

Changes in the Jugular Haematocrit of Sheep during Feeding

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Abstract

Intact and splenectomized sheep with and without a rumen fistula were used to investigate changes in the jugular blood haematocrit and plasma osmolality during hourly and once-daily feeding regimes. Osmolality was also estimated in the ruminal fluid of fistulated sheep with spleens.

Haematocrit decreased in sheep with spleens before they were given a once-daily feed; it increased when these sheep started to feed, reaching a maximum increase of 13% after 30 min of feeding; it decreased during the remaining 45 min of feeding time and usually continued to decrease after feeding stopped. These changes were not due to diurnal influences. Splenectomized sheep fed once daily showed only small decreases in haematocrit before they were fed. Increases occurred with the onset of eating but they were smaller (7%) than in intact sheep and were of shorter duration. In hourly fed sheep with spleens, haematocrit decreased in the early stages of sampling in a manner similar to that for sheep fed once daily. The changes in haematocrit that did occur were not related in any obvious manner to the feeding regime. The haematocrit in splenectomized sheep fed hourly was stable throughout feeding.

Variations in the haematocrit in splenectomized sheep, equivalent to a range of 13% in one of them, were observed in a series of blood samples obtained during a 5-h period remote from the feeding time.

Large increases occurred in osmolality of ruminal fluid when sheep were fed daily and this was abolished by hourly feeding. Plasma osmolality in sheep fed once daily increased slowly. Maxima occurred after 100 min from the start of eating and were 7% greater than prefeeding values. Only minor changes were observed when these sheep were fed hourly.

Introduction

When a sheep eats a single daily ration of dry chaff there are large reductions in the volumes of several body fluid compartments (Ternouth 1968; Blair-West and Brook 1969; Christopherson and Webster 1972) which are probably brought about by the shift of fluid into the gut. The decrease in one of these compartments, the plasma, has the effect of increasing the haematocrit of the jugular venous blood, but another factor contributing to this increase is a contraction of the spleen (Dooley *et al.* 1972).

The experiments reported here attempted to investigate the activity of the spleen of the sheep during feeding and to assess splenic activity from changes in haematocrit. Haematocrit was measured in successive samples of jugular blood taken before, during and after feeding in intact and splenectomized sheep fed hourly or daily. The interpretation of these values relative to splenic contraction is based on the reciprocal relationship between the haematocrit of jugular blood and the red cell content of the spleen (Turner and Hodgetts 1959) when plasma volume is stable.

Materials and Methods

Sheep

Fourteen mature Merino sheep with a liveweight of 36.2 kg (s.d. 3.6) were used. Sheep Nos 562, 565, 581 and 1886 had no surgery performed on them other than tail-docking and castration as lambs. These four sheep constituted the control group. In the treated group three sheep (Nos 538, 586 and 606) had a rumen fistula, five sheep (Nos 555, 569, 576, 580 and 600) had been splenectomized about 9 months previously, and two sheep (Nos 500 and 572) were splenectomized and had a rumen fistula. The term 'intact' sheep refers to sheep with spleens.

All sheep had been accustomed to handling in the weeks preceding the experiments so that blood and ruminal fluid could be sampled without causing noticeable disturbance.

Blood Samples

Using an aseptic technique, a polyethylene cannula was inserted through a 13-gauge needle into a jugular vein of each sheep at least 12 h before an experiment. Blood samples (5 ml), taken via the cannula into dry syringes, were added to bottles containing dried sodium heparin (20 i.u./ml blood). Haematocrit was determined within 1 h of collecting the blood sample by centrifugation at a relative centrifugal force of 10 000 *g* for 8 min in a Hawksley microhaematocrit centrifuge (Dooley *et al.* 1974).

The remainder of each blood sample was centrifuged at 1500 *g* for 15 min and the supernatant plasma was removed and stored in a stoppered bottle in iced water for up to 12 h before osmolality was measured on 2.0-ml plasma samples with a Fiske osmometer (Fiske Assoc., Bethel, Conn.). Reproducibility was acceptable (mean = 306.5 m-osmol/kg; s.d. 0.9; *n* = 10). Storage for 12 h decreased the osmolality by less than 3%.

