MEASUREMENT OF DENSITY AND MEDULLATION IN WOOL

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Density is one of the fundamental physical characteristics of wool which is assumed to remain constant, yet medullation, when present, is an obvious source of variation in the density of wool fibres. It was proposed that a standardised and commercially viable method of measuring the density of wool fibres should be developed. Adaptation of an existing method of measuring the volume and density of finely powdered and porous solids, using a rapid, automated helium pycnometer, was pursued. Preliminary studies had identified sample mass constraints and the number of sequential measurements required for the technique. In this thesis, the effect of fibre length on the measurement of volume and density of wool fibres was investigated, to complete the development of the standardised measurement method. The helium pycnometer was then used to explore: an hypothesised relationship between medullation and fibre density; seasonal differences in fibre volume and fibre density of four types of wool, with varying degrees of medullation; and an unsubstantiated suggestion of a difference in specific gravity between lambswool and that of the adult sheep.

A rapid, standardised method for measuring the volume and density of wool fibres was developed. Recommendations constituted use of: a sample mass between one and ten grams; fibre lengths greater than or equal to ten millimetres; and the fourth sequential sample measurement, for the measurement of the volume and density of wool fibre.

The fibre density of non-medullated wool, measured using a helium pycnometer (1.29 g/cm^3), was similar to the density and specific gravity, measured using a density gradient column or benzene pycnometer (1.30 g/cm^3). Fibre density of medullated wool was significantly lower than that of non-medullated wool. There was a significant ($p<0.001$) relationship between percent medullation by volume and fibre density. However, prediction of medullation using helium pycnometer measurements of fibre volume was less accurate than using the projection microscope, OFDA, WRONZ Medullameter or NIRA methods of measuring medullation. There was significant seasonal variation in fibre volume and fibre density, for both non-medullated and medullated wools. The degree of medullation also varied significantly between seasons. Seasonal variation in fibre density was only partially explained by variation in medulla content. The maximum fibre volume and percent medullation by volume occurred during the summer months, while the minimum for both occurred during the winter months. The reverse situation applied for fibre density. The fibre density of lambswool did not differ from that of the adult ewe, once the effect of medullation was accounted for.

This study has contributed new work to the area of wool metrology. This includes: a rapid and economically viable method of measuring the fibre density of medullated and non-medullated wool types; identification of seasonal trends in fibre volume and fibre density; and it questions the assumption that the density of wool keratin is constant.

Key words: wool; keratin density; fibre density; fibre volume; specific gravity; medullation; helium pycnometer; Merino; Crossbred; Cheviot; Drysdale.

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1. Introduction

The density of a substance is defined as mass per unit volume. Barker (1931) described density as one of the fundamental, physical characteristics of wool which remains constant. Assuming this to be true, density is an integral component of many physical equations. One commercial application of such an equation forms the basis of the airflow method of measuring fibre diameter (Cassie, 1942; IWTO, 1982). While accurate for a wide range of wool types, the fibre diameter of medullated, lambs and dyed wools is not able to be accurately measured using the Airflow method. This is possibly because these wools violate the assumption of constant density (van Luijk, 1984).

The accepted value for wool density (1.30 g/cm^3), determined and corroborated by various methods (King, 1926; Barker and King, 1926; Van Wyk and Nel, 1940; Abbott and Goodings, 1949; Connell and Andrews, 1974), was established in wool types expressing little or no medullation. Many breeds of sheep, however, express varying degrees of medullation. The amount of medullation present varies with both season and genotype (Tibbits, 1959; Story and Ross, 1960; Scobie *et al.*, 1993). The cortex of medullated fibres are interrupted by areas of vacuolated cells. Clearly, the density of medullated fibres should differ from the density of fibres with a fully keratinised cortex, as the vacuolated cells that comprise the medulla contribute to the volume of the fibre but not the mass.

The existing gravimetric, volumetric and gradient column methods for determining density are time consuming, tedious and use hazardous liquids as the displacement medium. No commercially viable method for measuring density has yet been developed. The commercial relevance and processing implications of significant variations in density remain largely unexplored. The development of an automated gas comparison pycnometer, used to measure the volume of powders and porous solids, has created an opportunity to develop a rapid method for measuring the volume of a mass of wool fibres. Initial experiments have established constraints for sample mass and the number of sequential measurements required for the measurement of wool (Merrick *et al.*, in press), (Appendix I). Further work is needed to complete development of an appropriate method.

The research detailed in this thesis was undertaken with the following objectives:

(i) Complete the development of a rapid method to measure the density of wool, using a gas comparison pycnometer.
(ii) Examine the relationship between density and medullation of wool
(iii) Examine seasonal differences in density and medullation of wool
(iv) Substantiate differences in density between lambswool and that of the adult ewe

2. Literature Review

2.1 Structure of the wool fibre

The skin of sheep (*Ovis aries*) consists of two main layers, a thin epidermis overlying a thick dermis. Wool fibres grow from follicles, in the dermal layer, each follicle being a protrusion of epidermal tissue into the dermis. A wool fibre is a complex matrix of filamentous protein, the structure of which is stabilised by the cross-linking of disulphide bonds. The main components of a wool fibre are the cuticle, cortex and sometimes a central medulla. These components differ in size, function and composition. The fully formed fibre is an inert, chemically resistant unit protruding from the skin surface (Morton and Hearle, 1962).

2.1.1 The wool follicle

Different regions within the follicle are responsible for the proliferation and differentiation of the fibre forming cells. Cell proliferation occurs near the dermal papilla at the base of the follicle. As cells migrate outward and upward they differentiate to form cuticle, cortex, medulla and inner root sheath cell types. Through the next region, the keratogenous zone, fibre proteins are synthesised, the fibre is formed and begins to harden. Fine structures such as the inner and outer root sheaths and a vitreous membrane are present on the outer surface of the developing fibre. In the next region, hardening of the fibre is completed and the fine structures begin to degrade. As the fibre continues towards the skin surface it passes through a zone of sloughing, where degradation of the fine structures continues. The fully formed fibre then enters the pilary canal before emerging through the epidermis (Fraser, 1965; Chapman, 1971; Chapman and Gemmell, 1971; Gemmell and Chapman, 1971; Chapman and Ward, 1979; Black, 1987).

2.1.2 Fibre cuticle

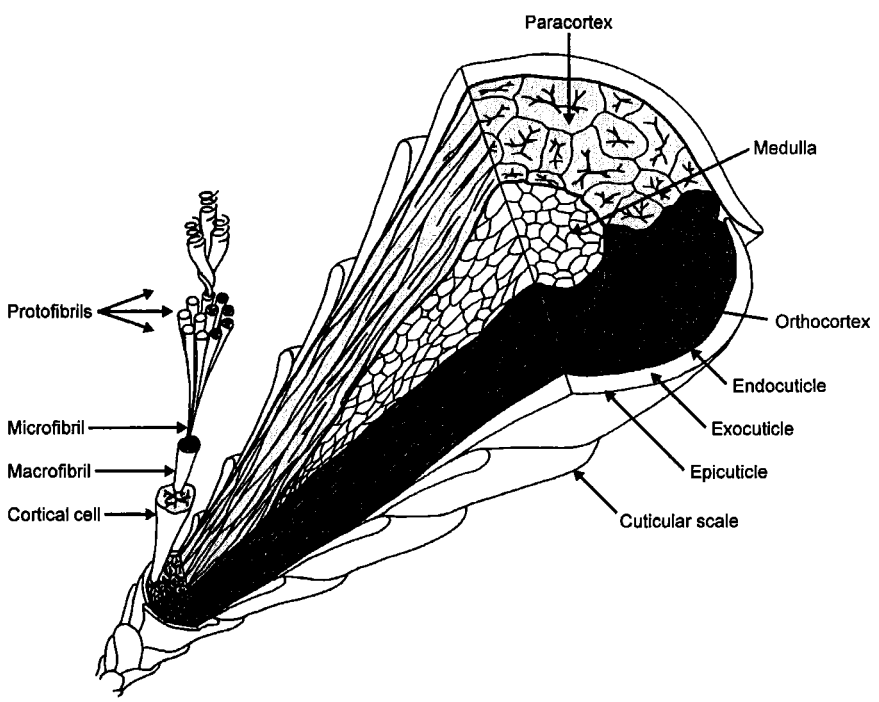
The cuticle is a thin layer of flattened cells on the outside of the wool fibre. The cells overlap around the fibre and along the length of the fibre to produce the characteristic scale pattern associated with wool fibres. Each cuticular cell is a complex laminated structure, consisting of an epicuticle, an endocuticle and an exocuticle. The cuticle strongly influences the surface properties of the fibre and acts as a barrier between the cortex and the external environment (Morton and Hearle, 1962; MacKinnon *et al.*,

1990). In particular, the epicuticle resists abrasion and chemical degradation (Ryder and Stephenson, 1968), and acts as a deterrent to the absorption of liquid (Lofts and Truter, 1969).

2.1.3 Fibre cortex

The cortex accounts for the major portion of the fibre. The cortex is composed of filamentous bundles of keratin. At the finest level, α-helical molecular chains twist together to form protofibrils, which then combine to form microfibrils. The microfibrils are packed together like multi-stranded ropes and cemented in place by a non-fibrous protein matrix, forming macrofibrils. The macrofibrils are the main structural component of the cortex (Figure 2.1), (Ryder and Stephenson, 1968; Chapman and Ward, 1979; Powell and Rogers, 1990).

Figure 2.1 Diagrammatic representation of the structure of a medullated fibre, adapted from Ryder and Stephenson (1968).



The cortex can be divided into regions of orthocortex and paracortex, the cells of which differ in size, shape and chemical composition. Orthocortical cells are larger in cross-section and have smaller, discrete macrofibrils, which are arranged in a whorl-like pattern (Chapman and Gemmell, 1971; Kaplin and Whiteley, 1978). The smaller cells of the paracortex have fused, poorly-defined macrofibrils (Kaplin and Whiteley, 1978), appear to be more heavily keratinised (Fraser and Short, 1960) and

the macrofibrils are arranged in hexagonal arrays (Rogers, 1959). Intermediate mesocortical cells, with macrofibrils less-defined than the paracortex, have also been reported (Brown and Onions, 1960; Kaplin and Whiteley, 1978; Orwin, 1988; Orwin and Bailey, 1988; Powell *et al.*, 1988). The proportion and distribution of cortical cell types varies within and between fibres (Chapman, 1976; Orwin *et al.*, 1980; Orwin *et al.*, 1984).

