CHAPTER 1

1. INTRODUCTION

The aims of the research that is presented in this thesis were principally twofold. One was to examine whether 40 years of single trait divergent selection, for or against clean fleece weight, had produced sheep that differed in the yield of microbial nitrogen per unit feed intake. Associated with this, the importance of yield of microbial nitrogen from the rumen and the efficiency of utilisation of absorbed amino acids, in accounting for differences between the selection lines in wool growth rate was estimated. The other aim was to investigate whether genetic variation existed between fine-wool Merino bloodlines, grazing at pasture, in the response of wool growth rate, average fibre diameter and live weight to increasing amino acid intake.

Before these aims could be successfully completed it was necessary to establish and investigate appropriate methods. Accordingly, the suitability of using the urinary excretion of purine derivatives to estimate the yield of microbial nitrogen from the rumen, and, lithium chloride to estimate supplement intake in grazing sheep, were investigated as part of the research that is reported in this thesis. Urinary excretion of purine derivatives (principally allantoin) has become accepted as a non-invasive and indirect measure of the yield of microbial nitrogen from the rumen. However, close examination of the literature suggested that the fraction of absorbed purines excreted in urine is neither complete nor constant. Hence, prior to using this technique in the studies that are described later in this thesis, the metabolism of allantoin, the end-product of purine degradation in ruminants, was investigated by the use of ^{14}C-allantoin.

When grazing sheep are fed supplements, it is common for large differences in intake of the supplement to exist between sheep. Research conducted recently at the University of New England has indicated that lithium chloride is a satisfactory marker of supplement intake with penned sheep (Suharyono 1992). However, the suitability of lithium chloride as an intake marker had not been investigated with grazing sheep. So, as part of the research presented in this thesis, lithium chloride was evaluated as a marker of supplement intake with grazing sheep. The protocol developed from this research was then used to estimate the degree of genetic

variation between fine-wool Merino bloodlines in the response of various wool and body traits to increasing intakes of a supplementary protein.

The literature that was reviewed provided sufficient background on the research presented in this thesis. Thus, the literature review provides information on the areas of 1) wool growth, 2) protein supply to the ruminant, 3) microbial growth efficiency, 4) nitrogen requirements of rumen micro-organisms, 5) methods to estimate the yield of microbial cells from the rumen and 6) the prediction of the yield of microbial cells from the rumen using the urinary excretion of purine derivatives.

CHAPTER 2

2. WOOL GROWTH

2.1 Biology of wool growth

2.1.1 Introduction

The development of wool follicles in sheep is completed *in utero* and has been described in detail by Hardy and Lyne (1956). The function of the mature wool follicle, the structure of the wool fibre and the process of keratinization have been discussed by Chapman and Ward (1979). Wool growth is the outcome of an extremely complex biological process which is still not fully understood. The factors that control both fibre output and composition have been outlined in the comprehensive reviews of Black and Reis (1979) and Black (1987).

Rather than attempting to review the area of wool growth in its entirety, only those factors most important in determining fibre output, and that are influenced by nutrient supply, are discussed in the following text.

2.1.2 Effects of nutrition on the biology of wool growth

2.1.2.1 Initiation of wool follicles

Wool follicles are composed of two major types; the first follicles to develop are termed *primary* and the latter *secondary*. Secondary follicles of Merino sheep can be further divided into *secondary original* and *secondary derived* with the latter resulting from branching from secondary original follicles. Where branching of secondary original follicles has occurred, more than one fibre is projected through the follicle opening at the skin surface (Hardy and Lyne 1956). Histologically, secondary follicles differ from primaries in that they do not possess a sweat gland nor an arrector pili muscle (Hardy and Lyne 1956).

It has been proposed (Nagorcka 1995a; Nagorcka 1995b) that the spatial organisation of primary and secondary follicles in the skin of sheep is controlled by the concentrations and diffusiveness of certain chemicals within the epidermal and dermal layers of the skin: this biochemical process is referred to as a reaction-diffusion system. As yet, the nature of these

chemicals has not been elucidated but in general they have been termed morphogens because of their ability to control morphogenesis of wool follicles.

An alternative theory of follicle initiation was put forward by Moore *et al.* (1989). These authors suggested that sheep have a "genetically determined developmental capacity" for wool follicles and that the capacity is controlled by the population size of pre-papilla cells which are direct ancestors of dermal papillae. In turn, fibre characteristics are controlled by the number of pre-papilla cells that are required for each follicle initiation event: the more cells the greater the fibre diameter. Thus, sheep with broader wool will have fewer follicles per unit skin area because more pre-papilla cells are involved in each follicle initiation event and consequently the population of these cells is more rapidly depleted.

Follicle development in sheep skin occurs *in utero* with initiation of primary wool follicles beginning at about day 60 of gestation and concluding *c.* 35 days later. Initiation of secondary wool follicles begins at about day 95 of gestation, and is largely complete by day 135 (Fraser 1954; Fraser and Short 1960). Thus the maximum number of wool follicles that a sheep can possess is fixed before birth. Maturation of secondary follicles, as measured by fibre production, continues after birth for 7–8 weeks (Schinckel 1955; Short 1955) with the result being that the ratio of mature secondary:primary follicles increases with lamb age in a sigmoid relationship.

As a consequence of *in utero* follicle initiation, the potential maximum number of fibre producing follicles is governed by genotype and by the placental-transfer of nutrients from the ewe to its lamb. Schinckel and Short (1961) concluded that adverse maternal nutrition, created by a severe restriction of feed (*c.* 300 g/d) during pregnancy, will reduce both the density and the total number of secondary follicles. These authors also observed that whilst restriction of feed intake of lambs from birth to 16 weeks of age retarded the maturation of secondary follicles, the delay in secondary maturation would not be permanent if the nutrition of the foetus had been adequate. That is, adequate nutrition following 16 weeks of restricted intake allowed maturation of follicles.

Mellor and Murray (1981) suggested that the affect of maternal undernutrition on the foetus is most likely mediated through a reduction in placental weight and a decrease in the blood concentrations of essential nutrients (e.g. glucose). In an attempt to define the period of foetal life when initiation of secondary follicles is most susceptible to maternal undernutrition, Hutchinson and Mellor (1983) imposed various degrees of maternal undernutrition (some followed by a period of refeeding) at different times of gestation. What was apparent from

this research was that inhibition of the initiation of secondary follicles, as estimated from the ratio of secondary:primary follicles, was most pronounced when ewes were severely underfed (0.3–0.6 ME requirements) from day 112–131 of pregnancy. In contrast, severe underfeeding of ewes from days 16–95 of pregnancy followed by a period of refeeding (1.0–1.8 ME requirements from day 117–142) did not depress initiation of secondary follicles. These observations highlight a 3 week 'window' where supplementation of the pregnant ewe may improve the life-time wool production of the lamb.

2.1.2.2 Follicle bulb, dermal papilla and cortical cells

The cellular components of the wool fibre and the inner and outer root sheath originate from mitotic activity concentrated around the dermal papilla in the follicle bulb (Schinckel 1961; Black 1987). Thus wool growth is in theory dependent on the mitotic activity within the follicle bulb. In an investigation of 10 major fleece phenotypes, including Merino and non-Merino breeds, Schinckel (1961) reported that there was a close linear relationship between the diameter of a follicle bulb (indirect measure of the number of cells within the bulb, assuming a constant bulb cell volume, (Wilson and Short 1979; Black 1987)) and the diameter of the fibre produced from that bulb. Sheep that possessed larger bulbs and a greater fibre diameter also had a greater mitotic activity, and mitotic activity$^{1/3}$ was closely related to fibre diameter. Given the moderate phenotypic and genetic correlation between fibre diameter and wool growth (g/d) (Walkley *et al.* 1987) it seems likely that mitotic activity is also related to fibre output. With the assumption that the volume rate of fibre growth was proportional to the cube of fibre diameter (later supported by the studies of Downes (1971)), Schinckel (1961) concluded that mitotic activity in the follicle bulb accounted for *c.* two-thirds of the variation in fibre output between fibres of differing diameter.