Ruminal Fluid Samples

A sampling device was inserted into the ventral sac of the rumen through the ruminal fistula 30 min before an experiment in order to avoid changing the gas phase during sampling. Samples of 3 ml were stored for 12–24 h at ambient temperature in bottles containing dried HgCl₂ as preservative (8.1 mg/ml ruminal fluid). The samples were centrifuged for 10 min at 1500 *g*, then 2.0-ml aliquots of the supernatant fluid were analysed immediately with the Fiske osmometer. Storage for 24 h decreased the osmolality by less than 3%.

Experimental Procedure

(i) *Daily feeding*

Sheep were allowed 75 min in which to eat up to 800 g lucerne chaff offered daily at 0810 h. A 10-day period of training to this feeding pattern preceded each experiment. Each experiment occupied about 460 min from 0720 to 1500 h. Four blood samples were usually taken in the 50 min before food was given to the sheep. Usually five blood samples were obtained during the feeding period, after which food residues were removed and weighed and the food intake was calculated. Another 12 samples were taken in the next 6 h. Thus, about 21 blood samples were obtained during each experiment.

Rumen fluid samples were taken from fistulated sheep after each blood sample.

Sheep were allowed water *ad libitum* during experiments. The time and volume of water intakes were recorded.

(ii) *Hourly feeding*

After the once-daily feeding experiments, the sheep were fed hourly by an automatic feeder (Minson and Cowper 1966) that delivered 33 g lucerne chaff every hour into the feeding trough. Sheep were allowed 7 days to become accustomed to this feeding pattern before another experiment was done. Sampling of blood and of ruminal fluid was similar to that for sheep fed daily, i.e. about 21 samples were obtained from each sheep between 0720 and 1500 h. When the hourly feeding experiments were completed the sheep were returned to the once-daily feeding pattern.

(iii) *Stability of haematocrit*

Haematocrit was studied in two intact and three splenectomized sheep fed daily during a period remote from feeding (1700–2200 h) so that variations in haematocrit other than those due to feeding could be investigated.

(iv) *Teasing with food*

Two intact sheep being fed daily were teased with food at 0810 h instead of being allowed to eat. Teasing consisted of holding a bucket of food just out of reach in front of the cage for about 15 min by which time the sheep had usually lost interest in it. Two hours after the start of teasing the sheep were allowed to eat. Fifteen blood samples were taken over 4 h starting 1 h before teasing and ending 1 h after the start of eating. This experiment was designed to see whether changes in haematocrit during feeding could be repeated by the psychological excitation effects of teasing with food.

(v) *Delay of feeding*

Blood was sampled from two intact sheep being fed daily which were fed 2 h after their normal feeding time. These animals were not teased though they could see other sheep eating. This experiment was designed to verify that eating and not a delayed effect of any stimulus acting before eating, or a time-conditioned reflex, was responsible for the increase in haematocrit during feeding.

(vi) *Addition of water to rumen*

One litre of tap water at room temperature was added to the rumens of each of two splenectomized rumen-fistulated sheep that had not eaten for 24 h to see whether a sudden decrease in osmolality of ruminal fluid, such as may occur when penned sheep drink, has any effect on haematocrit.

Results

Feeding Experiments

Results from intact rumen-fistulated sheep and from splenectomized sheep are shown in Figs 1 and 2 respectively. Results from the 'control' group have not been illustrated since they were indistinguishable from those obtained from the intact rumen-fistulated group.

(i) *Food and water intake*

Although sheep fed daily were offered 800 g of lucerne chaff, they actually consumed less than this. The mean intake of the six intact sheep was 510 g compared with a mean intake of 355 g by the three splenectomized sheep. Intakes were not related to liveweights. Sheep fed hourly ate each 33 g in about 6 min and left no residues.

Water intake during the experiments on sheep fed daily varied between 0 ml (three sheep) and 1900 ml. Two sheep drank first during feeding, three others drank soon after the end of feeding and another had its first drink about 3 h after the end of feeding. No relationship was found between the time of the first drink and the osmolalities of plasma or of ruminal fluid at that time. Sheep fed hourly drank 0–1300 ml during an experiment.

