Chapter 8

MICROBIAL ACTIVITY IN THE FORESTOMACH

8.1 Introduction

Microbial fermentation of ingested food in the forestomach of macropodines is clearly extensive. This results in the production of considerable quantities of VFA (Hume, 1977a), and the formation of ammonia which is a major source of nitrogen for bacterial protein synthesis (Kennedy and Hume, 1978). However, this fermentation has not been studied in detail. The low production of methane in M. eugenii (tammar wallaby) (Engelhardt, Wolter, Lawrenz and Hemsley, 1978) or lack of it in M. giganteus (eastern grey kangaroo) (Kempton, Murray and Leng, 1976), is just one aspect that suggests that the pattern of microbial fermentation in the macropodine forestomach may not be directly comparable to ruminal fermentation.

In the present experiments, it has been determined that the tubular flow of ingested food through the forestomach (Chapter 5) is coincident with changes in the composition of digesta along the length of the organ (Chapter 7). After ingestion, the more soluble dietary constituents are rapidly fermented in the cranial region of the forestomach. As digesta is transported along the tubiform forestomach, the rate of disappearance of organic matter apparently decreases, presumably because the fermentation becomes more restricted to digestion of the structural carbohydrates. This suggests a decreasing rate of fermentation.

The following experiments were undertaken to provide a more comprehensive understanding of microbial activity in the macropodine forestomach. The rates of production of VFA in vitro in stomach contents taken from successive sites along the forestomach of M. giganteus, T. thetis (red-necked pademelon) and M. eugenii were estimated. The rate of production of VFA was also estimated in vivo in T. thetis and M. eugenii. In addition, production and the fate of ammonia N were determined in T. thetis and M. eugenii.

Observations were also made on the concentration of VFA and ammonia N in forestomach fluid from *T. thetis* and *M. giganteus*, and from other species, shot in the field and forestomach contents examined for microflora. In the latter stages of this work it became feasible to measure the concentration of hydrogen and methane in stomach gas samples.

8.2 Materials and methods

8.2.1 Measurement of VFA and ammonia production

a) Experiment 7. Production of VFA *in vitro* in *M. giganteus*

The rate of production of VFA was measured in digesta samples, incubated *in vitro*, obtained from four sites along the forestomach, and from the caecum-proximal colon, of four adult male *M. giganteus* shot in the field. The animals were killed late in the evening or early morning on the assumption that they had been feeding for an extensive period up until the time of slaughter.

Each animal was quickly transported back to a temporary field station, weighed, and the stomach and caecum-proximal colon dissected free through a ventral midline incision. The forestomach was ligated as sacciform forestomach and three successive divisions of the tubiform forestomach (see Fig. 7.1). Each stomach segment, and the caecum-proximal colon, was weighed before and after removal of contents. The pH of digesta was recorded, and duplicate samples of digesta were quickly transferred to incubation bottles (wide-mouth, screw top, glass jars - 250 ml), without addition of substrate or buffer, and placed in a water bath maintained at 37°C. Zero-time samples, and successive 30 min samples (for a period of 2.5 h) from each incubation bottle were strained through gauze cloth into small bottles containing 0.5 ml of saturated mercuric chloride solution to stop further fermentation. The incubation bottles were

gassed with CO_2 at the commencement of incubation and after each sampling.

Samples of stomach digesta were retained for estimation of dry matter content. All samples were stored in ice until transported back to the laboratory and there stored at -10°C.

On each occasion, less than 30 min elapsed from the time the animals were shot until samples were placed in the water bath. Dissection, and removal of the digesta contents, were delayed until the animal had been transported to the field station to prevent unnecessary cooling of digesta.

b) Experiment 8. Production of VFA and ammonia *in vitro* in *T. thetis*, *M. eugenii* and sheep

The rate of production of VFA and ammonia *in vitro* was measured in sacciform and tubiform forestomach contents from two *T. thetis* and two *M. eugenii*, and in rumen contents obtained from one sheep.

The macropodines were adult animals and were fed chopped lucerne hay *ad libitum* from moving belt feeders, once hourly, up until the time of slaughter. Over the last five days dry matter intakes were recorded and feed offered and feed refusals and faeces collected over this period, were analysed to determine intake of nitrogen and digestible organic matter.

The animals were sedated with ketamine hydrochloride sedation and killed by pentobarbitone sodium euthanasia. Total wet contents from the two regions of the forestomach were weighed and samples were quickly transferred to incubation bottles, gassed with CO_2, and placed in a water bath at 37°C.

Duplicate pre-feeding samples of rumen digesta were obtained from one cannulated sheep and transferred to the water bath as above. The sheep was also fed chopped lucerne hay, but fed once daily.

Zero-time samples of digesta from the macropodines and the sheep were kept for estimation of dry matter and concentrations of VFA and ammonia.

To each of the incubation bottles was added 10 ml of a 0.9% solution of sodium chloride containing 10 μCi of (U-^{14}C) sodium acetate and 2 mg of (^{15}N) ammonium chloride (96 atoms ^{15}N/100 atoms N).

A sample was taken from each bottle at 30 min intervals for a total period of 4 h. Each sample was placed in a tared bottle containing 20 ml of 20% sulphosalicylic acid (SSA), reweighed, centrifuged at 15,000 g for 20 min and the supernatant stored at -10°C. The dilution factor for each sample was calculated with reference to the dry matter content of the zero-time sample.

At the end of the incubation period, the remaining digesta was diluted with 0.9% sodium chloride and a bacterial fraction isolated by differential centrifugation as described by Nolan and Leng (1972).

c) Experiment 9. Estimation of VFA and ammonia production *in vivo*

The rate of production of VFA was estimated in two *T. thetis* and two *M. eugenii*, *in vivo*, by a continuous infusion method and sampling at slaughter; and in the sheep used in the *in vitro* estimations, by a single injection technique and subsequent sampling of ruminal digesta over a 6 h period.

The rate of production of ammonia was estimated *in vivo* by ^{15}N-ammonia dilution in these five animals at the same time as the VFA measurements, and in two additional animals; one *T. thetis* and one *M. eugenii*.

1. The six macropodines, all adult animals, were surgically prepared with an infusion catheter into the sacciform forestomach (see Section 3.4.2) and were fed

chopped lucerne hay *ad libitum* from moving belt feeders once hourly. The animals were maintained on this diet for at least three weeks prior to surgery, and for a minimum period of 4 weeks post-operatively before the experiment.

The experiment consisted of a 5 day collection period during which daily dry matter intakes were recorded. Bulked samples of feed offered and feed refusals and faeces were used to determine intake of nitrogen and digestible organic matter.

During the last 48 h of the collection period, the animals were constantly infused with a solution containing ^{51}Cr-EDTA (2,000 cpm/ml), ^{103}Ru-P (1500 cpm/ml), (U-^{14}C) sodium acetate (1 μCi/ml) and (^{15}N) ammonium chloride (1.2 mg ^{15}N/ml, 96 atoms ^{15}N/100 atoms N), at a rate of 50 ml/d.

At the end of the infusion period, after which steady state conditions were assumed to apply, each animal was sedated with ketamine hydrochloride and killed by pentobarbitone sodium euthanasia. The digestive tract was quickly dissected free through a ventral midline incision. Samples of digesta were taken from the sacciform forestomach, the hindstomach and three successive sites along the tubiform forestomach, and added to tared bottles containing 20 ml of 20% SSA. The bottles were reweighed, and the contents centrifuged at 15,000 g and the supernatant stored at -10°C for later analysis of VFA and ammonia.

A bacterial sample from digesta contents of the sacciform forestomach, and one from the central region of the tubiform forestomach, were isolated by differential centrifugation. Samples of digesta were also kept for estimation of dry matter, organic matter, and radioactivity of ^{51}Cr-EDTA and ^{103}Ru-P. Blood samples were obtained by cardiac puncture and the plasma retained. All samples and the infusion solutions were stored at -10°C.

