

Chapter 5

THE FLOW OF DIGESTA ALONG THE DIGESTIVE TRACT5.1 Introduction

Since the macropodine forestomach is by far the largest region of the digestive tract, it may be expected to retain ingested food for a considerable time. Certainly, the passage of stained hay particles through the entire digestive tract of both Me rufa (red kangaroo) and M. giganteus (eastern grey kangaroo) is prolonged, although generally not as long as in sheep fed the same diets (see Section 2.2).

The steeper slopes of cumulative excretion curves determined for S. brachyurus (quokka), Me rufa and M. giganteus suggest that, in all three species, the rate of elimination of stained hay particles in the faeces is faster than in sheep. Calaby (1958) and McIntosh (1966) suggested that this was due to less efficient mixing of digesta in the macropodine stomach.

The macropodine stomach and the ruminant stomach are markedly different structures and it seems unlikely that the pattern of flow of digesta in these organs would be similar. Warner (1977) found a significant separation of the fluid and particulate phases of digesta in the stomach of M. eugenii (tamar wallaby) when fed a pelleted lucerne diet at hourly intervals. The fluid marker had a mean retention time of only 3 h in the stomach compared with 11 h for the particulate marker.

In the preceding chapter, differences in stomach structure among T. thetis (red-necked pademelon), M. eugenii and M. giganteus were found to be important in producing different patterns of initial dispersion of orally administered contrast medium. The following experiments were designed to define the pattern of flow and rate of

passage of fluid and particulate digesta along the digestive tract of these three species, and of M. robustus robustus (eastern wallaroo), and to compare the results with those obtained from sheep.

The markers $^{51}\text{Cr-EDTA}$ and $^{103}\text{Ru-P}$ were used to define the kinetics of the fluid and the particulate fractions of the digesta respectively. In Experiment 1, the recovery with time in the faeces of a single oral dose of both markers was used to define the pattern of flow and kinetics of the two markers in the entire digestive tract of the macropodines and the sheep. In Experiment 2, a single injection of the two markers into the hindstomach of T. thetis and M. eugenii and subsequent recovery in the faeces was used to define the kinetics of digesta flow in the intestine. In Experiment 3, the two markers were infused into the sacciform forestomach of T. thetis and of M. eugenii at a constant rate, and at equilibrium the animals were slaughtered and the concentrations of the markers in digesta were used to calculate the flow of both phases of digesta in each region of the digestive tract.

5.2 Materials and methods

5.2.1 Experiment 1

This experiment was in two parts:

- 1a - Twelve T. thetis, 8 M. eugenii, 8 M. giganteus, 4 M. robustus, and 4 sheep (Border Leicester x Merino castrated males) were fed chopped lucerne hay ad libitum,
- 1b - Four T. thetis and 5 M. eugenii were fed fresh Phalaris grass ad libitum. The Phalaris was harvested each day (see Section 3.2.2).

All animals were offered food every four hours and daily dry matter intakes were recorded. The two markers, $^{51}\text{Cr-EDTA}$ and $^{103}\text{Ru-P}$, were administered orally by offering each animal a small amount of crushed wheat grain (10 g each

for T. thetis and M. eugenii, 20 g each for M. giganteus, M. robustus, and the sheep) impregnated with approximately $2.5 \mu\text{Ci}^{51}\text{Cr-EDTA/g}$ and $1 \mu\text{Ci}^{103}\text{Ru-P/g}$. The marked wheat was prepared by mixing crushed wheat grain with a solution of the two markers and drying the slurry overnight in an oven at 70°C . The wheat was offered on top of some chopped lucerne in a feed tray and when most of the wheat had been consumed, this lucerne was replaced with the experimental diet. The marked wheat was always eaten within a 5 to 10 min period, and the mean time of consumption was recorded.

After a period of two hours, faeces, if present, were collected from trays under the metabolism cages every 30 min for 6 h, then hourly for 24 h, and then at 3 h intervals for a maximum period of 84 h. Time of each collection was recorded, the faeces weighed, and then stored at -10°C .

5.2.2 Experiment 2

Two T. thetis and two M. eugenii, each surgically prepared with a hindstomach infusion catheter (see Section 3.4.2), were fed chopped lucerne hay ad libitum offered at 4 h intervals. Daily dry matter intakes were recorded. A mixed solution (2 ml) of $^{51}\text{Cr-EDTA}$ ($10 \mu\text{Ci/ml}$) and $^{103}\text{Ru-P}$ ($5 \mu\text{Ci/ml}$) was injected into the hindstomach and the catheter slowly flushed with 2 ml of 0.9% NaCl solution. Time of infusion was recorded and faeces were subsequently collected, weighed, time of collection recorded and stored as in Section 5.2.1. The experiment was repeated in each animal.

5.2.3 Experiment 3

Four T. thetis and three M. eugenii, each surgically prepared with an infusion catheter into the sacciform forestomach (see Section 3.4.2), were fed chopped lucerne hay ad libitum each hour from automatic moving-belt feeders.

Daily dry matter intakes were recorded. The animals were continuously infused with a solution containing $^{51}\text{Cr-EDTA}$ (approx. 2,000 cpm/ml) and $^{103}\text{Ru-P}$ (approx. 1,500 cpm/ml) at an infusion rate of 50 ml/d for a minimum period of 48 h.

Each animal was sedated with ketamine hydrochloride then killed with an overdose of pentobarbitone sodium (see Section 3.4.1). The abdominal cavity was quickly opened through a ventral midline incision and the digestive tract was carefully dissected free and removed. Samples of digesta were obtained from a maximum of six sites along the stomach and from two sites along the distal colon. A combined sample was taken from the caecum-proximal colon. The small intestine was divided into three equal lengths and a sample of mixed digesta was obtained from each segment. The total wet weight of digesta from each region of the digestive tract was recorded. All samples were stored at -10°C .

5.2.4 Analytical procedures

Duplicate samples of faeces and digesta were packed to a constant height in tared plastic gamma counting tubes, weighed, and the radioactivity of $^{51}\text{Cr-EDTA}$ and $^{103}\text{Ru-P}$ in the samples measured as outlined in Section 3.5. Each of the infusion solutions used in Experiment 3 were diluted and 3 ml aliquots were counted in triplicate at the same time as the digesta samples. The tubes containing the faecal and digesta samples were then dried for 6 d at 70°C for calculation of dry matter content.

5.2.5 Calculations

The radioactivity of each marker in the faecal samples from each animal in Experiments 1a, 1b, and 2, was calculated as cpm/g dry matter. The time of each sample was taken to be the mid-point between the time of that sample and the previous sample. Transit time (TT) for each marker was taken to be the adjusted time of first appearance of each marker in the faeces after infusion.

The radioactivity of each marker, as cpm/g dry matter, was plotted against adjusted time after infusion and smooth curves fitted to the points by eye. From these marker excretion curves 10%, 50% and 90% excretion times (ET) were estimated by planimetry.

In Experiments 1a and 1b, the means of the successive 10% excretion times (0 to 100%) in each species were plotted against time to generate cumulative percentage recovery curves for each marker.

The major central component of the negative slope of each faecal marker excretion curve was used to calculate a half-time ($T_{\frac{1}{2}}$) for each marker. Each $T_{\frac{1}{2}}$ was calculated from the linear regression of the natural logarithm of concentration of marker against time, according to the relationship:

$$T_{\frac{1}{2}} = \frac{-0.693}{\text{regression coefficient}}$$

This value is the time required for the marker concentration in the largest pool to decrease by 50%. In studies with ruminants, this value is assumed to represent the $T_{\frac{1}{2}}$ of a marker in the ruminoreticulum (Grovm and Williams, 1973b, 1973c).

In Experiment 2, $T_{\frac{1}{2}}$ for each marker was calculated in each animal as representative of the $T_{\frac{1}{2}}$ of marker in the caecum-proximal colon.

In each animal in Experiment 3, the radioactivity of $^{51}\text{Cr-EDTA}$ and of $^{103}\text{Ru-P}$ in the infusion solution and in the digesta samples, were used to calculate the flow of fluid and of dry matter respectively, in each region of the digestive tract from the relationship:

$$\text{flow (g/d)} = \frac{\text{cpm/ml of infusate} \times \text{volume infused (ml/d)}}{\text{cpm/g of fluid (or dry matter)}}$$

The half-times of each marker in the sacciform forestomach, tubiform forestomach, hindstomach and caecum-proximal colon, and the approximate retention time (RT) in the distal colon,

were calculated from the wet weight and dry matter content of digesta in each region, and the flow rate of the respective phase of digesta, according to the relationships:

$$T_{\frac{1}{2}} \text{ (h)} = \frac{0.693 \times \text{weight of fluid, or dry matter (g)}}{\text{flow of fluid, or dry matter (g/h)}}$$

and;

$$RT \text{ (h)} = \frac{T_{\frac{1}{2}}}{0.693}.$$

5.2.6 Statistical analyses

In Experiments 1a and 1b, mean values for TT, 10% ET, 50% ET, and 90% ET, and $T_{\frac{1}{2}}$ of both markers in the digestive tract of each species and the dry matter intakes, were compared using a one-way analysis of variance (NECV programme, University of New England, Computer Centre). Because of unequal cell numbers, an approximate analysis (Snedecor and Cochran, p472, 1967) was carried out to examine diet x species interactions between T. thetis and M. eugenii. Differences between the rates of excretion and flow of the two markers in each species, and differences between the two species in Experiments 2 and 3, were compared using "Student's" t-test.

5.3 Results

5.3.1 Experiment 1

The pattern of appearance of the two markers $^{51}\text{Cr-EDTA}$ and $^{103}\text{Ru-P}$ in the faeces of one animal of each of the five species in Experiment 1a (single oral infusion) is illustrated in Fig. 5.1.

Mean values for TT, 10% ET, 50% ET and 90% ET in the entire digestive tract, and $T_{\frac{1}{2}}$, for each marker, for each species in Experiments 1a and 1b, and the mean dry matter intakes (expressed on a metabolic body weight basis, $W^{0.75}$)

Fig. 5.1. The pattern of appearance of the two markers $^{51}\text{Cr-EDTA}$ (●) and $^{103}\text{Ru-P}$ (○) in the faeces after a single oral infusion in sheep and four species of macropodines. The data are from one animal of each species in Experiment 1a. All animals were fed chopped lucerne hay ad libitum.

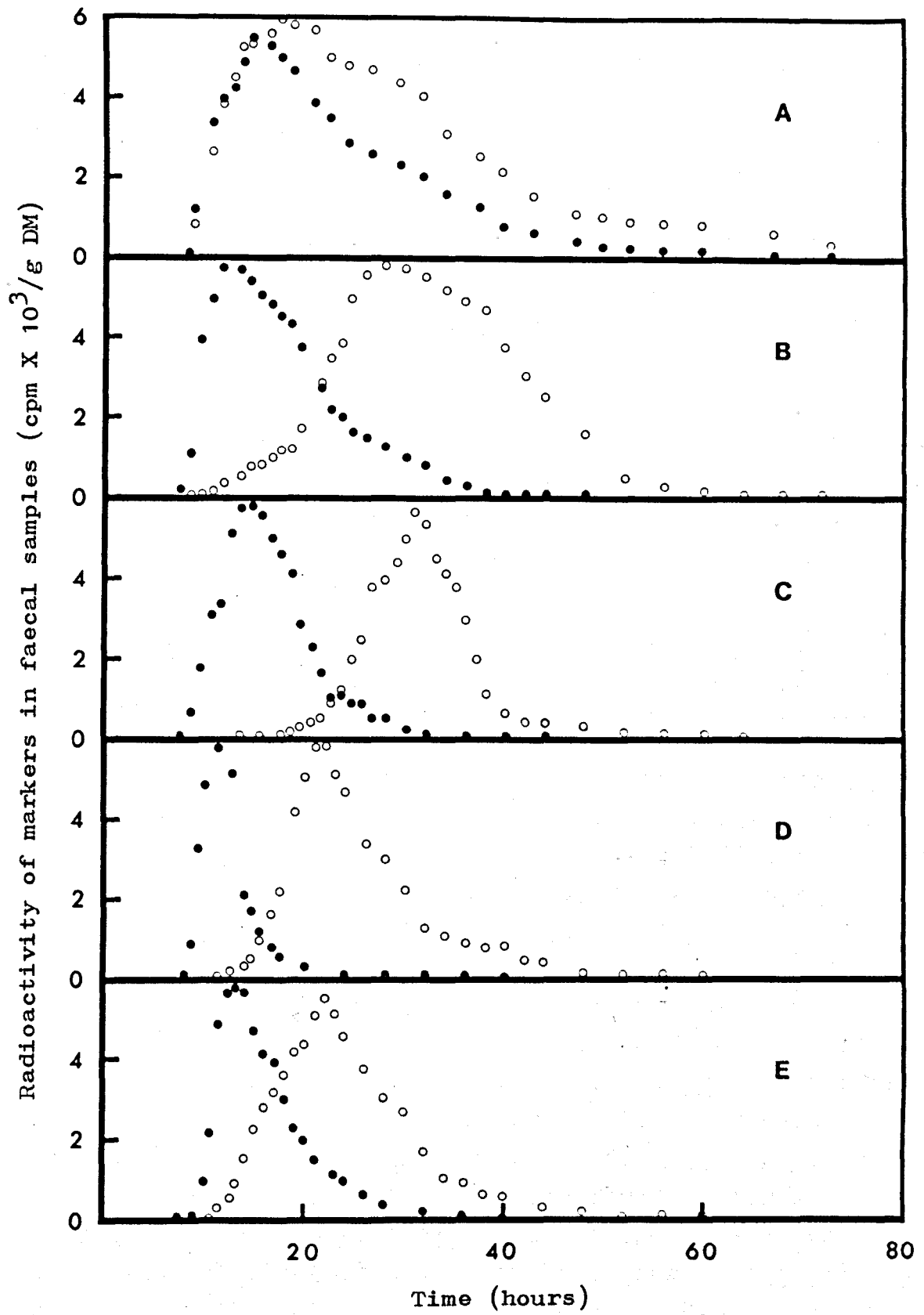
A = sheep,

B = M. robustus robustus,

C = M. giganteus,

D = T. thetis,

E = M. eugenii.



are presented in Table 5.1. Mean cumulative percentage recovery curves for each marker in each species are shown in Fig. 5.2.

a) Kinetics of the fluid marker

In Experiment 1a, mean TT for $^{51}\text{Cr-EDTA}$ was similar but variable in all species, ranging from 5.4 h in M. giganteus to 8.9 h in M. eugenii (Table 5.1). In Experiment 1b, TT was similar in T. thetis (5.9 h) and M. eugenii (7.1 h).

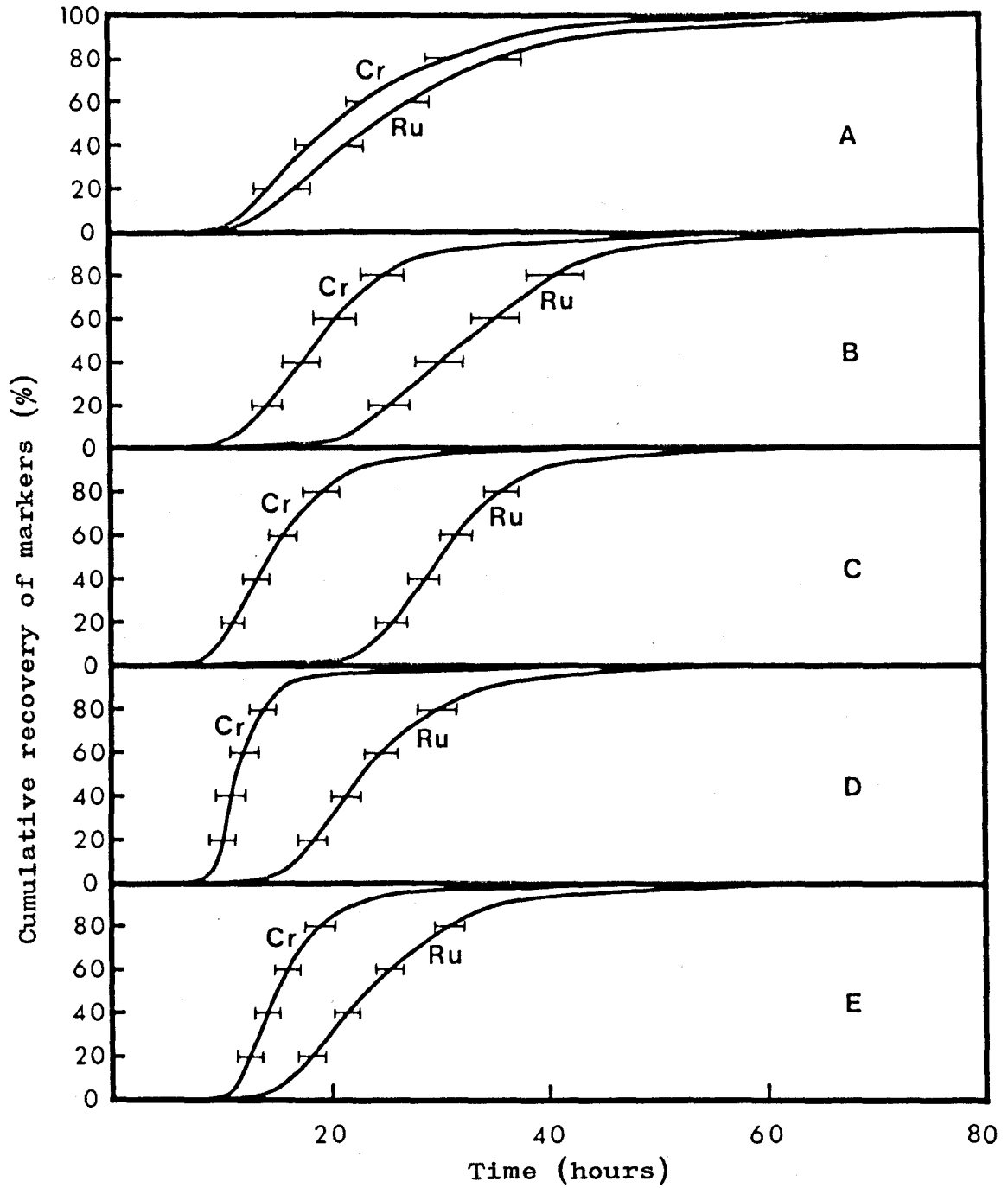
The mean 10% ET of $^{51}\text{Cr-EDTA}$ for all five species in Experiment 1a was within the range 9.4 to 12.6 h. However, 50% ET in the sheep (20.9 h) and in M. robustus (19.0 h) was longer ($P < 0.01$) than in M. giganteus (14.4 h), T. thetis (12.0 h) and M. eugenii (15.0 h); 90% ET was longer ($P < 0.01$) in the sheep (38.1 h) than in M. robustus (30.2 h), which in turn was longer ($P < 0.005$) than in the other three species (M. giganteus 22.6 h, T. thetis 18.7 h, M. eugenii 22.2 h). This is reflected in the steeper cumulative percentage recovery curves for the macropodine species (Fig. 5.2) and longer $T_{\frac{1}{2}}$ values for the sheep (Table 5.1). For the sheep, $T_{\frac{1}{2}}$ (7.9 h) was longer ($P < 0.001$) than in any of the macropodines (M. robustus 4.5 h, M. giganteus 3.4 h, T. thetis 2.4 h, M. eugenii 2.8 h).