(ii) *Osmolality of ruminal fluid*

Fig. 1 shows that osmolality of ruminal fluid increased in sheep fed daily from about 270 (hypotonic to plasma) to about 460 m-osmol/kg (hypertonic to plasma) within 2 h of the start of feeding. Maximum osmolalities did not appear to be related to the amount of food eaten: sheep No. 538, which ate 595 g, had a similar maximum to sheep 586 which ate 400 g; sheep 606, which showed the greatest osmolality, consumed 560 g. The osmolalities declined over the next 5 h to 300–340 m-osmol/kg, which was still higher than prefeeding values. The final samples were isotonic in two sheep and about 10% hypertonic to plasma in the third sheep.

The variations in osmolality of ruminal fluid were less pronounced when sheep were fed hourly. Osmolalities either decreased slightly or remained constant. The mean values were higher than prefeeding values for sheep fed daily.

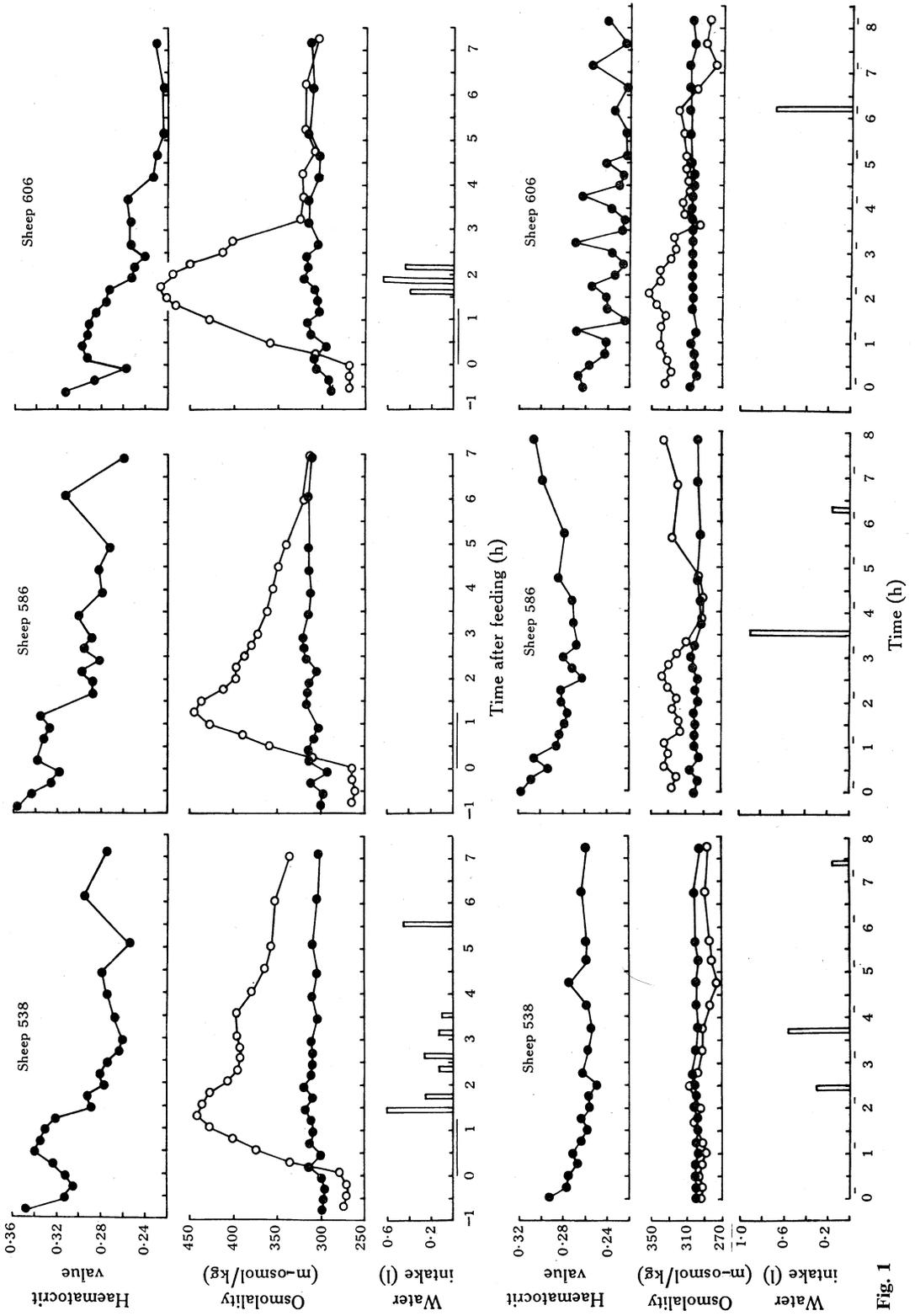


Fig. 1

(iii) *Osmolality of plasma*

Intact and splenectomized sheep fed daily usually had plasma osmolalities of 285–295 m-osmol/kg before feeding and these were increased to 300–320 m-osmol/kg by about 100 min after the start of feeding and remained elevated for the next 6 h. During hourly feeding of intact and splenectomized sheep the changes in osmolality were insignificant. The mean value was 305 m-osmol/kg.

(iv) *Changes in jugular haematocrit during the daily feed*

The first blood sample taken from each intact sheep fed daily had a high haematocrit value (Fig. 1), which generally decreased by about 0.04 in the remaining time before feeding. The start of feeding was associated with an increase in haematocrit, maximum values being attained after 30 min. The increase was 0.04 units, or 13% compared with the last prefeeding sample. The maximum value was not related to the rate at which the food was eaten, and maximum values were not maintained but gradually decreased, even though each sheep was still eating. At 6 h after the end of the feeding period the haematocrit value was between 9 and 19% lower than that of the last prefeeding sample.