2. The production of VFA and ammonia was estimated _in vivo_ in the same sheep. Immediately after the removal of the rumen contents for _in vitro_ incubation, 20 ml of a solution containing 200 mg (^{15}N) ammonium chloride and 20 μCi of (U-^{14}C) sodium acetate were injected, through the rumen cannula, into the ventral sac of the rumen and the contents were mixed with a plastic rod.

Samples of rumen digesta were taken at 20, 40 and 60 min, and then at 30 min intervals for a further three hours, and again at 5 h and 6 h. These samples were diluted with 20% SSA as above, and the supernatant samples were stored at -10°C.

8.2.2 Sampling of specimens in the field

As the occasion arose throughout this study, the wet weight of stomach contents, and organic matter content of stomach digesta were measured in animals shot in the field. Forestomach samples were also acidified and diluted in 20% SSA for later determination of VFA and ammonia concentration. Plasma samples were analysed for urea N concentration.

a) Stomach microflora

Sacciform and tubiform forestomach digesta samples were fixed in 4% formalin in 0.9% sodium chloride for identification of protozoa and fungi, and for bacterial counts. Samples from animals held in captivity fed chopped lucerne hay were also examined.

b) Stomach gas samples

Gas samples were obtained from the forestomach of animals shot in the field. The samples were collected into 20 ml glass syringes, previously moistened with water, by inserting a hypodermic needle through the forestomach wall into a localised gas cap, and slowly withdrawing the gas into the syringe. A rubber stopper was then placed over the needle to prevent differential loss of gases, and the

syringes stored in wet plastic bags on ice until transported back to the laboratory. All samples were analysed within 8 h of collection.

8.2.3 Analyses

Samples of feed offered and refusals, faeces and digesta were analysed for dry matter, organic matter and total N. The supernatant fractions of digesta samples were analysed for ammonia N and total VFA concentration and proportions of individual VFA. Plasma samples were analysed for urea N and gas samples were analysed for methane, hydrogen, nitrogen, carbon dioxide and oxygen. The analytical methods are outlined in Section 3.5.

Dilutions of infusates were measured for (U-^{14}C) sodium acetate, (^{15}N) ammonium chloride, ^{51}Cr-EDTA and ^{103}Ru-P.

Whole digesta samples from the in vivo continuous infusion experiment were measured for radioactivity of ^{51}Cr-EDTA and ^{103}Ru-P to allow estimation of the flow of the fluid and the particulate fractions of digesta. These results were presented earlier, in Chapter 5.

The specific activity of ^{14}C-labelled VFA, and the enrichment of ^{15}N in ammonia N, total N in digesta samples, plasma urea N and the bacterial N fractions were determined as outlined in Section 3.5.

Protozoa in the unstrained, diluted samples were counted using a counting chamber of 0.2 mm depth (Hawkesley Cristalite No. 13.s748). Samples of formalin preserved digesta were also examined for protozoa by Dr R.T.J. Clarke, and for bacterial populations and fungi by Dr T. Bauchop, of the Applied Biochemistry Division, DSIR, Palmerston North, New Zealand.

8.2.4 Calculations

a) Rate of production of VFA in vitro

Estimation of the rate of production of VFA *in vitro* for each animal was estimated by plotting the net increase of total VFA in each successive sample against sampling time and then estimating a zero-time production rate as described by Hume (1977a).

Production of VFA *in vitro* for *T. thetis* and *M. eugenii* was also estimated by reference to the specific activity of acetic and butyric acids and the known injected dose of (U-^{14}C) sodium acetate as outlined by Leng (1970). An estimate of the proportion of butyric acid that arose from acetic acid was obtained by comparing the specific activities of the two acids. Negligible amounts of the ^{14}C label appeared in propionic acid and in the calculations this was ignored.

b) Production of VFA and ammonia *in vivo*

The production of VFA and ammonia *in vivo* in the rumen of the sheep, after a single injection of the isotopes, was estimated as described by Leng (1970), and Nolan and Leng (1972), respectively.

Since total mixing of digesta does not occur in the macropodine forestomach the following procedure was adopted to estimate these production rates in *T. thetis* and *M. eugenii*.

The sacciform forestomach was considered to be a single pool of digesta, and since the isotopes were injected into this pool this region was therefore the primary pool. The net production rate (or irreversible loss) was thus estimated by the following relationship:

$$\text{Irreversible loss (I.L.)} = \frac{\text{Infusion rate}}{\text{Plateau specific activity, or enrichment}},$$

and therefore;

$$\text{I.L. of VFA (mM/d)} = \frac{\text{mCi } ^{14}\text{C infused/d}}{\text{m Ci/mM (acetic plus butyric acid)}},$$

$$\text{I.L. of } NH_3N \text{ (mg/d)} = \frac{\text{mg } ^{15}\text{N infused/d}}{\text{mg } ^{15}\text{N/mg total } NH_3N}.$$

The tubular forestomach was considered as three successive pools in line with the sacciform forestomach with no reversal of flow to a preceeding pool. Thus irreversible loss was determined for each pool from the following relationship:

$$\text{Irreversible loss}_{n+1} = \left(F_{n+1} \times \frac{C_n}{C_{n+1}} \right) - F_n ,$$

where F_n and F_{n+1} are the flows of VFA (mM/d) or NH_3 N (mg/d) in two adjacent pools and C_n and C_{n+1} are the specific activity of VFA (m μCi/μM, acetic plus butyric acids) or enrichment (mg ^{15}N/mg total NH_3 N) in these two pools.

Total irreversible loss from the forestomach is thus equal to the sum of the irreversible loss from all pools.

c) Incorporation of NH_3 N into plasma urea N and bacterial N

The proportion of plasma urea N, and of bacterial N, that arose from ammonia N were calculated as defined by Nolan and Leng (1972).

An estimate of the flow of bacterial N arising from ammonia N, that passed through the hindstomach was calculated from the relationship:

$$\text{Bacterial flow (g N/d)} = \frac{\text{NAN flow g/d x NAN enrichment (hindstomach)}}{\text{bacterial enrichment (tubular forestomach)}}.$$

This does not account for direct incorporation of dietary peptides and/or amino acids into bacterial protein, and therefore must be an underestimate of total bacterial protein flow.

8.3 Results

8.3.1 Experiment 7

The body weights, capacity of the stomachs, pH of digesta, initial concentrations and molar proportions of VFA and the estimates of production of VFA *in vitro* in the four *M. giganteus* are presented in Table 8.1. Up until the time of slaughter, these animals had been grazing under natural feeding conditions.

The capacity of the stomach relative to body weight ranged from 9.7% to 13.2% and the average fluid volume was 4.55 l (range 3.61 to 5.90 l).

The mean of the measurements of pH along the forestomach, and in the caecum-proximal colon, and the total concentration of VFA at slaughter were comparable to the data obtained from this species fed chopped lucerne hay (see Tables 7.1, 7.2).

The production of VFA was higher ($P<0.05$) in the sacciform forestomach than in any of the sites along the tubiform forestomach. However, the tubiform forestomach of this species has a greater capacity than the sacciform forestomach, and 84% of the VFA was produced in this region.

The average total production of VFA from the forestomach and the caecum-proximal colon was 2.44 M/d (range 1.92 to 2.86 M/d).

The proportions of the individual VFA in the forestomach fluid were similar among all four sites and did not change significantly during the incubation period. The proportions tabulated are the means of the four sample sites from all four animals, and the means of the proportions in the caecum-proximal colon, at the time of slaughter. Acetic acid comprised a lower proportion and propionic acid was higher ($P<0.005$) than in *M. giganteus* fed chopped lucerne hay (see Table 7.3). The proportions of

Table 8.1. Experiment 7. Production of VFA *in vitro* in the forestomach and the caecum-proximal colon of four *M. giganteus*. The animals were adult males grazing under natural feeding conditions until slaughter. All measurements made in late winter.