The differentiation process for cortical cell types is largely unknown. However, the presence of ortho- and paracortical cell types has been related to the external crimp of fibres (Horio and Kondo, 1953; Mercer, 1953; Mercer, 1954; Mercer *et al.*, 1954). Fine, highly-crimped fibres show definite bilateral segmentation of the ortho- and paracortex (Ryder and Stephenson, 1968; Orwin, 1979). In coarser fibres, with little crimp, segmentation is not as well-defined and areas of paracortex are replaced by mesocortex (Auber and Ryder, 1956; Kaplin and Whiteley, 1978; Orwin *et al.*, 1980). A known exception to these patterns of cortical cell distribution is 'steely' Merino wool. Such wool lacks crimp, due to a copper deficiency, yet expresses bilateral segmentation of the cortical cell types (Nott and Sikorski, 1965).

Non-keratinous protein surrounds the macrofibrils of the orthocortex but is found only in a few areas between the macrofibrils of the paracortex (Kulkarni *et al.*, 1971; Peters and Bradbury, 1976). The network structure of the macrofibrillar matrix of the orthocortex, aids movement of water and makes the orthocortex more wettable than the paracortex (Bradbury, 1973). Mercer (1954) and Mercer *et al.* (1954) proposed that fibre crimp results from these differences in the reactivity of the cell types to water, with the more reactive orthocortex always oriented on the outside of the curve. The curvature of crimped fibres means the orthocortex is covered with a single layer of slightly over-lapping cuticle cells while the paracortex, on the inside of the curve, is covered with multiple layers of more extensively overlapped cells (Kassenbeck, 1958 (cited in Brady, 1990)). The greater cuticle coverage of the paracortex hinders the uptake of water and dyes by these cells (Ryder and Stephenson 1968).

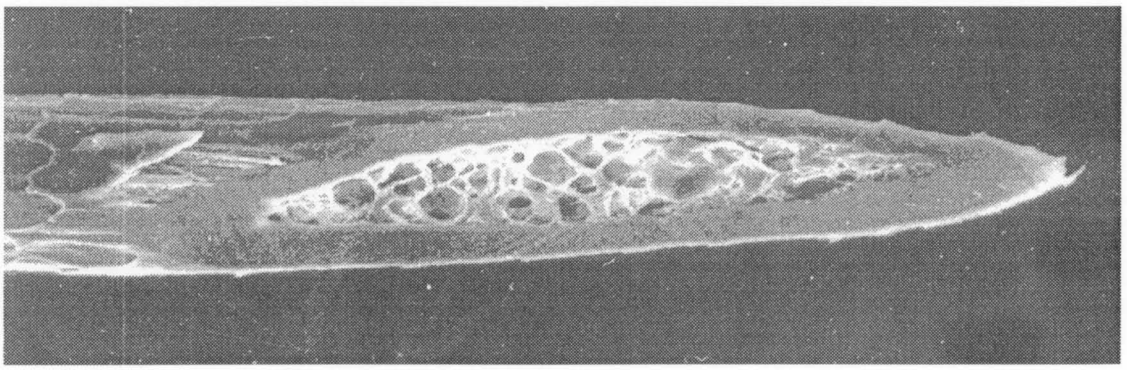
2.1.4 Medulla

The medulla is not an integral part of all wool fibres. The formation of a medulla may be continuous or intermittent and medullae may be unicellular or multicellular in width (Orwin, 1979). Medullae are typically associated with coarser fibres (Duerden, 1926; Lang, 1947; Skårman and Nõmmera, 1954; Orwin, 1979; Orwin, 1988), although Scobie *et al.* (1993) reviewed work in which medullae occurred in fibres ranging in

diameter from 14 μm to 70 μm. Fibres without a medulla, from the same samples, ranged between 10 μm and 68 μm in diameter.

The medulla is a tube-like cavity in the centre of the cortex (Auber, 1950). Generally, a single medulla is present, although instances of multiple medullae within an individual fibre have been reported (Auber and Ryder, 1956; Doney and Smith, 1962; Perkin and Appleyard, 1964). Two main types of medullae have been identified; latticed and non-latticed (Roberts, 1930; Wildman, 1954). Latticed medullae are most commonly associated with kemp and heavily medullated fibres, such as those from Drysdale sheep (Figure 2.2). In such fibres, the medulla occupies the major part of the cortex. The lattice structure results from the collapse of the cells, with the collapsed cell walls forming the lattice and vacuoles occurring between the cell walls (Ryder and Stephenson; 1968).

Figure 2.2 Scanning electron micrograph showing the vacuolar nature of the medulla of an obliquely cut, keratinised Drysdale fibre. x 675. (Orwin, 1979)



Non-latticed medulla are more widespread in their occurrence. The medulla does not occupy such a large part of the fibre. Wildman (1954) categorised non-latticed medullae as continuous, interrupted or fragmented, based on the proportion and frequency with which they occur. As the name suggests, continuous medullae form an unbroken tube within the cortex. Interrupted medullae occur when an otherwise continuous medulla is broken at irregular intervals by areas of cortical cells. The third category, fragmented medullae, occur when an almost continuous cortex is broken at intervals by areas of medulla. The presence and type of medullae vary with genotype (Tibbits, 1959), season and fibre diameter (Scobie *et al.*, 1993).

Brown and Onions (1960) reported the occurrence of mesocortical cells linking together areas of fragmented and interrupted medulla within the cortex, an observation

which suggested mesocortical cells could be considered as incipient medullation (Ryder, 1966). Scobie and Woods (1992) put forward a mathematical proposition that the cell types within the fibre form a continuum, from the fully keratinised paracortex through to non-keratinised medulla.

2.2 Composition of the wool fibre

Keratin is the principal constituent of wool. Keratin molecules are formed by the polymerisation of amino acids, resulting in long chain polyamide molecules with a multitude of side chains. The keratin molecules are held together with cystine links, which occur within and between the chains. Structural stability of the fibre is further aided by salt links and the weaker attractions of hydrogen bonds (Mercer, 1953; Bendit, 1956; Morton and Hearle, 1962).

The structure of keratin molecules has only been described in general terms as it varies between fibre components, along the length of the fibre and between fibres. The composition of the keratin also varies, depending on genotype, nutritional status and the physiological state of an animal (Alexander and Hudson, 1963; Marshall and Gillespie, 1988).

2.2.1 Cortex

Proteins within the cortex have been broadly identified and categorised as low sulphur, high sulphur, ultra-high sulphur or high tyrosine types. The low sulphur proteins account for approximately two thirds of the protein in wool and are the main component of microfibrils. The high sulphur proteins constitute the major part of the matrix which surrounds the microfibrils. Ultra-high sulphur proteins are also present in this matrix (Fraser *et al.*, 1972; Fraser *et al.*, 1973; Reis, 1979; Black, 1987). The high tyrosine proteins are found mostly in the matrix between the macrofibrils. The bilateral structure of the cortex also reflects a difference in composition. The paracortex has a higher cystine content than cells of the orthocortex (Mercer *et al.*, 1954; Fraser and Short, 1960) and also a greater proportion of non-keratinous material (Mercer, 1953).

2.2.2 Medulla

Medullae are comprised of cell walls and intracellular vacuoles (Section 2.1.4), the vacuoles accounting for a substantial portion of the volume of the medullae. The

composition of the gas enclosed in the vacuoles is unknown, but is most probably air (Ryder and Stephenson, 1968).

The cells of medullae are believed to be cortical cells in which keratin synthesis has failed to occur (Auber, 1950; Nott and Sikorski, 1965), possibly when the rate of cell division exceeds the capacity of the follicle to form keratin (Scobie *et al.*, 1993). Three main components have been identified in cells isolated from medullae; carbohydrate, lipid and protein. The carbohydrate and lipid components account for a small portion of the medullary substance. The nature of the carbohydrate component is unknown while the lipid component is known to contain cholesterol and small quantities of phospholipids. While protein comprises approximately 96% of the medullary mass (Matoltsy, 1953), the major portion of medulla volume is occupied by gas.

The protein component of medullae contains amino acids similar to those of the cortex. Medullary proteins, however, are characterised by the presence of citrulline (Matoltsy, 1953; Rogers, 1962; Steinert *et al.*, 1969), a high glutamic acid content and a low cystine content (Harris *et al.*, 1942 (cited in Bendit, 1956); Earland *et al.*, 1962; Steinert *et al.*, 1969; Keogh and Haylett, 1973). Citrulline has not been found in the cortical cells of medullated or non-medullated wools (Bradbury and O'Shea, 1969). Medullary cells, isolated from divergent sources (porcupine quill, fibres from rabbit, platypus, kangaroo, and chinchilla fur) are remarkably similar in composition (Matoltsy, 1953; Bradbury and O'Shea, 1969; Keogh and Haylett; 1973).

2.2.3 Cuticle

The composition of the cuticle is elusive. The epicuticle, containing keratinous proteins and lipids, is chemically inert (Lofts and Truter, 1969; Bradbury *et al.*, 1971). The endocuticle and exocuticle appear to contain the same amino acids as the fibre cortex, except that the proportions are different (Alexander and Earland, 1950). MacKinnon *et al.* (1990) suggest the cuticle contains a higher proportion of ultra-high sulphur proteins than the cortex.

2.3 Physical dimensions of the wool fibre

The Textile Institute defines a fibre as a unit of matter characterised by flexibility, fineness and a high ratio of length to thickness (Morton and Hearle, 1962). The physical dimensions encompassed by this definition are important to the processing

performance of wool fibres. The accurate measurement of fibre dimensions allows the processing performance of a mass of fibres to be predicted. While it is theoretically possible to measure every fibre dimension, or those of a mass of fibres, it is not always practical, nor economically viable to do so. Certain fibre dimensions are related in such a way that the measurement of one dimension forms the basis for the calculation of another. The most important dimensions are outlined below.