Splenectomized sheep showed only minor decreases in haematocrit before feeding (Fig. 2). The haematocrit in the sample obtained immediately before feeding in these sheep (mean = 25.6) was generally lower in value than that of the comparable sample obtained from intact sheep (mean = 29.5). The increases initiated by feeding were smaller and more transient than those observed in intact sheep. The haematocrit at 30 min was 5–10% greater, while that at 6 h after the end of the feeding period was about 8% smaller, than the value immediately before feeding.

(v) *Jugular haematocrit during hourly feeding*

Haematocrit showed only minor fluctuations in intact sheep fed hourly (Fig. 1). There was often a reduction of up to 0.02 units during the first 30–40 min of sampling. The haematocrit of intact sheep after 7 h was between 8% less than and 4% greater than the 30-min value. Haematocrit in splenectomized sheep (Fig. 2) fluctuated less than that in the intact sheep, only slight reductions being observed.

Stability of Haematocrit

Results shown in Fig. 3 seem to indicate that in most sheep, both intact and splenectomized, there was a gradual increase in haematocrit after about 1800 h. Only one sheep (No. 580, splenectomized) failed to demonstrate this haemoconcentration. The increase in haematocrit in the splenectomized sheep, though completely unexpected, was noticeably different in nature from the more rapid, larger and more transient changes in haematocrit that occurred when splenectomized sheep ate their daily ration of food (Fig. 2).

No general statement about haematocrit changes in the first hour of study (between 1700 and 1800 h) can be made since trends between sheep were variable in this period. The most notable change that occurred was the 11% decrease in haematocrit in 25 min in an intact sheep (1886).

Fig. 1. Changes in haematocrit, the osmolalities of plasma (●) and ruminal fluid (○) and water intake in three intact rumen-fistulated sheep fed daily (upper series of curves) and hourly (lower series of curves). The horizontal bars beneath the axes indicate the feeding periods. Each sheep ate about 520 g of lucerne chaff when being fed once daily.