On both the lucerne diet (Experiment 1a) and the fresh Phalaris diet (Experiment 1b), 10% ET and 50% ET of the $^{51}\text{Cr-EDTA}$ were shorter ($P < 0.05$) in T. thetis than in M. eugenii but 90% ET was similar. Although there was no within species effect of diet on TT, 10% ET, 50% ET or 90% ET in either species, $T_{\frac{1}{2}}$ of the fluid marker was shorter ($P < 0.01$) in T. thetis fed lucerne (2.4 h) than when fed Phalaris (4.1 h). Similarly for M. eugenii, $T_{\frac{1}{2}}$ was shorter ($P < 0.05$) on the lucerne diet (2.8 h) than the Phalaris diet (3.6 h).

Table 5.1. Experiment 1. Transit times (TT), 10% excretion times (ET), 50% ET, 90% ET and half-times ($T_{1/2}$) of the two markers $^{51}\text{Cr-EDTA}$ and $^{103}\text{Ru-P}$ (values in hours), and dry matter intakes of sheep and four species of macropodines fed chopped lucerne hay, and in two species of macropodines fed fresh *Phalaris* grass. All animals were fed ad libitum. Values are the means of each species.

Diet	Experiment 1a.					Experiment 1b.			
	Chopped lucerne hay					Fresh <i>Phalaris</i>			
Species	Sheep	<u>M.</u> <u>robustus</u>	<u>M.</u> <u>gigantous</u>	<u>T.</u> <u>thetis</u>	<u>M.</u> <u>eugenii</u>	SEM	<u>T.</u> <u>thetis</u>	<u>M.</u> <u>eugenii</u>	SEM
Number of animals	4	4	8	12	8		4	5	
Body weight (kg)	49.5 ± 2.5	16.6 ± 1.0	20.8 ± 1.1	4.9 ± 0.5	4.8 ± 0.2		3.2 ± 0.5	4.8 ± 0.4	
Dry matter intake (g/kg ^{0.75} /d)	68.1 ^a	42.2 ^b	55.4 ^c	51.9 ^c	37.1 ^b	7.2	46.6 ^x	34.8 ^y	4.6
$^{51}\text{Cr-EDTA}$									
TT	8.0 ^{a*}	7.1 ^a	5.4 ^a	7.4 ^a	8.9 ^a	2.6	5.9 ^x	7.1 ^x	1.2
10% ET	12.6 ^a	12.4 ^{ab}	9.7 ^{bc}	9.4 ^c	11.7 ^{ab}	2.2	7.6 ^x	10.5 ^y	1.1
50% ET	20.9 ^a	19.0 ^a	14.4 ^{bc}	12.0 ^b	15.0 ^c	2.7	11.8 ^x	14.9 ^y	1.5
90% ET	30.1 ^a	30.2 ^b	22.6 ^c	18.7 ^d	22.2 ^{cd}	4.0	21.8 ^x	24.4 ^x	3.0
$T_{1/2}$	7.9 ^a	4.5 ^b	3.4 ^{bc}	2.4 ^c	2.8 ^c	0.8	4.1 ^x	3.6 ^x	0.5
$^{103}\text{Ru-P}$									
TT	8.4 ^a	7.1 ^a	7.1 ^a	8.5 ^a	9.4 ^a	2.5	6.0 ^x	7.3 ^x	1.2
10% ET	14.2 ^a	22.6 ^b	23.8 ^b	16.6 ^a	16.2 ^a	2.9	13.1 ^x	16.1 ^y	1.1
50% ET	24.5 ^a	33.1 ^b	30.3 ^b	22.9 ^a	23.6 ^a	3.5	21.8 ^x	25.9 ^y	1.5
90% ET	44.1 ^a	45.7 ^a	39.7 ^b	34.9 ^c	35.0 ^c	3.8	34.9 ^x	37.4 ^x	2.8
$T_{1/2}$	8.9 ^a	5.6 ^b	4.2 ^b	5.2 ^b	4.5 ^b	1.0	5.4 ^x	5.4 ^x	0.7

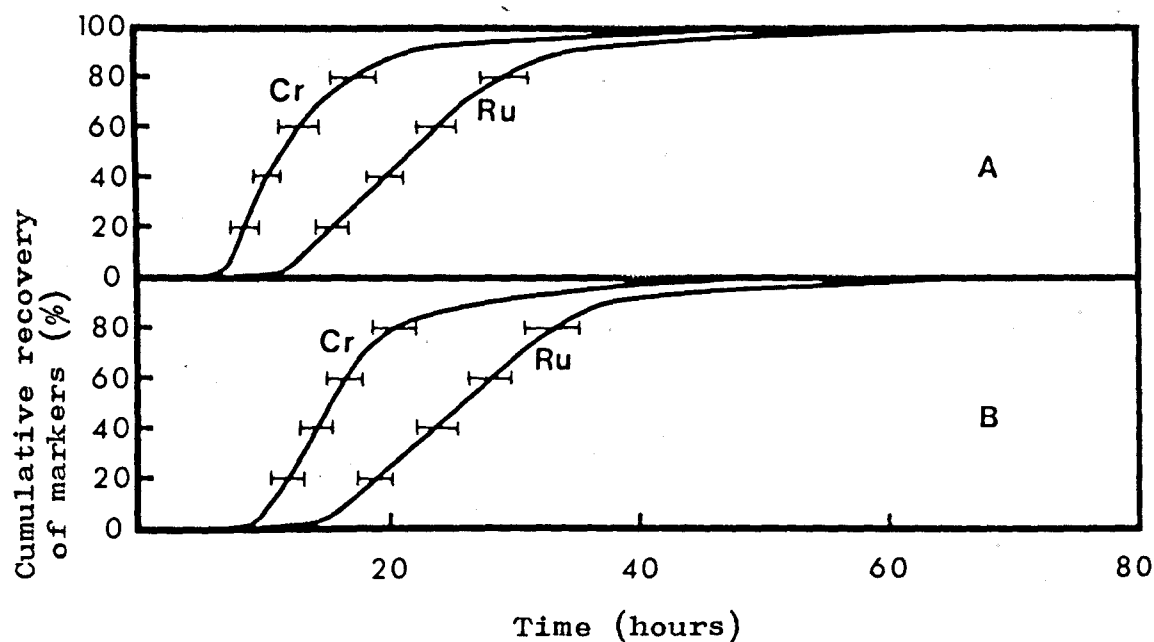
* Significant differences among species for each parameter are indicated by the superscripts a,b,c,d in Experiment 1a, and by x and y in Experiment 1b (P<0.05).



Experiment 1a; diet, chopped lucerne hay.

A = sheep, B = *M. robustus robustus*,

C = *M. giganteus*, D = *T. thetis*, E = *M. eugenii*.



Experiment 1b; diet, fresh Phalaris grass.

A = T. thetis, B = M. eugenii.

Fig. 5.2. Experiment 1a, 1b. Mean cumulative percentage recovery curves for the two markers, $^{51}\text{Cr-EDTA}$ (Cr) and $^{103}\text{Ru-P}$ (Ru), in each species.

(Since marker ogives are conventionally drawn as presented, and that 10% cumulative excretion times were measured with time as the variable, the bars representing s.e. are drawn horizontally.)

b) Kinetics of the particulate marker

The mean TT for $^{103}\text{Ru-P}$ in all five species in Experiment 1a was similar and ranged from 7.1 h in both M. robustus and M. giganteus to 9.4 h in M. eugenii. In Experiment 1b, TT was similar in both T. thetis (6.0 h) and M. eugenii (7.3 h).

In Experiment 1a, both 10% ET and 50% ET of the $^{103}\text{Ru-P}$ were similar in the sheep, T. thetis and M. eugenii, but shorter ($P < 0.005$) than in M. robustus and M. giganteus. However, 90% ET was longer ($P < 0.01$) in the sheep (44.1 h) and M. robustus (45.7 h) than in M. giganteus (39.7 h), which in turn was longer ($P < 0.01$) than in T. thetis (34.9 h) and M. eugenii (35.0 h). Again this is reflected in the steeper cumulative percentage recovery curves for the macropodines (Fig. 5.2) and longer ($P < 0.001$) $T_{\frac{1}{2}}$ in the sheep (8.9 h) than in the macropodines (M. robustus 5.6 h, M. giganteus 4.2 h, T. thetis 5.2 h, M. eugenii 4.5 h).

The excretion pattern of the particulate marker in T. thetis and M. eugenii fed the lucerne diet (Experiment 1a) was similar as measured by 10% ET, 50% ET, 90% ET and $T_{\frac{1}{2}}$. On the fresh Phalaris diet (Experiment 1b), although 10% ET and 50% ET were shorter in T. thetis ($P < 0.05$) than in M. eugenii, both 90% ET and $T_{\frac{1}{2}}$ were similar in both species, and overall there was no significant species x diet interaction for any parameter of the particulate marker.

c) Differential flow of the two markers

In the sheep the pattern of flow of $^{51}\text{Cr-EDTA}$ and $^{103}\text{Ru-P}$ through the digestive tract was similar (Fig. 5.1). The difference in 50% ET between the two markers was only 3.6 ± 0.2 h, and there was no significant difference between the $T_{\frac{1}{2}}$ values for $^{51}\text{Cr-EDTA}$ (7.9 ± 0.3 h) and $^{103}\text{Ru-P}$ (8.9 ± 0.6 h).

Despite the similar transit times for both markers in

each species, there was a marked separation of the two markers in all macropodines (Fig. 5.1), with the fluid marker passing through the digestive tract more rapidly than the particulate marker. The difference in 50% ET between the two markers was 14.1 ± 2.8 h in M. robustus, 15.7 ± 1.3 h in M. giganteus, 10.7 ± 0.6 h in T. thetis and 8.6 ± 1.0 h in M. eugenii. On the Phalaris diet, the difference in 50% ET between the two markers was 10.0 ± 1.3 h in T. thetis and 11.0 ± 1.1 h in M. eugenii; results similar to those of Experiment 1a.

d) Food intake

Mean ad libitum food intakes (Table 5.1) varied among the species, but the dry matter intakes of the sheep, M. giganteus, T. thetis and M. eugenii were such that the animals maintained body weight and were in nitrogen balance (see Chapter 6). A separate balance experiment with the four M. robustus fed the same diet (I.D. Hume, pers. comm.) indicated that the dry matter intakes of these animals, in the present experiment, were possibly lower than those required for maintenance of body weight and nitrogen balance. This may account for the longer 90% ET for both markers in this species.

Although the individual ad libitum dry matter intakes varied among animals within species, the differences were not great enough to allow prediction of relationships between rate of passage parameters and dry matter intake.

5.3.2 Experiment 2. Kinetics of the two markers in the intestine

The patterns of appearance of $^{51}\text{Cr-EDTA}$ and $^{103}\text{Ru-P}$ in the faeces, after a single injection of both markers into the hindstomach, were similar for both markers for each observation in each animal (Fig. 5.3). Cumulative excretion times for both markers were also similar.

The mean TT, 50% ET and $T_{\frac{1}{2}}$ of both markers, and dry

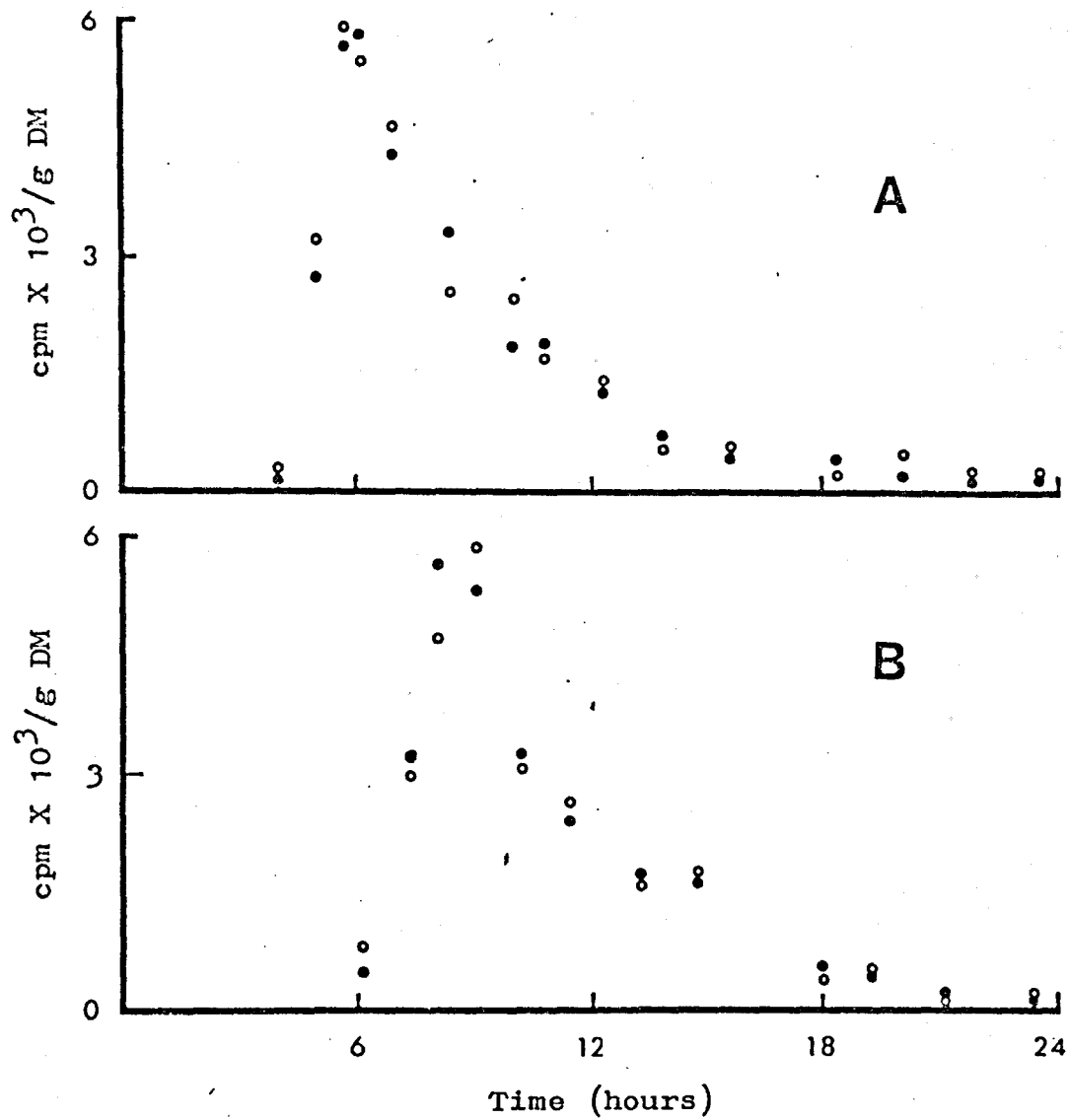


Fig. 5.3. Experiment 2. The pattern of excretion of ⁵¹Cr-EDTA (●) and ¹⁰³Ru-P (○) in the faeces of one *T. thetis* (A) and one *M. eugenii* (B) after a single injection of both markers into the hindstomach.

matter intake, in T. thetis and M. eugenii are presented in Table 5.2. The TT of both markers were identical in T. thetis (4.0 ± 0.7 h) and similarly in M. eugenii (6.8 ± 1.0 h). There was no significant difference between $^{51}\text{Cr-EDTA}$ and $^{103}\text{Ru-P}$ as measured by 50% ET in either T. thetis (8.8 and 9.0 h, respectively) or M. eugenii (12.6 and 12.7 h, respectively), or by $T_{\frac{1}{2}}$ in either T. thetis (2.8 and 2.7 h, respectively) or M. eugenii (3.0 and 3.1 h, respectively).

Thus both markers exhibited similar flow characteristics in both species, and there was no evidence of differential flow of the two phases of digesta in the intestine. The faster rate of passage of digesta in T. thetis is presumably due to the higher ($P < 0.01$) mean dry matter intake in this species ($34.4 \text{ g/kgW}^{0.75}/\text{d}$) compared to M. eugenii ($26.9 \text{ g/kgW}^{0.75}/\text{d}$).