In support of this earlier research, Hynd (1989) and Hynd and Everett (1990) reported that mitotic activity accounted for *c.* 80% of the phenotypic differences in wool growth between sheep, and that wool growth, the volume of the germinative region of the follicle bulb and the mitotic activity within the bulb are stimulated by improved nutrition. In contrast, Williams and Winston (1987) reported that the diameter of follicle bulbs and the mitotic rate were not influenced by either the genetic ability of sheep to grow wool (Merino ewes from the Trangie fleece plus and fleece minus selection lines) or by the doubling of feed intake. However the area of mitotically active tissue has been found to be greater (10%) in fleece plus as opposed to fleece minus ewes (Williams and Winston 1987) and in strong-wool as opposed to fine-wool Merinos (Hocking Edwards and Hynd 1992).

In an attempt to establish the biological mechanisms responsible for the length growth rate (L) and diameter (D) of wool fibres, Hynd (1994) selected sheep from a common flock that had the same fibre diameter but different staple length or the same staple length with different fibre diameters and fed these animals either at 1.0 (low) or 2.5 (high) x maintenance. All fibre and follicle characteristics were increased when the high replaced the low diet. When the data from all sheep were analysed by linear regression, Hynd (1994) reported that cortical cell length accounted for *c.* 60% of the between-sheep variation in fibre length, whilst cortical cell volume and papilla area accounted for *c.* 90% of the between-sheep variation in fibre diameter. Interestingly, in the studies reported by Hynd (1994), mitotic activity only accounted for 10% of the between sheep variation in fibre length, as opposed to fibre output, leading Hynd (1994) to suggest that length growth rate of fibre is predominately controlled by the post bulb activities of inner root sheath hardening and the duration and rate of keratinization

The close association between papilla dimensions and fibre diameter (Hynd 1994) support the earlier observations for wool (Rudall 1956; Henderson 1965) and hair (Ibrahim and Wright 1982) and such a relationship is also predicted by reaction-diffusion (RD) theory (Nagorcka and Mooney 1982). Using RD theory, Nagorcka and Mooney (1982) predicted that the dimensions of the dermal papilla and the follicle bulb, and the resulting follicular concentrations of various putative morphogens were the major factors in determining the commitment of migrating bulb cells to either fibre or root sheath cells. The importance of the dermal papilla in follicle development and fibre formation has also been suggested by Moore *et al.* (1991).

2.1.2.3 Proportion of follicle bulb cells that differentiate to form fibre

A small but variable proportion of cells (9–42%) that migrate from the follicle enter the fibre cortex (Short *et al.* 1965; Wilson and Short 1979; Hynd 1989) in Merino sheep. The remainder of the migrating cells contribute to the inner root sheath. The efficiency of this process, in terms of fibre production per migrating bulb cell, is low and is largely unaffected by feed intake (Wilson and Short 1979; Hynd 1989). In contrast, it appears that this process is genetically determined (Wilson and Short 1979). In support of this, Black (1987), quoting the unpublished results of P.I.Hynd and D.R.Scobie, provided evidence that *c.* 35–50% and 10–16% of bulb cells entered the fibre cortex of Lincoln and Suffolk sheep respectively. In Romney rams, 30–40% of bulb cells enter the fibre (Kelly *et al.* 1993).

Genetic differences in the proportion of bulb cells that enter the fibre is also evident from the Trangie fleece selection lines. Williams and Winston (1987) estimated that whilst the number of cortical cells produced per day was 17% greater in fleece plus ewes, mitotic density in the follicle bulb was on average 16% lower than that of ewes from the fleece minus flock. This suggests that more cortical cells are formed per mitotic event in fleece plus ewes and from this it can be inferred that the proportion of bulb cells entering fibre was also greater in these ewes. In support of this inference, Hynd (1989), using the data of Williams and Winston (1987), calculated that *c.* 40% more bulb cells entered the fibre of fleece plus as opposed to fleece minus sheep and that this process was unaffected by feed intake. Although the migration of bulb cells to fibre cortex is an inefficient process, Hynd (1989) reported that it explained a small (*c.* 6%) but statistically significant proportion of the between-sheep variation in fibre output.

2.2 Rate of wool growth

2.2.1 Introduction

It has long been known by both graziers and researchers that an increase in the quantity of feed available for the grazing sheep will result in a greater rate of wool growth. Wool growth increases with feed intake (Marston 1948; Allden 1979) and is primarily controlled by the protein content of the diet (Marston 1948). However, Ferguson (1959) disputed the importance of protein intake in controlling the rate of wool growth. Ferguson's conclusion, that wool growth was independent of protein content when the diet contained more than 8% crude protein, was based on an experiment were sheep were fed diets with a differing protein content (*c.* 8–30%). In those studies, wool growth was found to be insensitive to protein intake but responsive to feed intake.

In 1952, McDonald clearly demonstrated that dietary protein gives rise to ammonia in the rumen and that microbial degradation of dietary protein lowers the protein value of the diet. That is, increasing protein intake may not increase the supply of protein to the intestines. Hogan and Weston (1967) clearly illustrated this when they showed that whilst the intake of nitrogen differed between 2 of the diets used by Ferguson (1959), the amount of non-ammonia nitrogen leaving the abomasum did not. Thus, while the conclusions of Ferguson (1959) may have been technically correct, that is, the rate of wool growth may be relatively insensitive to protein intake, any inferences that suggested that wool production was unrelated to the supply of protein to the intestine were unsubstantiated.

2.2.1.1 Stimulation of wool growth by amino acids

Wool is a proteinaceous fibre with a predominance of the sulphur amino acid cystine. The requirement for cystine in the wool fibre can be met by either cystine, cysteine or by methionine following its conversion to cysteine via the transsulphuration pathway (Finkelstein and Mudd 1967). In this pathway, methionine is reversibly metabolised to yield homocysteine which in turn is irreversibly metabolised to cysteine. This process is regulated by cystathionine β-synthase (E.C. 4.2.1.22) and the concentrations of methionine and cysteine regulate its activity (Finkelstein and Mudd 1967). Transsulphuration can occur in both the liver and the skin but is less easily saturated in the latter (Pisulewski and Buttery 1985). Consequently, when Pisulewski and Buttery (1985) infused L-methionine into the duodenum of sheep at the rate of 0–5 g/d they reported that the fraction of cystine-sulphur (S) that originated from methionine was *c.* 5–20% for free amino acid in plasma, 15–50% in plasma albumin but 50–80% for wool. Reis *et al.* (1989) also reported that *c.* 80% of the cystine-S in wool originated from methionine.

Marston (1935) demonstrated that subcutaneous injection of L-cysteine increased wool growth and this stimulated Reis and Schinckel (1963) to observe the effects of abomasal infusions of either L-cysteine, DL methionine or casein on wool growth. These authors reported that the wool growth of sheep consuming a chaff diet was increased by 35–130% when either 2 g/d L-cysteine or 2.46 g/d DL-methionine was continuously infused into the abomasum. Provision of 60 g/d casein (providing *c.* 1.8 g/d sulphur amino acids) into the abomasum also stimulated wool growth (*c.* 90%) and the recovery of dietary casein sulphur in wool (*c.* 45%) was greater than that for the amino acids.