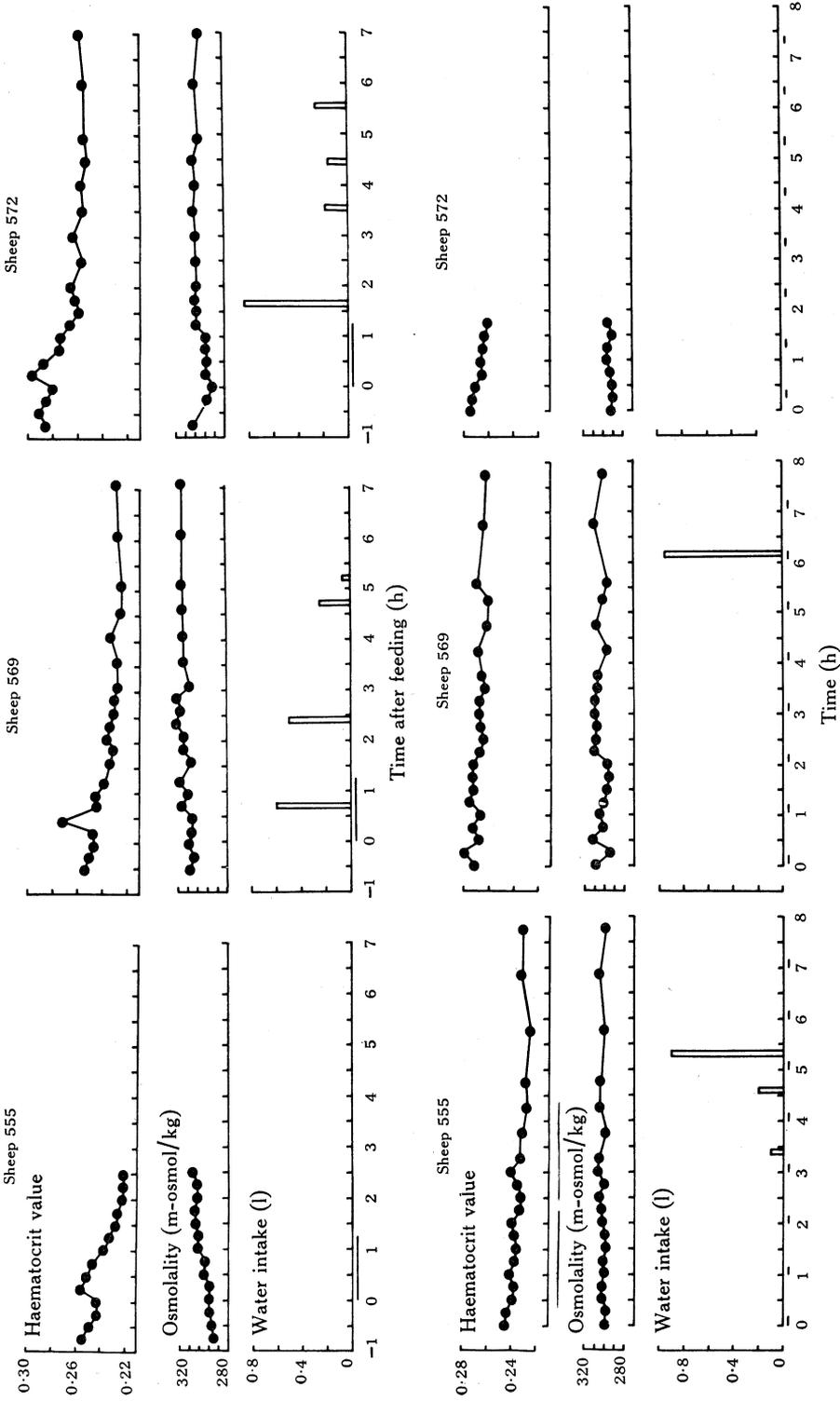


Fig. 2. Changes in haematocrit and in plasma osmolality and water intake in three splenectomized sheep fed daily (upper series of curves) and hourly (lower series of curves). The horizontal bars beneath the axes indicate the feeding periods. Each sheep ate about 350 g of lucerne chaff when being fed once daily.

The coefficient of variation (c.v.) of haematocrit was 1% in sheep 580; in the other sheep it ranged between 2 and 5%.