5.3.3 Experiment 3. Flow of digesta along the digestive tract

Mean values for dry matter intake, body weight, flow of fluid and dry matter, and dry matter content of digesta in each region of the digestive tract for T. thetis and M. eugenii are presented in Table 5.3.a. The $T_{\frac{1}{2}}$ for each marker in the three main regions of the stomach (sacciform forestomach, tubiform forestomach and hindstomach) and in the caecum-proximal colon, and the RT in the colon, of both species are presented in Table 5.3.b.

The net apparent flow of fluid in the sacciform forestomach and in the tubiform forestomach of T. thetis (approx. 1.9 l/d) was more than three times the rate of flow that would be predicted from reference to the particulate marker. Similarly, in M. eugenii the flow of fluid in the forestomach (approx. 1.2 l/d) was more than twice the flow that would be expected if both phases of digesta flowed at the same rate. The faster rates of flow of fluid resulted in much shorter $T_{\frac{1}{2}}$ values for the fluid in

Table 5.2 Experiment 2. Kinetics of digesta flow in the intestine of *T. thetis* and *M. eugenii*.

Data were derived from the pattern of appearance of the markers in the faeces after a single infusion into the hindstomach. Values (in hours) are the means (+ s.e.) of TT, 50% ET, and $T_{\frac{1}{2}}$ of both $^{51}\text{Cr-EDTA}$ and $^{103}\text{Ru-P}$, and dry matter intakes of two observations in two animals of each species. The animals were fed chopped lucerne hay ad libitum at 4 h intervals.

	<u><i>T. thetis</i></u>	<u><i>M. eugenii</i></u>
Body weight (kg)	5.1 ± 0.3	4.5 ± 0.7
Dry matter intake (g/kgW ^{0.75} /d)	34.4 ± 3.5	26.9 ± 2.1
TT : fluid	4.0 ± 0.7	6.8 ± 1.0
dry matter	4.0 ± 0.7	6.8 ± 1.0
50% ET : fluid	8.8 ± 1.7	12.6 ± 2.0
dry matter	9.0 ± 1.8	12.7 ± 2.1
$T_{\frac{1}{2}}$: fluid	2.8 ± 0.5	3.0 ± 0.3
dry matter	2.7 ± 0.4	3.1 ± 0.3

Table 5.3.a. Experiment 3. Dry matter intake and water intakes, flow of the fluid and the particulate phases of digesta along the digestive tract, and dry matter content of digesta, in T. thetis and M. eugenii. Values are the means (\pm s.e.) of estimates obtained from four T. thetis and three M. eugenii fed chopped lucerne hay each hour.

	<u>T. thetis</u>			<u>M. eugenii</u>		
	fluid flow (ml/d)	D.M.* flow (g/d)	DM (%)	fluid flow (ml/d)	DM flow (g/d)	DM (%)
DMI (g/d)		122 \pm 5			99 \pm 12	
DMI** (g/kgW ^{0.75} /d)		45 \pm 5			30 \pm 2	
Water intake: feed (ml/d)	14 \pm 2			10 \pm 4		
drinking (ml/d)	315 \pm 18			155 \pm 25		
SFS ⁽¹⁾	1900 \pm 115	114 \pm 6	20.0 \pm 1.0	1280 \pm 69	91 \pm 11	15.1 \pm 0.5
TFS	1915 \pm 110	93 \pm 8	18.5 \pm 0.5	1240 \pm 64	76 \pm 10	17.4 \pm 0.6
HS	2513 \pm 151	84 \pm 4	16.8 \pm 0.5	2220 \pm 156	69 \pm 9	16.5 \pm 0.4
SI.1	1180 \pm 130	82 \pm 5	7.5 \pm 0.9	843 \pm 51	66 \pm 8	6.5 \pm 0.7
SI.2	735 \pm 47	72 \pm 2	9.8 \pm 1.0	663 \pm 150	56 \pm 13	8.1 \pm 0.5
SI.3	540 \pm 52	62 \pm 3	11.2 \pm 0.9	380 \pm 25	42 \pm 8	10.8 \pm 1.4
CPC	304 \pm 19	58 \pm 3	16.4 \pm 0.3	250 \pm 43	46 \pm 8	15.9 \pm 0.4
DC.1	223 \pm 11	57 \pm 3	21.6 \pm 1.0	159 \pm 53	42 \pm 9	22.8 \pm 2.0
DC.2	170 \pm 27	54 \pm 3	25.0 \pm 1.7	79 \pm 5	43 \pm 8	33.6 \pm 3.1
Faeces	140 \pm 16	58 \pm 3	30.1 \pm 1.9	87 \pm 13	45 \pm 7	35.4 \pm 2.4

(*DM = dry matter; **DMI = dry matter intake; (1), SFS = sacciform forestomach; TFS = tubiform forestomach; HS = hindstomach; SI = small intestine; CPC = caecum-proximal colon; DC = distal colon).

Table 5.3.b. Experiment 3. Means (+ s.e.) of the half-time (T_2) for each marker in the three main regions of the stomach, and the caecum-proximal colon, and approximate retention time (RT) in the colon, of T. thetis and M. eugenii. Values are in hours.

Body weight (kg)	<u>T. thetis</u>		<u>M. eugenii</u>	
	$^{51}\text{Cr-EDTA}$	$^{103}\text{Ru-P}$	$^{51}\text{Cr-EDTA}$	$^{103}\text{Ru-P}$
SFS	1.4 ± 0.3	5.8 ± 0.8	0.9 ± 0.2	2.3 ± 0.4
TFS	1.1 ± 0.1	4.9 ± 0.1	2.0 ± 0.4	7.1 ± 1.6
HS	0.2 ± 0.1	1.4 ± 0.1	0.2 ± 0.1	1.4 ± 0.3
CPC	2.2 ± 0.6	2.2 ± 0.5	2.2 ± 0.3	2.3 ± 0.2
Colon (RT)	3.3 ± 1.1	3.4 ± 1.3	4.5 ± 2.2	5.1 ± 2.3

comparison to the particulate phase within all three regions of the stomach.

In the hindstomach of both species the differential flow of the markers was further increased by a net increase in fluid flow.

Calculation of total wet digesta flow in regions of the intestine, from reference to either marker, yielded similar results. As in Experiment 2, there was no evidence of differential flow of the two markers in the intestine.

Differential flow in the stomach resulted in much shorter ($P < 0.001$) $T_{\frac{1}{2}}$ values for fluid than for particulate digesta (Table 5.3.b). In contrast, the $T_{\frac{1}{2}}$ values for both markers in the caecum-proximal colon, and RT values in the colon were similar in both species.

5.4 Discussion

5.4.1 Validity of the dual phase marker technique

The markers $^{51}\text{Cr-EDTA}$ and $^{103}\text{Ru-P}$ are considered adequate, and superior to other marker systems, for the concurrent measurement of the flow of fluid and particulate fractions of digesta, and their use in ruminant nutrition studies is well established (Faichney, 1975a).

A small proportion of administered $^{51}\text{Cr-EDTA}$ (less than 5%) is absorbed from the digestive tract of sheep and is excreted in the urine (Downes and McDonald, 1964). Warner (1977) determined that some 2% of orally administered $^{51}\text{Cr-EDTA}$ was excreted in the urine of M. eugenii fed continuously. In the present experiments, less than 1.5% of the infused dose was calculated to be excreted in the urine and therefore no correction for absorption was made. No $^{103}\text{Ru-P}$ was detected in the urine.

Small amounts of the $^{51}\text{Cr-EDTA}$ may also be adsorbed onto the particulate matter in the ruminoreticulum (Warner,

1969) and in the intestine of sheep (Faichney, 1975a). This would not affect the flow rates calculated for fluid and particles in the intestine as flows of both markers were similar. However, as there was a marked differential of flow of the two phases in the stomach, distribution of the two markers in stomach digesta was examined. Stomach digesta samples from the animals used in Experiment 3 were centrifuged at 15,000 g, and the particulate material was washed twice and resuspended in 0.9% sodium chloride and recentrifuged. No $^{103}\text{Ru-P}$ was detected in the initial supernatant fraction, nor was there any $^{51}\text{Cr-EDTA}$ adsorbed on to the washed particulate material.

The method of oral administration of markers in Experiments 1a and 1b was adopted because the crushed wheat was always quickly consumed and there was no requirement to physically restrain and handle the animals. The quantities of wheat used (less than 5% of daily dry matter intake) were unlikely to cause serious disturbances of intake or flow patterns. Tan, Weston and Hogan (1971) found that in sheep the $^{103}\text{Ru-P}$ does not remain absorbed onto the originally marked particles but undergoes redistribution to other solids.

Although these two markers are not strictly ideal for the measurement of digesta flow, in that $^{51}\text{Cr-EDTA}$ undergoes some absorption from the gut and $^{103}\text{Ru-Phenanthroline}$ distribution is a function of particle size (Faichney, 1975a), their use in the present experiments to obtain information on the pattern of flow and the kinetics of digesta in each species, and for comparison among these species, is considered justified.

5.4.2 The pattern of appearance of the markers in the faeces

The pattern of appearance of the two markers in the faeces of sheep is largely due to the kinetics of digesta in the stomach (Grofum and Williams, 1973c), particularly the ruminoreticulum.

a) Differential flow of the two markers

Differential flow of the fluid and the particulate phases of digesta in sheep has often been observed (Weller, Pilgrim and Gray, 1962; Ellis and Hutton, 1968; Grofum

and Williams, 1973a, 1973b; Faichney, 1975a). This is related to a longer retention of particles in the ruminoreticulum and, to a lesser extent, a faster flow of digesta fluid in the abomasum (Grovm and Williams, 1973b; Faichney, 1975b).

Fluid and particulate material flow through the intestine of sheep at similar rates and have similar mean retention times (Coombe and Kay, 1965; Grovm and Williams, 1973a; MacRae, Reid, Dellow and Wyburn, 1973; Faichney, 1975a, 1975b). In the present experiments it was shown that, at least in T. thetis and M. eugenii, the same situation holds. Since the caecum-proximal colon of both M. giganteus and M. robustus robustus is also a simple tubular structure, similar to that of the sheep, and the colon has a narrow diameter, it is most unlikely that there would be any differential flow of the two markers along the intestine of these two species either. Thus the separation of the two markers within the digestive tract of the macropodine species is considered to occur only in the stomach.

b) Transit time

Flow of digesta in the ileum of sheep is infrequent and pulsatile (Hogan and Phillipson, 1960) and defaecation is discontinuous; both factors probably contribute significantly to variability in measurement of transit time in any animal.

Theoretically, the transit time of digesta in sheep is that of the intestine alone, since transit time across the ruminoreticulum and the caecum-proximal colon should approach zero if each region is considered to be a single pool of digesta. In fact, this is not entirely true since mixing of digesta in the ruminoreticulum is not immediate (Warner and Stacy, 1968), there is some delay and mixing of digesta in the abomasum (Grovm and Williams, 1973b), and mixing of digesta in the caecum-proximal colon is not always effective nor complete (Faichney, 1969;

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Hecker, 1971; Grovum and Williams, 1973b).

It is probable that the pattern of digesta flow through the caecum-proximal colon of the macropodines is similar to that in the sheep, but as the macropodine stomach is a long, wide tubular structure, transit time of digesta through this organ should be significant.

c) The rate constants

Theoretically, the faecal marker excretion curves of sheep should be similar for any digestive system with two significant pools of digesta, one pool larger than the other, providing that within each pool the digesta is well mixed and mixing is complete within a short time. Under these conditions, the slopes of the curve are dependent on the relative sizes of the two pools and of the turnover time of digesta in each pool.

The $T_{\frac{1}{2}}$ derived from the major component of the negative slope of the faecal marker excretion curve is independent of the second rate constant and is representative of the largest pool (Shipley and Clark, 1972). In the sheep this $T_{\frac{1}{2}}$ represents the kinetics of a marker in the ruminoreticulum. The second rate constant, obtained by normal curve analysis and regression of the points on the early rising part of the curve, has been shown to apply to the kinetics of marker in the caecum-proximal colon (Grovum and Williams, 1973c, Grovum and Phillips, 1973).

In Experiment 1 the $T_{\frac{1}{2}}$ calculated for $^{103}\text{Ru-P}$ should thus apply to the kinetics of the particulate phase of digesta in the stomach. The results for the sheep are in good agreement with those of Grovum and Williams (1973b), considering that the sheep in the present experiment were on a higher intake than the sheep used in Grovum's study. However, the $T_{\frac{1}{2}}$ calculated for $^{103}\text{Ru-P}$ in each of the macropodines was far shorter than for the sheep. Assuming

that the mean capacity of the forestomach of each macropodine species in Experiment 1a was similar to the data in Table 4.1, and that approximately 60% of the digestible organic matter was apparently digested in the forestomach (see Chapter 7), then at the mean intakes of these species in Experiment 1a, the $T_{\frac{1}{2}}$ should approximate 17 h in M. giganteus, 11 h in T. thetis and 10 h in M. eugenii. These theoretical values are two to three times longer than the actual estimates of $T_{\frac{1}{2}}$ for $^{103}\text{Ru-P}$ (Table 5.1).

This suggests that in all four species of macropodines, the forestomach cannot be considered as a single pool of digesta. The general shape of the faecal marker excretion curves, and the phasic time shift of the $^{103}\text{Ru-P}$ curve with respect to the $^{51}\text{Cr-EDTA}$ curve, both suggest that there is more than one pool of digesta in the macropodine forestomach and that the pattern of flow of digesta includes elements of tubular or bolus flow (Shipley and Clark, 1972). It is therefore not realistic to derive a second rate constant from the macropodine data. Such a constant may well contain elements of the kinetics of a marker from more than one pool of digesta and thus may have little biological meaning.

5.4.3 Stomach structure and flow of digesta

Since differences have been found in both stomach structure and digesta flow between macropodines and sheep, and also among macropodines, it is pertinent to discuss the pattern and mode of flow of stomach digesta in relation to stomach structure.

a) The sheep

The sacciform structure of the ruminoreticulum (Nickel, Schummer and Seiferle, 1973) ensures that ingested food is effectively removed from the polarised flow of digesta along the digestive tract, and mixes with a large volume of digesta containing residues from previous meals.

By definition, the transit time of an orally infused marker across the ruminoreticulum may still approach zero if the marker mixes thoroughly with all of the digesta and the outflow of digesta to the omasum is frequent.

Differential flow of the fluid and particulate phases of digesta arises, in part, from a longer retention of larger food particles in the ruminoreticulum. Events, as yet not thoroughly understood, preclude the outflow of particles through the reticulo-omasal orifice until they have been reduced to a minimum size by microbial attack, remastication during rumination and detrition (Reid, Ulyatt and Monro, 1977).

b) The macropodines

The radiological observations indicate that, in contrast to the ruminant stomach, ingested food enters the cranial regions of the forestomach, mixes with digesta in that region, and then is progressively transported along the tubiform forestomach. Although local mixing of digesta appears to be effective, total mixing of all digesta throughout the forestomach does not occur.

This tubular concept of digesta flow is supported by the results from the digesta flow experiments. Three further factors support the concept of tubular flow:

1. Macropodines do not ruminate (Barker, Brown and Calaby, 1963).
2. There is no anatomical feature within the macropodine stomach analogous to the reticulo-omasal orifice that could play a role in the differential flow of digesta particles of different size.
3. In all species examined there was no evidence of stratification of digesta particles within the forestomach.

The evidence suggests that all particles are transported along the stomach at a similar rate.

The gastric sulcus.

The gastric sulcus may be effective in adult animals in directing the initial flow of some ingesta, or at least ingested fluid, some distance along the lesser curvature of the tubiform forestomach. However, this structure does not extend the full length of the tubiform forestomach, and there was no evidence of rapid transport of contrast medium or of marked digesta directly to the hindstomach.

These observations suggest that in the macropodines, unlike the sheep, there must be a significant transit time for both digesta phases through the forestomach. In this aspect it is of interest to compare T. thetis, a species in which there is no gastric sulcus, and M. eugenii, in which the gastric sulcus is well defined. Although both species exhibited lower dry matter intakes in Experiment 2 (Table 5.2) than in Experiment 1 (Table 5.1), the TT for ⁵¹Cr-EDTA in the entire digestive tract of T. thetis was 3.4 h longer than TT in the intestine alone. In M. eugenii the difference was 2.1 h. Since the hindstomach contains only 7 to 15% of the total stomach digesta (Chapter 4, Table 4.1) and the T_2 of fluid in this region is short (0.2 h, Table 5.3.b), the TT of fluid digesta, and of particulate matter, in the forestomach must be considerable, even in species in which a gastric sulcus is present.

Differential flow of digesta in the stomach.

The differential flow of fluid and particulate digesta in the macropodine stomach does not result from the selective retention of the larger dietary particles, as in the ruminant stomach. Rather, it arises from a higher net flow of the fluid with respect to the particulate matter.

The measured fluid flow rates in the forestomach of both T. thetis and M. eugenii in Experiment 3 were considerably higher than the daily intake of drinking and food water. The net increase in fluid flow presumably

arose mainly from salivary secretions. The parotid salivary glands of the macropodines are large (Forbes and Tribe, 1969), and secretion rates are probably high (Brown, 1964). Fluid flow may also be complemented by secretions from the cardiac glandular epithelium of the forestomach.

Both the fluid and the particulate phases of digesta are subjected to tubular flow along the stomach and the extrusion of the fluid through the particulate material is presumably effected by contractions of the haustrations and the semi-lunar folds of the forestomach wall. Differential flow of the fluid with respect to the particulate phase is further increased in the hindstomach, no doubt as a result of gastric secretion.