Further research (Reis 1967) indicated that when sheep were consuming 800 g/d of a lucerne/wheaten (1:1) chaff diet, maximal wool growth responses were achieved when *c.* 1–2 g/d sulphur amino acids were infused into the abomasum. When greater amounts of methionine were infused, wool growth was either unchanged or depressed; this response was not observed with cysteine. Reis (1969) suggested that maximal rates of wool growth will occur in sheep, consuming 400 g/d roughage, when 100–120 g/d casein is infused into the abomasum. Hynd and Allden (1985) reported that the rate of wool growth was maximised when the flow of non-ammonia nitrogen (NAN) from the abomasum was *c.* 32 g/d. Assuming that the vast majority of the NAN was of microbial origin and that *c.* 0.85 of microbial nitrogen was protein nitrogen (Standing Committee on Agriculture 1990) then it can be calculated that

in the studies of Hynd and Allden (1985) wool growth rate was maximised when *c.* 170 g/d protein was available for absorption from the intestines of sheep weighing from 40–60 kg.

The importance of the amino acid composition of digesta flowing to the intestines for stimulation of wool growth was determined by Reis and co-workers (1990) when they infused a mixture of either 5 (isoleucine, leucine, lysine, methionine and glycine) or 10 essential amino acids (EAA) into the abomasum of sheep. In the same experiment, the effect on wool growth of various amino acid omissions from the infusion mixture was determined. Infusion of both amino acid mixtures stimulated wool growth above that of control sheep but when all 10 EAA were infused, wool growth was a factor of 1.8 that when only 5 EAA were supplied. Omission of methionine from both amino acid mixtures depressed wool growth rates to a value that was similar to control sheep and this depression was not overcome by substituting cysteine for methionine. However, when cysteine replaced only 0.66 of the methionine, wool growth rate was unaffected. These results led Reis *et al.* (1990) to conclude that methionine has a specific role, beyond provision of cysteine, in regulating wool growth.

The implicit need for methionine in a mixture of amino acids infused into the abomasum is at odds with the similarity of wool growth response to equimolar abomasal infusions of L-cysteine and DL-methionine. The difference between the two observations might be accounted for when it is considered that abomasal infusion of amino acid mixtures changed the ratio of non-sulphur amino acids:sulphur amino acids in plasma and also raised the concentration in plasma of all essential amino acids (Reis *et al.* 1990). Whether these factors are important in controlling wool growth has not been determined.

2.2.1.2 A specific role for methionine: polyamines

Reis (1989) suggested that the specific role of methionine in regulating the rate of wool growth may relate to the role it plays in the formation of S-adenosyl-L-methionine (SAM), an intermediate in the transulphuration pathway (Finklestein and Mudd 1967) and a metabolically active form of methionine. SAM serves as a methyl donor and also as a donor of propylamino groups in the synthesis of polyamines (Mathews and van Holde 1990). It is the role of SAM in polyamine synthesis that Reis (1989) suggested may account for the specific role of methionine in the regulation of wool growth.

The polyamines are cationic cell components that are abundant in rapidly dividing cells and play a role in the neutralisation of the negative charge associated with nucleic acids (Mathews and van Holde 1990). Transfer of propylamino groups by SAM is required for the biosynthesis of the polyamines, spermidine and spermine. Another polyamine, in fact a

diamine, putrescine, is synthesised directly from ornithine by the highly regulated ornithine decarboxylase. Putrescine is also nvolved in the synthesis of the other polyamines (Mathews and van Holde 1990).

In support of the notion that polyamines play an important role in controlling wool growth, Reis (1989) reported that inhibition of ornithine decarboxylase by the inhibitor, α-Difluromethylornithine reduced the length growth rate of wool fibres (L), resulted in a small increase in fibre diameter (D) and consequently changed the L/D ratio. However, nutritional stimulation of wool growth is not associated with a change in the activity of ornithine decarboxylase (Jarvis *et al.* 1990) thus casting doubt on the involvement of this enzyme in the stimulation of wool growth. In contrast, the activity of SAM decarboxylase, involved in the formation of spermidine and spermine, was more than doubled by nutritional stimulation of wool growth. This led Jarvis *et al.* (1990) to suggest that it is the cellular levels of SAM decarboxylase, rather than putrescine that regulate wool growth. This suggestion is supported by the difference in wool growth response as a result of inhibition of ornithine decarboxylase and methionine insufficiency (Reis 1989).

The stimulation of wool growth rate and SAM decarboxylase activity reported by Jarvis *et al.* (1990) may be associated changes in response to improved nutrition. That is, they may not necessarily be cause and effect. A more direct approach to determining the importance of SAM decarboxylase activity for wool growth may be achieved by inhibition of cystathionine β-synthase activity in the transulphuration pathway. However, sufficient cysteine would need to be supplied to compensate for the reduction in methionine transulphuration.

2.2.2 Effects of protein and energy supply on wool growth

As a consequence of microbial activity in the rumen, it is difficult to assess whether the response in animal production (e.g. wool, live weight) to a particular diet is due to the digestible crude protein fraction or the non-protein fraction of digestible organic matter intake. Increasing the intake of the non-protein fraction of a diet will increase the yield of microbial protein to the animal, assuming that there are sufficient amounts of other nutrients and minerals (e.g. N, S, Co, Mg). Consequently, when dietary supply is via the mouth, as opposed to the abomasum, increasing intake of the non-protein fraction of a diet may also be associated with an increased protein supply to the animal. For this reason, much of the research that examined the effects of nutrient supply on wool growth utilised the procedure of abomasal infusion. This research highlighted the importance of the sulphur amino acids and lysine for wool growth. However, because of the microbial activity in the rumen, the dietary intake of these

amino acids does not indicate the increased supply to the sheep. Unfortunately, commercially viable systems for increasing the supply of these nutrients to grazing sheep have not yet been developed.

Ferguson (1972) acknowledged that digestion of organic matter in the rumen contributes microbial protein to the animal and used partial regression procedures to estimate the relationship between wool growth and the yield of digestible microbial protein. In this approach he argued that,

$$W = k_p DCPI + k_{np} DNPOMI$$

where wool growth (W) is a function of digestible crude protein intake (DCPI) and microbial yield resulting from the non-protein fraction of digestible organic matter intake (DNPOMI). The coefficients k_p and k_{np} indicate the efficiency with which DCPI and DNPOMI (used here as an indirect measure of microbial protein yield) are each used for wool growth. A criticism of this approach is that dietary proteins can contribute to the yield of microbial protein and thus confound the predicted values of k_p and k_{np}. However, when formaldehyde-treated diets are used, protein degradability in the rumen is largely eliminated and this approach would have some merit in delineating the response of wool growth to microbial protein supply.

Nevertheless, Ferguson (1972) reported that the non-protein component of digestible organic matter intake had a relatively constant effect on wool growth and values for k_{np} were in the range 1.4–1.7 g and 1.1–1.3 g clean wool/100g DNPOMI when diets were either untreated or treated with formaldehyde. The greater values for k_{np} with untreated diets suggest that the ruminal degradation of dietary protein contributed to microbial protein yield.

In an attempt to increase the gross energy content of the diet without changing the availability of microbial protein to the animal, Ball *et al.* (1972) fed 100 ml/d of either linseed, safflower or cottonseed oil to Merino ewes receiving 800 g/d lucerne chaff. These authors reported that supplementation with oil increased live weight gain but had no effect on the rate of wool growth and on this basis suggested that wool growth is unresponsive to increases in the supply of acetogenic substrates to the animal. However, care should be taken in interpreting these results because the inclusion of more than 3–4% fat in the diet generally depresses cellulolytic activity and reduces the yield of microbial protein to the animal (Scott and Ashes 1993). Nevertheless, in the experiment conducted by Ball *et al.* (1972) neither the total concentration of volatile fatty acids nor the molar proportions of individual acids were affected by oil supplementation. On this basis it can be argued that VFA production rate

(Leng and Brett 1966) and hence ATP supply and the yield of microbial protein from the rumen were also unchanged.

The interaction between energy and protein on wool growth and nitrogen retention has been examined by several authors (Walker and Norton 1971; Black *et al.* 1973; Kempton *et al.* 1978; Reis *et al.* 1992). In general, these studies have provided liquid diets directly to the abomasum and for experimental purposes the dietary nutrients have been dramatically different to that experienced by grazing animals. Nevertheless, the data from these studies indicate that whilst wool growth rate is primarily determined by the abomasal supply of digestible protein, the response in wool growth rate to digestible energy is dependent on the level of digestible protein reaching the abomasum.

