Assay and digestion of ^{14}C-labelled condensed tannins in the gastrointestinal tract of sheep

BY T. H. TERRILL,[1]*, G. C. WAGHORN[2]†, D. J. WOOLLEY[3], W. C. McNABB[1]‡ AND T. N. BARRY[1]

[1] *Department of Animal Science, Massey University, Palmerston North, New Zealand*
[2] *AgResearch Grasslands, Private Bag 11008, Palmerston North, New Zealand*
[3] *Department of Plant Science, Massey University, Palmerston North, New Zealand*

(*Received 22 June 1993 – Revised 9 December 1993 – Accepted 14 January 1994*)

Three experiments were conducted to determine the fate of condensed tannins (CT) during digestion in sheep. CT were measured as extractable, protein-bound and fibre-bound fractions using the butanol–HCl procedure. In Expt 1, purified CT were added to digesta from different parts of the digestive tract obtained from a pasture-fed sheep. Recoveries of CT after 0 and 4 h of anaerobic incubation at 39° averaged: rumen 78·9 and 57·5%; abomasum 50·9 and 49·0%; duodenum 64·4 and 46·0% and ileum 43·4 and 38·8%. In Expt 2, [^{14}C]CT was given *per abomasum* over a 6·5 h period at 15 min intervals to a sheep previously fed on *Lotus pedunculatus* (which contains CT). The sheep was killed at the end of the period and 92·4% of the label was recovered. Virtually all of the label was in the digesta, and none was detected in the blood, so that the CT-carbon appeared not to be absorbed from the small intestine. In Expt 3, rumen, abomasal and ileal digesta and faeces samples from sheep fed on *Lotus pedunculatus* were analysed for CT and CT flow along the digestive tract calculated from reference to indigestible markers. Values were low in all digesta samples, indicating disappearance of CT across the rumen and small intestine, and CT recovery in faeces was only about 15% of intake. However, the ^{14}C results from Expt 2 suggested that little if any CT-carbon was absorbed and the low recoveries in Expt 1 are considered to be a consequence of either conformational changes to the CT molecule such that it is no longer detectable by colorimetric methods, an inability of the analytical method to release bound CT for the butanol–HCl assay, or interference from other digesta constituents. It is concluded that the butanol–HCl method of CT analysis is appropriate for quantifying CT in herbages but not in digesta or faeces, and that a substantial part of CT released during protein digestion in the small intestine may not be detectable by normal CT analytical methods.

[^{14}C]Condensed tannin: Digesta tannins: Tannin digestion

Condensed tannins (CT) are polyphenolic compounds occurring in a wide range of plants eaten by ruminants (Jones *et al.* 1976; Sarkar *et al.* 1976; Terrill *et al.* 1992*b*). They may be either beneficial or detrimental to the animals' nutrition depending on concentration in the forage, astringency and pH-dependent protein-binding characteristics. The concentration of CT may be as high as 200 g/kg plant dry matter (DM) but most of the herbages ingested by ruminants contain 20–100 g CT/kg DM (Terrill *et al.* 1992*b*). Evaluation of the role played by CT in ruminant nutrition has been hindered by analytical difficulties, especially the quantification of CT in digesta and faecal material. There are two commonly used procedures for CT quantification; the vanillin–HCl method (Broadhurst & Jones, 1978) and the *n*-butanol–HCl method (Bate-Smith 1973; Porter *et al.* 1986). Both methods are semi-quantitative (Mangan, 1988) so that considerable variation can exist

* Present address: Fort Valley State College, Fort Valley, CA 31030, USA. † For reprints.
‡ Present address: AgResearch Grasslands, Private Bag 11008, Palmerston North, New Zealand.

between assays. The *n*-butanol method, however, used in conjunction with an acetone–water–diethyl ether (4·7:2·0:3·3, by vol.) extraction followed by boiling sodium dodecyl sulphate (SDS) extraction of protein, enables unbound, protein-bound and fibre-bound CT to be measured (Terrill *et al.* 1992*b*). In view of the consistency and effectiveness of the modified butanol–HCl assay in identifying total and bound CT in herbage, it was decided to evaluate this technique for measuring CT in digesta and faeces. This was carried out as part of an investigation into the fate of CT during digestion by sheep.

METHODS

The work was carried out in three parts. Expt 1 examined the recovery of purified CT which were added to digesta obtained from several sites along the gastrointestinal tract of sheep; Expt 2 involved the administration of ^{14}C-labelled CT *per abomasum* and the recovery of radioactivity from the gastrointestinal tract at slaughter, whilst Expt 3 involved the quantitation of CT in abomasal and ileal contents and faeces of sheep fed on *Lotus pedunculatus* in which the digesta flow rates and digestibilities were known. The CT added to digesta in Expt 1, the ^{14}C-labelled CT given to a sheep in Expt 2, and all CT standards for colorimetry were extracted from fresh frozen *Lotus pedunculatus* and purified on Sephadex LH-20 using the method of Terrill *et al.* 1990.