Animal	1	2	3	4
Location	Jeogla[a]	Jeogla	Cherry Hill[b]	Cherry Hill
Time of slaughter	9.30 pm	10.30 pm	9.00 pm	5.30 am
Body weight (kg)	41.0	53.1	55.5	47.3
Wet weight of stomach contents / Body weight (%)	13.1	13.2	9.7	11.7

Sampling site[c]	SFS	TFS1	TFS2	TFS3	CPC
pH	6.7 ± 0.2	6.9 ± 0.2	6.6 ± 0.1	6.6 ± 0.2	7.2 ± 0.1
Total conc. of VFA (μM/ml)	118 ± 9	125 ± 10	122 ± 4	99 ± 12	63 ± 4
VFA production rate (μM/ml/h)	29.3 ± 3.6	23.3 ± 3.9	19.8 ± 3.3	18.5 ± 2.6	10.5 ± 1.0
Average fluid volume/site(ml)	528	1326	1401	1293	350
Average VFA production/ site (mM/d)	371	741	666	574	88

	Acetic	Propionic	n-Butyric	n-Valeric
Mean Molar proportions of VFA (%)[d]				
Forestomach	62.5 ± 1.3	27.7 ± 1.2	7.9 ± 0.4	0.8 ± 0.2
Caecum-proximal colon	75.5 ± 3.3	18.5 ± 2.4	4.8 ± 1.0	0.5 ± 0.2

a, Jeogla is 65 km east of Armidale, NSW.

b, Cherry Hill Station is 24 km SW of Armidale, NSW

c, SFS = sacciform forestomach,
TFS1 = cranial region of tubular forestomach,
TFS2 = central region of tubular forestomach,
TFS3 = distal region of tubular forestomach,
CPC = caecum-proximal colon (see Fig. 7.1).

d, at both sites, the mean molar proportions of both iso-butyric and iso-valeric acids were less than 1.0%.

the individual VFA in the caecum-proximal colon were similar to those determined in this species fed the lucerne diet. Insignificant proportions of branched-chain VFA were present in the digesta fluid. Compared with the forestomach, the molar proportion of acetic acid was higher ($P<0.01$) and the proportions of propionic acid and butyric acid lower ($P<0.01$) in the caecum-proximal colon.

8.3.2 Experiment 8

The body weights, intakes of dry matter, digestible organic matter and nitrogen, forestomach fluid volumes, initial concentration and production *in vitro* of VFA and ammonia in two *T. thetis* and two *M. eugenii*, and in the one sheep, are presented in Table 8.2.

The one sheep used in this experiment, and again in Experiment 9, consumed 720 g dry matter daily. However, the food was offered only once daily and the animal consumed this within 4 h. The experiments described herein were conducted in this animal sixteen hours after last feeding. Thus the results cannot be directly compared with those from the macropodines, which were fed hourly up until the time of slaughter. However, it was possible to measure the production of VFA and ammonia both *in vitro* and *in vivo* simultaneously in the sheep and the results are included as a comparison of the two methods.

a) Production of VFA *in vitro*

Production of VFA in the macropodines was faster in the sacciform forestomach than in the tubiform forestomach. Since the sacciform forestomach of *T. thetis* has a greater capacity than the tubiform forestomach (see Table 4.1), more VFA was produced in this region. In *M. eugenii* the tubiform forestomach is larger than the sacciform forestomach, and thus in terms of daily production of VFA, the two regions produced similar amounts.

The mean total production of VFA as a proportion of

Table 8.2. Experiment 8. Production of VFA and NH_3 *in vitro* in digesta from the forestomach of two *T. thetis*, two *M. eugenii* and one sheep. All animals were fed chopped lucerne hay.

		T. thetis		*M. eugenii*		Sheep
Animal		1	2	1	2	
Body weight (kg)		3.89	3.13	4.02	4.50	29.0
Dry matter intake (g/d)		153	147	76	80	720
DOMI[a] (g/d)		85	81	45	47	432
Nitrogen intake (g/d)		5.1	4.9	2.5	2.7	20
Fluid volume (ml)	SFS[b]	165	125	111	131	Rumen
	TFS	141	115	150	180	2000[c]
Initial concentration VFA(µM/ml)	SFS	157	211	86	64	27
	TFS	102	142	77	57	
NH_3(mgN/l)	SFS	353	245	304	280	133
	TFS	424	200	182	172	
VFA production (µM/ml/h)	SFS	38.1	36.3	21.4	23.2	14.1
	TFS	26.2	20.2	17.4	12.0	
(mM/d)	SFS	151	109	57	73	672
	TFS	89	56	63	52	
mM VFA/d / DOMI g/d		2.82	2.04	2.67	2.66	0.6
NH_3 production (mg N/l/h)	SFS	56	91	54	62	31
	TFS	82	114	65	49	
(mg N/d)	SFS	222	273	144	195	1488
	TFS	277	315	234	212	
mg NH_3 N/d / Nitrogen intake(g N/d)		99	120	151	151	70

a, DOMI = digestible organic matter intake.
b, SFS, TFS, sampling site notation is defined in Fig. 7.1.
c, the estimate of rumen fluid volume was obtained from the relationship; NH_3 N pool size (mg N) ÷ NH_3 N conc. (mg N/l) see Experiment 9.

digestible organic matter intake was similar in both species (*T. thetis*, 2.43 mM/g; *M. eugenii*, 2.67 mM/g).

Estimation of VFA production by reference to dilution of (U-^{14}C) sodium acetate gave rise to similar results. A high, but variable, proportion of the acetic acid was converted to butyric acid (range 24% to 41%) among all samples but negligible amounts of the label were detected in propionic acid.

The proportions of the individual VFA in the macropodine forestomach samples did not change significantly during the incubations and were similar to those reported in Table 7.3.

b) Production of ammonia *in vitro*

The net daily production of ammonia N in the tubiform forestomach of both species was greater than that produced in the sacciform forestomach, but the estimate does not include the ammonia N produced and incorporated into microbial protein.

The mean ratio of the production of ammonia N (mg N/d) to nitrogen intake (g N/d) was 110 mg/g in *T. thetis* and 151 mg/g in *M. eugenii* and 70 mg/g in the sheep.

At the start of each incubation, ^{15}N ammonium chloride was added to the digesta. At the end of the four-hour incubation period, some 5% to 20% of the label was incorporated into the non-ammonia N and bacterial N fraction. However, estimates of the true irreversible loss were not possible as the incorporation of ^{15}N in the early samples was difficult to measure with any degree of accuracy.

8.3.3 Experiment 9

a) Production of VFA *in vivo*

The body weights, intakes of dry matter, digestible organic matter, pool size and production of VFA in the

forestomach of the five animals are presented in Table 8.3.

The mean net production of VFA *in vivo* in the forestomach was 646 mM/d in *T. thetis*, 530 mM/d in *M. eugenii* and 1210 mM/d in the rumen of the sheep. These estimates are much higher than those obtained with the *in vitro* method.

The mean of the ratio of VFA produced (mM/d) to intake of digestible organic matter (g/d) was similar in both species; 10.0 mM/g in *T. thetis* and 9.3 mM/g in *M. eugenii*.

The production of VFA as a ratio of intake of digestible organic matter was lower in the sheep (2.80 mM/g), a reflection of the time elapsed since the animal had been last fed.

b) Production of ammonia *in vivo*

The means ($\pm$ s.e.) of the body weights, intakes of dry matter and nitrogen, ammonia pool size and irreversible loss in three *T. thetis* and three *M. eugenii*, and in the one sheep are presented in Table 8.4.