Tubular flow of particulate digesta.

The tubular flow of digesta in the macropodine stomach cannot be considered as ideal bolus flow since the digesta is not transported along the forestomach in discrete boli. Under normal feeding conditions, whether fed continuously, or at intervals of several hours, the stomach always contains digesta. It is visualised that as each bolus of ingested food enters the forestomach it is thoroughly mixed with digesta as a primary pool close to the cardia, and digesta from this pool is gradually transported along the tubiform forestomach. At some short distance from the cardia, dietary particles ingested earlier are no longer mixed with more recently ingested food. The contractions of the stomach wall result in effective local mixing of digesta along the forestomach, but since the macropodine stomach is not structurally divided into compartments, this must result in some interface mixing such that discrete pooling of boli does not occur.

5.5 Conclusion

The present experiments provide evidence that the mode of flow of digesta through the macropodine stomach is quite different from that in the ruminant forestomach.

Further, the tubular flow of stomach digesta was apparent in T. thetis and M. eugenii when fed either the chopped lucerne hay or the fresh Phalaris diets.

By definition, tubular flow should have a significant influence on the mode of microbial digestion in the stomach. A significant transit time in the forestomach should result in little of the soluble and rapidly fermentable dietary substrates escaping microbial attack. In addition, the very nature of the tubular flow should result in a changing pattern of microbial fermentation along the forestomach as some dietary substrates will be more rapidly digested than others. The effect of this tubular flow on microbial digestion and activity is discussed in later chapters.

Chapter 6

THE APPARENT DIGESTION OF A DRIED AND A FRESH HERBAGE DIET,
AND THE DYNAMICS OF ^{14}C -UREA AND WATER METABOLISM.

6.1 Introduction

Intake and digestion of dried roughage diets in the larger macropodine species have usually been estimated in experiments designed for direct comparison with sheep (see Section 2.3). Similar comparisons among species of macropodines have received less attention. Forbes and Tribe (1970) concluded that Me. rufa (red kangaroo) and M. giganteus (eastern grey kangaroo) were similar in utilisation of roughage diets. However, Hume (1974) determined that M. robustus cervinus (western euro) was superior to Me. rufa, and equal to the sheep, in digesting acid-detergent fibre, and in retaining nitrogen and sulphur. No such comparisons have so far been published involving the smaller species of macropodines, or species from more contrasting habitats; nor has the digestion of fresh herbage diets been investigated.

Recycling of plasma urea to the fermentative regions of the digestive tract and metabolism of urea by the micro-organisms increases the efficiency of utilisation and retention of dietary nitrogen. This is well documented for ruminants, and has been demonstrated in S. brachyurus (quokka) (Brown, 1964), M. robustus cervinus (Brown, 1969), and M. eugenii (tammar wallaby) (Kinnear and Main, 1975; Kennedy and Hume, 1978). The dynamics of water metabolism have also been examined in several macropodine species (Denny and Dawson, 1975). Urea and water metabolism have not been examined simultaneously in macropodines under conditions of known food and water intake.

The following balance experiments were undertaken to

examine the digestion of a chopped lucerne hay diet in M. giganteus, T. thetis (red-necked pademelon), M. eugenii, and sheep, and the digestion of a fresh herbage diet (Phalaris aquatica) in T. thetis and M. eugenii. The dynamics of ^{14}C -urea and tritiated water (THO) were also examined in T. thetis and M. eugenii fed both the dried and the fresh diets.

6.2 Materials and methods

6.2.1 Experiments 4a, 4b ; the balance experiments

In Experiment 4a, seven sheep (Border Leicester x Merino wethers), 8 M. giganteus, 9 T. thetis, and 8 M. eugenii were fed chopped lucerne hay ad libitum and in Experiment 4b, 4 T. thetis and 5 M. eugenii were fed fresh Phalaris grass ad libitum. In both experiments the following parameters were measured in each animal: the intake of dry matter (DM), organic matter (OM), digestible organic matter (DOM), digestible energy (DE), nitrogen (N), and water; the excretion of urinary N and faecal N; N balance; the digestibility of DM, OM, crude protein (CP), and acid-detergent fibre (ADF).

All animals were fed the respective experimental diet for a minimum period of three weeks before the experiments, and at least 4 days prior to and during an 8-day balance period the food was offered twice daily. For feeding and housing procedures, see Section 3.3.

The animals were weighed at the start of the experimental period and again on the eighth day and the mean body weights recorded. Food intake, and urine and faecal output were recorded daily. Urine and faecal samples were kept each day and stored at -10°C . Drinking water was made available ad libitum and daily water intakes were recorded. Evaporation was taken into account and estimated daily by reference to a similar container of water kept in the same room.

During Experiment 4a, a 100 g sample of the lucerne on offer each day was retained and daily food refusals were weighed and bulked over the 8-day period for each animal. Subsamples of the lucerne offered, and refusals, were dried at 70°C to allow calculation of dry matter intake. Because of the practice of some individual animals of selecting against the more fibrous particles of the chopped lucerne (see Section 3.2.1), analyses of both the lucerne offered, and the food refusals of each animal were required to obtain actual intake values of DM, OM, N, ADF and gross energy.

In Experiment 4b, a 200 g sample of the Phalaris harvested each day was stored at -10°C and a second sample was dried in a microwave oven to obtain daily dry matter content. The Phalaris was harvested at an early stage of growth and consisted of leaf material only. Since no stem material was present, selection of food by the animals was not possible. The daily food refusals from each animal were dried in a microwave oven, the dry weight recorded, and the refusals then discarded.

Dried samples of lucerne offered, refusals, Phalaris offered, and faeces were analysed for DM, OM, N, ADF and gross energy content. Urine samples were analysed for total N. For details of sample collection, preparation and analytical methods see Chapter 3.

6.2.2 Experiments 5a, 5b; ¹⁴C-urea and THO metabolism

In Experiments 5a and 5b, the dynamics of ¹⁴C-urea and THO metabolism were examined in 6 T. thetis and 6 M. eugenii fed chopped lucerne hay ad libitum, and in 4 T. thetis and 5 M. eugenii fed Phalaris grass ad libitum, respectively.

Food and water intake and urine and faeces output were measured, and DMI and N intake estimated, as outlined for Experiment 4, for each animal over a period of 7 days.

On Day 1 of the experimental period, each animal was

weighed and given an intramuscular injection of 1 ml of a solution containing 5 μ Ci of THO/ml and 15 μ Ci of ^{14}C -urea/ml in sterile saline. Each syringe and needle was weighed before and after injection to obtain the weight of the injected dose. Samples of the solution were stored at -10°C for later determination of the actual THO and ^{14}C -urea radioactivity. The animals were returned to their cages with access to food and water.

Discrete urine samples from each animal over the subsequent 24 h were collected into small bottles containing 1 ml of glacial acetic acid, and the time of urination and sample volume recorded. Further discrete urine samples from each animal were collected daily over the next 6 days, and the time recorded. All samples were stored at -10°C . On Day 7, a blood sample was obtained from each animal and the plasma stored at -10°C .

Dilutions of the infusion solution were analysed for ^{14}C and tritium activity. The urine samples from each animal on Day 1 were analysed for urea N and the specific activity of the ^{14}C -urea. Plasma samples were analysed for urea N. Four of the Day 1 urine samples, and the subsequent daily urine samples, were measured for THO activity. The analytical methods are detailed in Chapter 3.

6.2.3 Calculations

a) Urea metabolism

For each animal, the natural logarithm of the specific activity of ^{14}C -urea in each of the urine samples from Day 1 was plotted against time (Fig. 6.1). The time of each sample was taken as the midpoint between the time of collection (actual time of urination) and the time of the previous sample. It was evident from these curves that the ^{14}C -urea reached equilibrium within 2 to 2.5 h; thus the points used in the regression to obtain values for the fractional rate of loss, k , and the zero-time

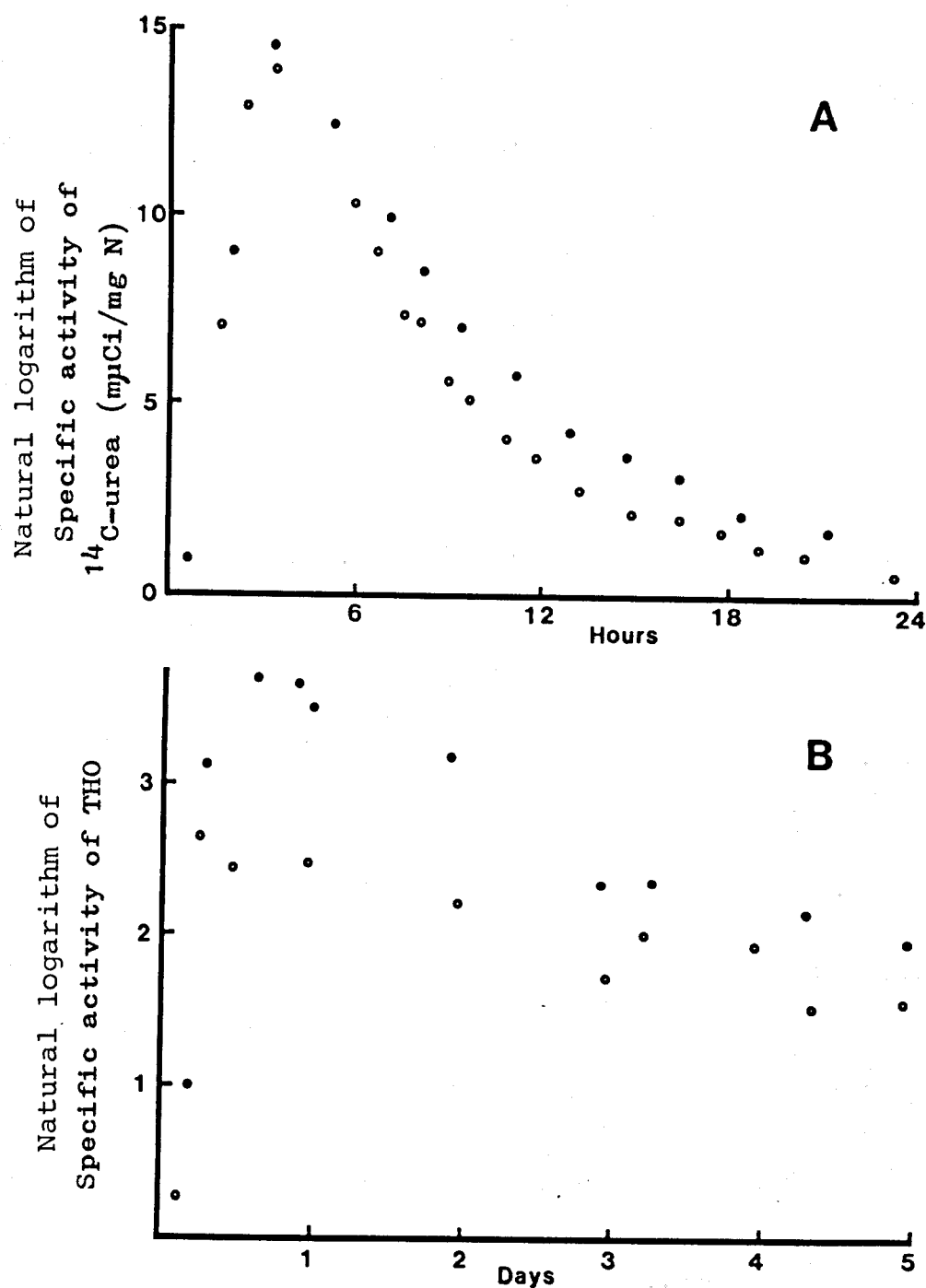


Fig. 6.1. Experiment 5. The pattern of urinary excretion of ^{14}C -urea (A) and THO (B) in one *T. thetis* (●) and one *M. eugenii* (o) after a single intramuscular injection.

For each isotope, in all animals, the natural logarithm of the specific activity of each sample was plotted against time; regression analysis determined k , the fractional rate constant.

intercept, were those within the 3 to 24 h period.

Parameters of ^{14}C -urea metabolism were estimated according to standard procedures (e.g. Baker and Rostami, 1969; Leng, 1970):

- (1) Body urea pool (gN)
- $$= \frac{{}^{14}\text{C-urea injected (m}\mu\text{Ci)} \times 10^{-3}}{\text{specific activity of } {}^{14}\text{C-urea at } T_0 \text{ (m}\mu\text{Ci/mgN)}}.$$
- (2) Urea N space (l)
- $$= \frac{\text{Body urea N pool (gN)}}{\text{Plasma urea N (gN/l)}}.$$
- (3) Urea N entry rate, i.e., irreversible loss (gN/d)
- $$= \text{Body urea N pool size (g)} \times -k(\text{d}^{-1}).$$
- (4) Urea N excretion rate (gN/d)
- $$= \text{total urea N excreted in the urine over the 24 h period.}$$
- (5) Urea N degradation rate, i.e., the quantity of urea N synthesized and appearing in the blood that is recycled to and degraded in the gut (gN/d)
- $$= \text{urea entry rate (gN/d)} - \text{urea N excretion rate (gN/d)}.$$
- (6) Urea N recycled (%)
- $$= \frac{\text{urea N degradation rate (gN/d)} \times 100}{\text{urea N entry rate (gN/d)}}.$$

b) THO metabolism

As in the analysis of ^{14}C -urea metabolism above, the natural logarithm of the specific activity of THO in the urine samples were plotted against time (Fig. 6.1) and regression analysis used to determine the fractional rate

constant, k , and the zero-time intercept. THO equilibration in the body appeared to take longer than for ^{14}C -urea, and was more variable among animals of both species on both diets. In most instances, the initial points used in the regression analyses were from samples taken 6 to 12 h after injection.

Parameters of THO metabolism were defined from the following relationships (e.g., Holleman and Dieterich, 1973):

- $$(1) \text{ THO space (l)} = \frac{\text{THO injected (}\mu\text{Ci)}}{\text{specific activity of THO at } T_0 \text{ (}\mu\text{Ci}\cdot\text{l}^{-1}\text{)}}.$$
- $$(2) \text{ Turnover time (d)} = \frac{1}{k \text{ (d}^{-1}\text{)}}.$$
- $$(3) \text{ Turnover rate (l/d)} = \frac{\text{THO space (l)}}{\text{turnover time (d)}}.$$
- (4) Turnover rate as $\text{ml/kgW}^{0.8}/\text{d}$ was calculated for interspecific comparisons.

6.2.4 Statistical analyses

Mean values of intake, excretion, and digestibility coefficients for each species were compared by one-way analysis of variance (NECV programme, University of New England, Computer Centre).

Because of unequal cell numbers, an approximate analysis (Snedecor and Cochran, p 472, 1967) was carried out to examine diet X species interactions between T. thetis and M. eugenii.

Differences between the means for each parameter measured in T. thetis and M. eugenii were compared by "Student's" t-test.

6.3 Results

Intake data and apparent digestibility coefficients for the four species fed the chopped lucerne hay, and for the two species fed Phalaris grass are presented in Table 6.1. Nitrogen intake, excretion and balance data from these experiments are presented in Table 6.2.

The data on ^{14}C -urea metabolism in T. thetis and M. eugenii fed both the lucerne and the Phalaris diets are presented in Table 6.3, and the data on THO metabolism in both species on both diets are in Table 6.4.

The values tabulated for each parameter are the means for each of the species. To allow interspecific comparisons, intake, nitrogen and ^{14}C -urea data are expressed on a metabolic body weight basis ($\text{kgW}^{0.75}$) (Kleiber, 1961) and water intake, excretion and turnover are expressed on the basis of $\text{kgW}^{0.8}$ (Richmond, Langham and Trujillo, 1962).

6.3.1 Balance experiments

a) Intake and digestion of chopped lucerne hay

Dry matter intake and organic matter intake were similar in the sheep and in M. giganteus, but since the sheep digested more ($P < 0.05$) of the dry matter and organic matter, digestible organic matter intake and digestible energy intake were higher ($P < 0.05$) in the sheep than in all three species of macropodines (Table 6.1).

All intake parameters for M. giganteus and T. thetis were similar and higher ($P < 0.005$) than those of M. eugenii.

M. eugenii digested a similar proportion of the dry matter consumed, and more ($P < 0.05$) of the organic matter and crude protein fractions, compared with the sheep, and both species digested more of these components ($P < 0.05$) than M. giganteus or T. thetis.

Table 6.1. Experiment 4. Intake and digestion in sheep and three species of macropodines fed chopped lucerne hay *ad libitum*, and in two species of macropodines fed fresh *Phalaris* grass *ad libitum*. Values are the means for each species.