Analysis of CT

Analyses of CT were by the *n*-butanol–HCl procedure based on that of Porter *et al.* (1986), modified by Terrill *et al.* (1992*b*) to measure extractable, protein-bound and fibre-bound CT. The first step involved an acetone–water–diethyl ether (4·7:2·0:3·3, by vol.) extraction, which was followed by the boiling SDS procedure to solubilize protein-bound CT, and then by boiling the residue with SDS and butanol–HCl to measure fibre-bound CT. Separate standard curves of purified CT were made up in either water or SDS solution, with extractable CT being estimated from CT made up in water and bound CT estimated from CT made up in SDS solution.

Expt 1: recovery of added CT

In this experiment the recoveries of known amounts of CT added to the digesta were determined in a sheep grazing a perennial ryegrass (*Lolium perenne*)/white clover (*Trifolium repens*) pasture. The sheep was brought off pasture and killed with an overdose of sodium pentobarbitone, the gastrointestinal tract removed and the contents of the rumen, abomasum, duodenum (0–90 cm from the pylorus) and ileum (0–100 cm from the ileo–caecal junction) were rapidly removed. Six weighed portions (15 ml) of each digesta fraction were placed in 50 ml tubes and measured quantities of purified CT were added (in duplicate) as follows: rumen, duodenum and ileum 0, 15 and 30 mg CT; abomasal (which has a lower DM content) had 0, 10 and 20 mg CT added. The CT were rapidly mixed into the digesta, and one of each sample type placed in ice and then frozen within about 30 min after removal from the sheep. The other tubes were gassed with CO_2, placed in an agitating water bath at 39° and incubated for 4 h before freezing. All tubes were capped to exclude air during the incubation, but those containing rumen digesta were fitted with a valve to allow fermentation gases to escape.

All digesta was freeze-dried before tannin extraction and analysis. Analyses of CT were performed in duplicate using a 1 ml sample with 6 ml butanol–HCl reagent. A further sample was used for DM determinations.

The recoveries of CT were evaluated by analysis of variance (AOV). Background values (no CT added) were tested for effects of digesta type (rumen, abomasal, duodenal and ileal)

and CT fractions (acetone-extractable, protein-bound and fibre-bound). The AOV for recovery of added CT in acetone-extractable, protein-bound and fibre-bound fractions used incubation time (0 and 4 h), digesta type and the amount of CT added (10 and 15 or 20 and 30 mg) as main effects. Interaction terms were included in both models.

Expt 2: fate of [^{14}C]CT in the digestive tract

Because of the low and variable recoveries of CT from digesta (Expt 1), it was decided to monitor the passage of CT-carbon through the gastrointestinal tract with ^{14}C-labelled material. This method enabled the carbon of the CT molecule to be monitored independently of assays for the CT molecule, and by measuring ^{14}C activity in whole blood, any loss of CT-^{14}C to degradation and absorption could be determined. The procedure involved labelling *Lotus pedunculatus* plants, extracting and purifying the CT, and administering the CT through an abomasal fistulae to a sheep given *Lotus pedunculatus* as a sole diet.

Preparation of labelled Lotus pedunculatus. *Lotus* plants were transplanted from the field into a plastic tray (370 × 780 × 50 mm) and allowed to establish over a 3-week period under a 12 h light/dark regime in an environmentally controlled plant growth unit.They were trimmed to 50 mm and allowed 3 d for recovery before being put into a sealed perspex chamber enabling the labelling process to be carried out using methods described by Lai *et al.* (1988).

The procedure involved sealing the chamber so that active photosynthesis reduced the CO_2 concentration to compensation point within 1 h. [^{14}C]Sodium bicarbonate (740 MBq) was then injected into 10 ml lactic acid (700 ml/l) via a rubber septum in the wall of the chamber in order to release $^{14}CO_2$, which was circulated by a small battery-powered fan located within the chamber. After 2 h photosynthesis, unlabelled CO_2 was injected into the growth chamber to bring atmospheric concentrations back to 340 μl/l, and further unlabelled CO_2 was added during daylight hours as necessary. CO_2 concentrations were monitored using an infra-red gas analyser (BINOS, Leybold-Heraeus GmbH, Hanau, Germany).