In *T. thetis* the ammonia N pool size in the sacciform forestomach and the irreversible loss of ammonia N from this region were greater ($P<0.01$) than from the tubiform forestomach.

As indicated earlier, the capacity of the sacciform forestomach of *M. eugenii* comprises only 30% of the total stomach contents (see Table 4.1). In this species the irreversible loss of ammonia N from the sacciform forestomach (1.1 g N/d) was similar to that from the tubiform forestomach (0.9 g N/d); the rate of production of ammonia N/g of digesta is clearly more rapid in the sacciform forestomach.

The ratio of irreversible loss of ammonia N (mg N/d) from the forestomach to nitrogen intake (g N/d) was similar

Table 8.3. Experiment 9. Production of VFA *in vivo* in two *T. thetis*, two *M. eugenii* and one sheep fed chopped lucerne hay.

	T. thetis		*M. eugenii*		Sheep
Animal	1	2	1	2	
Body weight (kg)	2.94	3.31	5.07	4.80	29.0
Dry matter intake (g/d)	131	113	106	114	720
Digestible organic matter intake (g/d)	67.2	62.0	52.5	61.6	432
Pool size (mM VFA):					Rumen
SFS[a]	18.56	16.11	4.36	3.56	54.6
TFS	15.69	12.15	6.90	10.40	
VFA production (mM/d):					
SFS	489	437	274	294	1210
TFS[b]	189	176	197	295	
Total forestomach production (mM/d)	678	613	471	589	1210
VFA production(mM/d) / Digestible organic matter intake (g/d)	10.09	9.89	8.97	9.56	2.80

a, SFS = sacciform forestomach

TFS = tubiform forestomach

b, This figure for VFA production in the TFS is the sum of the estimates determined from sequential samples along the TFS (see Fig. 8.1).

Table 8.4. Experiment 9. Net production (irreversible loss) of ammonia N in the forestomach of three T. thetis, three M. eugenii and one sheep fed chopped lucerne hay.

	T. thetis	M. eugenii	Sheep
Body weight (kg)	3.63 ± 0.52	4.80 ± 0.50	29.0
Dry matter intake (g/d)	124 ± 5	99 ± 12	720
Nitrogen intake (gN/d)	3.9 ± 0.3	3.2 ± 0.3	21.6
Ammonia pool size (mg N):			Rumen
SFS[a]	72 ± 9	19 ± 6	266
TFS	45 ± 6	38 ± 17	
Irreversible loss of NH_3N (g N/d):			
SFS	1.63 ± 0.50	1.08 ± 0.43	4.80[b]
TFS[c]	0.81 ± 0.40	0.93 ± 0.33	
Total forestomach	2.45 ± 0.27	2.02 ± 0.21	4.80
Irreversible loss of NH_3N (mg N/d) / Nitrogen intake (gN/d)	640 ± 110	660 ± 100	220

a, SFS = sacciform forestomach, TFS = tubiform forestomach.

b, total entry rate in the rumen was estimated as 8.22 g N/d and thus recycling of NH_3N = 3.42 g N/d.

c, This figure for NH_3N irreversible loss in the TFS is the sum of the estimates determined from sequential samples along the TFS (see Fig. 8.2).

in both species (640 mg/g in *T. thetis*; 660 mg/g in *M. eugenii*).

Irreversible loss of ammonia N from the rumen of the sheep as a proportion of nitrogen intake was 220 mg/g; lower than in the macropodines. Total entry rate of ammonia N in the forestomach of the sheep was 8.22 g N/d, and thus recycling of ammonia N to the rumen ammonia pool was 3.42 g N/d.

c) Transfer of ammonia N from the macropodine forestomach

The means (± s.e.) of the intake of organic matter and the amount of organic matter digested in the stomach, the proportion of plasma urea N and bacterial N that arose from ammonia, and the flow of bacterial N from the stomach of *T. thetis* and *M. eugenii* are presented in Table 8.5.

The proportion of plasma urea N that arose from ammonia N (26% in *T. thetis*, 16% in *M. eugenii*) was derived from the enrichments of plasma urea N and sacciform forestomach ammonia N, since the latter was the primary pool in this experiment.

The proportion of bacterial N that arose from ammonia N in the sacciform forestomach (44% in *T. thetis*, 40% in *M. eugenii*) was derived from the ratio of the enrichments of these two fractions in this pool. Similarly, the proportion of bacterial N that arose from ammonia N in the tubiform forestomach is the ratio of the enrichments of these two fractions in this region of the stomach. The latter ratio may not be strictly valid, since the enrichment of ^{15}N in ammonia decreased along the length of the forestomach.

Microbial N flowing from the stomach was estimated with reference to the enrichment of bacterial N in the tubiform forestomach on the assumption that this enrichment was unlikely to change significantly between the central region

Table 8.5. Experiment 9. The transfer of ammonia N from the macropodine stomach. These estimates were derived from the data obtained from three *T. thetis* and three *M. eugenii* fed chopped lucerne hay. (See Table 8.4 for other data from these animals).

		T. thetis	*M. eugenii*
Organic matter intake (g/d)		112 ± 4	89 ± 10
Organic matter apparently digested in the forestomach (g/d)		34 ± 3	25 ± 7
Proportion of plasma urea N derived from NH_3N in the forestomach (%)		25.6 ± 3.0	15.5 ± 1.4
Proportion of bacterial N derived from NH_3N in: (%)	SFS[a]	44.2 ± 6.9	40.4 ± 1.5
	TFS	73.9 ± 6.0	84.2 ± 4.9
Proportion of bacterial N in total NAN flowing from the stomach (%)		30.0 ± 3.5	31.0 ± 2.6
Flow of microbial N from the stomach[b] (g N/d)		0.84± 0.16	0.68± 0.12
Net synthesis of microbial matter (g N/kg OM apparently fermented in the stomach)		25.1 ± 3.6	27.3 ± 4.1

a, SFS = sacciform forestomach, TFS = tubiform forestomach, NAN = non-ammonia nitrogen, OM = organic matter.

b, This estimate refers to the proportion of microbial N that arose from ammonia N only.

of the tubiform forestomach and the hindstomach.

The proportions of bacterial N that arose from the ammonia pool in the sacciform forestomach indicate that 56% and 60% of the bacterial N (in *T. thetis* and *M. eugenii*, respectively) in this region arose from sources other than ammonia, such as peptides and amino acids. Presumably the majority of this was from dietary N sources.

The estimated flow of microbial N from the stomach (i.e., through the hindstomach), was 0.84 g N/d in *T. thetis* and 0.68 g N/d in *M. eugenii*. Net synthesis of microbial matter (g N microbial protein produced/kg of organic matter apparently fermented in the stomach) was similar in both species (25 g N/kg and 27 g N/kg of organic matter fermented in *T. thetis* and in *M. eugenii*, respectively).

8.3.4 Field observations

a) Stomach capacity and organic matter content of stomach digesta

The ratio of the wet weight of forestomach contents to body weight was measured in five species; *M. giganteus* (13.4 ± 0.5%, n = 15), *T. thetis* (12.1 ± 0.8%, n = 8), *M. rufogriseus* (red-necked wallaby) (11.7 ± 1.1%, n = 6), *W. bicolor* (swamp wallaby) (11.7 ± 0.8%, n = 11) and *M. robustus robustus* (eastern wallaroo) (12.8 ± 0.7%, n = 5).

Measurements varied among individuals and the variability was probably related to time of last feeding prior to slaughter, and voluntary food intake. There was no evidence of relatively greater stomach capacities in the larger grazing species compared to the browsers as has been determined for species of African ruminants (Hofmann, 1973; Hoppe, Qvortrup and Woodford, 1977).

These ratios are greater than those obtained from animals fed chopped lucerne hay *ad libitum* under laboratory conditions (Chapter 4) and are likely to be a reflection of higher voluntary food intake in the field.

