The effects of various forms of gastrointestinal cannulation on digestive measurements in sheep

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(*Received* 17 *August* 1976 – *Accepted* 7 *October* 1976)

1. There was little difference in digestive (voluntary food intake, dry matter digestibility and nitrogen balance) and blood measurements (venous concentrations of corticosteroids, serum aspartate aminotransferase (*EC* 2.6.1.1), protein-bound iodine, urea and glucose) of intact sheep (eight animals) and of sheep prepared with rumen cannulas (sixteen animals) and subsequently with either simple 'T-shaped' (eight animals) or re-entrant cannulas (eight animals) at the duodenum and ileum, when fed *ad lib.* a chopped, medium-quality-hay ration.

2. Wool growth rates of the intact sheep were similar to those in sheep with rumen cannulas and with rumen cannulas plus simple 'T-shaped' cannulas, but higher ($P < 0.01$) than those with rumen cannulas plus re-entrant cannulas.

3. When the sheep were subsequently given a restricted intake (800 g/d) of dried grass, retention times of solid- and liquid-phase digesta markers in the rumen and caecum were similar in all sheep.

4. The use of the different preparations in digestive physiology studies is discussed.

Ruminants prepared with gastrointestinal cannulas are used routinely to measure absorption of digesta constituents at various sites along the gastrointestinal tract. Designs of the cannulas are numerous, but basically they form two categories: (*a*) simple cannulas (usually 'T'-shaped) which provide a permanent fistula into the lumen of the tract, (*b*) re-entrant cannulas which divert the flow of the whole digesta exterior to the animal for a short distance.

Until recently the latter technique was favoured for measurement of flow of digesta into and out of the small intestine, because any difficulty experienced in preparation and maintenance of the animals was outweighed by the fact that the preparation provided a continuous supply of digesta from which representative samples could be taken (MacRae, 1975). However, since the development of certain rare-earth and transition elements as satisfactory particulate-phase markers the use of simple cannulas and dual-phase markers has become an attractive alternative (MacRae, 1974; Faichney, 1975).

Despite the widespread use of both forms of cannula, few attempts other than some digestibility measurements (Drori & Loosli, 1959; Reid, Shelton & Welch, 1961; Hayes, Little & Mitchell, 1964; Putman & Davis, 1965) have been made to evaluate whether surgical interference has any effects on subsequent digestive or metabolic measurements in such animals. The present study was designed to compare several pre- and postoperative digestive measurements in sheep prepared with a rumen cannula plus either simple ('T'-shaped) or re-entrant duodenal and ileal cannulas given a medium-quality-hay ration. Blood measurements and wool growth rates were also monitored.

Results from this study have been used in a review (MacRae, 1975).

EXPERIMENTAL

Twenty-four Scottish Blackface wethers of similar age (18 months) and provenance, ranging in live weight (W) from 35 to 47 kg, were used in the study. Two experiments were done.

Table 1. *Expt* 1 *and* 2. *Identification of groups of sheep by the type of surgery and feeding regimen used at each stage of the experiment*

Expt no. ...	1			2
Feeding regimen ...	Medium-quality mixed-pasture hay *ad lib.*			High-quality dried grass (restricted intake (800 g/d))
Stage of Expt	1	2	3	
Period of Expt	Feb.–Mar. 1973	Apr.–July 1973	Aug.–Dec. 1973	Jan.–Mar. 1974
Cannulation procedure	Intact (twenty-four sheep)	Group A (eight sheep): Intact (control)	→	→
		Group B (eight sheep): Rumen cannula	Duodenal plus ileal simple ('T'-shaped) cannulas	→
		Group C (eight sheep): Rumen cannula	Duodenal plus ileal re-entrant cannulas	→

Expt 1 consisted of a three-stage study of voluntary food intake (VFI) and digestibility of a chopped, medium-quality-pasture hay during which sixteen of the sheep underwent gastro-intestinal surgery. Expt 2 compared the retention times of rumen and caecal digesta in the sheep prepared with different cannulas. The experimental design is summarized in Table 1.

Expt 1. Each sheep was held individually in a metabolism crate and offered *ad lib.* chopped, medium-quality-pasture hay. Stage 1 consisted of a 4-week acclimatization period followed by a balanced period of 14 d during which VFI, ration dry matter (DM) digestibility and nitrogen balance were measured.