In the studies of Walker and Norton (1971), preruminant lambs of about 6 kg live weight and 4 days of age were given liquid diets providing from 0.63–1.46 MJ GE/kg$^{0.73}$/d (below maintenance to *ad libitum*) and containing either *c.* 12, 29 or 46% crude protein. Quite strikingly, wool growth rate was controlled by the ratio of protein to energy (P:E) (Kempton 1979) in the liquid diet. At the lower levels of protein intake, increasing energy (dried whole milk, butter oil and lactose) depressed wool growth. At the greater levels of protein intake, extra energy (dried whole milk) stimulated wool growth rate. The common factor between these effects was that at any level of protein, wool growth was maximised with a P:E of *c.* 12.7 g protein:MJ GE. Thus increases in energy intake at low levels of protein and increases in protein at high levels of energy moved the P:E farther away from its optima and depressed wool growth. In the studies reported by Walker and Norton (1971), nitrogen retention increased rapidly until the P:E that gave maximal wool growth rates (12.7:1) was reached. Thereafter, increases in protein stimulated nitrogen retention but at a lesser rate.

Black and co-workers (1973) infused into the abomasum of 1 yr old Merino wethers, a liquid formulation that provided 3–10 MJ GE/d and either 20, 60 or 100 g protein. In that experiment, increasing protein (milk casein) at any level of energy markedly stimulated wool growth but the wool growth response to increasing energy intake was dependent on the level of protein (Figure 2.1, p 15). When protein intake was 20 g/d, increasing 'energy' by the supply of glucose and butter oil depressed wool growth rate. However, when the protein intake was 60 g/d, increasing 'energy' by the supply of dried cow's milk stimulated wool growth but further addition of glucose and butter oil failed to affect wool growth. When the protein intake was 100 g/d, extra 'energy', provided by increasing the amount of dried cow's milk, glucose and butter oil, resulted in a linear increase in wool growth.

Analysis of the data of Black *et al.* (1973) indicated that, unlike the data of Walker and Norton (1971), there was no single P:E that was associated with maximal rates of wool growth. Maximum wool growth rates were achieved when the P:E was *c.* 7:1, 6:1 and 10:1 g protein:MJ GE for protein intakes of 20, 60 and 100 g/d. Similarly, the greatest response in nitrogen retention (wool-free) to protein was not associated with the P:E that resulted in the greatest rates of wool growth.

The interaction between protein and energy for wool growth was further confirmed by Reis *et al.* (1992), but these authors noted that the main effect of energy level was unimportant and the interaction between protein and energy was small relative to protein supply. Kempton *et al.* (1978) also reported that the abomasal supply (utilising the suckling reflex of the reticular groove) of 0–80 g/d glucose had no effect on the wool growth rate of 25 kg lambs consuming roughage diets. Stimulation of wool growth rate by the provision of extra 'energy' at high levels of protein (Black *et al.* 1973; Reis *et al.* 1992) may arise from the sparing of amino acids from catabolism. In support of this, Reis *et al.* (1992) reported that the blood concentration of urea and essential amino acids fell in response to increasing energy intake: energy intake was increased by the addition of differing amounts of glucose and glycerol. However, energy level had no effect on the concentration of methionine or cystine in plasma suggesting that in the experiment of Reis *et al.* (1992) methionine was first limiting.

In the studies reviewed in this section, the authors have used the term gross energy to quantify the heat of combustion of the diet but the nutrients that contributed to the gross energy of the diet differed both within and between experiments. In these experiments, the energy content of the diets was manipulated by the addition of acetogenic and glucogenic substrates. While the addition of these substrates to a diet will increase its energy content, the productive outcome arising from the addition of acetogenic or glucogenic substrates may be very different (Preston and Leng 1987). Thus, it is difficult to assess the effects of protein and energy on wool growth and nitrogen retention without knowing the nature of the compound that was used to manipulate the energy content of the diet. A further difficulty arises because of the metabolic and endocrinal interdependence of amino acids and acetogenic and glucogenic substrates (Kempton 1979; Preston and Leng 1987).

It is also difficult to relate the performance of animals on these diets with those consuming more normal diets of forage. For example, propionate is the major precursor of glucose in ruminants fed forage diets and very little glucose *per se* is absorbed from the digestive tract. Provision of glucose via abomasal infusions may alter the insulin response

which in turn can affect amino acid metabolism and lipogenesis. In fact, Black *et al.* (1973) speculated that changes in the endocrine status of the animal as a result of protein-energy supply may explain the depression of wool growth rate by the supply of extra energy at low levels of protein.

Thus the study of Walker and Norton (1971) suggests a P:E optima for maximal rates of wool growth which is independent of protein supply. However this was not supported by the observations of Black *et al.* (1973) and Reis *et al.* (1992). The difficulty in reconciling these observations is that not only did the age of the animal vary between experiments, which may influence the proportion of secondary follicles that are mature (see 2.1.2.1), but the range of protein and gross energy provided by Walker and Norton (1971) (4–28 g/kg$^{0.73}$/d and 0.63–1.46 MJ/kg$^{0.73}$/d) was many times that used by Black *et al.* (1973) (1.7–8.4 g/kg$^{0.73}$/d and 0.25–0.83MJ/kg$^{0.73}$/d) and Reis *et al.* (1992) (3.8–10.4 g/kg$^{0.73}$/d and 0.37–0.70 MJ/kg$^{0.73}$/d). Nevertheless, the finding consistent to all these authors was that wool growth rate is primarily dependent on the intestinal supply of protein. However, provision of acetogenic and glucogenic substrates may alter the way in which amino acids are metabolised within the animal and in certain situations may influence wool growth rate.

2.2.3 Divergent selection for clean fleece weight

Variation exists both between strains and between studs within strains within the Australian Merino flock for wool and body traits (Dunlop 1962; Dunlop 1963; Jackson and Roberts 1970). In an attempt to understand the genetic relationships between wool traits, numerous selection lines have been established and these animals have provided the opportunity to investigate the physiological consequences of selective breeding programs. In this section, emphasis will be given to those flocks that have been selected primarily on wool weight. Williams (1979, 1987) has provided excellent reviews in this area.

2.2.3.1 Feed intake and the efficiency of wool growth

Increased wool growth per head can arise from either an increase in feed intake or a greater efficiency with which feed is converted into wool. If feed intake is the primary determinant of wool growth differences between sheep, selection for increased wool weight will not alter the quantity of wool produced per land area. One of the earliest investigations conducted to examine the relationship between intake and wool growth (Weston 1959) indicated that strong-wool Merino sheep grazing at pasture produced more wool (*c.* 40%) than fine-wool sheep. When these sheep were fed in pens, the superior wool growth of the strong-wool

sheep arose because they were more efficient at converting feed to wool (18%) and also because of a greater voluntary feed intake (25%). However, correction of feed intake for the differences in live weight between the 2 strains indicated that the strong-wool sheep consumed 9% more feed.

Schinckel (1960) compared sheep chosen from the Cunnamulla control flock (Turner 1958) on the basis of either high, intermediate or low wool weight per unit body weight. In support of the findings of Weston (1959), sheep that had the greatest wool weight grew more wool per unit intake and had a greater feed intake when diets were offered *ad libitum*. These studies demonstrated that phenotypic differences in wool growth were associated with an increase in wool growth efficiency and possibly intake. However, they failed to indicate whether there was a genetic basis for differences in intake and the efficiency of wool growth.