The organic matter content of forestomach digesta samples from individuals of the five species ranged from 75 to 90% (overall mean, 84%). This was lower than that measured in laboratory macropodines fed chopped lucerne hay (range, 87 to 92%; overall mean, 90%).

b) Plasma urea, and ammonia and VFA in digesta fluid

In all species, plasma urea concentration ranged from 16 to 30 mgN/100 ml (overall mean, 26 mgN/100 ml) and forestomach digesta fluid ammonia concentration ranged from 9 to 33 mgN/100 ml (mean, 25 mgN/100 ml).

The concentration of total VFA in forestomach fluid was also variable and similar over all five species (range, 80 to 160 mM/ml) and comparable with values determined in laboratory animals fed chopped lucerne hay (see Table 7.2). A decrease in concentration of total VFA along the forestomach, as measured in laboratory animals (Table 7.2), was also determined in all field animals.

As determined in the four *M. giganteus* used in the field experiment (Experiment 7, Table 8.1), the proportion of propionic acid (26 to 33%) in forestomach fluid was higher and that of acetic acid (58 to 67%) lower in animals that were killed late in the evening (17.00 to 22.00 hours) than in animals killed during the day (10.00 to 15.00 hours). In the latter the proportions of propionic and acetic acids were comparable to those measured in animals fed chopped lucerne hay (see Table 7.3). This was observed in all five species.

c) Stomach microflora

Fungi

Fungal sporangia, similar to those found in ruminants (Bauchop, 1979a), were found in forestomach samples from all field animals except *T. thetis*. They were not present in samples from *T. thetis* or *M. eugenii* fed chopped lucerne hay.

Bacteria

Direct counts of bacteria in forestomach digesta ranged from 57 to 760 x 10^9/g in M. giganteus and 212 to 518 x 10^9/g in T. thetis. In forestomach digesta samples from T. thetis and M. eugenii fed chopped lucerne hay, direct counts of bacteria ranged from 66 to 511 x 10^9/g. In the sheep used in Experiments 8 and 9, the bacterial count was 443 x 10^9/g.

Protozoa

Ciliate protozoa were found in the forestomach of the majority of field and laboratory animals. Total numbers were variable, ranging from 15 to 150 x 10^3/g. Highest counts were recorded in samples from the sacciform forestomach and total numbers decreased along the length of the forestomach. In three W. bicolor, two M. rufogriseus and one M. giganteus protozoa were found in the sacciform and the cranial region of the tubiform forestomach only.

The majority of the protozoa structurally resembled the holotrichs Dasytricha and Isotricha. Heterotrichs, possibly two species, were also common, whereas spirotrich ciliates resembling Entodinium were only found in the sacciform forestomach of one M. rufogriseus and one M. robustus robustus.

d) Stomach gas composition

The composition of forestomach gas and pH of forestomach digesta fluid in adult macropodines shot in the field are presented in Table 8.6.

Up until the time of slaughter both the M. rufogriseus and the W. bicolor had been feeding for an extensive period whereas the M. robustus robustus and the M. giganteus were observed to be resting. Methane and hydrogen were present

Table 8.6. The composition of forestomach gas[1] and pH of forestomach digesta fluid in macropodines shot in the field.

Species	Animal	Time of slaughter (hours)	Methane (%)	Hydrogen (%)	pH
M. rufogriseus	1	18.30	7.2	0.6	-
	2	18.00	8.0	1.5	6.7
W. bicolor	1	20.00	9.5	9.8	6.0
	2	20.30	5.4	11.0	5.8
M. robustus robustus	1	12.00	0.5	2.2	6.2
	2	12.30	0.8	1.2	5.7
M. giganteus	1	14.00	1.8	2.8	6.1
	2	15.00	1.2	1.1	6.5

(1) In all gas samples; carbon dioxide > 70%
oxygen < 0.1%
nitrogen < 0.2%.

in all gas samples albeit in low concentrations. It is apparent that the production of significantly greater proportions of methane was associated with extensive feeding.

8.4 Discussion

8.4.1 Microbial production of VFA

In the macropodines, the in vivo estimation of the VFA produced as a ratio of the organic matter digested (9 mM/g to 10 mM/g) was similar to average estimates for ruminants (e.g. 8.3 mM/g; Czerkawski, 1978). This figure is four times higher than that estimated from the in vitro experiments for all three species. Estimation of VFA production in vitro did reflect the differences among the species, but they were clearly a gross underestimate.

The in vitro incubation method used in the present experiments, similar to that adopted by Hume (1977a), necessarily involves a significant time lag from the time the animal last consumed food until the incubation is begun. Initial rates of fermentation in the rumen immediately after ingestion of food are very high (Sutherland, 1963), and such an effect would not be observed in in vitro incubations such as in the present experiments.

The rate of VFA formation was higher in the sacciform forestomach of all three macropodine species, measured in vitro, than in the tubiform forestomach. This was more obvious in the in vivo experiments, particularly in T. thetis. In this species all of the ingested food is probably confined to the sacciform forestomach for an appreciable period (see Chapter 4), whereas in M. eugenii ingested food may enter either the sacciform forestomach or the cranial region of the tubiform forestomach.

The overall estimate of VFA production in vivo in the tubular region of the forestomach in T. thetis and M. eugenii (Table 8.3) was determined from successive samples obtained

along the length of the forestomach. In reality, the production of VFA *in vivo* decreases along the stomach. The pattern of the irreversible loss and flow of VFA along the stomach, as measured in the *in vivo* experiment, is presented schematically in Fig. 8.1. Concomitant with the observations on the pattern of disappearance of food components along the forestomach (Chapter 7), the production of VFA, and net absorption, also decreases along the forestomach. Some 18% to 24% of the VFA produced, in both species, flowed through the hindstomach and was apparently absorbed in the duodenum; only very low levels of VFA were detected in duodenal fluid (see Table 7.2).

8.4.2 Microbial production of ammonia

The net production of ammonia measured *in vitro* was variable and, like the VFA production estimates, considerably lower than the *in vivo* estimates in all three species. Production of ammonia *in vivo* is presumably enhanced, in animals that are not feeding, by continuous influx of endogenous nitrogen, particularly urea. This would seem apparent in the results from the sheep.

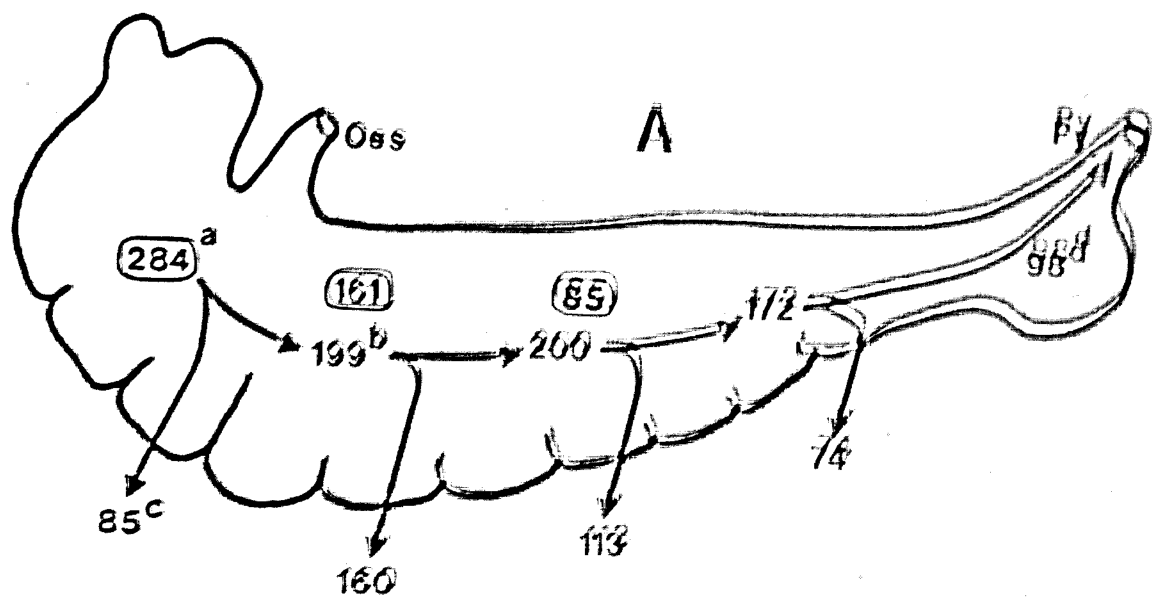
Total entry rate, or gross production, of ammonia N could not be estimated in the macropodines by the method employed in the present experiments, but it would be significantly higher than the irreversible loss estimates since recycling of ammonia N does occur. Significant quantities of nitrogen are recycled to the stomach of macropodines (Brown, 1964; Kinnear and Main, 1975; Kennedy and Hume, 1978) and as discussed earlier, this recycling is evident even when macropodines are fed diets adequate in nitrogen such as the lucerne used in the present experiments, and the diets used in Experiments 4 and 5 (Chapter 6).