On the basis of their VFI (g/kg $W^{0.75}$) in stage 1, the sheep were allocated to three equal groups (A, B, C) for stage 2. Group A sheep were kept intact (i.e. control group) whilst group B and C sheep were each prepared with a rumen cannula (Kay & McKenzie, 1968). After a recovery period of 4–5 weeks, all the sheep again underwent the same acclimatization and balance procedures as in stage 1.

In stage 3, group B sheep were each prepared with simple ('T'-shaped) cannulas in the duodenum and ileum (Hecker, 1974) and group C sheep were each prepared with re-entrant cannulas in the duodenum and ileum (Brown, Armstrong & MacRae, 1968). After a recovery period the sheep again underwent the acclimatization and balance procedures outlined for stage 1.

In each experiment the sheep undergoing surgery were given a high-quality-dried-grass ration for 2 weeks before the operation and for 3–4 weeks after the operation (1 kg/d, except immediately after surgery when on days 1–3 they were offered 250, 500 and 750 g respectively); food was withheld for 24 h before surgery.

Throughout the experiment the concentrations of corticosteroids, serum aspartate aminotransferase (*EC* 2.6.1.1) (SGOT), protein-bound iodine (PBI), glucose and urea were monitored in venous blood in an attempt to detect any stress conditions imposed by surgery.

In addition, wool growth rates were determined for the 14 d balance periods of stages 2 and 3.

Expt 2. To compare the rates of passage of particulate- and liquid-phase digesta markers

in the intact and surgically-prepared sheep, all animals received a limited amount (800 g/d) of chopped, dried grass (organic matter 890 g/kg, N content 36·8 g/kg, DM digestibility 0·75). During a 14 d period, digesta particulate- and liquid-phase markers (non-radioactive ruthenium-phenanthroline (Ru-P) (MacRae & Evans, 1974) and chromium-EDTA (Binnerts, Van't Klooster & Frens, 1968) respectively) were given as single intraruminal injections on two occasions (via the rumen cannula of group B and C sheep and via hypodermic puncture of the abdominal and rumen walls of group A sheep). After marker administration (09·00 hours in week 1 and 15.00 hours in week 2) faecal samples were collected at 12 h intervals (09.00 hours and 21.00 hours). This gave combined 6 h faecal marker concentrations from 12 to 120 h after rumen administration of marker; additional 12 h samples were also taken until 160 h after rumen administration of marker. The mean retention times of particulate- and liquid-phase markers in the rumen and caecum were obtained from the rate-constants of the two dependent exponential equations describing the relationship between faecal marker concentrations and time, using the curve-fitting programme of Grovum & Williams (1973).

Sampling and analyses. Samples of hay and dried grass were taken for analysis during ration preparation. Throughout the 14 d balance periods, daily collections were made of food refusals, faeces and urine. Urine (collected into 50 ml 0·3 M-sulphuric acid) was stored at −20°. The N content of food, faeces and urine were determined by a micro-Kjeldahl method (Allen, Grimshaw, Parkinson & Quarmby, 1974). After marker administration in Expt 2, faeces were collected every 12 h, weighed and subsampled. The subsamples were dried at 70°, ground in a ball-mill and analysed for Ru and Cr by X-ray spectrometry (Evans, MacRae & Wilson, 1977).

Blood was obtained once a week by venipuncture at 09.00 hours. Plasma for the measurement of corticosteroids (Bassett & Hinks, 1969), urea (Marsh, Fingerhut & Miller, 1965) and glucose (Gutteridge & Wright, 1968) was prepared by centrifuging at 3000 *g* for 20 min. Serum for measurement of SGOT (Kanai & Nomoto, 1970) and PBI (Riley & Gockman, 1964) was prepared from the clotted sample (obtained by maintaining blood at room temperature for 2 h) by centrifuging at 3000 *g* for 20 min. Wool growth was measured as the amount of greasy wool produced from a 2500 mm^2 'tattoo area' during the 14 d period of stages 2 and 3; the clipped wool was placed in a conditioning room at 18° and 65 % relative humidity for 48 h before being weighed.

RESULTS

VFI. The mean values for VFI of each group of sheep in Expt 1 are given in Table 2. Although the VFI values for group B, rumen-cannulated sheep in stage 2 was significantly lower ($P < 0·05$) than those for the control sheep (group A), no explanation can be offered for this finding because group C sheep, which underwent similar rumen surgery, had a mean VFI in stage 2 which was identical to that of the control animals. Indeed the mean VFI of all sixteen sheep prepared with rumen cannulas (59·5 ± 2·2 g/kg $W^{0·75}$) was not significantly different from the control value (Group A). The subsequent values for VFI of group B sheep prepared with simple 'T'-shaped cannulas (stage 3) was again significantly lower than that of the control sheep (group A) ($P < 0·05$), but the reason appeared to be due mainly to the effect of their rumen cannulation rather than their intestinal cannulation. Indeed intake changes consequent upon both types of intestinal surgery were not significantly different from changes in the control sheep between stages 2 and 3 (VFI (g/kg $W^{0·75}$) group A −8, group B −15, group C −14; SEM ±3·1).