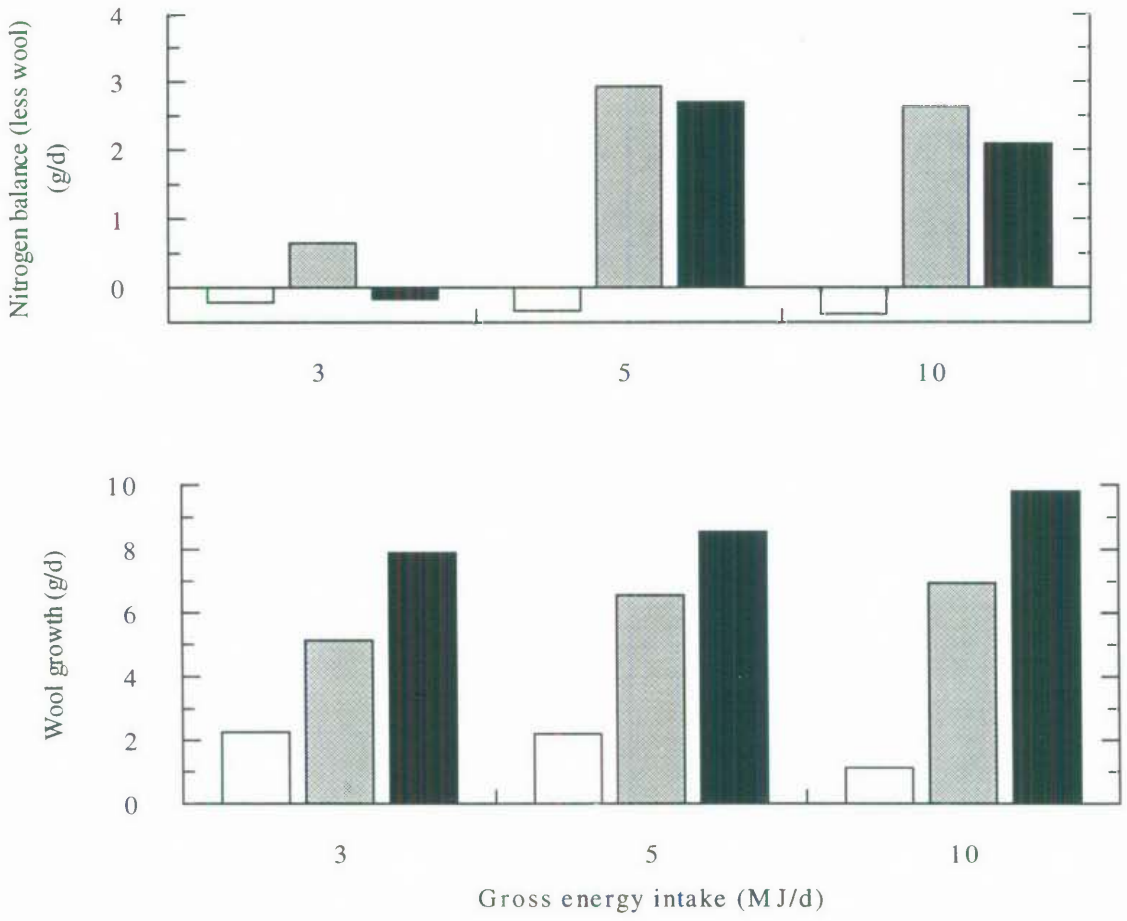

Figure 2.1. Effects of abomasal infusions of protein and energy on nitrogen balance and wool growth rate of adult Merino wethers. Prepared from the data of Black *et al.* (1973) with unfilled (20 g/d protein), hatched (60 g/d protein) and solid (100 g/d protein) histograms.

In 1951, 3 flocks were established by the N.S.W. Department of Agriculture at Trangie in which selection was for either high (fleece plus; F_p) or low (fleece minus; F_m) clean fleece weight at 15-16 months of age. The third flock was selected at random (F_r). Wool growth rate and efficiency are greatest in ewes from the F_p flock (Ahmed *et al.* 1963; Williams and Winston 1965) and the superior rate of wool growth of sheep from the F_p flock becomes more pronounced with increasing feed intake (Williams and Winston 1965). Ahmed *et al.* (1963) reported that feed intake was greatest and least for ewes from the F_p and F_m flocks respectively. However, when intake is adjusted for weight it can be shown that in the experiment of Ahmed *et al.* (1963) there were no differences in intake per unit live weight between the selection flocks. Williams and Winston (1965) reported a selection line x feeding level interaction for feed intake. Ewes from the F_m flock had the greatest and least intake at the lowest and highest feeding level respectively. Unfortunately it is difficult to interpret the differences between the selection lines in feed intake reported by Williams and Winston (1965). The difficulties arise because intake appears to have been restricted and also because intake was continually adjusted to compensate for body weight changes.

Selection of Merino sheep either for clean wool weight or at random was also conducted by the C.S.I.R.O at Cunnamulla. These flocks have been described by Turner (1958) but in brief, animals in the selected line (S) are chosen for greatest clean wool weight at 15-16 months of age after initial culling for excessive wrinkling and high fibre diameter. In agreement with the results from the Trangie fleece selection lines, ewes from the S group had a greater rate of wool growth and were also more efficient at converting feed to wool (Dolling and Piper 1968; Piper and Dolling 1969a). In the trial of Piper and Dolling (1969a) the difference between the selection lines for wool growth rate and efficiency was dependent on feed intake and the protein content of the diet. The relative superiority (percentage difference) of ewes from the S group for wool growth rate and efficiency for diets containing low, medium and high amounts of crude protein was *c.* -3, 25 and 29% for wool growth and -9, 29 and 32% for efficiency (Piper and Dolling 1969a).

Hutchinson (1961) suggested that the physiological components of wool production can be expressed in the multiplicative model

$$W = W/I \,.\, I/B \,.\, B$$

where W = clean wool weight

I = feed intake

B = live weight

W/I = wool produced per unit intake

I/B = intake per unit live weight

Using the method of Henderson and Hayman (1960) various authors (Hutchinson 1961; Dolling and Piper 1968, Piper and Dolling 1969a), have utilised this model and estimated the proportion of variation in wool growth rate that is accounted for by the various physiological components. In general, W/I accounts for 70–100% of the differences in W both within and between selection lines. Feed intake which is a composite of I/B and B has been found to be of lesser importance and accounts for the remainder of the variation in W.

These experiments indicated that there is a genetic basis for the relationship between wool growth rate and the efficiency of converting feed to wool. Other selection experiments confirm these observations. For example, sheep selected for low crimp frequency have a greater wool growth rate and efficiency than sheep selected for high crimp frequency (Robards *et al.* 1974). This could be expected given the relationship between crimp frequency and fibre diameter and the moderately positive genetic correlation between fibre diameter and fleece weight (Walkley *et al.* 1987). However it seems that the genetic correlation between fleece weight and efficiency is greater than that between crimp frequency and efficiency given the findings that wool growth rate and efficiency of ewes from the F_p flock were greater than those for ewes selected for a low crimp frequency (Robards *et al.* 1974).

It appears that the wool follicles of sheep selected for clean fleece weight are more sensitive to nutritional changes. Conversely, it may be more appropriate to consider that selection for low clean fleece weight has produced sheep whose wool follicles are relatively insensitive to changes in nutrient availability. Williams *et al.* (1972a) illustrated this point when they infused either L-cystine (2 g/d) or DL-methionine (2.5 g/d) into the abomasum of ewes from the F_p and F_m flock. Whilst the abomasal supplement increased the wool growth of F_p ewes by 50–60%, the wool growth of ewes from the F_m flock was only stimulated by 12–18%. Other evidence supporting the notion that sheep selected against fleece weight are insensitive to changes in nutrient availability is evinced by the observation that the wool growth of F_m ewes is not greatly influenced by the effects of pregnancy, litter size and lactation (Williams 1979).