2.3.1 Fibre diameter

Fibre diameter is a measure of the width of a fibre. The shapes of wool fibres range from circular to elliptical, and may also be irregular in cross-section (Anderson and Benson, 1953). One source of variation in diameter, along the length of elliptical fibres, will be the orientation of the fibre through the plane of measurement. Strictly, a ratio between the diameters of the major and minor axes should be used, but the different axis measurements are difficult to obtain (Morton and Hearle, 1962). Diameter is measured as the distance between the outside cuticle edges, across the centre of the fibre, ignoring plane of orientation. Practices for minimising measurement bias are described in Section 2.5. Estimates of the circumference and cross-sectional area of the fibre can be calculated from the measurement of fibre diameter.

2.3.2 Fibre length

The length of a fibre is simply the distance between the two ends of the fibre. The visco-elastic nature of a wool fibre under extension (Gourdie, 1989), and the extensibility due to fibre crimp, make it difficult to accurately measure the length of a raw wool fibre. The measurement of fibre length is a laborious process involving the manual straightening of individual fibres and measurement of their length directly against a rule, for long fibres (Morton and Hearle, 1962) or the projected images of short fibres (Fraser and Short, 1960). The manual straightening of fibres, however, introduces inaccuracies due to variation in tensions applied by different operators. A less laborious approach uses a digitiser tablet and pen to trace the length of fibres, mounted in a relaxed state, on a transparent backing (Denis O'Connell, *pers. comm.*).

2.3.3 Fibre density, mass and volume

Fibre density is the mass of fibres per unit volume, measured in grams per cubic centimetre. As such, fibre density can be derived from measurements of fibre mass and estimates of volume. Direct and indirect methods of measuring density are

described in Section 2.6. The terms density and specific gravity are often used interchangeably. While they are numerically equivalent, specific gravity is strictly a ratio of the density of a substance to that of water, and is therefore devoid of units.

For the purpose of this research, fibre mass will be measured as the weight of fibre, and expressed in grams. Fibre volume is defined as the space occupied by a given mass of fibre, and is usually expressed in cubic centimetres. The main components of fibre volume are the volume of keratin and, if present, the volume of medullae. The volume of a fibre can be estimated from measurements of fibre diameter and fibre length. This approach is not generally used, due to the laborious methods for measuring length (Section 2.3.2). In the case of non-medullated fibres, volume can also be estimated from measurements of mass, assuming constant density.

2.3.4 Dimensional changes with fibre growth

The rate of growth of continuously growing wool fibres varies with genotype, season and level of nutrition (Story and Ross, 1960; Nagorcka, 1979; White *et al.*, 1979; Black, 1987). Changes in growth rate are represented as changes in length and diameter along the length of individual fibres. Typically, a fibre of twelve months growth will exhibit maximum diameter and length growth in summer and minimum growth in winter (Story and Ross, 1960). Fibre composition also shows seasonal fluctuations. The cortex tends to contain a greater proportion of orthocortical cells (Black, 1987) and a medulla is more likely to be present as the diameter of the fibre increases during summer (Lang, 1947; Skårman and Nõmmera, 1954; Orwin, 1988).

2.3.5 Moisture absorption and changes in fibre dimensions

The fibre dimensions outlined in the previous section are established as the fibre grows. Changes in the diameter and length of a fibre can also be brought about when the fibre swells as a result of moisture absorption. Dimensional changes due to fibre swelling, translate to reversible changes in fibre volume (Morton and Hearle, 1962).

An appreciation of how moisture penetrates the cuticle and diffuses through the fibre can be gained from studies of dye uptake since dyestuffs are administered as an aqueous liquor. Diffusion into the fibre is highly influenced by the fibre structure, the initial uptake being directly related to cuticle structure (Brady, 1990; Schafer, 1994).

Dyes enter the fibre at the junctions between the cuticle cells and diffuse along the cell membrane complex (Ito *et al.*, 1987; Leeder *et al.*, 1990; Lewis, 1990; Schafer, 1994). The cell membrane complex, which is non-keratinous has more pronounced swelling properties than the keratinised cortical cells (Swift, 1992), thus aiding the movement of moisture into and through the fibre (Schafer, 1994).

Speakman (1944) proposed that, once inside the fibre, water molecules are bound firstly to hydrophilic sites in the side chains, then to amide groups in the main chain of the keratin molecules. Such absorption changes the forces between the molecules and breaks apart the hydrogen and ionic bonds within the fibre structure. The covalent bonds prevail and maintain the gross fibre structure. As further absorption occurs, water molecules attach in layers between the broken bonds, expanding the fibre dimensions (Pierce, 1929; Speakman, 1944). With desorption the reverse process occurs. The loosely bound layers of water molecules are the first to evaporate. Cross-links reform as the more tightly bound moisture is removed and the fibre returns to its dehydrated dimensions.

The same principles of absorption for a single fibre apply to a mass of fibres, except that the moisture in the air must first diffuse to the surface of the fibre mass, then into the spaces between the fibres. Moisture then moves from the interstices to the surface of individual fibres and diffuses to the interior of the fibres.

2.4 Characteristics of medullated fibres

Medullation is one of six major characteristics, of New Zealand wools, considered significant to processors (Ross, 1991). Medullation is desirable for the properties it imparts to finished carpets, giving an acceptable crisp handle, decreased lustre (Ross, 1988), improved carpet compression properties (Ince and Ryder, 1984), lower abrasion resistance and improved retention of appearance (Story, 1978).

Medullated wools are, however, undesirable from a mechanical processing point of view. They are associated with higher card wastage and lower yarn yields, compared to non-medullated wools. They are also more liable to break during processing, thus altering spinning efficiency (Ross, 1990; Ross, 1991). Identification and measurement of the degree of medullation is important so that the effect medullated fibres have on mechanical processing can be pre-empted. Medullated fibres impart lower strength and extensibility to yarn (Ince, 1977). The strength of continuously medullated fibres is lower than non-medullated fibres (Arora and Gupta, 1977; Rama Rao and Chopra,

1987; Ross, 1990) but the strength of fibres with interrupted or fragmented medullae may be similar to that of non-medullated fibres (Rama Rao and Chopra, 1987). Coarse, continuously medullated fibres are often clearly visible to the eye, whereas fibres containing interrupted and fragmented medullae may not be apparent (Ross, 1990). Methods for determining the degree of medullation in a wool sample are described in the following section (Section 2.5).

Medullated fibres are also undesirable in apparel fabrics. During processing, medullated fibres migrate to the outer surface of the yarn. This, and the generally greater mean diameter associated with medullated fibres, contributes to an undesirable 'prickle' effect. An exception is the inclusion of highly medullated or kemp fibres in tweed fabrics, where medullated fibres are desirable, for the speckled, natural look they give to the fabric (Ross, 1990). Medullae change the light reflective properties of non-pigmented fibres so they appear white and chalky in raw wool (Orwin, 1979). Additionally, the presence of medullae in processed wools alters dye uptake (Peryman, 1952) and the uniformity of colour (Duerden, 1926).

2.5 Methods of measuring medullation and fibre diameter

The amount of medullation in a wool sample can be expressed as percent by number, percent by area or percent by volume (Ranford *et al.*, 1990), depending on the measurement method employed. Care is needed, though, when interpreting the measurements. For example, a small number of heavily medullated fibres and a large number of fibres with little medullation would have different medullation when expressed as percent by number, but conceivably the same percent by volume. The differences, in practical terms, are that a small number of kemp or heavily medullated fibres will alter the appearance and handle of processed fabrics. In comparison, fibres containing interrupted or fragmented medullae, with the same degree of medullation (percent by number), may have little apparent effect on processing or product performance (Ross, 1990).

2.5.1 Projection microscope

The projection microscope provides a reference method, approved to international standards (IWTO, 1989), for measuring medullation and fibre diameter. When a medulla is present at the point of measurement, the diameters of the medulla and the fibre are measured concurrently. The type of medulla, whether continuous or fragmented, is also noted. The number of medullated fibres measured in a sample are

expressed as a percentage of the total number of fibres measured. The percent volume of fibre occupied by medullae can be calculated from the number, mean diameter and standard deviation of diameter of the medullated and non-medullated fibres, in accordance with Bray's formula (Bray, 1942; Appendix II).

Measurements are made by mounting fibre snippets on a glass slide, in a viscous medium which has a refractive index lower than that of wool (1.43 to 1.53 versus 1.55 for wool). The image, created by the material interfaces and the difference in refractive indexes of the mounting medium and the wool fibre, is magnified 500 times and projected onto a translucent screen. Fibre diameter and, if present, medulla diameter is read directly from the fibre image, using a graduated scale positioned at right angles to the fibre. Two slides per sample are prepared and measured, independently, by two operators. Each slide is traversed in a pre-determined step-wise progression until 250 fibres have been measured. Snippet lengths of 0.8 mm are recommended for fibres greater than 27 µm in diameter (Anderson and Palmer, 1951; IWTO, 1989). The measured diameter, particularly of finer fibres may be over-estimated, as short snippet lengths tend to lie in the direction of the major axis of ellipticity (Anderson and Palmer, 1951; Ryder and Stephenson, 1968). To allow mean fibre diameter to be measured without significant bias, Edmunds (1992a) recommended that snippet lengths of 2 mm be adopted for fibres with diameters of 30 µm and finer. In contrast to this, Browne and Hindson (1982) and Nissen-Wooller *et al.* (1994) found no significant difference in the mean fibre diameter of snippet lengths greater than 0.6 mm, or between snippets of 0.8 mm and 2 mm in length, respectively.

Measurements of medullation and fibre diameter by projection microscope are slow, operator dependent (Edmunds, 1992b) and relatively imprecise (Baxter *et al.*, 1991). This makes the method unsuitable for objective specification of large numbers of wool samples, which require rapid, accurate and economic measurement.

2.5.2 Airflow measurement of fibre diameter

The airflow method of measuring fibre diameter is widely used in the trading of raw wool. The method is standardised, simple and economical to execute. A wool sample of fixed mass is placed in a chamber of standard dimensions. Air is then forced through the packed mass of fibres. Mean fibre diameter can be calibrated to the decrease in pressure through the mass of fibres when a constant flow of air is applied. Alternately, the instrument can be set to constant pressure and mean diameter calibrated to the variation in air flow (IWTO, 1982).