The tray of plants was removed from the chamber 24 h after ^{14}C labelling and maintained for a further 8 d to allow conversion of the photosynthate to CT. Preliminary tests showed that the maximum concentration of [^{14}C]CT activity was reached after 9 d, so that at this time the plants were cut at 10 mm above soil level and stored at −20° for extraction. This procedure yielded 120 g of foliage DM. The CT were extracted and purified (Terrill *et al.* 1990), taken up in 100 ml water and stored at 4°. A 0·2 ml portion was added to 9·8 ml triton–toluene (2:1, v/v) scintillant for radioactive counting.

The procedure for extracting CT (Terrill *et al.* 1990) excluded proteins and structural fibre, and lipids were removed with diethyl ether. The aqueous fraction was washed through Sephadex LH20 resin which enables all soluble carbohydrates to be removed. The CT are adsorbed onto Sephadex LH20, and although other aromatic species may be present, chemical evidence (Foo *et al.* 1982) and the high concentration of CT in *Lotus pedunculatus* (74 g/kg DM; Terrill *et al.* 1992*b*) suggests that nearly all of the ^{14}C label would be in the CT.

Administration of [^{14}C]CT and analysis of digesta. Three sheep were used in this experiment. One was killed in order to establish the technique and to obtain intestinal digesta samples for background determination of radioactivity and Cr concentrations. One had been prepared with a rumen fistula and was used as a donor to provide rumen fluid, which was mixed with labelled CT before administration into the abomasum of the third sheep. The third animal was prepared with an abomasal cannula through which the CT were given over a period of 400 min, and then killed in order to obtain digesta and tissue

samples for analysis. All sheep were held indoors in metabolism cages, and had been given *Lotus pedunculatus* as a sole diet in hourly increments for 3 weeks before administration of the [^{14}C]CT.

At 24 h before commencement of the CT administration, samples of abomasal digesta (100 ml) and jugular blood (20 ml) were taken for determination of background radioactivity. An abomasal infusion of the liquid digesta marker Cr-EDTA (13·15 ml/h containing 784 μg Cr/ml) commenced 24 h before administration of [^{14}C]CT and was maintained until slaughter. Faeces and urine were collected for 24 h before infusion (for determination of background radioactivity and Cr concentrations) and at each defecation and urination during the period of [^{14}C]CT administration.

The administration of [^{14}C]CT required that 25 ml portions of the [^{14}C]CT solution be mixed with about 180 ml strained rumen fluid at approximately 2 h intervals. This solution was maintained at 39° in an agitating water bath to allow binding between the CT and digesta components at rumen pH before placement in the abomasum. The CT–digesta preparation was made on three occasions during the 400 min period over which [^{14}C]CT was given to the sheep. The mixture was heterogeneous in nature and could not be infused, so that weighed 20 ml aliquots were given at 15-min intervals, and 10 ml aliquots at 7·5-min intervals for 30 min before slaughter, in order to reduce effects from the pulsatile nature of the administration. The final dose of [^{14}C]CT was given 3·5 min before slaughter and Cr-EDTA infusion was maintained until 2 min before slaughter.

The labelled CT were given at a rate equivalent to $5{\cdot}4 \times 10^5$ disintegrations/min (dpm) per min, so that a total of $2{\cdot}17 \times 10^8$ dpm was given over 400 min until slaughter. The period of infusion was calculated to allow sufficient time for the label to reach the large intestine, after which digestion and absorption of CT was considered unlikely. It was assumed that the abomasal digesta pool size would be about 300 ml and the flux of digesta would be about 750 ml/h, so that the abomasal digesta pool would turn over every 24 min. On this basis an adequate mixing of label given at 15 min intervals should have occurred by the time it reached the jejunum.

Immediately before slaughter a blood sample (jugular) was taken for determination of CO_2 radioactivity (Hinks *et al.* 1966). The sheep was killed, the abdomen was opened, the pylorus and rectum tied off and the intestine removed. Two people removed the contents from the digestive tract. Digesta from the rumen, abomasum, small intestine, large intestine, caecum and rectum were weighed, subsampled and the pH determined. The small intestine was carefully but rapidly removed from the mesentery and digesta collected from sections (m from the pylorus) as follows: 0–1, 1–2, 2–3, 3–4, 4–6, 6–8, 8–10, 10–12, 12–15, 15–19, 19–21. Digesta were gently milked from each section, and both the digesta and the intestinal tissue were frozen for later analyses. The entire contents of the digestive tract distal to the rumen was collected for determination of Cr concentrations and radioactivity.