Diet Species	Experiment 4a				Experiment 4b			SEM
	Sheep	Chopped lucerne hay			<i>Phalaris aquatica</i>			
		<u>M.</u> <u>giganteus</u>	<u>T.</u> <u>thetis</u>	<u>M.</u> <u>eugenii</u>		<u>T.</u> <u>thetis</u>	<u>K.</u> <u>eugenii</u>	
Number of animals	7	8	9	8		4	5	
Body weight(kg)	48.2 ± 2.5	20.8 ± 1.1	5.0 ± 0.5	4.8 ± 0.2		3.2 ± 0.5	4.8 ± 0.4	
DMI ⁽¹⁾ (g/d)	1109	549	179	94		114	115	
(g/kgW ^{0.75} /d)	60.3 ^{a(2)}	56.7 ^{ab}	52.7 ^b	29.4 ^c	6.6	46.6 ^x	34.8 ^y	6.8
OMI								
(g/kgW ^{0.75} /d)	54.9 ^a	51.5 ^{ab}	47.9 ^b	26.8 ^c	6.0	38.5 ^x	28.6 ^y	5.6
DOMI								
(g/kgW ^{0.75} /d)	32.4 ^a	28.7 ^b	26.6 ^b	16.3 ^c	3.2	23.9 ^x	19.0 ^{y*}	3.2
DEI								
(MJ/kgW ^{0.75} /d)	0.63 ^a	0.57 ^b	0.53 ^b	0.32 ^c	0.06	0.50 ^x	0.39 ^y	0.07
DM apparent digestibility(%)	59.0 ^a	55.4 ^b	55.4 ^b	59.7 ^a	1.8	56.3 ^x	59.9 ^{y*}	2.5
OM apparent digestibility(%)	59.0 ^a	55.9 ^b	55.8 ^b	60.9 ^c	1.9	62.3 ^x	66.1 ^{y*}	2.6
CP apparent digestibility(%)	74.2 ^a	73.0 ^{ab}	71.7 ^b	77.3 ^c	2.2	74.5 ^x	72.5 ^x	4.2
ADF digestibility(%)	49.1 ^a	38.6 ^b	37.8 ^b	38.7 ^b	3.4	36.7 ^x	36.2 ^x	4.9
Total water intake								
(ml/kgW ^{0.8} /d)	160 ^a	129 ^b	126 ^b	52 ^c	24	167 ^x	103 ^y	22

(1), DM(I) = dry matter (intake), OM(I) = organic matter (intake), DE(I) = digestible energy (intake), CP = crude protein, ADF = acid-detergent fibre.

(2), superscripts a,b,c, and x,y denote significant differences among species for each parameter in Experiments 4a and 4b, respectively (P<0.05).

* , denotes significant differences between the two species in Experiment 4b for P<0.10 only

Digestibility of the acid-detergent fibre fraction was similar in all three species of macropodines and lower ($P < 0.05$) than in the sheep.

b) Intake and digestion of Phalaris

As on the lucerne diet,

T. thetis consumed more dry matter, organic matter and digestible energy than M. eugenii ($P < 0.05$) (Table 6.1).

Differences between the two species in digestible organic matter intake and dry matter and organic matter apparent digestibility on this diet were significant only at the 10% level of probability. Crude protein digestibility was similar, and both species digested similar proportions of the acid-detergent fibre.

c) Comparison of the two diets

Although T. thetis consumed somewhat less of the Phalaris than the lucerne diet as measured by all parameters, and M. eugenii consumed more, the differences within each species between the two diets were not significant. Efficiency of utilisation of the dry matter, and the acid-detergent fibre fraction, were also similar within each species on both diets.

In contrast, both species digested a higher ($P < 0.05$) proportion of the organic matter of the Phalaris than the lucerne, a reflection of the higher readily fermented carbohydrate content of the fresh diet (Table 3.1). T. thetis digested a similar proportion of the crude protein fraction of the fresh herbage diet, whereas M. eugenii digested a higher proportion ($P < 0.05$) of the crude protein fraction of the lucerne compared to Phalaris.

d) Nitrogen intake, excretion and balance

On the lucerne diet, nitrogen intake and faecal nitrogen excretion were both similar in the sheep, M. giganteus and

T. thetis but lower ($P < 0.01$) in M. eugenii (Table 6.2). Urinary nitrogen excretion in the sheep was lower ($P < 0.05$) than in M. giganteus but higher ($P < 0.05$) than in M. eugenii. In urinary N excretion T. thetis did not differ significantly from M. giganteus and the sheep.

Nitrogen balance was positive and similar in all four species.

Similarly, on the Phalaris diet, T. thetis consumed more nitrogen ($P < 0.05$) than M. eugenii and excreted more nitrogen ($P < 0.05$) in both the faeces and the urine.

Nitrogen balance was positive and similar in both species.

T. thetis consumed more nitrogen ($P < 0.05$) when fed the lucerne in comparison to the Phalaris diet, and excreted more nitrogen ($P < 0.05$) in the faeces and urine. M. eugenii also consumed more nitrogen ($P < 0.05$) when fed the lucerne diet, but urinary and faecal nitrogen losses were similar on both diets.

The fact that both species consumed more nitrogen when fed the lucerne diet is due to the higher nitrogen content of the lucerne in comparison to the Phalaris (see Table 3.1). Both species retained more nitrogen ($P < 0.05$) when fed the lucerne diet.

6.3.2 Urea metabolism

a) The chopped lucerne hay diet

T. thetis consumed more nitrogen ($P < 0.05$) than M. eugenii, (Table 6.3); plasma urea N levels were higher ($P < 0.05$), and both urea N entry rate and urea N excretion rate were higher ($P < 0.05$). On the other hand, urea N degradation rate, body urea N pool size, and the ratio of body urea N space to body weight were similar in both species. Thus in T. thetis, there was a faster

Table 6.2 Experiment 4. Nitrogen intake, excretion and retention in sheep and three species of macropodines fed chopped lucerne hay *ad libitum*, and in two species of macropodines fed fresh *Phalaris* grass *ad libitum*. Values are the means for each species.

Diet	Experiment 4a				Experiment 4b		SEM
	Sheep	Chopped lucerne hay			<i>Phalaris aquatica</i>		
Species		<i>M. giganteus</i>	<i>T. thetis</i>	<i>M. eugenii</i>	<i>T. thetis</i>	<i>M. eugenii</i>	
Number of animals	7	8	9	8	4	5	
Body weight (kg)	48.2 ± 2.5	20.8 ± 1.1	5.0 ± 0.5	4.8 ± 0.2	3.2 ± 0.5	4.8 ± 0.4	
Nitrogen intake (g/d)	30.1	16.5	5.9	3.5	2.8	2.7	
(g/kgW ^{0.75} /d)	1.63 ^{a(1)}	1.69 ^a	1.72 ^a	1.09 ^b	0.23 1.13 ^x	0.84 ^y	0.16
Urinary nitrogen (g/d)	15.9	9.8	3.2	1.6	1.7	1.7	
(g/kgW ^{0.75} /d)	0.86 ^a	1.01 ^b	0.94 ^{ab}	0.50 ^c	0.12 0.68 ^x	0.51 ^{y*}	0.14
Faecal nitrogen (g/d)	7.9	4.5	1.7	0.8	0.7	0.7	
(g/kgW ^{0.75} /d)	0.43 ^a	0.46 ^a	0.49 ^a	0.25 ^b	0.08 0.27 ^x	0.22 ^y	0.03
Nitrogen balance (g/d)	+6.4	+2.2	+1.05	+1.09	+0.40	+0.29	
(g/kgW ^{0.75} /d)	+0.34 ^a	+0.23 ^a	+0.30 ^a	+0.34 ^a	0.12 +0.18 ^x	+0.10 ^{y*}	0.07

(1), superscripts a,b,c and x,y denote significant differences among species for each parameter in Experiments 4a and 4b, respectively (P<0.05).

• , denotes significant differences between the two species in Experiment 4b for P<0.10 only.

Table 6.3. Experiment 5. Nitrogen intake and ^{14}C -urea kinetics in two species of macropodines, *T. thetis* and *M. eugenii*, fed a chopped lucerne hay diet and a fresh herbage diet (*Phalaris aquatica*), ad libitum.

Diet Species	Experiment 5a		Experiment 5b			
	Lucerne		Phalaris			
	<i>T. thetis</i>	<i>M. eugenii</i>	<i>T. thetis</i>	<i>M. eugenii</i>		
Number of animals	6	6	4	4		
Body weight (kg)	3.52 ± 0.34	5.42 ± 0.32	3.21 ± 0.53	5.11 ± 0.32		
			SEM			SEM
Nitrogen intake (g/kgW ^{0.75} /d)	1.55 ^{a(1)}	1.21 ^b	0.13	1.13 ^x	0.84 ^y	0.20
Plasma urea-N (mgN/100 ml)	33.5 ^a	24.9 ^b	2.5	40.0 ^x	19.8 ^y	3.1
Body urea-N pool size: (g)	0.80	1.00		0.91	0.72	
(mg/kgW ^{0.75})	310 ^a	284 ^a	44	379 ^x	214 ^y	31
Body urea-N space Body weight (%)	68.0 ^a	75.2 ^a	6.8	72.1 ^x	72.9 ^x	13.0
Urea-N entry rate; (gN/d)	3.78	4.25		2.84	2.84	
(gN/kgW ^{0.75} /d)	1.47 ^a	1.20 ^b	0.19	1.16 ^x	0.86 ^y	0.17
Urea-N excretion rate; (gN/d)	1.73	2.03		1.28	1.34	
(gN/kgW ^{0.75} /d)	0.67 ^a	0.57 ^b	0.08	0.51 ^x	0.41 ^x	0.13
Urea-N degradation rate; (gN/d)	2.06	2.22		1.56	1.50	
(gN/kgW ^{0.75} /d)	0.80 ^a	0.63 ^a	0.17	0.65 ^x	0.45 ^y	0.12
Urea recycled (%)	54.0 ^a	52.4 ^a	6.4	56.1 ^x	52.4 ^x	8.2

(1), superscripts a,b, and x,y denote significant differences between species for each parameter in Experiments 5a, 5b, respectively (P<0.05).

turnover of nitrogen as urea N than in M. eugenii but in both species the proportion of urea N synthesized that was recycled to and degraded in the gut was similar.

b) The Phalaris diet

The pattern of nitrogen metabolism was similar for both species on both the lucerne and the fresh diet. Nitrogen intake and plasma urea N levels were higher ($P < 0.05$) and body urea N pool size larger ($P < 0.05$) in T. thetis whereas body urea N space, expressed as a percentage of body weight, was similar in both species.

Urea N entry rate was higher ($P < 0.05$) in T. thetis than in M. eugenii, and since urea excretion rates were similar in both species, urea N degradation rate was also higher ($P < 0.05$) in T. thetis. However, the proportion of urea N synthesized that was recycled to the gut was again similar in both species.

c) Effect of diet on urea metabolism

As the experiments were undertaken on the basis of ad libitum food intake, the range of intakes exhibited among individual animals of both species on both diets allowed an estimate of correlation of nitrogen intake with each of the parameters of urea metabolism.

In both species, there was a significant correlation between nitrogen intake and urea N entry rate (Fig. 6.2.a) and between nitrogen intake and urea N excretion rate (Fig. 6.2.b). In both comparisons there were no significant differences between the regression coefficients or the intercepts, indicating that the relationships between nitrogen intake and urea synthesis, and between nitrogen intake and urea excretion, are similar in both macropodine species.

There was also a significant relationship between nitrogen intake and urea N degradation rate in M. eugenii

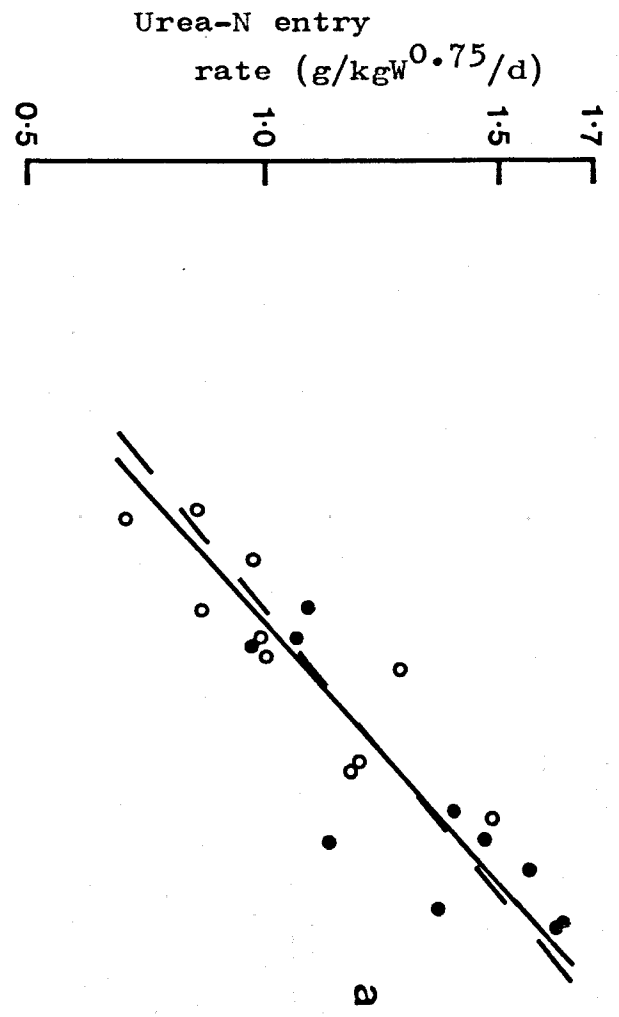
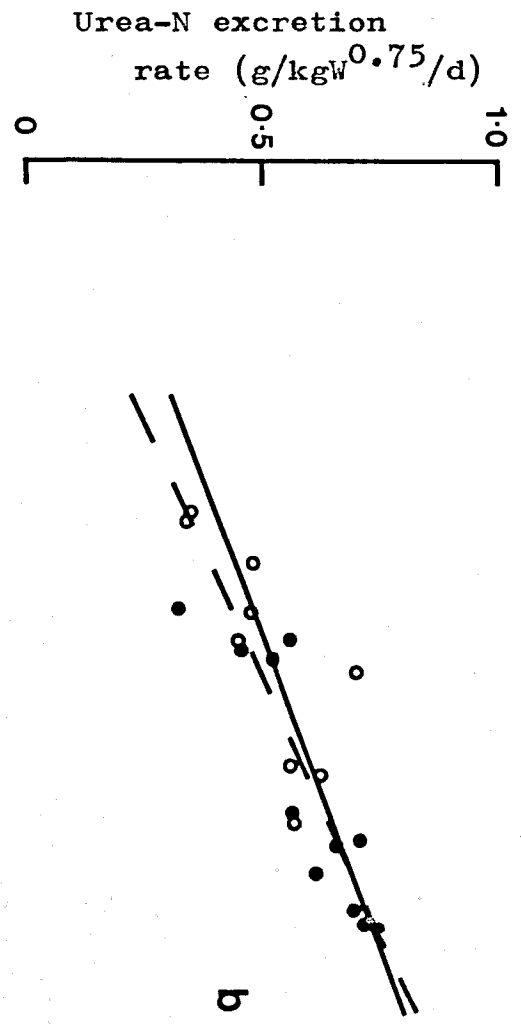
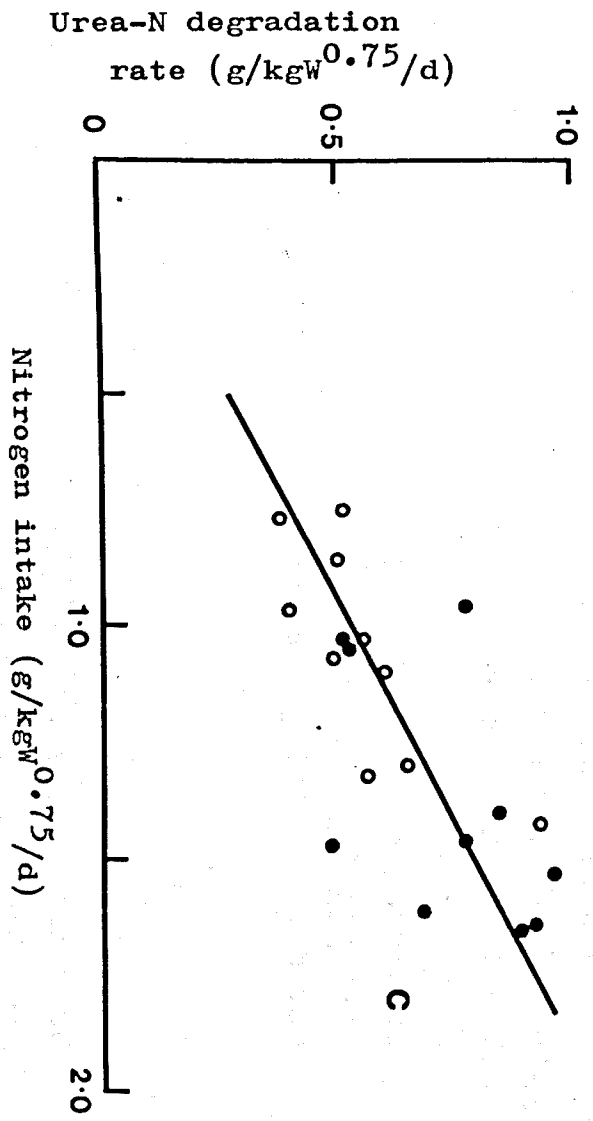
Fig. 6.2. Experiment 5. Urea metabolism in T. thetis and M. eugenii. Regression relationships between:
(a), nitrogen intake and urea entry rate;
(b), nitrogen intake and urea excretion rate;
(c), nitrogen intake and urea degradation rate.

Regression equations:

(a) <u>T. thetis</u>	$y = 0.80x + 0.23$ (S.E. = 0.12)
	$r = 0.86, P < 0.01;$
<u>M. eugenii</u>	$y = 0.89x + 0.12$ (S.E. = 0.10)
	$r = 0.88, P < 0.01;$
(b) <u>T. thetis</u>	$y = 0.45x - 0.01$ (S.E. = 0.06)
	$r = 0.89, P < 0.01;$
<u>M. eugenii</u>	$y = 0.38x + 0.11$ (S.E. = 0.07)
	$r = 0.76, P < 0.01;$
(c) <u>T. thetis</u>	$y = 0.36x + 0.25$ (S.E. = 0.14)
	$r = 0.53, NS$;
<u>M. eugenii</u>	$y = 0.52x + 0.01$ (S.E. = 0.09)
	$r = 0.77, P < 0.01 .$

Differences between regression coefficients and between intercepts in Fig. 6.1(a) and Fig. 6.1(b) are not significant.