Mean fibre diameter, the only characteristic measured by this method, is derived from the measurement of the specific surface of the fibres (Cassie, 1942). The specific surface of fibres being the surface area per unit volume of fibre. The flow of air, from which fibre diameter is indirectly measured, is influenced by fibre characteristics such as the variability of fibre diameter (James and David, 1968) and is also thought to be affected by specific gravity (van Luijk, 1984). Potentially these factors contribute to the poor measurement accuracy for medullated wools and lambswool.

2.5.3 Optical Fibre Diameter Analyser (OFDA)

The OFDA operates like an automated projection microscope. It is a video image analysis system which transforms optical images from a microscope into digital signals for computer processing. The software is critical to the success of the instrument as it synchronises a video camera, a strobe light source and positioning of a slide on a motor driven stage. Baxter *et al.* (1991) describe how a light emitting diode provides a bright field image, to which a sequence of algorithms are applied to automatically recognise and measure fibre diameter. Medullation is measured through fibre opacity, that is, the ability of the fibre to transmit light perpendicular to its length. Zero opacity occurs when the amount of light transmitted by the fibre is the same as that transmitted by a glass rod of the same diameter. Conversely, a metal rod is totally opaque, having a value of 100 % opacity (Turpie and Steenkamp, 1995). The internal homogeneity of a fibre affects the ability of the fibre to transmit light. The greater the ratio of medulla diameter to the external fibre diameter, the greater the opacity due to internal reflection caused by the medulla. Opacity measurements are carried out in conditions of dark field illumination, created by an annulus of five equally spaced light-emitting diodes (Brims and Peterson, 1994). Fibres are classified as medullated when opacity at the measurement site exceeds a threshold of 80 %.

The OFDA determines and records three categories of medullation; total medullation, objectionable medullation and flat medullated fibres. The OFDA records total medullation as all fibres which have opacities above a threshold value of 80%. Objectionable medullation is defined, by the OFDA, as fibres with diameter greater than 25 microns, and for which opacity exceeds 94%. The OFDA measurement of objectionable medullated fibres is highly correlated to the manual measurement of this same trait (Lee *et al.*, 1996). Objectionable medullation is defined as fibres that appear optically different to surrounding fibres, when viewed with the naked eye under controlled light conditions (Turpie and Steenkamp, 1995). Such fibres are usually

undesirable in processed products. Fibres that do not stand out as optically different may also contain medullation, hence the reference to non-objectionable medullation. The third category, flat medullated fibres, identifies ribbon-shaped fibres with a high ratio of medulla diameter to fibre diameter. The collapsed structure of such fibres generally gives rise to an opacity of less than 80%, but produce a wide light band under dark field illumination. A further criterion for the detection of flat medullated fibres by OFDA is that they must have a diameter greater than 60 microns (Turpie and Steenkamp, 1995; IWTO, 1996).

The OFDA is a rapid system, capable of measuring up to 10,000 fibres per minute. Measurement time varies depending on the measurement options selected and the capacity of the computing hardware. Like the projection microscope, the OFDA records medullation as percent by number and calculates an approximate percent by volume. The OFDA uses automation to minimise variation and bias due to operators. Relatively poor precision at individual measurement sites is overcome by making a large number of measurements per sample (Peter Maher, *pers. comm.*). Potential bias of diameter measurements due to fibre ellipticity is overcome using longer snippet lengths (2 mm), removing the tendency of short fibres to settle on their major axis. The methods for measuring mean fibre diameter, standard deviation of diameter (IWTO, 1995) and medullation (IWTO, 1996) have international approval as test methods. The development of new software which enables the OFDA to measure further fibre characteristics is an ongoing process.

The main advantages of the OFDA, compared with measurement by the airflow method, are the ability to measure the variability of fibre diameter and estimate medulla content. The OFDA also has the ability to measure frequency distributions of fibre diameter and medullation. Unlike the airflow method, the OFDA is not dependent on an assumption of constant density.

2.5.4 WRONZ Medullameter

The Wool Research Organisation of New Zealand (WRONZ) medullameter was designed for rapid measurement of medullation of a wool sample, without regard for individual fibre characteristics. The principle is not new. It was first used by Elphick (1932), developed into a working instrument by McMahon (1937), improved by Belin and Goldstone (1951) then further improved by Lappage and Bedford (1983).

The wool sample is immersed in a medium with a refractive index close to that of wool. Fibres without a medulla are essentially invisible in such a medium, as there is no reflection of light at the interface between the liquid and the surface of the wool fibres. When fibres are medullated, light is refracted at the interface between the keratin and the vacuolated space. The amount of light refracted is related to the proportion of the fibres occupied by the medullae. The intensity of the refracted light is detected by a photosensitive cell and the signal output measured as an electrical current. The output, by convention, is calibrated to projection microscope results of percent medullation by number. Alternatively, the output could be calibrated to projection microscope measurements of percent medullation by volume.

Although medullation measurement using the WRONZ medullameter is rapid relative to the projection microscope, it is slower than using the OFDA. It is also a destructive technique and involves the use of unpleasant chemicals.

2.5.5 Near-Infrared Reflectance Analyser (NIRA)

Estimates of medullation can be made using reflectance in the near-infrared region (1100 to 2500 nm) (Hammersley *et al.*, 1995). The proportion of light absorbed at known wavelengths in the near-infrared spectrum is proportional to the volume of wool fibre occupied by medullae. Thus results are expressed as percent medullation by volume. The results can also be expressed as percent by number of medullated fibres when calibrated against projection microscope.

2.6 Measurement of density

Since the early nineteenth century, attempts have been made to measure the density of wool, either directly, or from measurements of sample mass and volume. Barker (1931) states that density is one of the fundamental characteristics of wool that appears to be relatively constant and emphasised the importance of an accurate method to measure density. However, at the time of writing this thesis there was no standardised or commercially viable method of measuring the density of wool.

2.6.1 Liquid displacement methods

The earliest records of specific gravity measurements for wool date from the early nineteenth century. Applying Archimedes' principle, Ure (cited by Barker, 1931) measured the volume of a wool sample in a flask of water. Such values, obtained in water, are apparent specific gravity as wool fibres absorb moisture. The true volume, from which specific gravity is derived, will vary with different displacement liquids, depending on the degree to which the liquids are absorbed by the fibre and the contact between the liquid and the fibre surface (Davidson, 1927). King (1926) found absorption was minimised when organic liquids such as benzene, toluene, nitrobenzene, olive oil or oleic acid were used as displacement media. Thus, the true specific gravity of non-medullated wool, in benzene, was reported as 1.304. A tendency towards lower values in kemp fibres was noted (King, 1926).

2.6.2 Specific gravity bottles

Van Wyk and Nel (1940) and Fraser and MacRae (1957) developed a method using specific gravity bottles to gravimetrically determine the specific gravity of wool. This method, sometimes referred to as a benzene pycnometer method, was time consuming. Specific gravity was derived from the difference in weight between sealed flasks containing benzene, benzene plus wool, and water. Values of specific gravity measured by this method agreed well with those of the earlier liquid displacement methods.

2.6.3 Density gradient column

To determine the density of individual fibres or small bundles of fibres, Abbott and Goodings (1949) developed a floatation method, whereby fibres were chopped up and centrifuged in a liquid, or mixture of liquids with a density similar to that of the fibres. The fibres remained uniformly spread throughout the liquid when the densities of the fibres and liquid were the same. The fibres accumulated in a single group when the densities of the fibres and liquid were different. The group of fibres floated when the density of the fibre was less than the density of the liquid, and sank in the reverse situation.

The density gradient column is an adaptation of the method of Abbott and Goodings (1949). The column contains a heavy liquid (eg. pentachlorethane, 1.7 g/cm^3) at the

bottom, a light liquid (eg. xylol, 0.9 g/cm^3) at the top and a gradually varying mixture of the two in between (Preston and Nimkar, 1950; Stock and Scofield, 1951; Austin and Roberts, 1956). Fibres placed in the top of the column sink to the point at which the density of the surrounding liquid equals that of the fibres. The density gradient column provides a direct measure of density for small quantities of wool. The method is relatively slow, requiring approximately 16 hours for a sample to reach equilibrium.

Under standard measurement conditions and using a column containing a mixture of t-butanol and tetrachloroethene, Connell and Andrews (1974) recorded a density of 1.305 g/cm^3 for non-medullated wool, the same as measured by the previously described methods.

2.6.4 Gas displacement methods

As an alternative to using liquid as the displacement media, Davidson (1927) explored the use of helium gas to measure the volume of raw cotton. By measuring changes in volume, temperature and pressure when helium was introduced to a chamber containing the cotton sample, specific volume could be measured. Volume remained unchanged after 24 hours, indicating helium was not absorbed by the cotton and that the true specific volume was being measured.

A rapid, automated device, using the same principles as Davidson (1927) has been developed to measure the volume of finely powdered and porous solids. The helium pycnometer (Horiba Instruments, Kyoto, Japan) consists of two chambers connected by a series of valves. The volume of a sample of known weight is determined by measuring the volume of gas displaced within a chamber when the sample is introduced. This is achieved by equalising the pressure difference that arises when gas is displaced by the sample. The density of the sample can be calculated from the sample mass and volume measurements.

A reference method exists in the paint manufacturing industry, for determining the density of coating powders, using a helium pycnometer (ISO 8130-2, 1992). Preliminary work, developing the application of a helium pycnometer to the measurement of wool volume, has established sequential measurement and sample mass requirements (Merrick *et al.*, in press). Further work is needed to complete development of an appropriate method.

3. General Materials and Methods

3.1 Principle of the helium pycnometer

The gas pycnometer measures the volume of a sample by application of a gas phase displacement method. The pycnometer used for the research presented in this thesis was a helium pycnometer, manufactured by Stec Incorporated (No.2 Kisshoin, Minami-Ku, Kyoto, Japan). The helium pycnometer consists of two chambers, of known volume, connected by a series of valves. The sample is sealed in the measurement chamber, the system purged to atmospheric pressure (P_A), helium gas pumped into the measurement chamber and the pressure (P_1) measured. The gas is then released to the second chamber, where it expands to the volume of the chamber. The resultant pressure (P_2) is measured and the gas expelled.