Analyses and calculations. Six portions of blood (1 ml) collected 24 h before and at the end of the [^{14}C]CT administration, were stored under paraffin. Triplicate samples were digested in protozol tissue solubilizer (New England Nuclear, Boston, Mass., USA) at 80 and 120 μg blood/ml protozol in scintillation vials and, after digestion, 9 ml scintillant were added for determination of blood radioactivity. The radioactivity associated with digesta fractions collected distal to the omasum, in urine, faeces, liver and intestinal tissue were also determined. This required the intestinal tissue segments and liver to be homogenized and subsampled, and the digesta to be mixed and subsampled. Radioactivity was determined in homogenized samples which were oxidized (OX300; Harvey, Hillsdale, NJ, USA) and the $^{14}CO_2$ absorbed in scintillant (Carbomax; Lumac, 6372 AE Landgraaf, Netherlands) for quantitation by liquid scintillation counting (Tricarb 4000; Packard).The Cr content was determined in freeze-dried digesta by atomic absorption spectrometry.

The fate of CT during digestion was calculated on the basis of total dpm given and dpm recovered in digesta, urine, faeces, tissue and blood fractions. Cr concentrations in digesta and faecal DM enabled digesta DM flows and losses of digesta DM to be calculated at various sites distal to the omasum, which assisted in the validation and interpretation of digesta specific activity.

Expt 3: unlabelled CT digestion in sheep fed on Lotus pedunculatus

A group of six young wether sheep given *Lotus pedunculatus* as a sole diet in hourly increments for 31 d were part of a trial investigating effects of CT on digestion of protein, carbohydrate and minerals (Waghorn *et al.* 1994*a*, *b*) and sulphur amino acids (McNabb *et al.* 1993). The sheep had been prepared with rumen and abomasal fistulas and digesta flow rates through the abomasum were measured in conjunction with digestibility and balance measurements, using the indigestible markers ^{51}Cr-EDTA and ^{103}Ru-phenanthroline.

At slaughter the rumen pool size was measured and ileal digesta obtained to estimate flow rate at that site. Samples of feed, rumen, abomasal and ileal digesta and faeces were freeze-dried, ground and analysed for extractable and bound CT to quantify CT passage through the gastrointestinal tract.

RESULTS

Expt 1

Background readings for the CT assay of digesta (no CT added) showed small differences between digesta types ($P < 0{\cdot}05$; Table 1). Values for total CT ranged between 4·39 and 6·82 mg/g DM, with abomasal and ileal digesta giving the highest readings. Background assays did show significant ($P < 0{\cdot}001$) differences between CT fractions (acetone-extractable, protein-bound, fibre-bound) and these values (Table 1) were subtracted from the values for CT recovery, for presentation in Table 2. The amount of CT added to the various digesta types did not have a major effect on recovery, although increasing the amount of CT added did tend to increase the percentage recovered, especially with the 4 h incubation ($P = 0{\cdot}109$; Table 2).

Recovery of added CT was affected by digesta type ($P = 0{\cdot}06$) with highest total recoveries from rumen digesta (53·1–82·3%) and lowest recoveries from ileal digesta (35·9–45·7%). The effects of digesta type on CT recovery were greatest for protein-bound ($P < 0{\cdot}01$) and acetone-extractable ($P < 0{\cdot}05$) fractions (Table 2).

The principal effect of the 4 h incubation was to reduce the total recovery of acetone-extractable CT ($P < 0{\cdot}05$) which was a major component of the rumen and abomasal fractions before incubation (Table 2). This reduction in recovery of acetone-extractable CT was not associated with changes in recovery of protein-bound or fibre-bound fractions. Hence the recovery of total CT was reduced by increasing the incubation time ($P = 0{\cdot}06$) and was lower in digesta from more distal parts of the intestinal tract ($P = 0{\cdot}06$).

Expt 2

The [^{14}C]CT administered *per abomasum* over 400 min totalled $2{\cdot}17 \times 10^8$ dpm, of which $2{\cdot}01 \times 10^8$ dpm was recovered in digesta, intestinal tissues, faeces and urine (Table 3). There was no detectable radioactivity in either whole blood or blood CO_2, and only 0·0008 of the infused dpm was recovered in the liver. Net recovery of label was 92·4%, of which 96·4% was in the digesta recovered from the abomasum, small and large intestines and caecum, 3·5% was in intestinal tissues and 0·05% each in faeces and urine (Table 3).