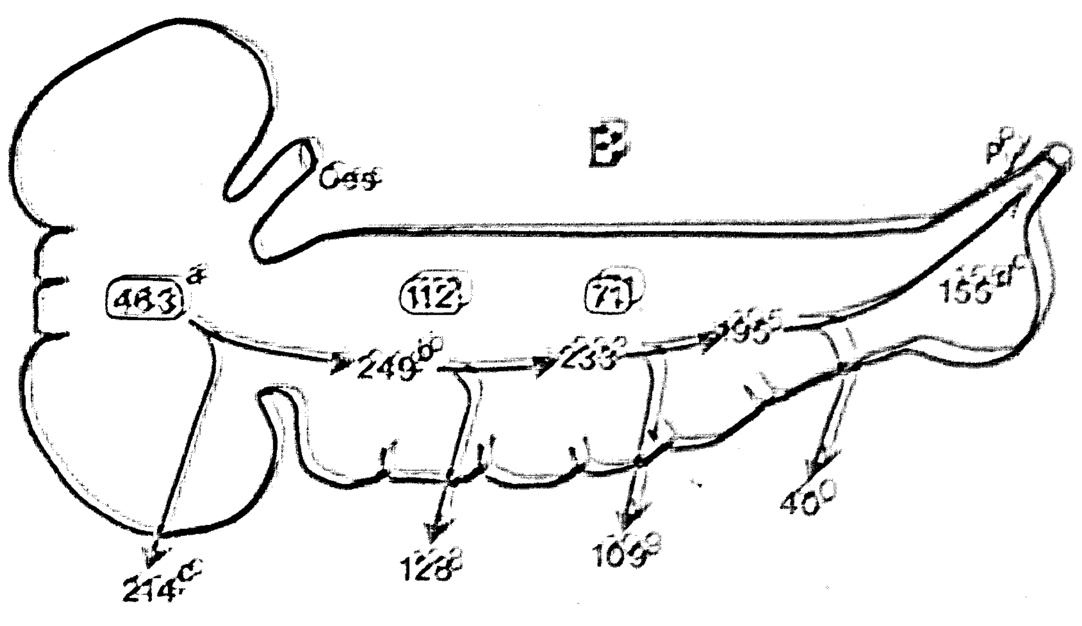
As noted earlier, the experiments with the sheep were conducted some 16 h after the animal was last fed. At this time, it is of interest to note that 71% of the ammonia N actually produced was recycled to the rumen ammonia N pool. This is much higher than estimates obtained from sheep fed lucerne hourly (Nolan and Leng, 1972), and is an indication

Fig. 8.1. Schematic representation of the production, absorption and flow of VFA (mM/d) in the stomach of; A = *M. eugenii*, B = *T. thetis*. Data are the means of values determined *in vivo* in Experiment 9 (see Table 8.3).

a = net production (irreversible loss).
b = flow of VFA along the forestomach.
c = net absorption.
d = flow of VFA through the hindstomach.

oes = oesophagus, py = pylorus.


A
Oss
Py
284 a
161
85
199 b
200
172
98 d
85 c
160
113
74


B
Oss
Py
483 a
112
77
249
233
195
155
214
128
109
40

of the importance of continued recycling of nitrogen to the forestomach pool when animals are not feeding at frequent intervals.

The irreversible loss and flow of ammonia N in the stomach of T. thetis and M. eugenii is presented schematically in Fig. 8.2. These are the data derived from the in vivo experiment. The irreversible loss of ammonia N was higher in the sacciform forestomach, particularly in T. thetis, and decreased along the length of the forestomach. This does not necessarily indicate that the rate of gross production of ammonia was decreasing. Since the more readily fermentable dietary constituents are digested in the cranial region of the forestomach (Chapter 7), it is likely that as digesta is transported further along the forestomach, the recycling of nitrogen to the ammonia pool becomes more significant.

8.4.3 Irreversible loss of ammonia N from the forestomach

Irreversible loss of ammonia N represents the portion of ammonia N that is synthesized that does not return to the primary fluid ammonia pool (Nolan and Leng, 1972). It is assumed that the main avenues of flux of ammonia N from the forestomach fluid are via incorporation of N into bacterial protein, absorption, and flow of ammonia into the small intestine. The portion that is irreversibly lost will be excreted in the faeces and urine or incorporated into tissue protein.

Clearly, ammonia N was absorbed and converted to urea and some of this was lost in the urine but, in the present experiments, ammonia N was also incorporated into microbial N. The estimates of net synthesis of microbial matter (per kg organic matter fermented) leaving the stomach are within the range of such estimates for ruminants (Czerkawski, 1978) but are likely to be low.

The value derived for the proportion of bacterial N

Fig. 8.2. Schematic representation of the irreversible loss and net flow of ammonia N (mg/day) in the stomach of; A = *M. eugenii*, B = *T. thetis*. Data are the means of values determined *in vivo* in Experiment 9 (see Table 8.4).

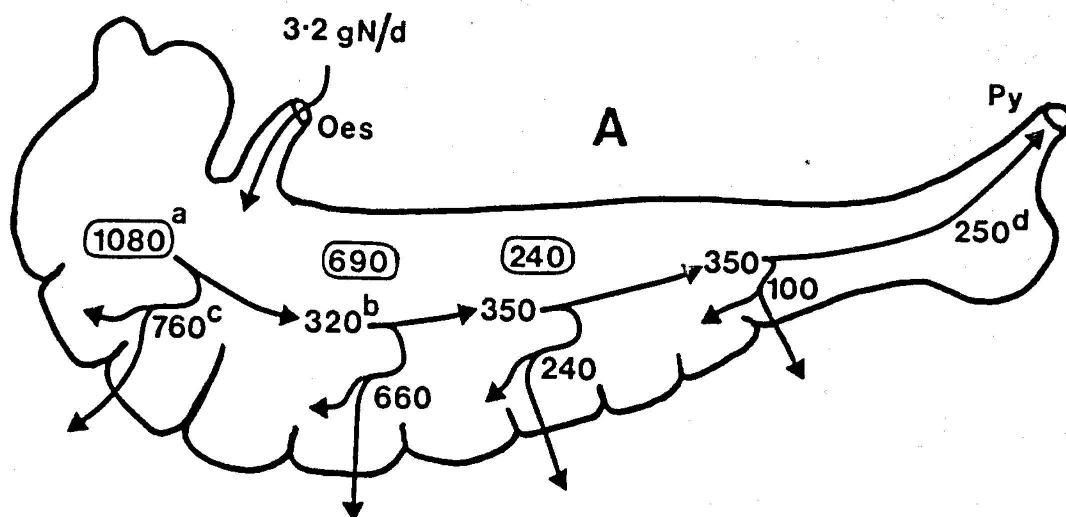
a = irreversible loss.