Two common gas laws form the basis for the calculation of sample volume.

These are: (i) the ideal gas law, $PV = nRT$

and (ii) Boyle's law, which states that, at a given temperature, the product of pressure and volume of a definite mass of gas is constant,

i.e. $P_1V_1 = P_2V_2$

where: P = pressure; V = volume; n = moles of gas;

R = universal gas constant; T = temperature

Sample volume is calculated according to the equation:

$$V_X = V_1 + \frac{1}{1 - \frac{P_1 - P_A}{P_2 - P_A}} \cdot V_2$$

The full derivation of the equation can be found in Appendix III.

The variables V_X, V_1 and V_2 represent the volumes of the sample, measurement chamber and second chamber, respectively. Clearly, when six of the variables are known the equation can be solved for the remaining variable.

3.2 Operation of the helium pycnometer

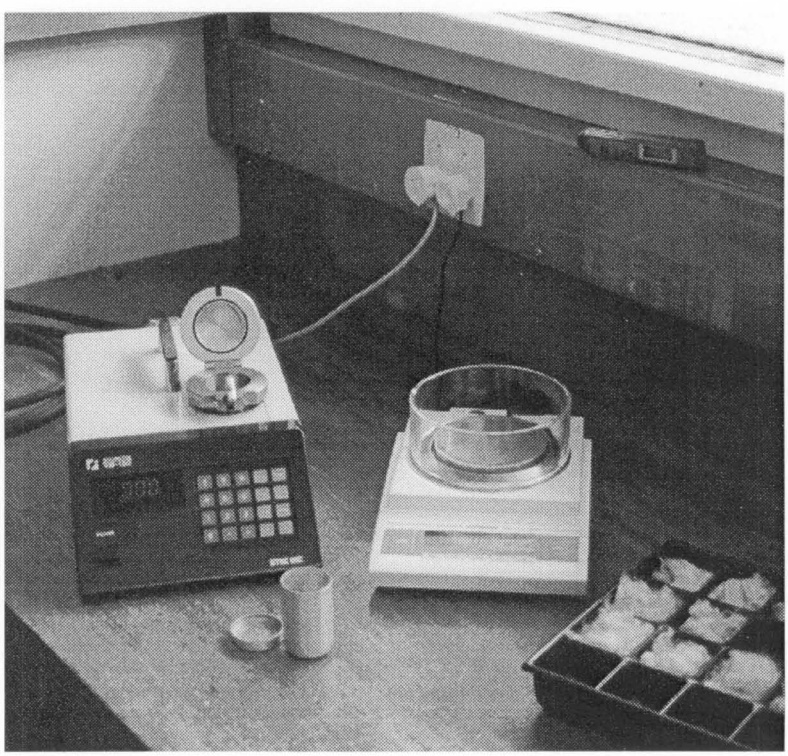
Operation of the pycnometer is semi-automated. Once connected to a supply of helium, with a purity no less than 99.99 % and supply pressure regulated to approximately 20 psi, the pycnometer requires calibration at the zero and upper limits. When initiated, the automated calibration sequence makes four sequential

measurements at each limit and requires around fifteen minutes to complete the procedure. The limits are represented by the empty sample cup and the sample cup plus a stainless steel ball of known volume.

To make the volume measurement, the mass of the conditioned sample is recorded and the sample placed in the sample cup in the measurement chamber. The required number of sequential measurements is entered on the numeric key pad and the automated measurement process started. Individual measurement cycles are completed in ninety-nine seconds. For the experiments in this thesis the recommendations of Merrick *et al.*, (in press) have been adopted. A sample mass between one and ten grams and the fourth sequential volume measurement have been used.

When measurement is completed, sample volume (cm^3) is displayed. The density of the sample (g/cm^3) can then be calculated as sample mass divided by sample volume. This calculation can be performed automatically by this model of pycnometer.

Figure 3.1 The helium pycnometer (on the left) and Sartorius balance (centre) ready for the measurement of wool samples (right). The measurement cup is in the foreground and at the top right of the figure is a thermo-hygrometer, used to record temperature and humidity.



3.3 Standard measurement conditions

Moisture regain in wool, and the rate at which it occurs, varies with humidity and temperature (Watt *et al.*, 1959; Morton and Hearle, 1962; Wortmann and de Jong, 1985). To minimise the effects of humidity and temperature on fibre dimensions, measurements were carried out under constant environmental conditions. Standard conditions of 20 ± 2 °C and 65 ± 2 % relative humidity (Warburton, 1947; Morton and Hearle, 1962) were used for all experiments presented in this thesis.

3.4 Procedures for measurement of medullation and fibre diameter

There are two main ways in which measurements of medullation can be expressed:

(i) percent by number (% by number) i.e. the number of medullated fibres measured in a sample, expressed as a percentage of the total number of fibres measured.

(ii) percent by volume (% by volume) i.e. the proportion of fibre volume occupied by medullae.

Measurements of medullation (% by number) and fibre diameter made using the projection microscope were carried out in accordance with IWTO-8-89(E). Bray's formula (Bray, 1942) was used to calculate percent medullation by volume values from projection microscope measurements of percent medullation by number, medullae diameter and fibre diameter. Medullation measurements made using the WRONZ Medullameter followed WRONZ internal guidelines (Lappage and Bedford, 1983; WRONZ Internal Method 01.20, 1989). OFDA measurements adhered to International Wool Textile Organisation (IWTO) guidelines IWTO-47-95 and IWTO Draft TM-57-96 for fibre diameter and medullation measurements, respectively. NIRA measurements of medullation followed the procedure outlined by Ranford *et al.* (1990) and Hammersley *et al.* (1995).

3.5 Statistical analyses

Statistical analyses were carried out using Genstat® for Windows, Release 4.1, ©Lawes Agricultural Trust, IACR - Rothamsted. Analyses applied to specific experiments are detailed in the materials and methods section of each experiment.

4. The effect of sample length on the measurement of volume and estimates of density

4.1 Introduction

The preliminary development of a method to measure the volume of wool samples, using a helium pycnometer, has been reported by Merrick *et al.* (in press). The effects of sequential measurement, conditioning humidity and sample mass on the volume measurement have been determined. The fourth measurement from a cycle of four, at standard conditions (65 % relative humidity, 20 °C) and using a sample mass between two and ten grams were recommended. The effect of sample length was not investigated.

The aim of this experiment was to determine the effect that fibre length within a sample had on measured volume and density, and to recommend the use of appropriate fibre lengths for the measurement of wool using a helium pycnometer. The hypothesis tested was that the measured volume of a wool sample, of constant weight, was not affected by the length of fibres within the sample.

4.2 Materials and methods

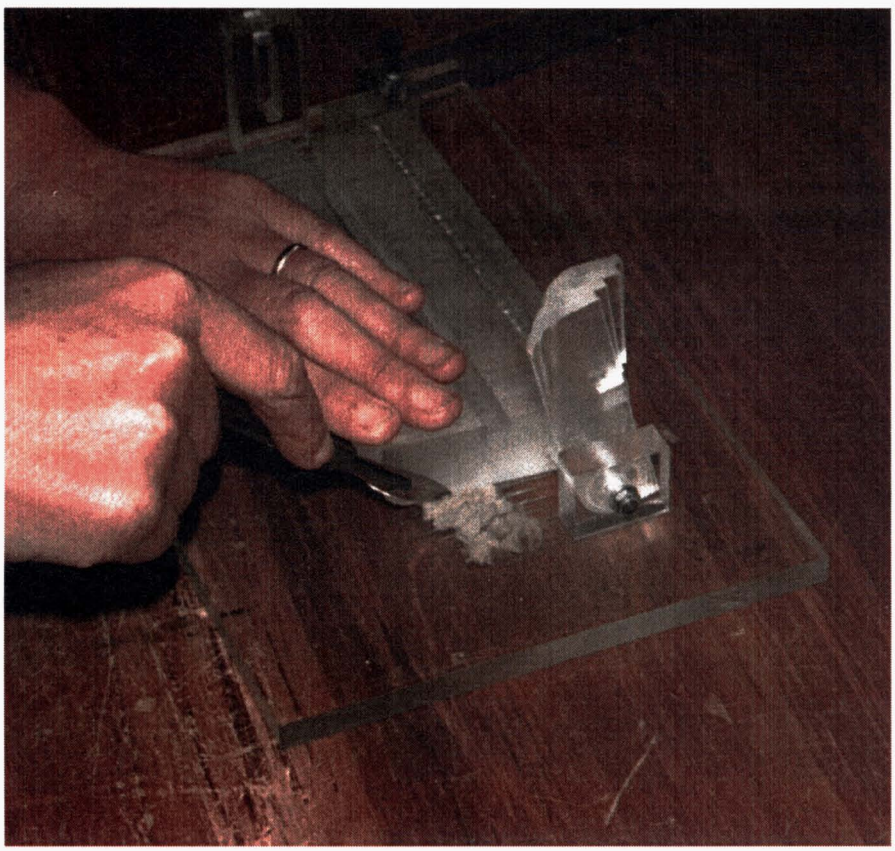
Midside wool samples of twelve months growth were harvested from twenty one sheep, representing fine, coarse and two specialist wool types; medullated and high bulk. The sheep breeds used were Merino (n=5), Crossbred (n=5 {Romney (n=3) and Coopworth (n=2)}), Cheviot (n=6) and Drysdale (n=5). Three replicates, of each length treatment group, were established for each animal. Homogenous treatment groups were created by extracting a block of staples from the centre of the mid-side sample of greasy fleece. Individual staples were randomly allocated to the treatment groups. The extraction of blocks and randomisation of staples was repeated, using adjacent blocks of staples and distinct randomisation sequences, until each treatment group contained five grams of greasy wool. When the first replicate was complete the process was repeated for the second and third replicates.