T. thetis ●, ---; M. eugenii, ○, ——— .



(Fig. 6.2.c), but this relationship was not significant for T. thetis. The data from T. thetis are included to indicate the similarity to M. eugenii.

6.3.3 Intake and metabolism of water

The mean total water intake (feed water plus ad libitum drinking water) for each species in the balance experiments, 4a and 4b, are shown in Table 6.1. The mean dry matter intake, total water intake, urine and faecal water excretion, THO space to body weight ratio, and THO turnover time and rate, for T. thetis and for M. eugenii in Experiments 5a and 5b are presented in Table 6.4.

On the chopped lucerne hay diet (Experiment 4a), total water intake was higher ($P < 0.05$) in the sheep than in the three macropodines, and M. eugenii consumed water ($P < 0.001$) than either M. giganteus or T. thetis. On the Phalaris diet, T. thetis consumed more water ($P < 0.05$) than M. eugenii.

In Experiment 4, T. thetis and M. eugenii both consumed more water when fed the fresh Phalaris diet ($P < 0.05$). The chopped lucerne contained approximately 11% water (Table 3.1) and on this diet food water only contributed 5 to 6% of the total water intake in both T. thetis and M. eugenii. On the other hand, the Phalaris diet contained approximately 78% water and contributed 82% of the total water intake in T. thetis and 96% of the total water intake in M. eugenii. Thus the higher water intakes exhibited by both species when fed Phalaris were largely due to a higher obligatory water intake with the food.

a) Experiment 5a

On the chopped lucerne hay diet, T. thetis consumed more water than M. eugenii ($P < 0.05$) (Table 6.4) and urine excretion and faecal water excretion were also higher ($P < 0.05$). These observations were concordant with the THO

Table 6.4 Experiment 5. Intake and excretion of water and body water kinetics in two species of macropodines, *T. thetis* and *M. eugenii*, fed a chopped lucerne hay diet and a fresh herbage diet (*Phalaris australis*), *ad libitum*.

Diet	Experiment 5a		Experiment 5b		SEM
	Lucerne		Phalaris		
Species	<i>T. thetis</i>	<i>M. eugenii</i>	<i>T. thetis</i>	<i>M. eugenii</i>	
Number of animals	6	6	4	4	
Body weight (kg)	3.52 ± 0.34	5.42 ± 0.32	3.21 ± 0.53	5.11 ± 0.32	
Dry matter intake (g/kgW ^{0.75} /d)	51 ^{a(1)}	39 ^b	4 47 ^x	35 ^y	7
Total water intake (ml/kgW ^{0.8} /d)	131 ^a	74 ^b	21 160 ^x	103 ^y	13
Urine output (ml/kgW ^{0.8} /d)	47 ^a	20 ^b	7 38 ^x	39 ^x	14
Faecal water (ml/kgW ^{0.8} /d)	47 ^a	24 ^b	9 80 ^x	34 ^y	13
THO space (2)					
Body weight (%)	83 ^a	77 ^a	6 88 ^x	77 ^x	13
Turnover time (d)	5.7 ^a	8.1 ^b	1.1 5.3 ^x	8.0 ^y	1.4
Turnover rate (ml/d)	520 ^a	520 ^a	116 535 ^x	502 ^x	133
(ml/kgW ^{0.8} /d)	191 ^a	136 ^b	28 210 ^x	138 ^y	24

- (1), superscripts a,b and x,y denote significant differences between species for each parameter in Experiments 5a and 5b, respectively (P<0.05).
- (2), THO space/body weight, turnover time and turnover rate were calculated from reference to a single intramuscular injection of tritiated water and subsequent collection of discrete urine samples over a 7-day balance period.

data; THO

turnover time was shorter ($P < 0.05$) and THO turnover rate ($\text{ml}/\text{kgW}^{0.8}/\text{d}$) was higher ($P < 0.05$) in T. thetis. THO space as a ratio of body weight was variable but similar in both species.

b) Experiment 5b

T. thetis also had a higher intake ($P < 0.05$) and total excretion of water than did M. eugenii when fed the Phalaris diet. Although urine excretion was similar in both species, faecal water excretion increased in T. thetis so that the sum of urine and faecal water excretion was higher ($P < 0.05$). THO turnover time was shorter ($P < 0.05$) and turnover rate higher ($P < 0.05$) in T. thetis.

As in Experiment 5a, the ratio of THO space to body weight was variable but similar in both species.

c) Effect of diet on water metabolism

The ratio of THO space to body weight tended to be higher in T. thetis than in M. eugenii on both diets; however, the difference was not significant. This ratio was also similar within each species on both diets.

Although T. thetis appeared to consume more water when fed the fresh Phalaris diet (mean, $160 \text{ ml}/\text{kgW}^{0.8}/\text{d}$; Table 6.4), mainly due to the higher obligatory water intake, than when fed lucerne ($131 \text{ ml}/\text{kgW}^{0.8}/\text{d}$), the difference was not significant in this experiment. Urine output was also similar but faecal water excretion was higher ($P < 0.05$) on the Phalaris diet.

On the Phalaris diet M. eugenii consumed more water ($P < 0.05$) than on the lucerne diet, excreted more water in the faeces ($P < 0.05$) and had a higher output of urine ($P < 0.05$); however, these differences were not reflected in the THO results.

On both the lucerne and the Phalaris diets, THO turnover time was lower ($P < 0.05$) and THO turnover rate (as $\text{ml/kgW}^{0.8}/\text{d}$) was higher ($P < 0.05$) in T. thetis than in M. eugenii, but there was no effect of diet in either species.

6.4 Discussion

6.4.1 Digestion of lucerne in sheep and macropodines

In the present experiments, both the sheep and M. giganteus consumed similar amounts of dry matter and organic matter (on a metabolic body weight basis) but, as determined in the experiments of Forbes and Tribe (1970) and Kempton, Murray and Leng (1976), digestion of dry matter and organic matter was less complete in M. giganteus than in sheep. Thus the sheep were consuming more digestible organic matter and digestible energy.

T. thetis was similar to M. giganteus in all parameters of intake and digestion of this diet, and also less efficient than sheep in digestion of dry matter and organic matter. The notable exception was M. eugenii. As found by Hume (1977b), M. eugenii consumed less dry matter and organic matter than T. thetis, and yet maintained nitrogen balance and body weight. M. eugenii digested dry matter as effectively as the sheep, and was superior in apparent digestion of organic matter and crude protein. However, this was not achieved by increased digestion of acid-detergent fibre.

a) Digestion of dietary fibre

All three species of the macropodines digested similar proportions of the fibre fraction of this diet but were less efficient than the sheep. Similarly, it has been demonstrated in other studies that although M. giganteus (Forbes and Tribe, 1970; Kempton, Murray and Leng, 1976) and also Me. rufa (Foot and Romberg, 1965; McIntosh, 1966; Forbes and Tribe, 1970; Hume 1974) digest significant proportions of the fibrous fraction of high roughage diets,

they are less efficient than sheep.

As described earlier, the mode of flow of digesta through the ruminoreticulum of the sheep differs from that observed in the macropodine forestomach. In sheep, the efficiency of microbial digestion of the structural carbohydrates of a diet is enhanced by the selective retention of larger dietary particles in the ruminoreticulum until decreased in size by rumination and microbial attack. Additional comminution of previously ingested food material during rumination presumably also favours more efficient microbial utilisation of larger particles by increasing their surface area.

Macropodines do not ruminate. Further, as discussed in Chapter 4, there is no apparent mechanism for selective retention of larger particles within the macropodine forestomach. Thus the less complete digestion of fibre in macropodines compared to sheep appears to be due to the fact that larger particles are (a) retained in the stomach for a shorter time, and (b) only reduced further in size by microbial attack. It would be expected that the macropodines could digest the cellulose fraction of a diet more efficiently if it was fed in a ground and pelleted state rather than in a chopped or long hay form. This has not been tested experimentally.

Hume (1974) compared M. robustus cervinus and Me. rufa with sheep fed roughage diets, and determined that, although all three species digested hemicellulose equally well, M. robustus cervinus and sheep utilised the less tractable acid-detergent fibre fraction more efficiently than Me. rufa.

In an extensive study on the evolutionary relationships of teeth structure and function in macropodines, Sanson (1977) provided evidence indicating that the cheek teeth of the grazing species are better adapted for cutting and the

comminution of abrasive and fibrous savannah grasses. Since M. robustus cervinus is a more sedentary grazer (Ealey, Bentley and Main, 1965), while Me. rufa is a more mobile (Bailey, 1971) and highly selective feeder (Storr, 1968), it would be of interest to compare the efficiency of comminution of the same roughage diet in these two species.

6.4.2 Digestion of Phalaris in T. thetis and M. eugenii

As

on the lucerne diet, T. thetis consumed more organic matter, nitrogen and digestible energy than M. eugenii to maintain body weight and nitrogen balance. However, both species digested similar proportions of fibre.

Both species digested the organic matter fraction of Phalaris more extensively than that of lucerne. This can be attributed to the higher readily fermentable carbohydrate content of the fresh diet. However, overall each species was similar in its digestion of the two diets.

Digestion of fresh herbage diets by the macropodines has not been previously reported, but it is of interest to compare the present results with those of Hogan, Weston and Lindsay (1969) who studied the digestion in sheep of Phalaris harvested at three successive stages of maturity. Although the earlier harvest was dried prior to feeding, and had been fertilised at seeding and during growth with ammonium salts which presumably gave rise to the higher crude protein content, the composition of the diet was otherwise similar to that fed to the macropodines in the present experiment. Crude protein digestibility of the Phalaris in sheep was comparable to that in the macropodines. However, the sheep digested a higher proportion of the cell wall constituent fraction, and thus a higher proportion of the organic matter. The comparison suggests that sheep may also digest grasses in the young vegetative state more efficiently than the macropodines but direct comparisons

between sheep and macropodines fed fresh diets should be undertaken to determine if this is so.

6.4.3 Validity of the ^{14}C -urea and THO techniques

In a preliminary experiment, lateral tail vein catheters were inserted in eight M. eugenii, under ketamine hydrochloride sedation, and in each animal, an outer sleeve on the catheter wall was sewn to subcutaneous connective tissue. The presence of the catheters obviously caused some discomfort and all animals had either bitten through the catheter, or removed it entirely, within 24 hours. It was therefore decided to estimate the decline in specific activity of ^{14}C -urea and THO in discrete urine samples. In experiments with rabbits, Rubsamen, Nolda and Engelhardt (1979) have shown that THO pool size can be measured with reference to the specific activity of THO in either plasma or in urine with similar results.

Neither T. thetis nor M. eugenii usually void large volumes of urine at one time, and thus at ad libitum water intakes urination occurs frequently throughout the day. In Experiment 5, a mean of 10 discrete urine samples were obtained from T. thetis and 9 samples obtained from M. eugenii, on the lucerne diet, over the initial 24 hour period. On the Phalaris diet, a mean of 12 urine samples from T. thetis and a mean of 20 samples from M. eugenii were obtained during a similar period.

The urine samples used in the estimation of ^{14}C -urea turnover were those collected in the period 3 to 24 h, as equilibration of the ^{14}C -urea with the body urea pool was apparent after 2.5 h (see Fig. 6.1). The regression of the specific activity of ^{14}C -urea in urine against adjusted time of collection, for all animals, had a correlation coefficient not less than $r = 0.95$; thus the relationship accounted for 90% of the variation over all animals. Estimation of urea turnover from discrete urine samples can therefore be considered valid.

Measurement of THO specific activity in urine samples over the 7-day period was more variable in some animals, so a minimum of 8 samples was used to define THO turnover in each animal (mean correlation coefficient for all animals, $r = 0.98 \pm 0.01$).

Determination of ^{14}C -urea turnover time and THO turnover time is independent of the dose injected. Thus variations in the estimates of urea pool size and entry rate, and THO space and THO turnover rate, are more likely to be caused by loss of label at the time of injection and during equilibration. The interspecific comparisons of urea entry rate in T. thetis and M. euseanii can be considered valid, since ^{14}C -urea equilibration time was short and similar in both species.

Estimates of THO space were higher in T. thetis (although not significantly different) than in M. euseanii. The estimates may be realistic, but since THO turnover time was considerably shorter in T. thetis, yet equilibration time (6 to 12 h) was similar in both species, the higher estimate of THO space in T. thetis is likely to be due to a greater loss of label prior to equilibration. Denny and Dawson (1975), in a study of water turnover in five species of macropodines, determined that up to 3.9% of the injected THO may be lost prior to equilibration.

In the present experiments, turnover time (TT) was significantly correlated with turnover rate (TR) (expressed as $\text{ml}/\text{kg}^{0.8}/\text{d}$) in each species:

$$\text{T. thetis } y_{\text{TR}} = -24.18x_{\text{TT}} + 352 \text{ (S.E. = 12.65),}$$

$$r = -0.85, P < 0.01 ;$$

$$\text{M. euseanii } y_{\text{TR}} = -15.30x_{\text{TT}} + 260 \text{ (S.E. = 15.15),}$$

$$r = -0.80, P < 0.01 .$$

The differences in both the slopes and the intercepts are significant ($P < 0.05$), which does suggest that in T. thetis more THO was lost during equilibration than in M. euseanii, and thus the THO space to body weight ratio for T. thetis is

overestimated to a greater extent than the ratio derived for M. eugenii. THO space is usually considered to be approximately 70% of body weight in ruminant herbivores (Macfarlane, 1965). The significant correlations between THO turnover and THO turnover rate in both species further indicate that any error in estimation of THO space arose from loss during equilibration rather than loss of label at injection.

6.4.4 Nitrogen and urea metabolism

M. eugenii maintained nitrogen balance on a considerably lower nitrogen intake than the sheep, M. giganteus or T. thetis on the lucerne diet, and similarly in comparison with T. thetis on the Phalaris diet. This is consistent with the findings of Hume (1977b), who determined a maintenance nitrogen requirement of $0.24 \text{ g/kgW}^{0.75}/\text{d}$ of dietary nitrogen for M. eugenii and $0.60 \text{ g/kgW}^{0.75}/\text{d}$ of dietary nitrogen for T. thetis. The former estimate of maintenance nitrogen requirement for M. eugenii is similar to that obtained for this species by Barker (1968).

Urea metabolism in T. thetis and M. eugenii in the present experiments correlated well with nitrogen intake. The rate of urea synthesis, the rate of urea excretion, and the proportion of urea that was recycled to and degraded in the digestive tract were similar in both species. These results are comparable to similar estimates in the sheep on equivalent nitrogen intakes (Cocimano and Leng, 1967; Nolan and Leng, 1972).

Although M. eugenii has a much lower maintenance requirement for nitrogen than T. thetis, it is apparent, at least on diets containing adequate levels of nitrogen for maintenance, that this is not due to more efficient microbial utilisation of urea.

6.4.5 Water and THO metabolism

Total water intake, as measured in these experiments, included the sum of feed water and ad libitum drinking water. Total water intakes in all species were a reflection of dry matter intakes, but there was no significant correlation between these parameters due to variation among individual animals in free water intake.

The measurements of turnover time and turnover rate in T. thetis and M. eugenii indicate that there is a difference in water metabolism between the two species. The higher water turnover rate in T. thetis is clearly related to the higher ad libitum food intake recorded in this species. This was independent of the diet; whether there is a difference independent of dry matter intake cannot be determined from the present experiments.

Since intake and digestibility parameters and total water intake were similar in M. giganteus and T. thetis fed the lucerne diet, it is likely that water turnover would also be similar in these two species under ad libitum feeding conditions.

Denny and Dawson (1975) determined water turnover in five macropodine species. Their values for water turnover rate were much lower (e.g., M. eugenii, $65.2 \text{ ml/kgW}^{0.8}/\text{d}$; M. giganteus, $120 \text{ ml/kgW}^{0.8}/\text{d}$). Food and water were provided ad libitum in their experiments after the equilibration period, but were not recorded. The results suggest that food and water intake were lower in their animals than observed in the present experiments.

6.5 Conclusion

Fibre digestion was less complete in the three species of macropodines examined than in the sheep. This finding appears to be related to the mode of flow of particulate digesta through the macropodine stomach compared with the ruminant stomach.

M. eugenii had a lower requirement for digestible energy and nitrogen and a concomitant lower water turnover than T. thetis. However, this does not appear to be due to differences in efficiency of microbial utilisation of diet since urea metabolism in the two species was similar. Macfarlane, Howard, Haines and Kennedy (1971) pointed out that animals with a low metabolic rate also have a low water turnover. The present results suggest that the lower estimates of maintenance nitrogen requirement, water turnover and voluntary food intake for M. eugenii, in comparison to T. thetis, may be due to a lower standard metabolic rate rather than a more efficient microbial digestion.

Chapter 7

DIGESTION IN THE STOMACH AND IN THE INTESTINE7.1 Introduction

Estimation of the proportional apparent loss of dietary components within the stomach, small intestine and large intestine of herbivores is a means of defining the relative importance of microbial fermentation to the overall digestive process.

In hindgut fermenters such as the horse, the rate of passage of ingested food through the relatively small stomach is rapid (Robinson and Slade, 1974), and apparent loss of dietary constituents occurs only in the intestine (Hintz, Hogue, Walker, Lowe and Schryver, 1971). More than 50% of both the soluble carbohydrate and the digestible crude protein of a roughage diet are apparently digested and absorbed in the small intestine. The majority of neutral detergent fibre is digested through microbial fermentation in the caecum and colon (Hintz, Hogue, Walker, Lowe and Schryver, 1971).

On the other hand, microbial fermentation in the ruminant stomach is extensive. In sheep fed roughage diets, some 60 to 75% of the digestible organic matter consumed is digested in the ruminoreticulum (Hogan and Weston, 1967; Egan, Walker, Nader and Storer, 1975). This forestomach fermentation results in the digestion of virtually all of the soluble carbohydrates and some 68 to 94% of the digestible portion of the plant cell wall constituents (Hogan and Weston, 1967).