2.2.3.2 Metabolism of cystine

It is generally accepted that wool growth rate is determined by the availability of sulphur amino acids and increasing intake has been shown to increase cystine availability (Williams *et al.* 1972b) and wool growth (Allden 1979). Similarly wool growth and plasma cystine concentration are linearly related when plasma cystine ranges from *c.* 30–45 μmol/l (Lee and Williams 1993). However, for some flocks curvilinearity is apparent (Lee and Williams 1993). The relationship between intake and cystine availability occurs because ruminal microbial yield (Kahn 1991; Chen *et al.* 1992a) and hence microbial sulphur amino acids increases linearly with feed intake and also because some dietary sulphur amino acids may escape rumen fermentation and thus contribute to the pool of amino acids available for absorption.

Given the preceding discussion, it could be expected that the availability of cystine is greater in sheep genetically superior in wool production. Surprisingly this is not the case. In fact the plasma concentration of cystine is greater in F_m as compared to F_p ewes and the rate of entry of cystine does not differ between the selection lines (Williams *et al.* 1972b). However, Williams (1973) has reported that the entry rate of cystine may at times be 25% greater in F_m ewes.

These observations suggest that the differences in wool growth rate and efficiency between F_p and F_m sheep are not related to the apparent availability of cystine and thus differ fundamentally from the relationship between feed intake and wool growth rate.

2.2.3.3 Digestibility and rumen function

The rate of wool growth is primarily determined by the availability of sulphur amino acids to the sheep and the efficiency with which the wool follicle competes for and then utilises these compounds (Black and Reis 1979; Cronjé and Smuts 1994). However, abomasal infusion of a mixture of essential amino acids in addition to methionine and cystine further stimulates wool growth rate (Reis *et al.* 1990). The potential availability of amino acids to the sheep will be governed by intestinal supply, digestion and absorption, and variation in any of these could in theory account for some of the phenotypic and genetic differences in wool growth rate that have been reported in the literature

Determination of apparent whole-tract digestibility in sheep has been used to elucidate whether the digestion and absorption of nutrients is related to wool growth rate. The results from a large number of these determinations indicates that variation in the apparent digestibility of DM (Hutchinson 1961; Piper and Dolling 1969b), OM (Weston 1959; Piper and Dolling

1969b), N (Hutchinson 1961; Piper and Dolling 1969b) and S (Piper and Dolling 1969b) in the whole-tract is unrelated to genetic and phenotypic differences in the rate of wool growth.

Thus the processes involved with digestion and absorption have been shown to be unrelated to wool growth rate. In contrast, there are very few reports indicating whether differences in wool growth rate, at constant feed intake, are related to variation in the intestinal supply of protein. With most diets, a large fraction of the dietary protein is fermented in the rumen as a result of microbial activity (see Chapter 3). Consequently, little dietary protein is available for digestion and absorption and the majority of protein that is absorbed from the digestive tract of ruminants is of microbial origin (see Chapter 3). Thus in many instances it is the yield of microbial protein that governs the rate of growth and composition of wool.

The amino acid composition of microbial protein is relatively constant and little affected by diet (see Chapter 3). Hence, stimulation of wool growth rate can be achieved by either increasing the yield of microbial protein from the rumen or by increasing the protein content of microbial cells. Genetic and phenotypic differences in wool growth rate, at constant feed intake, may reflect differences in the yield of microbial protein from the rumen. Unfortunately there have been no reports directly examining whether such differences exist.

Indirect information is available from the data of Lush *et al.* (1991) who measured the net portal absorption (NPA) of α-amino nitrogen (AAN) (estimate of amino acid nitrogen) in F_p and F_m sheep fed lucerne chaff calculated to provide from 0.7–1.3 of maintenance requirements. In that experiment, NPA of AAN increased in a linear fashion with feed intake and whilst being *c.* 25% greater in F_p sheep at all levels of intake, the differences between the selection lines were not statistically significant.

Because the proportional contribution of dietary and microbial amino acids were not determined by Lush *et al.* (1991) the cause of the greater NPA of AAN can not be determined. Other references to selection line differences in rumen fermentation are limited, but indicate that sheep from the F_p and F_m flocks do not differ in the rumen concentration of ammonia nor in the concentration and proportions of volatile fatty acids (Williams 1979).

2.2.3.4 Variation in the sulphur content of wool

The rate of wool growth and the sulphur content of wool increase in response to abomasal infusions of L-cystine, DL-methionine or casein (Reis and Schinckel 1963; Reis 1967; Williams *et al.* 1972a). However, when comparisons are made between sheep, an inverse relationship exists between wool production and the sulphur content of the wool (Reis *et al.* 1967). That is, within a flock, those sheep with the greatest rates of wool growth will produce wool with

the lowest sulphur content. Piper and Dolling (1966) demonstrated that this relationship was genetic when they reported that the wool grown by ewes from the Cunnamulla high clean fleece weight selection line had a lower sulphur content than that grown by ewes from the low clean fleece weight selection line: 3.0% and 3.5% sulphur respectively. This observation was consistent with the differences between animals from the F_p and F_m Trangie selection lines. The sulphur content of clean dry wool of ewes from the F_p flock is less than that of ewes from the F_m flock (Reis *et al.* 1967; Williams *et al.* 1972a) but the difference between the selection lines decreases when L-cystine and DL-methionine are infused into the abomasum (Williams *et al.* 1972a).

The increase in the sulphur content of wool that accompanies an increased supply of sulphur amino acids to the intestines, and presumably to the wool follicle bulb, results from an increase in the proportion of the high-sulphur proteins in the matrix of the wool fibre (Gillespie and Reis 1966). In addition, the sulphur content of the high-sulphur proteins is also increased (Gillespie and Reis 1966) and this is associated with the synthesis of ultra-high-sulphur proteins (Reis 1979). These changes also account for the differences in the sulphur content of wool grown by sheep with differing capacities to grow wool. When the wool from 1 high and 1 low producing sheep was compared (Gillespie and Reis 1966) the latter wool had a higher sulphur content and a greater proportion of high- and ultra-high-sulphur proteins.

Presumably, changes to the proportions of the high- and ultra-high-sulphur proteins also account for the lower sulphur content of wool grown by sheep selected for, as opposed to against, clean fleece weight (Piper and Dolling 1966; Reis *et al.* 1967; Williams *et al.* 1972a). When it is considered that the concentration of cystine in plasma is greater in F_m sheep, the entry rate of cystine to the blood plasma pool is independent of the genetic capacity of the sheep to grow wool (Williams *et al.* 1972b) and the total output of sulphur in wool is greater in high producing sheep (Williams *et al.* 1972a) it appears that genetic differences in the S content of wool are unrelated to the apparent availability of cystine to the follicle bulb.

Various biological mechanisms have been suggested to explain the synthesis of wool keratins (Chapman and Ward 1979) but these fail to account for differences in the composition of wool proteins observed in genetic comparisons. Nevertheless, the ability of sheep selected for high rates of wool growth to grow wool of a lower sulphur content contributes to the greater efficiency of wool growth by these animals.

2.3 Conclusion

The rate of wool growth and its composition are largely controlled by the nutrition of the sheep and lifetime wool production is sensitive to maternal nutrition during pregnancy. This occurs because maternal undernutrition between days 112–131 of gestation, (the period corresponding to initiation of secondary follicles in the foetus) can permanently reduce the number of secondary wool follicles per unit skin area of the unborn lamb. Thus strict attention to the nutrition of the pregnant ewe for a short period of time during late pregnancy can alter the lifetime wool production of the lamb.

The rate of wool growth increases with the supply of digestible protein to the intestines and this occurs because increasing the supply of digestible protein stimulates mitotic activity in the wool follicle bulb. It is has been estimated that variation in mitotic activity accounts for *c.* 80% of the phenotypic differences in wool growth rate. However, fibre diameter and the length growth rate of wool appear to be under different controls. Of the components of digestible protein, it is the availability of sulphur amino acids at the intestines that is most responsible for the stimulation of wool growth. Based on abomasal amounts, there is some suggestion that the wool growth response to DL-methionine is greater than that to L-cystine but the differences are not significant.