Two groups of samples from each replicate were used for the full length and 10 mm treatments. Staples from the 10 mm treatment were cut to length using a cutting board modified from the design of Hansford *et al.* (1985). Forty perspex slides, each 5 mm wide, and with a hole drilled through one corner, were threaded onto a bolt and suspended between two supports attached to a perspex base. Staples were placed

between the base and slides, held intact by applying downward hand pressure, and cut to length using a scalpel blade (Figure 4.1). The entire length of all staples within a treatment were cut to the required length. This avoided any bias of fibre characteristics varying along the length of the staples. The length of staples were measured against a rule, prior to cutting.

Two further groups of samples were prepared and measured, one for percent medullation by volume, using a WRONZ Medullameter (WRONZ Internal Method 01.20, 1989), the other was mini-cored and measured for fibre diameter, using an OFDA and IWTO-47-95 (IWTO, 1995). These measurements were made to describe the characteristics of the samples.

Figure 4.1 The perspex cutting board, used to prepare 10 mm length treatments, modified from the design of Hansford *et al.* (1985).



The length treatments, and samples for measurement of medullation and fibre diameter, were detergent scoured using a three minute warm wash (40°C) in an aqueous solution of Teric GN9 (0.15%), rinsed twice in warm water (35°C), oven dried for 24 hours at 45°C and conditioned for 24 hours at 20 ± 2°C and 65 ± 2% relative humidity.

The volume of each sample in each length treatment group was measured with a helium pycnometer, under standard conditions (20 ± 2°C, 65 ± 2% relative humidity) and using the fourth sequential measurement, as recommended by Merrick *et al.* (in press). A mass of 2.50 ± 0.05g was used for all samples. The order of measurement was randomised across wool types and animals. Length treatments were randomised within animals. Replicates were measured in sequential order, with each replicate having a unique randomisation sequence. The effect of fibre length on the measured fibre volume, and fibre density, was determined using a paired t-test. Fibre density was calculated from the measured mass and measured volume of each sample.

4.3 Results

The wool types used to investigate the effect of sample length on measured volume differed significantly in staple length, mean fibre diameter and percent medullation by volume (Table 4.1). Merino and Drysdale wool represented extremes for these characteristics. Crossbred wool was intermediate for all characteristics. Cheviot wool was similar to Crossbred wool in fibre diameter, but had a medulla content closer to that of Drysdale wool.

Table 4.1 Mean staple length, mean fibre diameter and medulla content of full length wool staples, for the four breeds used to determine the effect of sample length on measured volume.

	Wool Type				
Fibre Characteristics	Merino	Crossbred	Cheviot	Drysdale	lsd $_{0.05}$
mean staple length (mm)	91	180	117	353	23
mean fibre diameter (μm)	18.9	35.0	38.7	54.4	3.4
medullation (% by volume)	0	3	33	44	8

Neither volume nor density differed significantly (p=0.261 and 0.224, respectively) between 10mm and full length samples, for all wool types (Table 4.2). Significant differences in fibre volume and fibre density between wool types were apparent. The fibre volume of Drysdale wool was significantly greater than Merino, Crossbred and Cheviot wool (p<0.001) for both 10 mm and full length treatments. Drysdale and Cheviot wool had significantly lower density (p<0.001) than wool with little or no medullation, for 10 mm and full length treatments. Breed comparisons not mentioned were non-significant.

Table 4.2 Mean fibre volume and mean fibre density measurements of 10 mm and full length treatments, of three replicates of a 2.5 g sample, for four wool types.

		Merino	Crossbred	Cheviot	Drysdale
Volume	10 mm	2.009	2.019	2.104	2.643
(cm^3)	full length	2.041	2.032	2.136	2.704
	$sem_{0.95}$	0.026	0.028	0.048	0.090
Density	10 mm	1.247	1.244	1.193	0.935
(g/cm^3)	full length	1.227	1.235	1.177	0.957
	$sem_{0.95}$	0.016	0.017	0.023	0.032

4.4 Discussion

There was no significant difference between volume or density of wool from the same animals when measured either on full length staples or those cut into nominal 10 mm sections. It would seem reasonable to assume that intermediate lengths would not affect fibre volume or fibre density measurements either. The absolute length to which samples were cut would vary with the angle of the fibres between cutting points, the amount of crimp in both the staple and individual fibres and the movement of fibres within the cutting device.

The volume and density values for the different breeds are indicative. The breeds were chosen for the range of characteristics they typically possess. Similarly, animals within each breed were selected to represent a wide range of fibre characteristics for those breeds.

The density of non-medullated wool (1.238 g/cm^3) was similar to that previously measured (1.304 g/cm^3, van Wyk and Nel, 1940; 1.312 g/cm^3, WIRA, 1973; 1.305 g/cm^3, Connell and Andrews, 1974; 1.307 g/cm^3, Merrick and Scobie, 1997). The density of the medullated wools was significantly lower than that of non-medullated wools, decreasing as the level of medullation increased (1.185 and 0.946 g/cm^3 for Cheviot and Drysdale, respectively). This concurred with the findings of Merrick and Scobie (1997) whereby fibre density was related to medulla content. The relationship between medulla content and fibre density was not clear and will be investigated in a later chapter (Chapter 6).

The published density of non-medullated wool translates to a volume of approximately two cubic centimetres, for a sample mass of two and a half grams. The manufacturers

specify the sample volume range, within which the helium pycnometer should be operated, as zero to twenty cubic centimetres. The helium pycnometer was clearly working at the low end of its range when measuring wool volume in these experiments. Even if the ten gram maximum mass of wool were measured (Merrick *et al.,* in press), the sample volume (7.6 cm^3) would remain below half the recommended capacity of the helium pycnometer.

Prior to the experiment reported above, a more extensive set of length treatments were created from the same wools. The results for the six treatment lengths from that experiment are presented below, because they support the conclusions about length which were based only on 10 mm and full length samples. Full length, 50 mm, 20 mm, 10 mm and 5 mm treatment lengths were prepared, using the cutting board described above (Figure 4.1). Snippet lengths of 2 mm were prepared using a guillotine designed specifically for the preparation of slides for measurement by a Sirolan-Laserscan (Appendix IV). Length treatments were randomised within animals, animals randomised within breeds, and wool types measured as blocks. The effect of fibre length on measured volume was determined using analysis of variance.

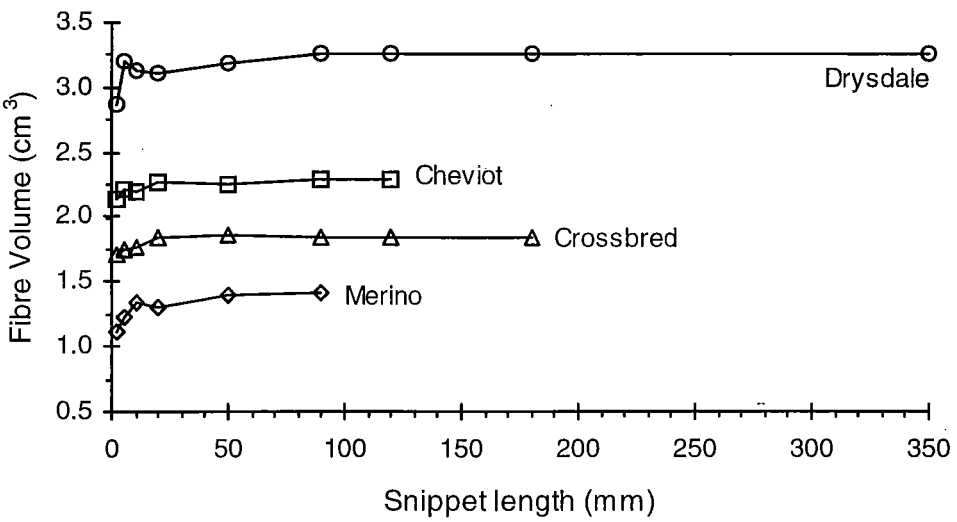
On completion of the volume measurements for all six length treatments, calculation of density revealed inconsistencies between the density of the non-medullated Merino wool (1.707 g/cm^3) and previous measurements for the same breed, made on full length wool (1.304 g/cm^3, van Wyk and Nel, 1940; 1.305 g/cm^3, Connell and Andrews, 1974; 1.307 g/cm^3, Merrick and Scobie, 1997). The helium pycnometer was returned to the manufacturer for servicing, and the internal calibration carefully checked, prior to measurement of the full length and 10 mm treatments reported in Section 4.3. The mean volume of samples did not differ significantly from that of the full length and 10 mm treatments measured prior to servicing ($p=0.542$). However, the coefficient of variation of the volume measurements reduced significantly, from 13% to 7% ($p=0.023$). A combination of the relatively low density of wool and short fibre lengths (<10 mm) appear to have enabled fibres to move from the measurement chamber with the release of the gas. This potentially obstructed filters and/or valves, and resulted in erroneous measurements. The cause of the fault could not be confirmed by the manufacturer, but fibres were lodged in a filter, examined using a light microscope.

Given the progressive nature of the failure of the helium pycnometer, the actual volume and density values for the larger set of length samples should be regarded with caution. Comparisons of sample length within wool types, for all six length treatments, remained valid as there was no significant interaction between fibre length and the

order of sample measurement within wool type (p=0.534), nor between wool type and fibre length (p=0.781).

The volume measurements for the six treatment lengths, at equal mass, are presented below (Figure 4.3). Volume did not differ significantly, within wool type, for treatment lengths equal to or greater than 10 mm (p=0.157). The volume of 5 mm and 2 mm treatments, however, was significantly less than the volume of treatments 10 mm and above (p<0.001), for all wool types. The decline in volume of the 5 mm and 2 mm treatment lengths appeared greater for the Merino wool type.

Figure 4.2 Changes in measured fibre volume with increasing length of a 2.5 g sample for Merino, Crossbred, Cheviot and Drysdale wool types (pre-service measurements).



It was expected that as shorter fibre lengths were measured the density of medullated wools would approach that of non-medullated wools. Initially, an experiment to determine the density of keratin, by grinding the wool fibres to a powder, was considered. Grinding the fibres would fracture the medullae within the sample and minimise or eliminate the effect of medullation on fibre volume and fibre density. The idea was not pursued when it became apparent that the helium pycnometer was unable to handle short fibre lengths.

