 100 min of feeding in splenectomized sheep, implicates an expanding plasma volume. Water must be added to the plasma from another fluid compartment (such as the alimentary canal) at a greater rate than it is being removed from the plasma by salivary glands.

The changes in turnover of plasma water that followed the sudden fall in plasma volume were not detectable by changes in the concentration of labelled albumin. Loss of tracer during this period (phase 3) was not significantly different from loss before feeding commenced (phase 1), so the net gain of water during phase 3, restoring plasma volume to normal, must have been accompanied by a reduced loss of tracer.

Besides the demands of the salivary glands on plasma water during the feeding period there are also the demands of the pancreas (Taylor 1958) and the abomasum (Hill 1960; McLeay and Titchen 1970) commencing within an hour or two of the start of feeding, i.e. during phase 4 of tracer loss. Increased reabsorption of water from the kidney tubules (Stacy and Brook 1964, 1965) and the alimentary canal offset these demands. Redistribution of fluid must be taking place, along with the actual escape of tracer from the circulation, during phase 4 of tracer disappearance, and these processes interact, with the result that tracer disappears at a faster rate in fed sheep than during the corresponding period after injection of tracer in fasted animals. Haemodilution in the post-feeding period (Fig. 2) is consistent with this possibility, though the data do not permit more precise statements to be made.

A possible model describing the disappearance of tracer from the plasma of fasted sheep would include two compartments: plasma and the extravascular spaces. It may be assumed that the volume of neither compartment would change during the course of the experiment. Hence, the first exponential function defined in the present study would describe loss of tracer to the extravascular spaces with minimal recycling and metabolic loss, whilst the second exponent would reflect primarily the recycling of tracer into the plasma pool, plus metabolic loss. In fed sheep, the same basic model would apply but with the added complication of changes in the volumes of both compartments.

Intact Sheep

The disappearance of iodinated albumin from the plasma of intact sheep and splenectomized sheep was the same during feeding experiments, and the previous interpretation of changes in the concentration of tracer in splenectomized sheep applies also to the intact sheep. Plasma volumes, however, expressed on a body weight basis were lower in the splenectomized group by 5·5 ml/kg due to removal of the splenic plasma pool. The difference was not significant. Theoretically, no differences in tracer loss would be expected. Much of the blood in the spleen consists of sequestered red blood cells stored at a high haematocrit, but the plasma pool exchanges rapidly with the rest of the circulating plasma (Dooley *et al.* 1971).

Haematocrit increases in intact sheep were twice the increases measured in the splenectomized sheep. Only half of the increase in haematocrit could be ascribed to the reduced plasma volume (Table 3). Contraction of the spleen and the ensuing addition of its sequestered red blood cells to the circulation (Dooley *et al.* 1972) was probably responsible for the rest of the increase.

The spleen does not remain in a contracted state for the duration of the feeding period, but gradually relaxes. This is evident from the changes in haematocrit (Fig. 1) and from the change in thickness of the spleen (Dooley *et al.* 1972). The result is a similar pattern of haematocrit change as in splenectomized sheep. The time course of the relaxation of the spleen appears to be contemporaneous with the re-establishment of the plasma volume and the possibility arises that the two changes are linked in some way, possibly through stimulation of volume receptors, to ensure a constant volume of blood within the circulatory system. If reduced plasma stimulates a contraction of the spleen, red blood cells will be added to the circulation and blood volume remains constant. Further, as the plasma volume returns to normal the spleen may relax and withdraw red blood cells from the circulation and blood volume would then stay the same. Balance between the volumes of plasma and red blood cells could thereby ensure an adequate volume of blood in the circulation. Another advantage of the elevated haematocrit would be the improved oxygen transport which would help satisfy the additional oxygen demand during feeding (Blaxter and Joyce 1963).

Any testing of these hypotheses would need to grapple with the problem that splenectomized sheep do not seem to be disadvantaged by the lack of a spleen. Certainly their appetites and live weights are not reduced (Tables 1 and 3).

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References

Bailey, C. B. (1961). Saliva secretion and its relation to feeding in cattle. *Br. J. Nutr.* **15**, 443.

Blair-West, J. R., and Brook, A. H. (1969). Circulatory changes and renin secretion in sheep in response to feeding. *J. Physiol.* (*London*) **204**, 15.

Blaxter, K. L., and Joyce, J. P. (1963). The accuracy and ease with which measurements of respiratory metabolism can be made with tracheostomized sheep. *Br. J. Nutr.* **17**, 523.

Christopherson, R. J., and Webster, A. J. F. (1972). Changes during eating in oxygen consumption, cardiac function and body fluids of sheep. *J. Physiol.* (*London*) **221**, 441.

Dooley, P. C., Hecker, J. F., and Webster, M. E. D. (1972). Contraction of the sheep's spleen. *Aust. J. Exp. Biol. Med. Sci.* **50**, 745.

Dooley, P. C., Morris, R. J. H., and Harris, L. R. (1971). Distribution volumes of labelled red blood cells and labelled protein and F cells in intact and splenectomized HbA and HbB type sheep. *Aust. J. Exp. Biol. Med. Sci.* **49**, 129.

Dooley, P. C., Morris, R. J. H., Williams, V. J., and Bofinger, V. J. (1974). An investigation into the precision of micro-haematocrit determinations of sheep blood. *Aust. J. Exp. Biol. Med. Sci.* **52**, 663.

Dooley, P. C., and Williams, V. J. (1975). Changes in the jugular haematocrit of sheep during feeding. *Aust. J. Biol. Sci.* **28**, 43.

Hill, K. J. (1960). Abomasal secretion in the sheep. *J. Physiol.* (*London*) **154**, 115.

McLeay, L. M., and Titchen, D. A. (1970). Abomasal secretory responses to teasing with food and feeding in the sheep. *J. Physiol.* (*London*) **206**, 605.
Snedecor, G. W., and Cochrane, W. G. (1967). 'Statistical Methods'. 6th Edn. (Iowa State University Press: Iowa, U.S.A.)
Stacy, B. D., and Brook, A. H. (1964). The renal response of sheep to feeding. *Aust. J. Agric. Res.* **15**, 289.
Stacy, B. D., and Brook, A. H. (1965). Antidiuretic hormone activity in sheep after feeding. *Q. J. Exp. Physiol.* **50**, 65.
Stacy, B. D., and Warner, A. C. I. (1966). Balances of water and sodium in the rumen during feeding: osmotic stimulation of sodium absorption in sheep. *Q. J. Exp. Physiol.* **51**, 79.
Taylor, R. B. (1958). Pancreatic secretion in the conscious sheep. *J. Physiol.* (*London*) **143**, 81P.
Ternouth, J. H. (1968). Changes in the thiosulphate space and some constituents of the blood of sheep after feeding. *Res. Vet. Sci.* **9**, 345.
Turner, A. W., and Hodgetts, V. E. (1959). The dynamic red cell storage function of the spleen in sheep. I. Relationship to fluctuations of jugular haematocrit. *Aust. J. Exp. Biol. Med. Sci.* **37**, 399.
van Beaumont, W. (1972). Evaluation of haemoconcentration from haematocrit measurements. *J. Appl. Physiol.* **32**, 712.
Vogel, A. I. (1961). 'A Textbook of Quantitative Inorganic Analysis'. 3rd Edn. p. 710. (Longmans: London.)

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