DM *digestibility and N balance.* The mean DM digestibility values and N balance results obtained in Expt 1 are given in Table 3. Surgical preparation of rumen fistulas or of double intestinal fistulas had no significant effect on either measurement.

Table 2. *Expt* 1. *Voluntary feed intake (g/kg* $W^{0.75}$*) of chopped, medium-quality-hay ration given to intact sheep (group A, stages* 1–3; *groups B and C, stage* 1), *to sheep prepared with a rumen cannula (groups B and C, stage* 2) *and to the same sheep subsequently prepared with either duodenal and ileal simple ('T'-shaped) cannulas (group B, stage* 3) *or duodenal and ileal re-entrant cannulas (group C, stage* 3).*

Group ...	A	B	C	SEM
Stage of Expt				
1	48	48	48	0·9
2	64	55	64	2·2
3	56	40	50	2·9

* For details of groups experimental procedures, see p. 66 and Table 1.

Table 3. *Expt* 1. *Dry matter* (DM) *digestibility and nitrogen balance (g/d) values for intact sheep (group A, stages* 1–3; *groups B and C, stage* 1), *for sheep prepared with a rumen cannula (groups B and C, stage* 2) *and for the same sheep subsequently prepared with either duodenal and ileal simple ('T'-shaped) cannulas (group B, stage* 3) *or duodenal and ileal re-entrant cannulas (group C, stage* 3), *given a chopped, medium-quality-hay ration**

	DM digestibility				N balance			
Group ...	A	B	C	SEM	A	B	C	SEM
Stage of Expt								
1	0·61	0·62	0·61	0·011	−0·34	−0·78	−0·42	0·176
2	0·59	0·61	0·62	0·009	+0·95	+0·81	−0·69	0·329
3	0·58	0·62	0·60	0·008	−1·64	−2·21	−1·28	0·630

* For details of groups and experimental procedures, see p. 66 and Table 1.

Table 4. *Expt* 2. *Mean retention time (h) of particulate-(ruthenium–phenanthroline (Ru-P)) and liquid (chromium-EDTA)-phase digesta markers in the rumen and the caecum of intact sheep (group A), of sheep prepared with a rumen cannula plus duodenal and ileal simple ('T'-shaped) cannulas (group B) and of sheep prepared with a rumen cannula plus duodenal and ileal re-entrant cannulas (group C), given chopped dried grass* (800 *g/d*)*

	Mean retention time of:			
	Ru-P		Cr-EDTA	
	Rumen	Caecum	Rumen	Caecum
Group				
A	44·1	11·5	36·7	10·7
B	46·1	11·2	34·7	9·6
C	49·2	12·0	39·6	11·5
SEM	3·12	0·60	2·76	0·72

* For details of groups and experimental procedures, see p. 67 and Table 1.

Retention times of digesta particulate- and liquid-phase markers. The estimated mean retention times of Ru-P and Cr-EDTA in the rumen and caecum of sheep in Expt 2 are given in Table 4. Although the liquid-phase marker left the rumen consistently faster than the particulate-phase marker in all groups there was no significant 'between group' differences in the release of individual markers from either the rumen or the caecum.

Venous blood measurements. There were no major changes in the concentrations of cortico-

Table 5. *Expt* 1. *Concentrations of plasma corticosteroids, glucose and urea and serum aspartate aminotransferase* (EC 2.6.1.1) (*SGOT*) *and protein-bound iodine* (*PBI*) *in jugular blood taken from intact sheep and from sheep prepared with either rumen cannula only, rumen cannula plus duodenal and ileal simple cannulas or rumen cannula plus duodenal and ileal re-entrant cannulas in stages* 1–3 *of the experiment**

Position of cannula ...	None	Rumen	Rumen plus duodenal and ileal simple ('T'-shaped)	Rumen plus duodenal and ileal re-entrant	SEM
Measurement					
Cortisol (nmol/ml)	9·6	11·9	9·3	4·7	1·36
Glucose (mmol/l)	1·94	1·78	1·78	1·61	0·078
Urea (mmol/l)	1·22	1·15	1·58	1·58	0·092
SGOT (munit†/ml)	27	21	21	22	1·5
PBI (μg/l)	58	55	55	65	2·9

* For details of groups and experimental procedures, see p. 66 and Table 1.