There was a progressive increase in dpm/g digesta DM (specific activity; SA) as it passed toward the terminal ileum, after which SA diminished (Table 3). The increase in SA was

Table 1. *Expt 1. Condensed tannin content (mg/g dry matter) of digesta samples obtained from a sheep fed on perennial ryegrass* (Lolium perenne)*/white clover* (Trifolium repens) *pasture ('background' values)*

(Mean values from two assays)

Gut segment	Condensed tannins			
	Acetone-extractable	Protein-bound	Fibre-bound	Total
Rumen	2·88	1·09	0·95	4·92
Abomasum	3·44	2·04	1·13	6·61
Duodenum	1·94	1·26	1·19	4·39
Ileum	3·43	1·88	1·51	6·82

Table 2. *Expt 1. Recovery* of condensed tannins (CT) added to 15 ml digesta obtained from the rumen, abomasum, duodenum or ileum of a sheep fed on perennial ryegrass* (Lolium perenne)*/white clover* (Trifolium repens) *pasture, after 0 and 4 h incubation*†

Gut segment	CT added (mg)	Concentration of CT in digesta (mg/g DM)	Recovery* of CT (% of CT added)			
			Acetone-extractable	Protein-bound	Fibre-bound	Total
No incubation:						
Rumen	15	17·5	31·5	46·6	4·2	82·3
	30	37·5	34·6	38·6	2·3	75·5
Abomasum	10	9·7	32·1	12·8	0·6	45·5
	20	19·8	41·9	13·1	1·2	56·2
Duodenum	15	12·4	18·6	47·7	3·5	69·8
	30	22·4	13·2	43·6	2·1	58·9
Ileum	15	11·3	10·3	33·1	2·3	45·7
	30	21·4	3·3	34·6	3·2	41·0
4 h incubation:						
Rumen	15	19·5	13·5	38·7	0·9	53·1
	30	38·5	16·3	42·3	3·3	61·9
Abomasum	10	10·4	6·3	12·5	1·6	20·4
	20	14·5	44·6	26·7	6·2	77·5
Duodenum	15	13·2	2·4	30·9	6·8	40·1
	30	26·1	11·1	34·9	5·8	51·8
Ileum	15	11·2	1·3	34·6	0	35·9
	30	22·6	4·5	35·1	2·0	41·6

DM, dry matter.
* Corrected for background values.
† For details of procedures, see pp. 468–469.

a consequence of DM loss to absorption (indicated by increasing concentrations of Cr in the DM; Table 4) and the lower values in the large intestine and caecum due to insufficient time being available for label to reach those areas. The dpm in digesta in the large intestine and caecum accounted for 29% of the total recovered.

The SA approximately doubled between the proximal and distal small intestine (Table 3), a distance of about 19 m. The similar extent to which both the SA of digesta DM, and Cr concentration increased in digesta passing down the small intestine is illustrated in Table 4. Values of dpm/μg Cr in digesta were similar at 1–2 m and 15–19 m down the small

Table 3. *Expt 2. Weights and radioactivity (disintegrations/min, dpm) of digesta, faeces, urine and tissues from a sheep given 2·17 × 10⁸ dpm of [¹⁴C]condensed tannins* per abomasum *at 15-min intervals over 400 min**

Gut segment	Digesta wet† (g)	Digesta dry (g)	Digesta specific activity: Total dpm (× 10⁶)†	Digesta specific activity: dpm/g DM (× 10⁵)	Gut tissue: Wet wt (g)	Gut tissue: Total dpm (× 10⁵)
Rumen	5431	760·3	0·54	0·01	ND	ND
Abomasum	849	35·0	22·42	6·40	ND	ND
Small intestine (m distal to pylorus)						
0–1	44	2·25	1·13	5·00	72	0·54
1–2	42	2·08	1·17	5·62	52	0·35
2–3	28	1·53	0·91	5·95	52	0·56
3–4	65	3·59	1·93	5·38	50	0·66
4–6	72	4·58	2·79	6·08	73	0·70
6–8	61	4·12	3·17	7·68	74	0·51
8–10	100	6·36	5·66	8·90	80	0·91
10–12	124	9·87	8·72	8·83	80	1·52
12–15	119	9·72	10·01	10·30	173	15·78
15–19	346	40·31	46·57	11·55	158	16·13
19–21	215	23·24	29·50	12·69	141	19·71
Caecum	371	56·84	25·49	4·48	98	7·49
Large intestine						
Proximal	535	90·04	32·42	3·60	209	5·28
Distal	115	29·78	1·32	0·45	268	0·53
Rectum	Empty	—	—	—	115	0·09
Faeces‡						
0–240	292	104·9	0·06		—	—
240–390	118	35·3	0·06		—	—
390–400	69	23·1	0·19		—	—
Urine‡						
0–240	171	—	0·19		—	—
240–390	120	—	1·17		—	—
390–400	0	—	—		—	—
Total radioactivity			194·4			63·3

DM, dry matter; ND, not determined.
* For details of procedures, see pp. 469–470.
† Includes faeces and urine.
‡ Minutes after commencement of condensed tannin administration.

intestine, suggesting that little if any absorption of CT-carbon had taken place between these sites. Lower values in the terminal ileum and more distal portions of the gastrointestinal tract (Table 4) are a consequence of insufficient time being allowed for the label to reach these sites. High values in the abomasum may be due to sampling errors following the last pulse administration.