Microbial fermentation in the macropodine forestomach, like that in ruminants, is much more extensive than in the large intestine (Hume, 1977a). However, no estimates of the extent of apparent fermentation and digestion in various

regions of the macropodine digestive tract have been reported.

The following experiment (Experiment 6) was undertaken to estimate the extent of apparent digestion in the stomach, small intestine and large intestine in three species of macropodines; T. thetis (red-necked pademelon), M. eugenii (tammar wallaby) and M. giganteus (eastern grey kangaroo). The experiment was also conducted to obtain measurements of the relative capacities of defined regions of the digestive tract under controlled conditions and known food intake (these results were presented in Chapter 4), and to measure the concentration of the microbial fermentation end-products NH_3 and VFA, and pH and urea, in digesta in each region of the digestive tract.

The tubular flow pattern of digesta transport in the macropodine stomach (Chapter 5) suggests that ingested food is gradually transported along the length of the forestomach and that total mixing of the contents does not occur. Sequential digesta samples were therefore obtained from along the length of the forestomach and analysed to determine whether there is a gradient of apparent loss of dietary components.

7.2 Materials and methods

7.2.1 Experimental procedure

Four adult animals of each of the three species, T. thetis, M. eugenii and M. giganteus, were housed in metabolism cages and fed chopped lucerne hay ad libitum for a minimum period of 7 weeks. During the latter 2 weeks, chromic oxide (Cr_2O_3) powder (0.5%) was thoroughly mixed with the daily ration for each animal and the diet offered twice daily.

A balance experiment was conducted over the last five days, to allow calculation of mean intake and digestibility coefficients for each animal, and then the animals were

slaughtered. During the last two days of the collection period fresh portions of the diet were offered at more frequent intervals. This was done to minimise as far as possible any large fluctuations of digesta flow in the digestive tract prior to slaughter.

Three animals, one of each species, were slaughtered each morning and each afternoon during the next two days. Each animal was sedated with ketamine hydrochloride and then killed by pentobarbitone sodium euthanasia (see Section 3.4.1). The digestive tract was then carefully dissected free and removed through a ventral midline incision in the abdominal wall, and measurements and samples were obtained as quickly as possible. A blood sample was taken by cardiac puncture and the plasma stored for analysis of urea N.

The stomach was considered as three distinct regions; the sacciform forestomach, the tubiform forestomach and the hindstomach. A mixed sample of digesta was taken from the sacciform forestomach, and another from the hindstomach. Up to three sequential samples were obtained from approximately equidistant sites along the length of the tubiform forestomach. The small intestine was divided into three approximately equal lengths and the digesta from each segment was thoroughly mixed prior to subsampling. A mixed sample of digesta was taken from the caecum-proximal colon, and two sequential samples from the equally divided distal colon.

The total wet weight of digesta in each defined region of the digestive tract was obtained by weighing the gut segment before and after the removal of digesta. The pH was recorded immediately, and subsamples of digesta were stored at -10°C . Further subsamples of digesta were added to a known weight of sulphosalicylic acid solution (20%), reweighed, centrifuged at 13 000 g for 30 min, and the supernatant stored at -10°C .

7.2.2 Analyses.

Samples of the lucerne offered, feed refusals and faeces from the collection period were analysed for Cr_2O_3 content, DM, OM, total N and ADF. Whole digesta samples were analysed for Cr_2O_3 , DM, OM, total N, and carbohydrate fractions (total soluble sugars, pectin, hemicellulose, cellulose and ADF).

Digesta supernatant samples were analysed for urea N, ammonia N, and total VFA and individual proportions of the acids (see Chapter 3 for analytical methods).

7.2.3 Calculations

The proportional apparent loss of substrate (e.g. OM) along the digestive tract was calculated with reference to the concentration of Cr_2O_3 in the feed consumed and that in the sample, e.g.;

$$\frac{\frac{\text{mg OM}}{\text{mg Cr}_2\text{O}_3} \text{ (in feed)} - \frac{\text{mg OM}}{\text{mg Cr}_2\text{O}_3} \text{ (in sample)}}{\frac{\text{mg OM}}{\text{mg Cr}_2\text{O}_3} \text{ (in feed)}}$$

In only four of the twelve animals did the recovery of Cr_2O_3 in the faeces, during the collection period, correlate with estimation of dry matter digestibility. In these animals, the estimation of dry matter digestibility by reference to total collection and by reference to recovery of Cr_2O_3 was similar ($\pm 1.2\%$).

In the other eight animals, more Cr_2O_3 was accounted for in the faeces than was calculated to be consumed on the basis of analysis of food offered and food refusals. For these animals, intake of the marker was calculated from the concentration in the faeces and by reference to dry matter digestibility.

The intake and digestibility data from the collection

period, and the calculated proportional apparent loss of dietary constituents in regions of the digestive tract, for each species, were compared by one-way analysis of variance.

7.3 Results

The regions of the digestive tract, the sites sampled, and the notation used for the tabulated results, are presented schematically in Fig. 7.1.

7.3.1 The pH and concentrations of VFA, urea and ammonia in digesta fluid

The pH and concentrations of total VFA in digesta fluid from the digestive tract of T. thetis, M. eugenii and M. giganteus are presented in Tables 7.1 and 7.2, respectively. The average proportions of the individual VFA in fluid from the forestomach and the caecum-proximal colon are in Table 7.3. The concentrations of urea N and ammonia N in digesta fluid are presented in Tables 7.4 and 7.5, respectively.

a) pH

The mean pH of digesta fluid in the forestomach of the four animals, in all three species, was within the range pH 6.4 to 7.4. There were no significant differences in pH along the forestomach.

Gastric secretion in the hindstomach increased ($P < 0.001$) the acidity (pH 2.7 to 3.3) of digesta flowing from the forestomach. This was neutralised in the duodenum (pH 6.5) and the pH increased further ($P < 0.01$) (pH 7.8 to 8.1) in the ileum. The pH of fluid in the caecum-proximal colon in M. giganteus (pH 6.9) was lower than in the ileum ($P < 0.05$).

b) VFA

In T. thetis, the concentration of total VFA in the sacciform forestomach was higher ($P < 0.01$) than in the

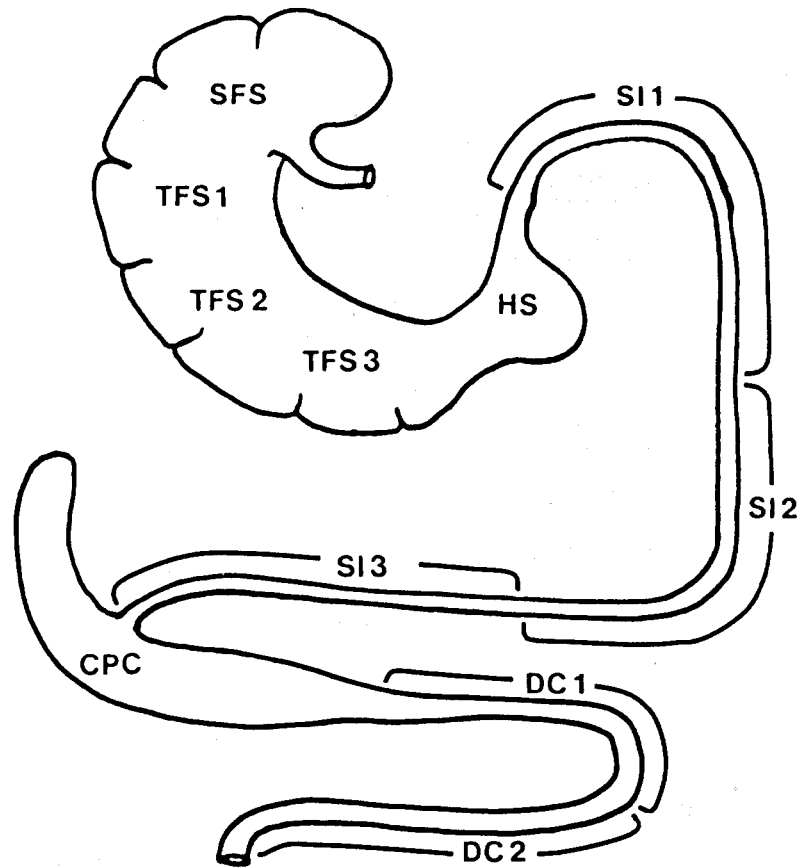


Fig. 7.1. Schematic representation of the major regions of the macropodine digestive tract, the sites sampled, and the notation used for the tabulated results.

SFS		=	sacciform forestomach.
TFS	1)	=	three equidistant sampling sites along
	2)		the tubiform forestomach.
	3)		
HS		=	hindstomach.
SI	1)	=	the small intestine; mixed samples were
	2)		obtained from each of the three segments.
	3)		
CPC		=	caecum-proximal colon
DC	1)	=	distal colon; mixed samples were obtained
	2)		from both segments.

Table 7.1. The pH of digesta in *T. thetis*, *M. eugenii* and *M. giganteus* fed chopped lucerne hay ad libitum.
Values are the means (+ s.e.) of four animals of each species.

Species	<u>T. thetis</u>	<u>M. eugenii</u>	<u>M. giganteus</u>
SFS ^a	7.4 ± 0.2	7.4 ± 0.1	6.4 ± 0.2
TFS 1			6.5 ± 0.1
2	7.0 ± 0.1	7.3 ± 0.2	6.8 ± 0.1
3			6.9 ± 0.1
HS	3.0 ± 0.5	3.3 ± 0.4	2.7 ± 0.4
SI 1	6.5 ^b	-	6.5 ± 0.2
2	7.4 ± 0.2	7.9 ± 0.1	7.6 ± 0.1
3	7.9 ± 0.1	8.1 ± 0.1	7.8 ± 0.1
CPC	7.7 ± 0.1	7.9 ± 0.2	6.9 ± 0.1

a = the sites of sampling and the notation used are described in Fig. 7.1.

b = one observation only.

Table 7.2. The concentration of total VFA ($\mu\text{M}/\text{ml}$) in digesta fluid in T. thetis, M. eugenii and M. giganteus fed chopped lucerne hay ad libitum. Values are the means (\pm s.e.) of four animals of each species.

Species	<u>T. thetis</u>	<u>M. eugenii</u>	<u>M. giganteus</u>
SFS ^a	165 \pm 9	109 \pm 12	136 \pm 13
TFS 1	124 \pm 6	102 \pm 4	131 \pm 9
2	99 \pm 5	72 \pm 5	102 \pm 6
3			77 \pm 5
HS	56 \pm 8	53 \pm 5	32 \pm 8
SI 1	22 \pm 11	-	8 \pm 3
2	7 \pm 4	16 \pm 4	6 \pm 1
3	18 \pm 1	14 \pm 2	6 \pm 3
CPC	61 \pm 8	63 \pm 5	60 \pm 6
DC 1	78 \pm 2	95 \pm 8	61 \pm 5
2	86 \pm 10	113 \pm 16	64 \pm 4

a = the sites of sampling and the notation used are described in Fig. 7.1.

cranial region of the tubiform forestomach, but this was not evident in M. eugenii or M. giganteus. However, in each of the three species, the concentration of VFA decreased ($P < 0.01$) between all sampling sites along the tubiform forestomach and the hindstomach.

Concentrations of VFA were low in digesta fluid from the small intestine, but increased again in the caecum-proximal colon. There was a trend for the concentration of VFA to increase along the large intestine in all species, but not significantly so.

The proportions of the individual volatile fatty acids in forestomach fluid (Table 7.3) are the average of samples taken from the sacciform forestomach and the tubiform forestomach.

The proportions of the individual acids in the digesta fluid from the forestomach and the caecum-proximal colon were similar in all three species. Acetic acid was predominant (71 to 75% in the forestomach; 76 to 77% in the caecum-proximal colon). The mean proportion of propionic acid, in all three species, ranged from 16 to 18% in the forestomach and from 13 to 16% in the caecum-proximal colon.

In both regions of the digestive tract, there was more butyric acid present in the fluid ($P < 0.01$) than valeric acid. The branched-chain isomers of butyric and valeric acids comprised less than 1% of the total VFA in both sites in all three species.

c) Urea

In all three species, virtually no urea N was detected in digesta fluid from either the forestomach or the large intestine (Table 7.4). On the other hand, urea N increased from a low concentration of 2 to 3 mgN/100 ml in the hindstomach to 15 to 17 mgN/100 ml in the duodenum. In the more distal regions of the small intestine, the

Table 7.3. The proportions (%) of the individual volatile fatty acids in digesta fluid from the forestomach and the caecum-proximal colon of *T. thetis*, *M. eugenii* and *M. giganteus* fed chopped lucerne hay ad libitum. Values are the means (+ s.e.) of four animals from each species.

	<u>T. thetis</u>	<u>M. eugenii</u>	<u>M. giganteus</u>
Forestomach ^a			
Acetic	74 ± 1.1	75 ± 0.7	71 ± 1.0
Propionic	16 ± 0.9	17 ± 0.5	18 ± 0.5
Iso-butyric	<1	<1	<1
Butyric	6 ± 0.4	5 ± 0.5	8 ± 0.3
Iso-valeric	<1	<1	<1
Valeric	2 ± 0.4	1 ± 0.3	2 ± 0.2
Caecum-proximal colon			
Acetic	76 ± 1.4	77 ± 0.8	76 ± 1.7
Propionic	14 ± 0.9	13 ± 0.3	16 ± 1.6
Iso-butyric	1 ± 0.3	2 ± 0.2	<1
Butyric	7 ± 0.9	7 ± 0.7	6 ± 0.7
Iso-valeric	<1	<1	<1
Valeric	1 ± 0.3	1 ± 0.1	1 ± 0.6

a = the mean forestomach values are calculated from the average of SFS and TFS samples combined, see text.

Table 7.4. The concentrations of urea-N (mgN/100 ml) in plasma and digesta fluid in T. thetis, M. eugenii and M. giganteus fed chopped lucerne hay ad libitum. Values are the means (+ s.e.) of four animals of each species.

	<u>T. thetis</u>	<u>M. eugenii</u>	<u>M. giganteus</u>
Plasma	31 ± 0.3	26 ± 1.3	24 ± 1.2
Forestomach	<1	<1	<1
HS ^a	3 ± 1	2 ± 1	2 ± 1
SI 1	17 ± 6	-	15 ± 1
2	33 ± 1	25 ± 1	21 ± 2
3	33 ± 2	26 ± 4	22 ± 3
CPC	<2	<2	<2

a = the sites of sampling and the notation used are described in Fig. 7.1.

concentration of urea N increased further to levels similar to those determined for plasma urea N.

d) Ammonia

The concentrations of ammonia N in forestomach digesta fluid were similar at all sites sampled in both T. thetis and in M. giganteus, but higher ($P < 0.01$) in T. thetis than in M. giganteus (Table 7.5). Concentrations of ammonia N in the sacciform forestomach of M. eugenii were similar to T. thetis, but lower ($P < 0.01$) in the tubiform forestomach.

In all three species, the concentration of ammonia N in the digesta fluid from the caecum-proximal colon was similar and higher ($P < 0.05$) than in digesta fluid from the distal colon.

7.3.2 Intake and apparent digestion in the digestive tract

Organic matter intake, nitrogen intake and acid-detergent fibre intake (expressed on a metabolic body weight basis) were similar in T. thetis and M. giganteus but higher ($P < 0.01$) than in M. eugenii (Table 7.6).

Organic matter apparent digestibility was similar in T. thetis and M. giganteus but lower ($P < 0.05$) than in M. eugenii. Crude protein apparent digestibility and acid-detergent fibre digestibility were similar in all three species.

a) Apparent digestion in the stomach

The proportion of each of the three fractions digestible organic matter, digestible crude protein and digestible acid-detergent fibre, that was apparently digested in the stomach was similar in all three species (Table 7.6).

Of the total amount of organic matter digested in the digestive tract, 62 to 65% was apparently digested in the stomach. This included 16.5 to 17.3% of the digestible

Table 7.5. The concentration of ammonia-N (mgN/100 ml)
in digesta fluid in T. thetis, M. eugenii and
M. giganteus fed chopped lucerne hay ad libitum.
Values are the means (+ s.e.) of four animals
of each species.

	<u>T. thetis</u>	<u>M. eugenii</u>	<u>M. giganteus</u>
SFS ^a	34 ± 2	30 ± 4	22 ± 2
TFS 1	33 ± 1	-	20 ± 1
2	32 ± 1	17 ± 1	19 ± 3
3	32 ± 1	-	24 ± 1
HS	23 ± 1	17 ± 2	18 ± 2
CPC	41 ± 2	33 ± 5	32 ± 5
DC 1	30 ± 1	25' ± 2	23 ± 1

a = the sites of sampling and the notation used are described in Fig. 7.1.

Table 7.6. Experiment 6. Intake and the apparent digestion of dietary constituents in the digestive tract of *T. thetis*, *M. eugenii* and *M. giganteus* fed chopped lucerne hay *ad libitum*. Values are the means of four animals of each species.

(1) Intake and digestibility coefficients were based on a 5-day collection period.

(2) Estimates of apparent loss in regions of the digestive tract were calculated from analysis of digesta samples taken at slaughter with reference to Cr_2O_3 .