The differences in fibre volume and density, for fibre lengths less than 10 mm, may have been due to blockages within the helium pycnometer. If not, then these differences could be explained by the absorption of the helium gas during measurement. Calculations of fibre volume, the surface area of the fibre and the ratio between these two, show that

changes in snippet length alter the surface area to volume ratio only marginally, assuming mean fibre diameter and sample mass remain constant. However, the same calculations of surface area to volume ratio show that if the mean fibre diameter is halved then the surface area to volume ratio of the fibre will almost double, at a constant fibre length. The fibre cuticle deters the absorption of liquids (Lofts and Truter, 1969) and, potentially, the absorption of gas, leaving the exposed or cut ends of the fibre as the most likely points of entry for helium. The absorption of helium by the fibre, and consequent measurement of volume and density, is likely to have been affected only marginally by changes in fibre length. The absorption of helium could differ between wool types that vary in mean fibre diameter. The entry of helium into the fibre was not investigated in this study, but Davidson (1927) reported that helium was not absorbed by raw cotton fibres over a period of 24 hours. It therefore seems reasonable to assume that helium was not absorbed by the wool fibres within the 7 to 8 minute exposure time these experiments used to measure volume. The uniform volume and density of samples longer than 10 mm indicates that the absorption of helium was either constant for all wool types or did not occur to the extent that it influenced density.

Loss of fibre during transfer of the sample between the balance and the sample cup could explain why the fibre density of the 2 mm and 5 mm sample lengths was significantly less than the longer treatment lengths. The potential for fibre loss is greater for shorter fibres. No measurement of fibre loss was made for this experiment. Measurement of sample mass immediately prior to and following the measurement of fibre volume is recommended for future experiments.

In medullated fibres, gas could easily flow into vacuolated medullary cells exposed at the ends of fibres, the degree of permeation depending on the lattice structure of the medullae (Section 2.1.4). The decrease in volume and increase in density of the 5 mm and 2 mm Drysdale samples was potentially due to the greater number of cut fibre ends exposing more vacuolated medullary cells. The gas did not appear to fill all medulla within the fibre, as the density of heavily medullated wool was significantly lower than that of non-medullated wool, even at the 2 mm snippet length. If the medulla were non-latticed, like an open tube, helium would enter the fibre, thus decreasing the fibre volume measurement and increasing the fibre density closer to that of non-medullated wool. Diffusion of helium through the medullae, from the fibre ends, would be a slow process, due to the small diameter of medullae. It is unlikely that the diffusion of helium would significantly affect the volume measurements within the short time required for measurement. The lesser change in fibre volume and fibre density for 5 mm and 2 mm lengths of Cheviot and Crossbred wool may reflect the lower level of medullation that

these wools possess. As the incidence of medullation decreases, the chance of exposing medullae at the cut fibre ends also decreases.

A reason for the significant decrease in volume, and corresponding increase in density, of the 5 mm and 2 mm treatment lengths of non-medullated wool is difficult to find. Fine diameter wool would contain a greater number of fibres than coarse wool, for a sample of constant mass and fibre length. The greater number of fibres would expose a greater number of fibre ends for gas to penetrate. Logically though, the total surface area of exposed fibre ends is likely to be the same as for a sample with a coarser fibre diameter, for samples of the same mass, fibre length and fibre density. Only the number of fibres in the sample will differ.

The main finding from this experiment was that the volume of wool samples 10 mm in length did not differ from that of full length wool. Therefore, the hypothesis that the volume of a wool sample, of constant weight, is not affected by the length of individual fibres within the sample was found to be true for fibre lengths greater than or equal to 10 mm. Fibres shorter than 10 mm in length appeared to obstruct filters in the helium pycnometer and cause erroneous measurements of volume and density. It is therefore recommended that only wool samples with fibre lengths greater than or equal to 10 mm be measured using a helium pycnometer.

5. Precision of individual measurements of volume and density

5.1 Introduction

Precision is defined as definite, careful in observance (Collins Concise Dictionary and Thesaurus, 1994). Precision measures the closeness of values, obtained from either repeat measurements of the same samples or from sub-sets of the samples.

Precision and accuracy are two terms often confused. *Accuracy* is defined as exact, correct, without errors. Development of methodology aims to minimise errors and obtain a measurement close to the true value. The accuracy of a procedure is determined by comparison of results from samples measured both by the new method and a standard method. Despite the existence of several methods of measuring density (Section 2.6), no standard is available for wool, therefore it is not possible to determine the accuracy of measurements of fibre volume and fibre density of wool, made using a helium pycnometer.

The objective of this experiment was to establish the precision, within one laboratory, of fibre volume and fibre density measurements made using a helium pycnometer.

5.2 Materials and methods

Scoured wool samples, from commercially shorn fleeces, were collected from wool scours throughout New Zealand. The samples, collected and supplied by staff from WRONZ, represented a variety of wool types used in commercial production. The fifty three samples ranged in mean fibre diameter from 26.3 μm to 47.2 μm and medullation from 0.8 percent by volume to 45.5 percent by volume, as determined by projection microscope. Samples were hand carded to eliminate short fibres (<10 mm), created by previous sampling for measurement of fibre diameter and medulla content. Fibre length was not measured, but was estimated to be greater than 10 mm. Samples were conditioned for 24 hours in a standard atmosphere before a mass of 4.00 ± 0.05 g per sample was placed in individual containers. Sample mass was increased from 2.50 ± 0.05 g, used in Chapter 4, to improve the ease of handling of the samples. There was only sufficient wool available for measurement of a single specimen of each sample. After measurement of volume and calculation of density, using previously established constraints, each sample was returned to its container.

Sample mass was recorded immediately before placement in, and on removal from, the measurement chamber. Care was taken to ensure limited loss of fibres, and cross-contamination of samples during transfer. Five replicate measurements of the fifty three samples were made, using a completely randomised block design, with samples conditioned for 24 hours between measurement of each replicate. Two days were required to measure each replicate.

The standard deviation, mean volume and mean density of individual samples was calculated, using measurements from all five replicates. An overall mean and pooled standard deviation was calculated, to estimate the precision of the helium pycnometer in measuring volume and density. Changes in sample mass within and between replicates were analysed using analysis of variance.

5.3 Results

The precision of individual measurements of volume and density, as measured using a helium pycnometer, and that of density, measured using a density gradient column (Connell and Andrews, 1974) and a benzene pycnometer (van Wyk and Nel, 1940) are presented in Table 5.1. The precision of the density measurements were 2.90 %, 1.00 % and 0.68 % of the mean density, for the helium pycnometer, density gradient column and benzene pycnometer, respectively. Re-measurement of the same sample would increase the precision of both volume and density measurements. Estimates of the increase in precision due to increasing the number of measurements of volume and density, by helium pycnometer, for the same sample, are presented in Table 5.2.

The standard deviation of the helium pycnometer method, for individual samples, did not differ significantly between wool with very little or high medullation, for either volume ($p=0.453$) or density ($p=0.520$), (Table 5.3). However, the volume of samples with little medullation was significantly lower ($p<0.001$), and the density significantly higher ($p<0.001$) than for wool with high medullation.

Table 5.1 Precision of fibre volume and fibre density measurements for a single sample, measured using a helium pycnometer; density measurements, measured using a gradient column; and specific gravity measurements, measured using a benzene pycnometer.

	Helium pycnometer		density gradient column[+]	benzene pycnometer[++]
measurement	volume (cm^3)	density (g/cm^3)	density (g/cm^3)	specific gravity
number of samples	203	203	174	54
mean	3.148	1.275	1.305	1.304
standard deviation	0.102	0.037	0.013	0.009
95% confidence value	0.014	0.005	0.002	0.002

[+] Connell and Andrews (1974)
[++] van Wyk and Nel (1940) in Connell and Andrews (1974)

Table 5.2 Increase in precision of the helium pycnometer measurement of fibre volume and fibre density, with an increase in the number of replicate measurements.

	fibre volume (cm^3)		fibre density (g/cm^3)	
No. of replicates	standard deviation	coefficient of variation (%)	standard deviation	coefficient of variation (%)
1	0.102	3.24	0.037	2.90
2	0.072	2.29	0.026	2.04
5	0.046	1.46	0.016	1.25
10	0.032	1.02	0.012	0.94

Table 5.3 Precision, as standard deviation (sd) and 95% confidence value (CV) of fibre volume and fibre density of a 4.00 g sample of wool, measured using a helium pycnometer, for wool with widely different levels of medullation.

Medulla content		Volume (cm^3)			Density (g/cm^3)		
(percent by volume)	no.	mean	sd	95%CV	mean	sd	95%CV
low (<2%)	33	3.120	0.103	0.037	1.287	0.038	0.014
high (22%)	20	3.195	0.100	0.046	1.255	0.035	0.017
		***	ns	ns	***	ns	ns

*** $p<0.001$
ns not significant at 5%

Changes in sample mass before and after the measurement of fibre volume and fibre density of five replicates of the same samples are presented in Table 5.4. The average reduction in sample mass was 0.023 g between the first and last of five repeat measurements ($p<0.001$). The masses of replicates three, four and five were significantly less than those of the first two replicates, both before placement in ($p<0.005$) and after removal from ($p<0.005$) the measurement chamber of the helium pycnometer. Wool with high levels of medullation lost significantly more mass than those with no medullation, before ($p<0.001$) and after ($p<0.001$) measurement of fibre volume and fibre density (not tabulated). There was a significant and consistent reduction in mass within replicates, during measurement, ($p<0.001$), regardless of the level of medullation. This reduction in mass was not fully recovered when samples were re-conditioned.

Table 5.4 Difference in sample mass before placement in and after removal from the measurement chamber of the helium pycnometer, and between five replicates, conditioned for 24 hours prior to each measurement.