Expt 3

Butanol–HCl extraction of the *Lotus pedunculatus* diet fed in Expt 3 resulted in 56·7 g CT/kg DM, of which 72% (40·8 g/kg DM) was acetone-extractable, 25·4% protein-bound and 2·6% fibre-bound (Table 5). Once the forage had been eaten the acetone-extractable CT became a minor component of digesta CT at all sites (Table 5). In contrast, the protein-bound CT was the major fraction at all sites, accounting for 55–90% of total CT recovered, whilst fibre-bound CT was 8–39% of the total recovered.

Table 4. *Expt 2. Digesta pH, dry matter (DM) content, Cr concentration, disintegrations/min (dpm) and digesta DM remaining (relative to abomasal DM flow) in the gastro-intestinal tract of a sheep given* [^{14}C]*condensed tannins* per abomasum*

Gut segment	Digesta pH	DM (%)	Cr concentration† (μg/g DM)	dpm/μg Cr (× 10^3)	Estimated DM remaining‡ (relative to 100)
Rumen	6·3	14·0	7		—
Abomasum	3·0	4·1	239	2·68	100
Small intestine (m from the pylorus)					
0–1	5·4	5·1	188	2·66	127
1–2	5·4	5·0	267	2·11	89
2–3	5·7	5·5	277	2·15	86
3–4	5·5	5·8	309	1·74	77
4–6	6·0	6·4	352	1·73	68
6–8	6·6	6·8	444	1·73	54
8–10	7·1	6·4	458	1·94	52
10–12	7·3	8·0	457	1·93	52
12–15	7·6	8·2	536	1·92	45
15–19	8·0	11·7	560	2·05	43
19–21	7·9	10·8	689	1·84	35
Caecum	7·1	15·3	627	0·72	38
Large intestine	ND	18·3	763	0·47	31
Faeces	ND	34·1	757	0·06	31

ND, not determined.
* For details of procedures, see pp. 469–471.
† Corrected for background Cr concentrations.
‡ Calculated from Cr concentration in the digesta DM.

Table 5. *Expt 3. Concentrations of condensed tannins (CT; g/kg dry matter) determined in acetone-extractable, protein-bound and fibre-bound fractions of feed, rumen, abomasal and ileal digesta and faeces of sheep fed on* Lotus pedunculatus*

(Mean values with their standard errors for six sheep)

	Concentration of CT							
	Acetone-extractable		Protein-bound		Fibre-bound		Total	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Feed	40·8	—	14·4	—	1·5	—	56·7	—
Digesta:								
Rumen	1·3	1·83	26·3	1·62	4·5	0·57	32·1	1·61
Abomasum	11·8	0·93	36·6	0·87	5·7	0·23	54·1	1·31
Ileum	0·8	0·07	16·6	3·36	2·6	0·74	20·0	4·07
Faeces	0·6	0·07	17·5	1·42	9·2	1·53	27·3	2·00

* For details of procedures, see p. 470.

Table 6. *Expt 3. Daily dry matter (DM) intakes, fluxes and apparent disappearance of condensed tannins (CT), from the gastrointestinal tract of sheep fed on* Lotus pedunculatus *as a sole diet**

(Mean values with their standard errors for six sheep)

	Mean	SE
DM flux (g/d):		
Intake	1200	31·0
Abomasum	802	19·2
Ileum	474	19·7
Faeces	380	7·4
CT flux (g/d):		
Intake	68·1	1·81
Abomasum	43·5	1·58
Ileum	9·6	1·80
Faeces	10·4	0·84
Apparent disappearance of CT (g/d):		
Rumen	24·6	3·02
Small intestine	33·9	3·11
Large intestine	−0·8	1·69
Total	57·7	1·04

* For details of procedures, see p. 470.

Colorimetric estimates of CT flux along the gastrointestinal tract (Table 6) show an apparent disappearance of 24·6 g/d of CT across the rumen, 33·9 g/d of CT disappeared in the small intestine and there was no loss of CT in the large intestine.

DISCUSSION

The principal finding of the present investigation was that little if any of the ^{14}C-labelled CT-carbon was absorbed from the small intestine (Table 3 and 4), whereas digesta flow studies based upon colorimetric determination of extractable and bound CT by the butanol–HCl method showed considerable disappearance of CT in both the rumen and the small intestine (Table 6). Possible reasons for this discrepancy are that the SDS procedure used to estimate bound CT (Terrill *et al.* 1992*b*) was not releasing all bound CT from digesta, that interfering substances were present in digesta which led to underestimation of CT by the butanol–HCl procedure, or that the conformation of the CT molecule was changed by passage through the gastrointestinal tract such that the CT-carbon was still present in digesta but the molecule was no longer chemically detectable as CT.