† One unit of enzyme activity was defined as the amount of enzyme which transforms 1 μmol substrate/min at 25°.

steroids, SGOT, PBI, glucose and urea in the jugular blood of the three groups of sheep, monitored throughout Expt 1 (see Table 5).

Wool growth rates. Wool growth rates for intact sheep, for sheep prepared with a rumen cannula plus duodenal and ileal simple ('T'-shaped) cannulas and with a rumen cannula plus duodenal and ileal re-entrant cannulas were 21·0, 22·5, 19·9 and 11·2 mg/2500 mm²/kg food per d respectively (SEM ± 2·24). Sheep prepared with rumen cannulas or with rumen cannulas plus simple cannulas in the duodenum and ileum had similar wool growth rates to the intact sheep. However, values for the sheep prepared with re-entrant cannulas were significantly ($P < 0·01$) lower than those of all the other sheep.

DISCUSSION

It was surprising that in Expt 1 the only significant effect on VFI directly attributable to surgery was in group B sheep during stage 2, where there was a reduction caused by rumen cannulation, the least severe of the surgical preparations. No reason can be offered for this finding. The fact that the mean VFI for all sixteen sheep prepared with rumen cannulas was not significantly different from the intact sheep would confirm observations made on three sheep prepared with larger rumen cannulas (Thornton & Minson, 1972). It would have been desirable from the biological point of view to have re-randomized groups B and C rumen-cannulated animals before intestinal surgery, but this was not possible because all sheep had previously been randomized on a block design with relation to their VFI in stage 1.

Several workers have shown that surgical preparation of various cannulas in sheep (Reid *et al.* 1961; MacRae, 1967; MacRae, Reid, Dellow & Wyburn, 1973), in calves (Putman & Davis, 1965) and in steers (Drori & Loosli, 1959; Hayes *et al.* 1964) has little effect on ration digestibility. The results of the present study are in agreement with these findings. A multitude of factors is, however, known to contribute to this measurement and it is thought that measurement of rates of passage of digesta through the gastrointestinal tract is probably a more sensitive index of any changes in gut motility brought about by surgical interference. In the present study, the retention times of the liquid- and solid-phase markers in the rumen and caecum showed no significant changes due to surgery. This was in general agreement with the findings of MacRae *et al.* (1973), reporting on caecum-cannulated sheep with markers administered through ileal cannulas. It is worth noting that they did report a

significant increase in rate of passage of digesta in one sheep where caecal function had been totally inhibited through surgery; but they could not detect any change in ration digestibility in this sheep.

Although small differences consequent upon surgical establishment of gastrointestinal cannulas cannot be ignored, particularly with respect to VFI values, there did not appear to be any large effects on any of the digestive measurements studied. It is therefore probable that cannulation of such animals does not grossly interfere with their ability to digest their rations.

None of the blood measurements (Table 5) showed any major changes during Expt 1. Caeruloplasmin levels did increase considerably after the preparation of rumen cannulas and simple cannulas and the results will be discussed in detail elsewhere (MacRae & Suttle, unpublished results). The implications of this phenomenon are not understood but are being studied further (N. D. Suttle, personal communication).

The only major change due to surgery observed in this study was the significant reduction in wool growth rates in the sheep prepared with re-entrant cannulas (group C, stage 3). Whilst it must be remembered that these experiments were designed specifically to compare digestive rather than metabolic measurements, the fact that wool production appeared to be reduced in re-entrant-cannulated sheep might suggest some alteration of metabolism in these animals. One reason for this might be that re-entrant cannulation was exerting some chronic stress on the animals which manifested itself in changes in the animals' ability to utilize the end-products of metabolism. This may partly explain why difficulties have been experienced in maintaining such sheep over long periods (MacRae & Ulyatt, 1972). This aspect obviously requires further study, however if substantiated it might seem appropriate to restrict the use of re-entrant-cannulated animals to determination of digestive measurements on high-quality rations. Where studies are undertaken on poorer-quality rations and especially where utilization of the end-products of digestion are under study, simple cannulas are possibly a more suitable preparation.

The authors wish to thank Messrs C. C. Evans and E. Skedd for analytical services, Mr W. F. Smith for the wool growth measurements and Mr A. R. Fawcett and Mrs C. Landale for assistance in the surgical and blood sampling programmes.

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Printed in Great Britain