	<u><i>T. thetis</i></u>	<u><i>M. eugenii</i></u>	<u><i>M. giganteus</i></u>	
Body weight (kg+s.e.)	5.85 ± 1.03	4.45 ± 0.26	19.05 ± 0.74	
(1)				
DMI ^a (g/d)	222 ± 43	98 ± 12	523 ± 51	
				SEM
OMI (g/kgW ^{0.75} /d)	52.8 ^{aA}	28.9 ^b	51.8 ^a	7.9
OM digestibility (%)	56.4 ^a	60.2 ^b	55.2 ^a	2.4
NI (g/kgW ^{0.75} /d)	1.90 ^a	1.15 ^b	1.69 ^a	0.25
CP digestibility (%)	70.3 ^a	72.5 ^a	72.3 ^a	2.6
ADFI (g/kgW ^{0.75} /d)	20.1 ^a	11.6 ^b	20.5 ^a	4.0
ADF digestibility (%)	38.8 ^a	36.9 ^a	39.4 ^a	3.4
(2)				
Digestible OM digested in the stomach (%)	65.0 ^a	62.0 ^a	62.2 ^a	7.9
Digestible OM digested in the intestine (%)	35.0	38.0	37.8	
Digestible CP digested in the stomach (%)	16.7 ^a	17.3 ^a	16.5 ^a	6.5
Digestible CP digested in the intestine (%)	83.3	82.7	83.5	
Digestible ADF digested in the stomach (%)	81.7 ^a	81.6 ^a	85.0 ^a	5.7
Digestible ADF digested in the intestine (%)	18.3	18.4	15.0	

a, DMI = dry matter intake; OM(I) = organic matter (intake); NI = nitrogen intake; CP = crude protein (N x 6.25); ADF(I) = acid-detergent fibre (intake).

A, superscripts a, b denote differences (P<0.05) among species for the same parameter.

crude protein and 81.6 to 85.0% of the digestible acid-detergent fibre.

Analyses of the individual carbohydrate fractions (total soluble sugars, pectin, hemicellulose and cellulose) in stomach digesta were more variable than analysis of the acid-detergent fibre fraction. However, virtually all of the total soluble sugars and approximately 90% of the pectin ingested were apparently digested in the forestomach of all three species.

b) The pattern of apparent digestion in the stomach

In all three species, it was evident from analyses of digesta samples taken from sequential sites along the stomach (Fig. 7.1) that there was a gradient loss of organic matter from the cardia to the hindstomach (Table 7.7).

Since M. giganteus is the largest of the three species, it was possible to obtain more, and larger, samples of stomach digesta and this allowed a clearer definition of the pattern of stomach digestion in this species.

The flow of organic matter, acid-detergent fibre, crude protein and total soluble sugars is represented graphically in Fig. 7.2. The sequential proportional (%) loss of the digestible fraction of these dietary components is represented graphically in Fig. 7.3. The data are the means for the four animals.

Nearly all of the total soluble sugars ($95 \pm 2\%$) were digested in the cranial region of the stomach, whereas the acid-detergent fibre was digested more slowly along the length of the forestomach. The flow of crude protein (total N x 6.25) was higher in the sacciform forestomach and cranial region of the tubiform forestomach than the actual intake of crude protein; this is indicative of endogenous recycling of N to the forestomach, and fixation as microbial N. The apparent digestion of digestible organic matter occurred to a greater extent in the sacciform forestomach and cranial region of the tubiform

Table 7.7. Experiment 6. The apparent flow of organic matter (g/d) along the stomach of *T. thetis*, *M. eugenii* and *M. giganteus* fed chopped lucerne hay ad libitum. Values are the means (\pm s.e.) for four animals of each species.

	<u><i>T. thetis</i></u>	<u><i>M. eugenii</i></u>	<u><i>M. giganteus</i></u>
Organic matter flow (g/d)			
Intake	203 \pm 39	89 \pm 11	474 \pm 46
SFS ^a	192 \pm 40	72 \pm 8	400 \pm 46
TFS 1			338 \pm 37
TFS 2	138 \pm 23	59 \pm 6	325 \pm 32
TFS 3			317 \pm 30
HS	128 \pm 23	56 \pm 7	310 \pm 28

a = The sites of sampling and the notation used is described in Fig. 7.1.

Fig. 7.2. Experiment 6. The apparent flow (g/d) of; (a) organic matter, (b) acid-detergent fibre, (c) crude protein, (d) total soluble sugars, along the stomach of M. giganteus fed chopped lucerne hay ad libitum. Data are the means from four animals (vertical bars represent the s.e.).

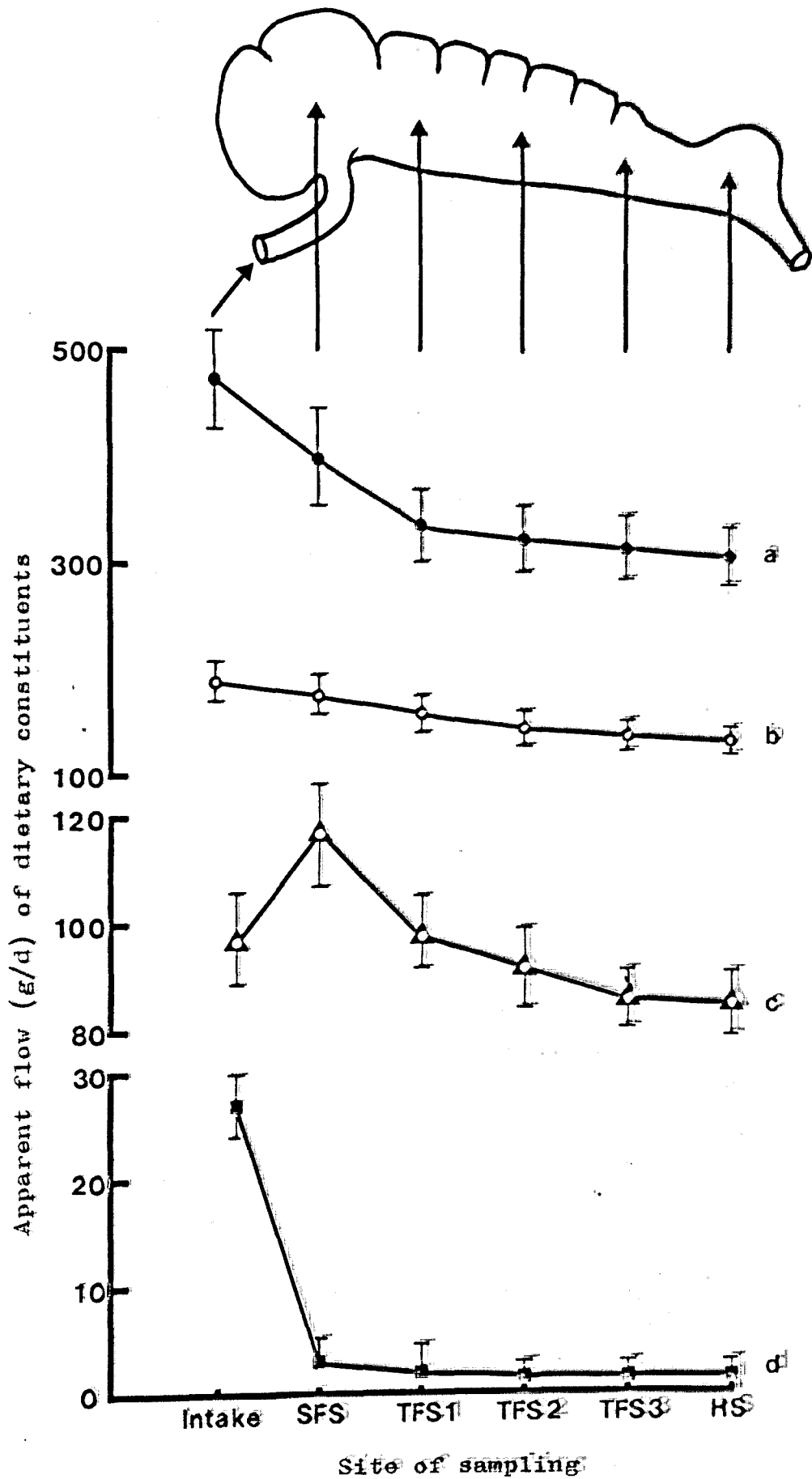
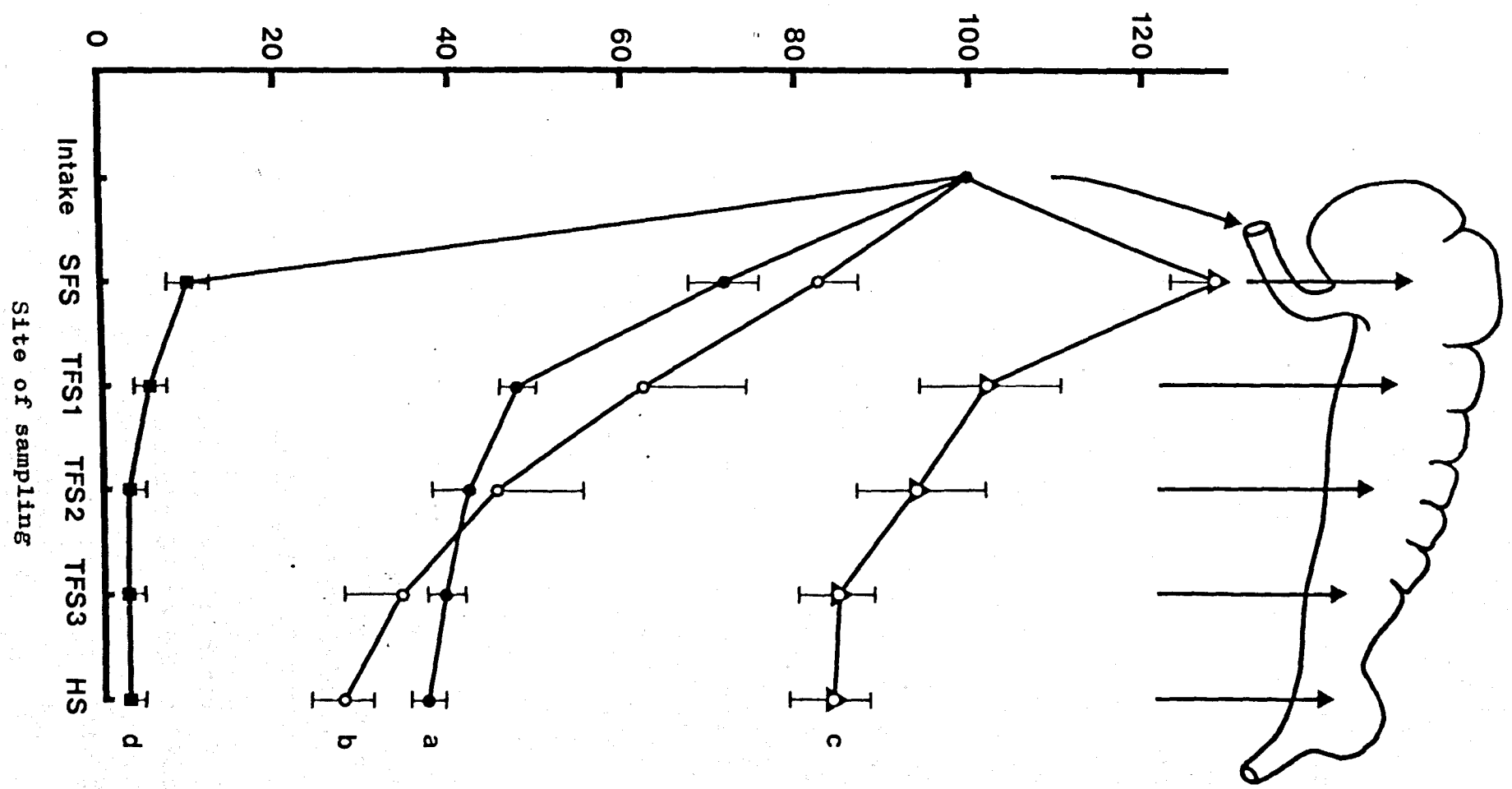


Fig. 7.3. Experiment 6. The apparent proportion remaining (%) of the digestible component of dietary constituents flowing along the stomach of M. giganteus fed chopped lucerne hay ad libitum: (a) organic matter, (b) acid-detergent fibre, (c) crude protein, (d) total soluble sugars. Data are the means from four animals (vertical bars represent the s.e.).

Apparent proportion remaining (%) of the digestible fraction of dietary constituents



forestomach than in the central and distal regions of the tubiform forestomach.

c) Digestion in the intestine

The apparent digestion in the intestine of the three fractions measured was similar in all three species (Table 7.6).

Analysis of flow parameters for the intestine, with reference to the Cr_2O_3 marker, gave rise to variable results which did not permit realistic evaluation of the partitioning of digestion within the small intestine and the large intestine separately. In seven of the twelve animals there was an apparent increase in organic matter flow in the duodenal segment. This is possibly due to shedding of epithelial tissue into the duodenal lumen, a phenomenon that is often observed in slaughter experiments and was first reported by Badawy, Campbell, Cuthbertson, Fell and Mackie, (1958). However, since urea concentrations in the ileal digesta fluid were high, and the concentration of VFA low, it can be assumed that microbial activity in the ileum was negligible. Thus the estimate of ADF digestion must be a reflection of microbial activity in the large intestine.

7.4 Discussion

7.4.1 Digestion in the macropodine stomach

Under the conditions of the present experiment, the extent of microbial digestion of ingested food in the forestomach was extensive, and similar, in all three species. As found in sheep fed chopped lucerne hay (Hogan and Weston, 1967; Egan, Walker, Nader and Storer, 1975), approximately 62 to 65% of the digestible organic matter was fermented in the macropodine forestomach. The forestomach is also the major site for digestion of the dietary carbohydrates.

Although the concentration of VFA in the forestomach

digesta was relatively high, the pH was approximately neutral. The digesta fluid is clearly a well-buffered medium for the support of microbial fermentation and growth.

a) The pattern of digestion

Because of the tubular nature of digesta flow in the macropodine forestomach sequential apparent digestion of organic matter along the length of the forestomach was observed. Digestion rate decreased as digesta was transported caudally.

Initial fermentation of ingested food, within the cranial region of the forestomach, involved rapid digestion of the soluble carbohydrates (Figs. 7.2 and 7.3) and, presumably, soluble protein. As digesta was transported along the tubiform forestomach, microbial fermentation was restricted to the less readily digested dietary constituents such as the structural carbohydrates. The decrease in the concentrations of the total VFA along the forestomach supports this.

Such a mode of microbial activity would be less evident in animals that had stopped feeding for a period of time before the time of sampling. In the present experiment, all the animals were encouraged to continue eating up until the time of slaughter.

b) Comparison among the species

This pattern of microbial fermentation in the forestomach was clearly established for M. giganteus. Although fewer digesta samples were obtained from the smaller species, a similar pattern of digestion was evident in both T. thetis and M. eugenii.

In T. thetis the majority of ingested soluble carbohydrate is probably fermented in the sacciform forestomach since there is a significant time delay before ingested material is detected in the tubiform forestomach

(Chapter 4). However, even in M. eugenii, and presumably in other macropodines with a well-developed gastric sulcus, transit time of digesta in the forestomach is likely to be significant (Chapter 5); the possibility of dietary soluble carbohydrates escaping microbial fermentation in the tubiform forestomach is remote.

The small amounts of soluble carbohydrates detected in samples from the distal tubiform forestomach and the hindstomach in all species may have been a measure of mucopolysaccharide (Topps, Kay and Goodall, 1968) rather than dietary carbohydrates.

c) Digestion of dietary protein

Only 17% of the apparently digestible crude protein was digested in the forestomach of all three species. The flow of N in the sacciform forestomach and the cranial region of the tubiform forestomach was higher than the dietary intake of N. This increase in the flow of N must be due to the endogenous secretion of N and subsequent microbial fixation.

In ruminants fed dried roughage and fresh herbage diets, considerable modification of dietary protein occurs in the ruminoreticulum through bacterial activity, with concomitant production of microbial protein (Walker, Egan, Nader, Ulyatt and Storer, 1975). This results in the flow of both dietary and microbial protein to the intestine.

Extensive incorporation of dietary N into microbial protein has been determined in M. eugenii (Lintern-Moore, 1973). In the same species Kennedy and Hume (1978) demonstrated that endogenous urea N is also incorporated into microbial protein.

d) VFA, ammonia and the flow of digesta fluid

No attempt was made to measure the flow of digesta fluid from reference to the Cr_2O_3 marker in the present

experiment. The rate of passage experiments (Chapter 5) determined that, although both the fluid and the particulate phases of digesta are subjected to tubular flow in the forestomach, the fluid is transported at a relatively much faster rate. Thus calculations of the flow of VFA and ammonia within and from the stomach in the present experiment would be grossly underestimated.

However, the significant decline in the concentration of VFA along the length of the stomach (Table 7.2) is not only an indication of a faster production of VFA in the cranial region of the forestomach, but also an indication of significant absorption.

7.4.2 Digestion in the intestine

The intestine was the major site of apparent digestion of protein in all three species. A minor but significant amount of the digestible acid-detergent fibre (15 to 18%) was also digested in the intestine. Since urea concentration in the ileal digesta fluid was high, and VFA low, it can be assumed that microbial fermentation of this acid-detergent fibre was confined to the large intestine.

7.5 Conclusion

Accurate estimation of the extent of digestion within defined regions of the digestive tract, from reference to the Cr_2O_3 marker and to samples taken at slaughter, may not be possible. Notwithstanding, the present results indicate that microbial fermentation in macropodines fed chopped lucerne hay plays a major role in the overall digestive process.

The apparent microbial digestion of ingested food in the forestomach was extensive, and similar, in all three species. It is probable that macropodines are as reliant as ruminants on absorption of VFA and the digestion and absorption of microbial protein for sources of energy and amino acids.

The patterns of apparent digestion of dietary constituents along the length of the stomach were related to the tubular pattern of digesta flow.