The high proportion of infused radioactivity which was recovered from the small intestine (0·924) suggests that the infused fraction contained little if any digestible material. The absence of significant digestion and absorbtion of CT-carbon is also supported by the relatively consistent dpm:Cr concentration ratio in digesta at intervals down the jejunum. Had the carbon been absorbed then the dpm:Cr concentration ratio would have declined in more distal parts of the intestine.

The choice of a liquid-phase marker to follow the fate of ^{14}C in the digesta may be questioned in view of the established binding between CT and protein or fibre fractions, but hydrolysis and absorption of protein during digestion will reduce the number of binding sites available. This may increase the amount of unbound (acetone-extractable) CT which would occupy the liquid phase of digesta but data are not available to indicate the digesta fraction with which the CT were associated *in vivo*. The *in vitro* incubations did not support

an increase in unbound (acetone-extractable) CT, but assay of added CT did not account for a large proportion of the CT, and *in vitro* incubations cannot be assumed to represent *in vivo* conditions. Hence the question of association of CT with solid or liquid phases of digesta and the choice of the most appropriate flow marker remains unresolved.

Factors affecting the recovery of CT from digesta

Although the reasons for the low recovery of CT from digesta have not been specifically addressed in these experiments, results from Expt 1 where the acetone-extractable and total CT were lower after 4 h than 0 h incubation are consistent with the hypothesis that the CT become progressively more bound to protein with time, and that the SDS treatment used was unable to release this bound CT. However, the lower binding affinity of CT under acidic conditions (Jones & Mangan, 1977) was evident when 20 mg was added to abomasal digesta, enabling over 40% to be recovered in the acetone-extractable fraction (Table 2). Nevertheless, recoveries from digesta were low compared with the 100% recovery of purified CT added to forages (Terrill *et al.* 1992*b*) or cottonseed (Yu *et al.* 1993). In Expt 3, where the forage was being digested, there also was a substantially greater recovery of the extractable and protein-bound CT from abomasal digesta than rumen, ileal or faecal fractions. Again this may have been due to the acidic conditions in the abomasum releasing CT from the protein.

The possibility that substances present in plants are able to interfere with CT assays has been indicated by Terrill *et al.* (1992*b*). Although these substances have not been identified they are absent from purified CT (Terrill *et al.* 1992*b*) but may have contributed to both the values in ryegrass/white clover pasture (3–4 mg CT/g DM; Terrill *et al.* 1992*a*) and to the background values for digesta in the present study (4–7 mg/g DM; Table 1).

Changes in the CT molecule as it passes down the gastrointestinal tract may well be an important explanation of the poor recoveries. If the abomasal and ileal flows of CT (Table 6) are corrected in accordance with recoveries of purified CT added to abomasal and ileal digesta (Table 2) then the abomasal CT flux is similar to CT intake and there would be little if any loss from the rumen. However, corrected fluxes show a substantial disappearance of CT in the small intestine (about 48 g/d). In this context, disappearance could include both absorption from the small intestine and also conformational changes such that CT can no longer be detected by colorimetric methods. As the [^{14}C]CT studies showed essentially no absorption of CT from the small intestine, it follows that conformational changes in CT are likely to have occurred during passage through the small intestine.

The net effect of binding, conformational changes and interference with the colorimetric assay is that a conventional technique (e.g. butanol–HCl) is unable to quantify CT in animal digesta or the flow of CT along the digestive tract. Given the phenolic nature of the CT molecule and its similarity to lignin it is unlikely that extensive microbial degradation would occur in the rumen or large intestine, and in fact faecal lignin assays can give unrealistically high values, when diets containing CT are fed (Waghorn, 1994*a*).

Implications for digestion

If the CT in forage are not digested by the ruminant then they represent a loss of potentially digestible nutrients. For example, the sheep in Expt 3 consumed 1200 g DM/d of which 68·1 g was CT. The forage DM had a digestibility coefficient of 0·683 but if the CT had been digested to a similar extent as carbohydrate in the forage then the digestibility of DM would have been about 0·720. Such losses must be compensated for by improvements in the utilization of other nutrients in ruminant diets, notably protein, or by a reduction in the concentration of CT in the feed if plants containing low concentrations of CT are to be incorporated into temperate agriculture (Barry, 1989; Terrill *et al.* 1989; Waghorn, 1990).

Mr I. D. Shelton and Miss R. A. Watson are thanked for technical assistance, and Massey University Research Fund for providing financial assistance. T.H.T. acknowledges post-doctoral scholarship support from the Organisation for Economic Co-operation and Development and from the New Zealand Wool Board. The authors wish to thank the Forest Research Institute in Rotorua, New Zealand for assistance with the ^{14}C analyses.