Teasing Experiments

Haematocrit increased when two intact sheep were teased with food (Fig. 4). One sheep responded with a prompt 7% increase while the response by the other was obscured by an increase that commenced 30 min before teasing. Hence, the increase in haematocrit in this second sheep may not have been due solely to the

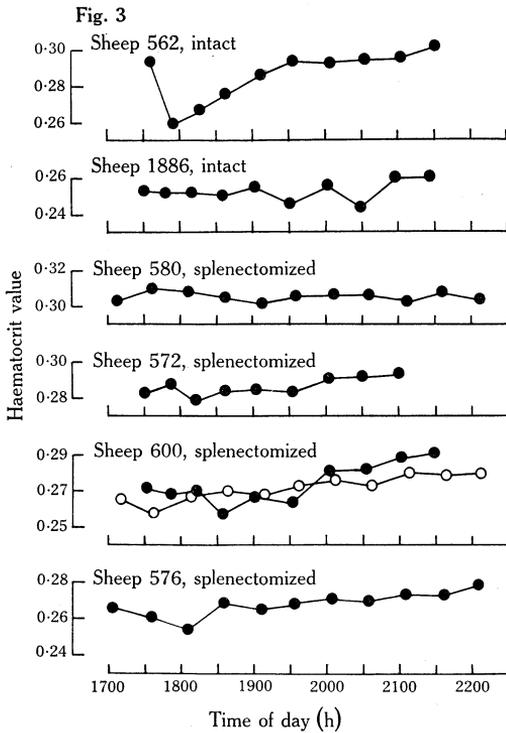
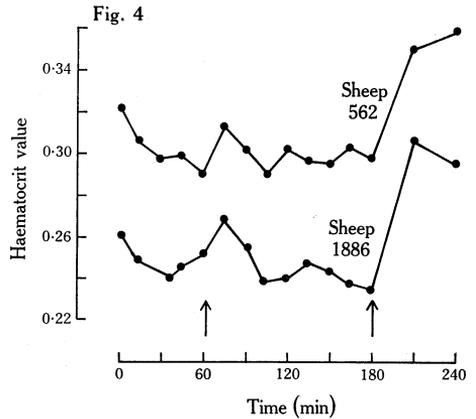


Fig. 3. Changes in haematocrit in intact and splenectomized sheep during a quiet period. Sheep 600 was studied twice (•, ○).

Fig. 4. Effect of teasing with food and of feeding on haematocrit values in two intact sheep. The arrow at 60 min indicates the time at which both sheep were teased with food, and that at 180 min indicates the time at which each sheep was allowed to start eating.



teasing. However, the haematocrit in both sheep returned to reasonably steady baseline values 30 min after the teasing. When these animals were later fed the increase in haematocrit was three times greater than that caused by teasing.

Delayed Feeding

Haematocrit varied to a small degree in two intact sheep in the period that normally preceded the daily feeding time (Fig. 5). Owing to the variability of the data in this period, it was difficult to decide whether or not the haematocrit was affected by withholding food, but there was possibly a 7% increase in one sheep. The effect, if any, was slight compared with the increase in haematocrit that occurred when these sheep were later allowed to eat.

The c.v. for the haematocrit of the 19 samples taken before feeding was 2.6% in sheep 562 and 5.0% in sheep 1886.

Effect of adding Water to Rumens of Splenectomized Sheep

Osmolality of ruminal fluid was reduced by 60 m-osmol/kg in one sheep and by 35 m-osmol/kg in the other 1 h after water loading (Fig. 6); osmolality then increased steadily in these sheep. Only slight changes were observed in plasma osmolality and in haematocrit.

Discussion

The first two samples of blood usually had a higher haematocrit than the third sample in intact sheep fed daily or hourly. Probably, the arrival of the experimentalist at the animal house at an unusually early hour disturbed the sheep and caused splenic contractions and associated increases in the haematocrit of jugular blood. Excitement was only temporary since the value then fell as the spleen expanded and filled with blood. This may be taken as evidence of the quiet temperament of these sheep. This interpretation of splenic involvement is based on the reciprocal relationship between haematocrit of jugular blood and the red cell content of the spleen (Turner and Hodgetts 1959) in the absence of plasma volume changes. The haematocrit of the blood sampled immediately before feeding was assumed to have been taken while the spleen was fully relaxed. This assumption was based upon the results of previous experiments in which it was observed that red cell concentration in jugular blood returned to control values 30 min after a near-maximum contraction of the spleen (Dooley *et al.* 1972).