When sheep are consuming maintenance amounts of a roughage diet, wool growth rate is maximised by the abomasal infusion of *c.* 1–2 g/d sulphur amino acids. However, it seems that further addition of other essential amino acids (EAA) stimulates wool growth to a greater extent. Similarly, a greater rate of wool growth is elicited when equivalent amounts of sulphur amino acids are supplied per abomasum as casein rather than as cystine or methionine. When the diet is completely via the mouth, wool growth rate is maximised when *c.* 150–170 g/d of protein are available for absorption at the intestines.

The studies of Reis (1967) which concluded that the wool growth rate of sheep consuming *c.* 800 g/d of a roughage diet is maximised by the abomasal infusion of *c.* 1–2 g/d sulphur amino acids, indicates that on these types of diets microbial supply of sulphur amino acids is insufficient for maximal rates of wool production. To calculate the probable supply of microbial sulphur amino acids in the sheep that were studied by Reis (1967) several assumptions need to be made because of lack of details concerning the ration: intake 800 g/d DM. Firstly it was assumed that the apparent digestibility of the ration (lucerne/wheaten chaff, 1:1, 2% N on DM basis) was 0.54, the gross energy of the diet was 18.2 MJ/kg, ME = 0.81DE and that 8.4 g microbial crude protein (MCP) were produced per MJ ME intake (Standing

Committee on Agriculture 1990). Given these assumptions it can be calculated that *c.* 54 g/d microbial crude protein (MCP) were produced and if 0.8 of this was true protein (Standing Committee on Agriculture 1990), this equates to *c.* 43 g/d microbial true protein available for digestion and absorption at the intestines. To calculate the flow of microbial sulphur amino acids to the intestines it is assumed that 1.1% and 2.8% of microbial amino acids are cysteine and methionine respectively (Purser and Buechler 1966). This was considered valid given the finding that the amino acid composition of microbial proteins is considered to be virtually constant (Weller 1957; Purser and Buechler 1966). Thus in the above example, *c.* 1.7 g/d of microbial cysteine + methionine would have been available for digestion and absorption. If wool growth rate was maximised with an additional 1–2 g/d of sulphur amino acids, then it seems that the optimal supply of sulphur amino acids for maximal rates of wool growth is in the range 2.7 to 3.7 g/d.

The discussion above indicates that the wool growth rate of sheep on maintenance amounts of a roughage diet will be *c.* 0.5–0.6 of genetic potential. The deficit in amino acid supply may be met by either increasing the yield of microbial protein derived from the feed or by providing extra protected digestible dietary protein. These aspects will be discussed in the following chapters. Alternatively, wool growth rates may be increased by genetic selection programs. For example, divergent selection for clean fleece weight (e.g. Trangie fleece selection lines) has produced sheep that grow wool at vastly different rates while consuming the same amount of feed. The greater rates of wool growth have generally been ascribed to a more efficient utilisation of sulphur amino acids by the wool follicles rather than to changes in the digestion and absorption of dietary DM, OM or N. However, there has been no published information indicating whether between-sheep differences in wool growth rate, at constant feed intake, are related to variation in the intestinal supply of microbial protein.

As part of the studies reported in this thesis, ewes from the Trangie F_p and F_m selection lines were used to investigate whether divergent selection for clean fleece weight was associated with a change in the yield of microbial protein from the rumen. In addition to this, the importance of yield of microbial protein from the rumen in accounting for differences between the selection lines in the rate of wool growth rate was estimated.

CHAPTER 3

3. PROTEIN SUPPLY TO THE RUMINANT

3.1 Microbial protein

3.1.1 Quantitative importance of microbial protein to the ruminant

Ruminants derive their protein supply from two major sources: rumen microbes and the diet. Generally, a large proportion of the dietary protein is degraded by ruminal micro-organisms to yield ammonia via intermediates which include peptides and amino acids (McDonald 1952; Annison 1956). As a consequence, it is not surprising that in many instances the majority of the amino acids and peptides absorbed from the digestive tract of ruminants are of microbial origin (McDonald and Hall 1957; Weller *et al.* 1958; Nolan and Leng 1972). In a summary of the published literature, Smith (1975) reinforced these earlier findings and concluded that with many diets (excluding tanniniferous and dried forages) microbial-N is the major contributor to the total non-ammonia nitrogen (NAN) flowing from the rumen to the proximal duodenum. Nolan (1975) provided yet further support for the quantitative importance of microbial-N to total NAN flow to the duodenum. Using the values of Weller and co-workers (1958) for rumen microbial-N enrichment, Nolan (1975) calculated that *c.* 60% of the duodenal NAN was microbial in origin when sheep were fed a lucerne diet. However, Leibholz (1972) reported that microbial protein could be as high as 97% of the daily flow of amino acids to the duodenum in sheep given a low protein diet (1.4 gN/d).

In general, the supply of microbial protein from a nutritionally balanced rumen will be sufficient to meet the nitrogen requirements of animals except those that are either 1) rapidly increasing in mass when young, 2) in the late stages of pregnancy 3) in lactation or 4) have high rates of wool growth (Ørskov 1970).

3.1.1.1 Amino acid composition of microbial protein

Purser and Buechler (1966) reported that the amino acid composition of 22 strains of rumen bacteria was essentially constant and Weller (1957) demonstrated that the amino acid composition of mixed rumen bacteria varies little regardless of the diet consumed by the ruminant host. These conclusions were substantiated by Leibholz (1972) who found that the

type and quantity of protein in the diet had virtually no effect on the amino acid composition of the bacterial fraction.

Because the amino acid composition in ruminal bacteria is relatively constant the biological value of bacterial protein is also quite constant (*c.* 70) and virtually the same as protozoal protein (Weller 1957; Purser and Buechler1966). Protozoal protein is higher in the concentrations of some essential amino acids (e.g. lysine). However, because protozoal protein has a greater true digestibil ty, its net protein utilisation value (0.73) is superior to that of bacterial protein (0.60) (Purser 1970).

When microbial protein is the sole source of dietary protein for growing steers, methionine has been found to be the first limiting amino acid and lysine and threonine the second and third limiting (Richardson and Hatfield 1978). This also appears to hold true for lambs (Storm and Ørskov 1984), with arginine and histidine, but not threonine also being implicated as potentially limiting amino acids in microbial protein. However, when animals are fed protein sources that have some resistance to microbial degradation, the limiting amino acids may also reflect characteristics of the dietary protein. For example, lysine is suggested as being first limiting when steers are fed corn-based diets (Merchen and Titgemeyer 1992), presumably because the bypass protein in corn is a poor source of lysine. The notion that other amino acids are probably either co-limiting or nearly as limiting as methionine (Titgemeyer and Merchen 1990) is supported by the observation that responses in nitrogen retention to a mixture of amino acids (Chalupa *et al.* 1973) are greater than to methionine alone.

3.1.1.2 Composition of bacterial cells

Whilst the amino acid composition of bacterial protein is relatively constant this does not imply that the protein content of the cell is also constant. Changes in bacterial composition occur in response to numerous factors, including; variations in specific growth rate (Isaacson *et al.* 1975; Maeng *et al.* 1976; Bergen *et al.* 1980; Russell 1986), time after feeding (Smith and McAllan 1974; Merry and McAllan 1983; Cecava *et al.* 1990a), faunation status (Smith and McAllan 1974), and also between fluid and particle associated bacteria (Cecava *et al.* 1990a). In contrast to these factors bacterial composition appears to be unaffected by the ratio of roughage:concentrate in the diet (Cecava *et al.* 1990a).