REFERENCES

Barry, T. N. (1989). Condensed tannins; their role in ruminant protein and carbohydrate digestion and possible effects upon the rumen ecosystem. In *The Role of Protozoa and Fungi in Ruminant Digestion*, pp. 153–169 [J. V. Nolan, R. A. Leng and D. I. Demeyer, editors]. Armidale, Australia: Penambal Books.

Bate-Smith, E. C. (1973). Tannins of herbaceous leguminosae. *Phytochemistry* **12**, 1809–1812.

Broadhurst, R. B. & Jones, W. T. (1978). Analysis of condensed tannins using acidified vanillin. *Journal of the Science of Food and Agriculture* **29**, 788–794.

Foo, L. Y., Jones, W. T., Porter, L. J. & Williams, V. M. (1982). Proanthocyanidin polymers of fodder legumes. *Phytochemistry* **21**, 933–935.

Hinks, N. T., Mills, S. C. & Setchell, B. P. (1966). A simple method for the determination of specific activity of carbon dioxide in blood. *Analytical Biochemistry* **17**, 551–553.

Jones, W. T., Broadhurst, R. B. & Lyttleton, J. W. (1976). The condensed tannins of pasture legume species. *Phytochemistry* **15**, 1407–1409.

Jones, W. T. & Mangan, J. L. (1977). Complexes of the condensed tannins of the sainfoin (*Onobrychis viciifolia* Scop.) with Fraction 1 leaf protein and with submaxillary muco-protein, and their reversal by polyethylene glycol and pH. *Journal of the Science of Food and Agriculture* **28**, 126–136.

Lai, R., Woolley, D. J. & Lawes, G. S. (1988). Patterns of assimilate transport from leaves to fruit within a kiwifruit (*Actinidia deliciosa*) lateral. *Journal of Horticultural Science* **63**, 725–730.

McNabb, W. C., Waghorn, G. C., Barry, T. N. & Shelton, I. D. (1993). The effect of condense tannins in *Lotus pedunculatus* on the digestion and metabolism of methionine, cystine and inorganic sulphur in sheep. *British Journal of Nutrition* **70**, 647–661.

Mangan, J. L. (1988). Nutritional effects of tannins in animal feeds. *Nutrition Research Reviews* **1**, 209–231.

Porter, L. J., Hrstich, L. N. & Chan, B. C. (1986). The conversion of procyanidins and prodelphinidins to cyanidin and delphinidin. *Phytochemistry* **25**, 223–230.

Sarkar, S. K., Howarth, R. E. & Golpen, B. P. (1976). Condensed tannins in herbaceous legumes. *Crop Science* **16**, 543–546.

Terrill, T. H., Douglas, G. B., Foote, A. G., Purchas, R. W., Wilson, G. F. & Barry, T. N. (1992*a*). Effect of condensed tannins upon body growth, wool growth and rumen metabolism in sheep grazing sulla (*Hedysarum coronarium*) and perennial pasture. *Journal of Agricultural Science, Cambridge* **119**, 265–273.

Terrill, T. H., Rowan, A. M., Douglas, G. B. & Barry, T. N. (1992*b*). Determination of extractable and bound condensed tannin concentrations in forage plants, protein concentrate meals and cereal grains. *Journal of the Science of Food and Agriculture* **58**, 321–329.

Terrill, T. H., Windham, W. R., Evans, J. J. & Hoveland, C. S. (1990). Condensed tannin concentrations in *Sericea lespedeza*, as influenced by preservation method. *Crop Science* **30**, 219–224.

Terrill, T. H., Windham, W. R., Hoveland, C. S. & Amos, H. E. (1989). Forage preservation method influences on tannin concentration, intake and digestibility of *Sericea lespedeza* by sheep. *Agronomy Journal* **81**, 435–439.

Waghorn, G. C., Shelton, I. D. & McNabb, W. C. (1994*a*). Effects of condensed tannins in *Lotus pedunculatus* on its nutritive value for sheep. 1. Non-nitrogenous aspects. *Journal of Agricultural Science, Cambridge* **123**, 99–107.

Waghorn, G. C., Shelton, I. D., McNabb, W. C. & McCutcheon, S. N. (1994*b*). Effects of condensed tannin in *Lotus pedunculatus* on its nutritive value for sheep. 2. Nitrogenous aspects. *Journal of Agricultural Science, Cambridge* **123**, 109–119.

Yu, F., Barry, T. N., Moughan, P. J. & Wilson, G. F. (1993). The concentration of condensed tannin and gossypol in cottonseed and in processed cottonseed meal. *Journal of the Science of Food and Agriculture* **63**, 7–15.

Printed in Great Britain