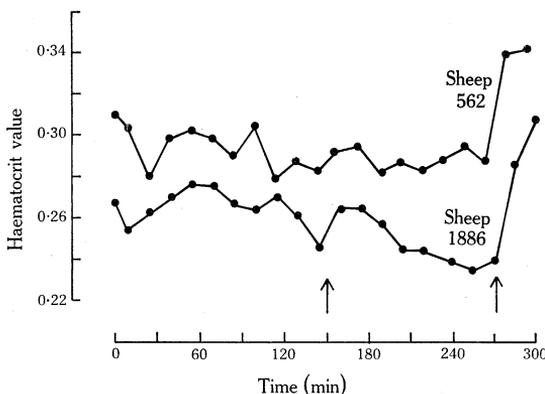


Fig. 5. Effect of a delay in feeding on haematocrit of two intact sheep. The arrow at 150 min indicates the normal feeding time for these sheep, and that at 270 min indicates the time at which both sheep were allowed to start eating their rations.

The data (Fig. 5) show that there is no substantial increase in haematocrit if the sheep are not fed. The apparent contraction of the spleen (seen as a decrease in spleen thickness) that was observed in sheep just before they were fed in a previous series of experiments (Dooley *et al.* 1972) was not observed in the present study as evidenced by changes in haematocrit (Fig. 1). The earlier work was done in a large animal house in which food was distributed to other sheep for 45–60 min before the experimental sheep were fed. The present experiments were done in a smaller animal house where there was no such disturbing factor. It is possible therefore that the sheep in the smaller animal house were less reactive to excitation effects before feeding than the sheep in the large animal house. Variations in temperament between sheep may also have been involved: Fig. 5 shows that sheep can respond differently to a temporary delay in feeding as far as their haematocrit values are concerned.

Haematocrit increased rapidly when intact fistulated sheep (Fig. 1), control sheep (not shown) and splenectomized sheep (Fig. 2) started to eat their single daily ration

of food. There were no obvious differences between control and intact fistulated sheep in their haematocrit responses to feeding. Differences existed, however, between the haematocrit response of intact and splenectomized sheep with respect to the magnitude, occurrence and duration of the response. The mean increase in intact sheep, 13.5%, occurred within 25–35 min of the start of feeding, but haematocrit increased by only 7.1% in splenectomized sheep and these maxima occurred within 15–27 min of the start of feeding.

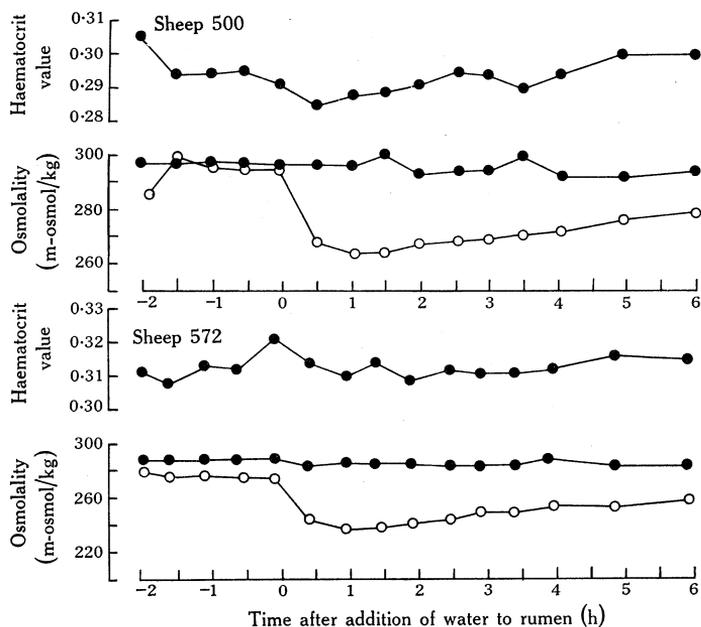


Fig. 6. Haematocrit and osmolality of plasma (●) and ruminal fluid (○) after addition of water to the rumens of two splenectomized rumen-fistulated sheep. At time 0, 1 litre of tap water was poured into the rumen of each sheep through the fistula.