Isaacson *et al.* (1975) reported that mixed rumen bacteria stored carbohydrate (Stouthamer 1979) and also increased the cellular proportion of nitrogen as the dilution rate of a chemostat was increased from 0.02 to 0.12 h^{-1}. Whilst this may be true for a mixed culture

not all rumen bacteria respond in th s way (Russell 1986). The increase in the nitrogen content of the cell with increasing dilution ·ate is accounted for by an increase in the RNA content of the cell (Maeng *et al.* 1976; Russell 1986) and a corresponding increase in the RNA:protein ratio (Bergen *et al.* 1980). It is interesting to note that whilst increasing dilution rate increases the content of RNA in the cell, p·esumed transcriptional and translational increases do not appear to result in the accumulation of cellular protein.

The overall conclusion from the preceding discussion has indicated that with many diets, microbial protein is often the major contributor to total protein flow from the rumen to the intestines. As a consequence of this, the amino acid composition of duodenal contents in ruminants is often unrelated to that of the diet. Thus, alterations to the availability of limiting amino acids for tissue accretion and wool growth are largely dependent on the amino acid composition of ruminal micro-orgar isms. The latter has been shown to be relatively constant, unaffected by diet and first limiting in methionine and lysine. The composition of bacterial cells can be manipulated and strategies that affect dilution rate and faunation status are probably the most potent means of achieving compositional changes. However, manipulation of these factors is not always practical let alone economic.

Increasing the yield of microbial protein per unit intake is probably the most practical and effective means of increasing the availability of limiting amino acids. However, when microbial yield has been maximised further stimulation of protein-requiring processes, such as wool growth, will require provision of dietary proteins that are partially resistant to rumen fermentation but are digestible in the intestines. In the remainder of this section the fate of dietary protein in the rumen and its subsequent feeding value to the animal will be addressed. In Chapters 4 and 5 the ATP and nitrogen requirements of ruminal micro-organisms will be examined. The growth requirements of ruminal microbes is covered in some detail because a clear understanding of this topic is required to devise strategies that stimulate microbial yields and hence increase the availability of limiting amino acids to the grazing animal.

3.2 Dietary protein

Once microbial yields have been maximised, increases in the total flow of protein to the intestines can only be achieved by supplementation with dietary protein that is protected from rumen degradation but is digestible in the intestines. Such protein sources also have the potential to increase the supply of amino acids that are first limiting to production. In ruminants, however, dietary proteins, peptides and amino acids are often subject to extensive

microbial degradation in the rumen and as a result the amino acid composition of duodenal contents is often little affected by diet. Whilst as a generalisation this is true, the potential degradability of a protein depends on factors such as its solubility and secondary and tertiary structure (Wallace and Cotta 1988). Generally, the degradability of a protein increases with its solubility, however this is not always true as the soluble proteins, casein and bovine albumin, differ in the extent to which they are degraded in the rumen (Annison 1956). In the studies of Annison (1956), casein was readily degraded whilst albumin was only slowly degraded.

Degradation of protein within the rumen (proteolysis) involves initial hydrolysis of peptide bonds by microbial proteases to yield oligopeptides and other shorter length peptides. The latter are then transported into the cell and degraded to yield amino acids which can be either incorporated into bacterial or protozoal protein or further metabolised to a range of products including ammonia. On diets of fresh ryegrass, fresh clover or lucerne hay a substantial fraction of the dietary protein is degraded in the rumen and more than half of the crude protein-N is contributed to the rumen ammonia pool (Nolan 1993).

Peptides and amino acids also differ in the extent to which they are degraded in the rumen. Hydrophobic peptides appear to be degraded less readily than hydrophilic peptides by rumen microbes (Chen *et al.* 1987) and of the essential amino acids, lysine is most rapidly degraded and methionine amongst the slowest (Chalupa 1976; Cottle and Velle 1989). Thus it could be expected that the dietary protein which flows to the small intestine will be relatively rich in hydrophobic amino acids, of which many are considered essential for the ruminant (Downes 1961).

To counter the proteolytic activity of the rumen microbes, many methods to lower the extent of degradation of proteins have been investigated and protein meals that partially escape rumen degradation (e.g. cottonseed meal) have been commercially available for some time. These methods rely on either structural or chemical changes to the protein or on physically protecting the protein from microbial activity. The most common methods of protein protection include; formaldehyde, condensed tannins, heat and heat-induced maillard reactions involving reducing sugars and ε-amino groups of lysine, or other cross-linking reactions. These areas of research have been comprehensively covered by various review articles including those of Ferguson (1975), Tamminga (1979) Satter (1986) and most recently by Broderick *et al.* (1991).

3.2.1 Proteolysis

It is generally accepted that most of the proteolytic activity in rumen fluid is associated with bacteria (Blackburn and Hobson 1960; Kopecny and Wallace 1982), suggesting that in populations of mixed rumen bacteria, specific proteases are generally cell associated. However, it appears that the cellular distribution of proteolytic activity differs amongst rumen bacteria and in some organisms is distinctly extracellular (Wallace and Brammall 1985). Protozoa also contribute to the proteolytic activity in the rumen and primarily degrade particulate proteins. Nevertheless bacteria are generally considered to be the most important contributors to proteolysis in the rumen (Cotta and Hespel 1986).

Wallace and Brammall (1985) suggested that the rumen bacteria which have the greatest proteolytic activity possess mainly serine proteases whereas mixed bacterial populations in the rumen have proteolytic activity that is predominately of the cysteine protease type (Kopecny and Wallace 1982). Based on this information, Wallace and Brammall (1985) concluded that within the rumen it is likely that those organisms that have a lower proteolytic activity but possess mainly cell-associated cysteine protease (e.g. *Bacteroides ruminicola*) are of major importance.

Proteolytic activity appears to be expressed constitutively (Wallace and Brammall 1985) and, as a consequence, proteolysis in the rumen is likely to governed by both the size of the bacterial population and the species composition. Moreover, Wallace and Brammall (1985) concluded that differences in the rate of hydrolysis of proteins are more directly attributable to the nature of the specific proteins rather than due to differences in proteolytic activity between organisms.

Given that proteases are cell-associated, the first stage in proteolysis by bacteria is adsorption of soluble protein to the cell surface of bacteria (Nugent and Mangan 1981). The resulting peptides can be transported into the cell via membrane carriers; however rumen bacteria also possess transport systems for amino acids (Russell *et al.* 1988). Which of these transport mechanisms predominates is unclear and it seems that quantitative data to establish the relative importance of peptide and amino acid transport is minimal (Broderick *et al.* 1991).

There are however, some indications that rumen bacteria metabolise peptides more rapidly than amino acids (Chen *et al.* 1987) and that peptides are also the preferred source of ruminal bacterial protein. In support of this, Wright (1967) reported that when ^{14}C-labelled peptides were added to an *in vitro* culture containing rumen fluid, *c.* 50% of the radioactivity recovered in VFA and bacterial protein was found in the latter product (Wright 1967). When

^{14}C-labelled amino acids were used, this value was only 25%. Consequently, Wright (1967) suggested that peptides rather than amino acids are the preferred source for bacterial protein synthesis whilst a greater fraction of amino acid-C is degraded to yield VFA. These results also imply that some peptide carbon is incorporated into bacterial protein without passage through the extracellular amino acid pool.

3.2.2 Conclusion

As a consequence of the proteolytic activity of rumen bacteria a large proportion of dietary proteins are degraded within the rumen. This process is detrimental to the nutrition of the animal because in many instances a significant fraction of the dietary protein-N is converted to ammonia in the rumen and eventually excreted in urine as urea. However, degradation of dietary proteins is beneficial to ruminal micro-organisms because the end-products of protein degradation; ammonia, peptides, amino acids and branched chain volatile fatty acids, may stimulate microbial growth.

The exact nature of ruminal microbial protein metabolism is complex and at present, only partially understood. For further reference in this and other associated areas the reader is directed to reviews in these fields (Allison 1970; Matthews and Payne 1975; Cotta and Hespell 1986; Wallace 1988; Broderick *et al.* 1991; Russell *et al.* 1991).