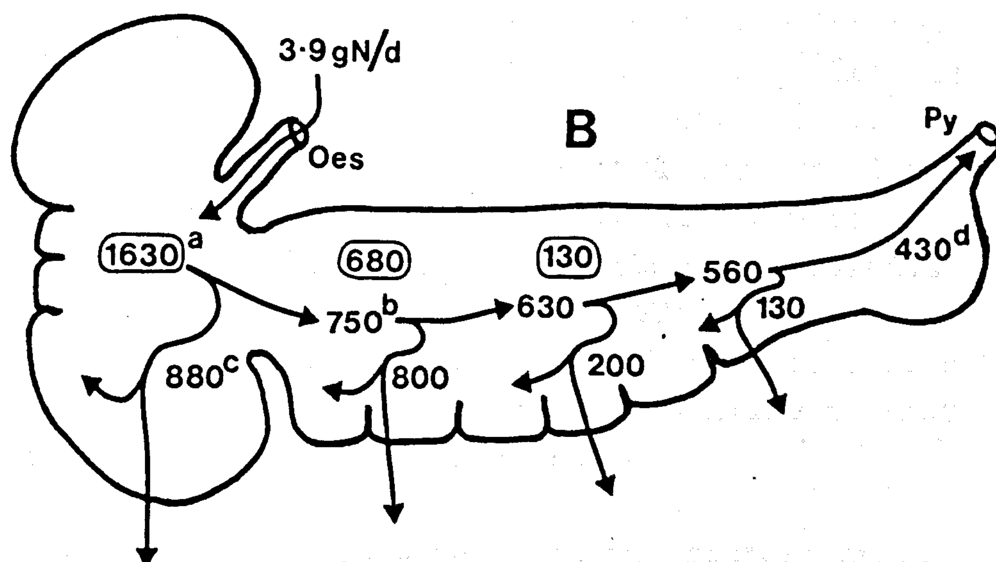
b = flow of ammonia N along the forestomach.

c = net loss (absorption plus incorporation into microbial N).

d = flow of ammonia N through the hindstomach

oes = oesophagus, py = pylorus.


3·2 gN/d
Oes
A
Py
1080[a]
690
240
760[c]
320[b]
350
350
250[d]
100
660
240


3·9 gN/d
Oes
B
Py
1630[a]
680
130
880[c]
750[b]
630
560
430[d]
130
800
200

in the tubiform forestomach arising from ammonia N in this region may be too high, since the enrichment of ammonia N decreased along the length of the forestomach. Conversely, as the enrichment of N in the bacterial fraction from the tubiform forestomach was greater than in the bacterial fraction from the sacciform forestomach, this suggests that progressively more of the microbial N was derived from the ammonia N pool. This would be difficult to determine accurately as the very nature of the tubular flow of digesta, and decreasing pattern of substrate availability and microbial activity, along the length of the forestomach in macropodines indicates a changing dynamic system, even when the animals are feeding at a constant rate.

8.4.4 Stomach microbial activity in field macropodines

Although the field observations of this study were undertaken with few animals it is clear that diurnal variations in stomach microbial activity occurred in all five species and this can be related to the evening and nocturnal extensive feeding periods. However, there are several facets of the field studies that parallel the laboratory findings. In all species, plasma urea levels and the concentration of ammonia and total VFA were comparable with those determined for laboratory animals. The decrease in concentration of total VFA along the forestomach of the field animals is also indicative of a pattern of digesta flow and fermentation similar to that determined in the laboratory animals.

The ciliate protozoa were found in greatest numbers in the cranial regions of the forestomach. As most of the soluble dietary constituents are fermented in the cranial forestomach, the ciliates apparently respond to a chemotaxic gradient. It is unlikely that protozoa contribute significantly to the flow of microbial protein from the macropodine stomach.

Large numbers of anaerobic phycomycetous fungi are

present in the ruminoreticulum of sheep and cattle fed roughage diets (Bauchop, 1979a). Sporangia are principally found attached to the more slowly digested fibrous material; they have not been observed in digesta from ruminants grazing soft leafy pastures (Bauchop, 1979b). Both *T. thetis* and *W. bicolor* are highly selective feeders (Johnson, 1977; P.J. Jarman, pers. comm.) and although grass stem and leaf fragments were present in digesta samples from both species, other leaf material was predominant. This may have precluded the colonisation of fungi in the forestomach of *T. thetis*, but sporangia were present in *W. bicolor*. Sporangia were not found in samples from either *T. thetis* or *M. eugenii* fed chopped lucerne hay but since all samples examined were preserved in formalin, culture tests for the presence of fungi could not be carried out.

The study of the ecology of fungi in the forestomach is very recent; factors other than fibre may also influence colonisation. The establishment of fungi, their role in cellulose digestion and their contribution to the overall microbial fermentation system deserves further investigation.

8.5 Conclusion

The results from the present experiments indicate that the extent of microbial activity in the forestomach of *T. thetis* and *M. eugenii* is similar. This is in support of the finding that apparent digestion of organic matter and plant fibre in the forestomach is also similar in these two species and in *M. giganteus* (Chapter 7). However, the values obtained for microbial activity and synthesis in Experiment 9 were estimated by one method only and, by necessity, with a small number of animals. Be that as it may, microbial activity in the macropodine forestomach appears to be extensive and as efficient as that determined for ruminant animals.

Chapter 9

GENERAL DISCUSSION AND CONCLUSIONS

The pioneer study on digestive function in *S. brachyurus* (quokka) by Moir, Somers and Waring (1956), and more recent investigations on other species of macropodines, suggested that fermentative digestion in the forestomach is extensive and comparable to that in the ruminants. This has been confirmed in the quantitative estimates of the extent of forestomach digestion and activity in the present experiments. In addition, this study has established that digesta within the macropodine forestomach are subjected to a tubular mode of flow and concomitant pattern of microbial digestion not previously described.

Several species-specific differences in gross structure of the forestomach and relative distribution of the squamous and cardiac glandular epithelia occur, but most of these variations are minor. The greatest differences in gross stomach structure among the macropodines examined are represented by *T. thetis* (red-necked pademelon) and *M. eugenii* (tammar wallaby).

Tubular flow and differential retention of the fluid and particulate phases of digesta in the stomach were most obvious in *M. giganteus* (eastern grey kangaroo). This can be attributed to two main factors; most of the ingested food is directed into a relatively small sacciform forestomach (thus the primary pool is smaller), and the tubiform forestomach is relatively longer and narrower than in *T. thetis* or *M. eugenii*. In *T. thetis* all ingested food is directed into the large sacciform forestomach whereas in *M. eugenii* ingested food is directed into both the sacciform forestomach and the cranial region of the tubiform forestomach. Evidence from the rate of passage experiments suggests that the size of the primary pool of digesta in these two species is similar. Digesta flowing from the primary pool is subjected to tubular flow along the tubiform forestomach in all species.

It has often been suggested that the function of the gastric sulcus of adult macropodines is to direct ingesta past the main fermentative region of the stomach. The inference is that the gastric sulcus may contribute to the efficiency of utilisation of food by the animal by directing soluble carbohydrate and protein directly to the hindstomach. The results from the present experiments refute this. In _M. eugenii_ the gastric sulcus is as well developed as that seen in many other species, but there was no evidence of direct transfer of contrast medium or of digesta to the hindstomach. Furthermore, the transit time of digesta in the forestomach of _M. eugenii_ appears comparable to that in _T. thetis_ and certainly longer than transit time in the ruminant forestomach.