The increase in haematocrit in splenectomized sheep was small and transient and was likely to have been caused by a reduction in plasma volume. Christopherson and Webster (1972) showed that the plasma volume was at a minimum 15 min after the sheep had eaten 317 g of hay and that plasma volume attained prefeeding values 30 min after feeding had stopped. The same rapid reduction in plasma volume associated with feeding was recorded by Blair-West and Brook (1969), but the minimum plasma volume was maintained for a longer period of time, possibly because food intake was almost double. Clearly the rise in haematocrit with feeding in splenectomized animals was most likely due to a decrease in plasma volume; the results also suggest that the return to prefeeding plasma volumes was as fast in these sheep as that recorded by Christopherson and Webster (1972).

The implication that arises from a comparison of the increases in haematocrit in intact and splenectomized sheep observed during the daily meal is that the increase in the haematocrit of blood of intact sheep is due to two events: firstly there is a contraction of the spleen; secondly there is a reduction in plasma volume. The effects on haematocrit of a reduced plasma volume in intact sheep are likely to be greater than that suggested from the increase in haematocrit in splenectomized sheep (Fig. 2), since the splenectomized sheep ate less chaff in the allotted time than the intact sheep.

The stimulus that contracts the spleen during the daily feed has not been elucidated. The act of eating *per se* does not seem to be important because there was no correlation between eating and increase in haematocrit in the animals fed hourly. If the quantity of food consumed influences the extent of splenic contraction the amount required must be small because the haematocrit was at a maximum about 30 min after the start of eating, even though at this juncture the animals were voraciously eating. It is unlikely that osmolality of the plasma was a stimulus to contraction because it had only risen minutely before haematocrit rose substantially. Excitation by anticipated feeding (Figs 4 and 5) could have initiated contraction, but teasing caused a smaller and faster response than that due to feeding.

Haematocrit was reduced in intact and splenectomized sheep after they had eaten their daily meal. The values in splenectomized sheep at the end of the meal time were 9% lower than the prefeeding values, but unlike those in intact sheep the haematocrit then remained constant. The tendency in intact sheep after feeding was for the occurrence of a continued reduction in haematocrit (amounting to a mean reduction of 15% compared to the prefeeding value) which lasted many hours. Comparison of the response after feeding in intact and splenectomized sheep indicates that the continued fall in haematocrit after feeding in intact sheep must be due to a splenic effect. This conclusion is in accordance with earlier work which recorded that the spleen of the sheep requires several hours to return to its prefeeding size after the contraction induced by eating the daily meal (Dooley *et al.* 1972).

Water intake did not modify the haematocrit. Visual appraisal of the data showed that neither the normal addition of water to the rumen by drinking, nor the addition of a large volume of cold water to the rumen via a fistula, altered the haematocrit.

It is generally accepted that splenectomized sheep have a stable haematocrit (Turner and Hodgetts 1959; Dooley *et al.* 1972). Variations in haematocrit in splenectomized sheep at a time remote from feeding (Fig. 3) were therefore unexpected. The value in one splenectomized sheep (No. 600) varied over a range of 13%. The trend was repeatable in this sheep on a second occasion, suggesting a physiological basis. An injection of adrenaline (10 $\mu\text{g}/\text{kg}$ liveweight) failed to increase the haematocrit in this sheep. Since haematocrit can be measured with an accuracy of 1% (Dooley *et al.* 1974), variations greater than this must be due to biological effects. The large increases in peripheral haematocrit produced in dogs by injections of adrenaline or noradrenaline are abolished by splenectomy, demonstrating that there are no red cell reservoirs in these dogs (Reeve *et al.* 1953; Hamilton and Horvath 1954; Chacalos and Moore 1963; Weisse *et al.* 1968). Peripheral haematocrit, however, is still subject to alterations under some conditions: shifts of plasma or red cells out of small vessels induced by infusions of vasoactive agents into anaesthetized splenectomized dogs alter the haematocrit in peripheral blood (Chacalos 1967). Whether there are any naturally occurring stimuli that act in a similar manner in conscious splenectomized dogs or sheep has not been determined.

In conclusion it must be emphasized that the results obtained are a direct consequence of the experimental design, i.e. they are directly applicable to sheep in pens fed a single daily ration of dry chaff or small quantities of dry chaff at hourly intervals. Therefore, there are probably no implications for better understanding of the physiological processes that operate in free-grazing sheep. However, there are limited implications for sheep in situations such as intermittent drought feeding.

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