Alternatively, the gastric sulcus may function as a means of directing more fluid ingesta to the central region of the tubiform forestomach to provide additional readily fermentable substrate for microbial activity and growth in digesta that has been in the forestomach for a significant period of time. This was not evident in the present experiments but such a mechanism could be advantageous in macropodines faced with a restricted selection and availability of diet, particularly for species adapted to less favourable habitats. The gastric sulcus presumably plays a major role in the developing digestive physiology of the pouch young. However, this has not been investigated and until this is done, any discussion on the function of the gastric sulcus in the young macropodines must be conjecture.

It is possible that some sequestration of particulate digesta may occur in the sacciform forestomach of species possessing a gastric sulcus, although it is unlikely to be of any functional significance. Richardson (in press) observed small amounts of contrast medium to be retained in the sacciform forestomach of _M. eugenii_ for several days, but only in three of fourteen animals examined. In the present experiments there was no evidence of sequestration of particulate digesta in _M. eugenii_, nor in any other species.

As observed in this and in previous studies, sheep can utilise the structural carbohydrates of a diet more efficiently than macropodines. More complete detrition of the larger dietary particles within the ruminoreticulum and remastication of particulate material during rumination are major factors in improving microbial utilisation of fibre. The macropodines do not ruminate, there is no evidence of preferential retention of larger dietary particles within the forestomach, nor are there any anatomical structures to impede the flow of larger dietary particles to the hindstomach. On this basis, Hume (1978) advanced the hypothesis that macropodines on high fibre diets may be able to compensate for less complete fibre digestion by increasing their food intake and thus increasing digesta flow. As yet the evidence for such a mechanism is slender and requires further experimentation. The results of the present study were restricted to a single diet of relatively high digestibility, and thus do not test the hypothesis. A similar strategy was proposed by Janis (1976) for the Equidae as some species can maintain themselves in the wild on a high fibre/low protein diet for extensive seasonal periods. For example, the zebra (Equus burchelli) consistently select the high fibre components of lower quality diets in comparison to ruminants grazing the same herb layer (Bell, 1970).

Evolutionary adaptation of teeth structure in the macropodines classified as grazers allows these species to cope with high silica content native grasses (Sanson, 1977) More complete comminution of such diets should predispose the dietary fibre to more rapid microbial colonisation and attack but this was not evident in M. eugenii. Both M. eugenii and T. thetis digested similar proportions of the fibre component of the fresh high silica content grass despite the former being a grazer and the latter a browsing species.

The efficiency of utlisation of fibre and recycled urea nitrogen by gut microorganisms in T. thetis was similar to M. eugenii when fed either the dried lucerne or the fresh grass diets. Furthermore, microbial production of VFA and protein, and microbial utilisation of ammonia from dietary and urea nitrogen sources, were also similar in both species when fed the chopped lucerne hay.

The maintenance nitrogen requirement of *T. thetis* is twice that of *M. eugenii* (Hume, 1977b). *T. thetis* must therefore have a higher voluntary food intake or consume a diet of higher nitrogen and digestible energy content than *M. eugenii*. There was no evidence from the present experiments that the higher maintenance requirement of *T. thetis* is due to a more limited microbial activity in the digestive tract. On the other hand, it is possible that *T. thetis* may have a higher basal metabolic rate than *M. eugenii*. The estimates of water turnover rate support this but the evidence is not conclusive. There is no evidence from the studies of Dawson and Hulbert (1970) that differences in basal metabolic rate occur among the macropodines.

In the present experiments, the dynamics of water metabolism in *T. thetis* and *M. eugenii* were apparently related to food and water intake rather than diet. Denny and Dawson (1975) found no correlation between water turnover and aridity of habitat in five species of macropodines, but comparative estimates of water metabolism under normal and stress conditions can only be accurately assessed under conditions of measured and constant food and water intake.

The results from the present and similar studies cannot be readily extrapolated to species adapted to different natural habitats when fed diets low in nitrogen and/or digestible energy content. The basal metabolic rate of macropodines is only 70% of that of homeothermic eutherian mammals, and many species have adapted physiologically and behaviourally to semi-arid and arid zone habitats. Such adaptations allow these species to cope with low water availability and diets low in nitrogen content. This is, in part, achieved by adaptive changes in renal physiology. The relative medullary thickness is

greater in some arid zone adapted species. For instance, it is greater in *M. eugenii* than in *T. thetis*, and *M. eugenii* is able to maintain nitrogen balance when offered only saline drinking water (1.25% NaCl) whereas *T. thetis* can not (Hume and Dunning, 1979). *M. eugenii* can also selectively reabsorb urea and thus efficiently conserve nitrogen in times of dietary stress (Kinnear and Main, 1975). The principal advantage of selective reabsorption of urea by the kidneys is to maintain ammonia nitrogen availability, through urea recycling, for microbial activity and protein production in the forestomach. It is probably this mechanism, rather than a higher fermentation rate *per se*, that allows semi-arid and arid zone macropodines to survive when faced with a selection of poor quality diet of low nitrogen content.

Results from studies of microbial fermentation and metabolism in animals fed good quality diets *ad libitum* with free access to water cannot be readily extrapolated to stress situations. In recent experiments with the rock hyrax (*Procavia habessinica*) (Hume, Rübsamen and Engelhardt, in press) the efficiency of microbial utilisation of recycled urea was 71% in animals fed a low protein diet; this increased to 98% with water restriction. Further comparative experimentation with macropodine species from different habitats is required. Studies with animals fed diets low in nitrogen content and with restricted access to water should allow a more complete understanding of the digestive physiology of macropodines adapted to semi-arid and arid zone habitats.

Tubular flow of digesta in the macropodine forestomach results in the more readily digested dietary constituents being rapidly fermented in the cranial regions of the forestomach and the rate of fermentation, and microbial growth, decreasing along the length of the organ. Such a pattern is probably common to all species although the quantitative significance

of microbial activity within either the sacciform or the tubiform regions of the forestomach will differ among some species. This is solely because of the differences in relative capacities of the two regions (e.g. *T. thetis* compared with *M. giganteus*.

The *in vivo* estimations of forestomach microbial activity in *T. thetis* and *M. eugenii* were similar and suggest that VFA and microbial protein production in the primary pool of digesta are highly efficient. Although the macropodines digested less fibre than the sheep, the overall efficiency of microbial fermentation and activity in the forestomach was similar to estimates for ruminants (e.g. Czerkawski, 1978). Direct counts of bacteria forestomach bacteria were also equal to such estimates for the sheep, and represent a significant biomass.

Rapid transit of bacteria through the forestomach to the hindstomach should increase the efficiency of microbial protein production. In sheep fed a similar diet, approximately 30% of microbial cells in the ruminoreticulum are degraded each day (Nolan and Leng, 1972). In the macropodine forestomach the mode of tubular transfer of bacterial cells, either in the fluid phase or attached to particulate material, could conceivably result in a more efficient net production of bacterial protein.

Contrary to the findings of Kempton, Murray and Leng (1976), methane was found, albeit in low concentrations, in the stomach gas samples taken from all four species of macropodines. Both methane and hydrogen were present in higher concentrations in animals that were observed to be feeding up until the time of slaughter. On the basis of their results, Kempton, Murray and Leng (1976) postulated that forestomach fermentation in *M. giganteus* is of limited significance in comparison to the sheep.

It must be stressed that the pattern of fermentation

in the macropodine forestomach is very different from that observed in the ruminants and virtually nothing is known of the microbial ecology of this system. Extrapolation of metabolic pathways and interactions determined for microorganisms in the ruminoreticulum to the macropodine forestomach may well be misleading and erroneous. The relatively short retention time of particulate digesta and rapid flow of the fluid phase in the forestomach could foreseeably preclude the establishment of species of microorganisms that have a low growth and reproduction rate. Clearly, there are many facets of the digestive physiology of macropodines that require more detailed investigation.