Sample mass (g)	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	$lsd_{0.05}$
before measurement	4.016	4.007	3.997	3.994	3.993	0.004
after measurement	4.005	3.997	3.986	3.984	3.983	0.004
difference (before - after)	0.011	0.010	0.011	0.009	0.009	0.001
$lsd_{0.05}$	0.002	0.002	0.001	0.001	0.001	

5.4 Discussion

The precision of density measurements by the helium pycnometer was poorer than that of either the gradient column or benzene pycnometer methods. However, in contrast to the other two methods, the helium pycnometer is a non-destructive method of measuring density. Repeat measurements of specimens and the measurement of the density of medullated wool is possible only with a helium pycnometer. In addition, averaging two or more replicate measurements would give improved precision for the helium pycnometer. Eight measurements of the same specimens would be required, using the helium pycnometer, to achieve similar precision of the density measurement to that achieved by the gradient column. Precision similar to that of a benzene pycnometer would require seventeen measurements of the same specimens. It is not possible to re-measure the same sample in a benzene pycnometer or gradient column,

due to the liquid displacement medium these methods use. Precision of the helium pycnometer measurement of density could be further improved by measuring a number of specimens of each sample. There was insufficient wool available, in this experiment, for the measurement of sub-samples using the helium pycnometer. The helium pycnometer is a rapid procedure, requiring approximately seven minutes to make the recommended number of sequential measurements. This compares to approximately sixteen hours for a single measurement using a density gradient column and even longer using a benzene pycnometer.

The mean density of wool with no or low medullation, measured using a helium pycnometer (1.287 g/cm^3) was close to that measured by Connell and Andrews (1974), (1.305 g/cm^3). The density of medullated wool does not appear to have been measured by methods other than the helium pycnometer. It would be interesting to re-measure the wools, from the experiment described above, using an alternative method of density measurement, to determine the accuracy of the helium pycnometer. Wool from South African Merino strains was measured using the benzene pycnometer method (van Wyk and Nel, 1940) while those measured using the gradient column method were Australian Merino (n=154) and Crossbred (n=20) sheep, (Connell and Andrews, 1974). The level of medullation in the Crossbred wool was presumably negligible, as the density did not differ markedly from that of the Merino samples. The lower density of medullated samples, as measured by the helium pycnometer is further investigated in the following chapter (Chapter 6).

There was a small but significant reduction in fibre mass between, and during, measurement of the five replicates. The reduction in mass between the measurement of replicates was probably due to fibre loss during handling. Short fibres, remaining in the sample after carding, were observed adhering to the balance and measurement cup. By the time the fourth and fifth replicates were measured the majority of the loose short fibres were probably no longer in the sample, as the reduction in mass was not significant between replicates three and four, and four and five. The significantly greater reduction in fibre mass between replicates from medullated samples, compared to samples with little medullation, was possibly due to the higher electrostatic charge of medullated fibres compared to non-medullated fibres (Besançon, 1974). Medullated fibres could have more frequently adhered to the container or balance during handling, due to this electrostatic charge. Potentially, fibre loss could be minimised by measuring specimens containing only relatively long wool fibres.

The significant reduction in fibre mass during measurement was unlikely to have been due to absorption of helium into the fibres. It was more likely to be due to moisture loss from the samples. As indicated in the previous chapter (Section 4.4), absorption of helium into the fibres probably did not occur. Samples had been conditioned at 65 % relative humidity for at least 24 hours prior to placement in the helium pycnometer. The helium gas used for measurement was a dry gas. Moisture was probably flushed from within and between fibres in the sample as the gas exited the measurement chamber (Merrick *et al.*, in press). Presumably, samples of similar mass would have held similar amounts of moisture, which would have accounted for the similar reduction in mass for each replicate and each wool type. The increase in mass between the 'after' measurement of the mass of one replicate and the 'before' measurement of mass of the next replicate was likely to have been a function of moisture regain when samples were reconditioned.

The poorer precision of the helium pycnometer, relative to the benzene pycnometer and density gradient column methods, is compensated for by the greatly reduced measurement time of individual samples. A greater number of measurements of the same specimen, or an increase in the number of specimens measured would improve the precision of the helium pycnometer. The former option is not possible with either the benzene pycnometer or density gradient column methods.

6. The effect of medullation on volume and density

6.1 Introduction

The density of wool is an integral component of several physical equations related to the measurement of wool characteristics, including the Airflow method of measuring fibre diameter (IWTO, 1982). In the development of the airflow method, Cassie (1942) extended the hydrodynamic equations of Kozney (1931, cited in Baxter, 1993) to cover airflow through fibres, and developed an application for wool. In such applications, the density of wool is assumed to be constant, yet one obvious source of variation in the density of wool is medullation.

Wool samples need to be completely free from entrapped air and moisture to measure keratin density (Fraser and MacRae, 1957). For non-medullated wool, measurement of the fibre density should equate to that of the wool keratin density. When measuring medullated wool with a helium pycnometer, fibre density is measured i.e. the combined density of the keratin and the air enclosed in the medulla cells within the fibres.

When medullae are present, the volume measured using the helium pycnometer will differ from volume calculated as the quotient of mass and keratin density (Merrick and Scobie, 1997). The hypothesis under test was that differences between measured and calculated volume, at the same wool mass, could be accounted for by the level of medullation in the wool. This section of work aimed to evaluate the helium pycnometer as an indirect method of measuring medulla content, and to quantify the relationship between medullation, volume and density of wool samples.

6.2 Materials and methods

Fibre volume and fibre density measurements from fifty three wool samples, measured to determine the precision of the helium pycnometer (Section 5.2), were also used to determine the relationship between fibre volume and medullation. The samples had previously been measured for medulla content using the projection microscope (percent by number), WRONZ Medullameter (percent by volume), NIRA (percent by volume) and OFDA (percent by number and percent by volume). The OFDA value for percent medullation by volume is an approximation, derived using an internal calculation. Percent medullation by volume was calculated from projection microscope results of percent medullation by number, and the mean and standard deviation of fibre

and medulla diameter, according to Bray's formula (Bray, 1942). All measurements were carried out in accordance with the international guidelines and standard procedures detailed in General Materials and Methods (Section 3.3).

Correlation coefficients were calculated, between volume measured using the helium pycnometer and volume predicted from the sample mass divided by the published density of wool (1.305 g/cm^3, Connell and Andrews, 1974). A predicted percent medullation by volume (predicted medullation) was calculated, in order to directly compare medullation, on a percent by volume basis, between the helium pycnometer and projection microscope, OFDA, WRONZ Medullameter and NIRA measurements. Predicted medullation was calculated from volume measured using a helium pycnometer and predicted volume, in accordance with the following equation.

$$\text{Predicted medullation} = \frac{\text{measured volume - predicted volume}}{\text{measured volume}} \times \frac{100}{1}$$

To determine the relationship between volume and medullation, linear and second order regressions were fitted to the helium pycnometer measurements of volume and projection microscope measurements of percent medullation by volume. The means of all five volume and density measurements, for each sample, from the experiment in Chapter 5 were correlated with medullation measurements (percent by volume) from the projection microscope, OFDA, WRONZ Medullameter, NIRA and predicted medullation.

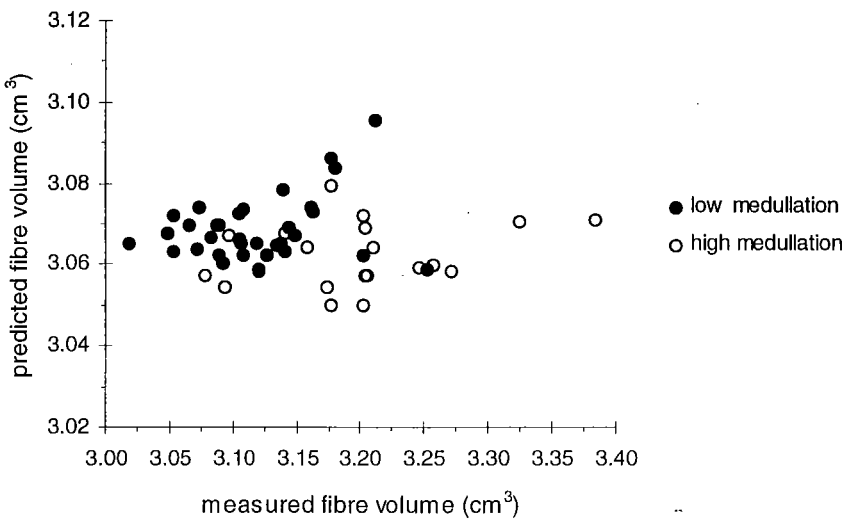
6.3 Results

The differences between predicted and measured volume, for wools with low or high medullation, are presented in Figure 6.1. The predicted volume of a 4.00 g sample of wool, calculated using constant density, obviously remained constant (3.065 cm^3), regardless of medulla content. The measured volume (3.120 cm^3) of wool with low medullation did not differ significantly from the predicted volume ($p=0.250$). For wool with high medullation, the measured volume (3.195 cm^3) was 3.1 % greater than the predicted volume ($p<0.001$), (not tabulated).

There was a significant difference between the mean measured volume of wool with low compared with high medullation, the latter having 2.4 % greater volume ($p<0.001$), but the spread of volume measurements overlapped markedly for the two levels of medullation (Figure 6.1). Measured volume ranged from 3.020 cm^3 to 3.203 cm^3 for

wool with low medullation and from 3.079 cm^3 to 3.384 cm^3 for wool with high medullation (not tabulated). The level of medullation, as determined using an OFDA, for wool with low medullation ranged from 0.0 % to 3.3 % by volume, and from 8.2 % to 33.8 % by volume for wool with high medullation (not tabulated). The mean percent volume medulla content differed significantly ($p<0.001$) between wools with low (2 %) and high (22 %) levels of medullation (not tabulated).

Figure 6.1 Volume of 4.00 g wool samples, measured using the helium pycnometer and predicted as the quotient of mass and the published density of wool (1.305 g/cm^3) for fifty three samples, categorised with low or high medullation.



Regression of measured fibre volume and percent medullation by volume showed that measured volume increased linearly with increasing medulla content ($p<0.001$). There was no indication of a quadratic relationship ($p=0.494$), (slope = 0.004 ± 0.001; intercept = 3.110 ± 0.011).

The correlation coefficients for measured volume, density, predicted medullation and medullation, measured using a projection microscope, OFDA, WRONZ Medullameter and NIRA, are presented in Table 6.1. Fibre volume, measured using the helium pycnometer, was positively correlated with the percent medullation by volume measured using a projection microscope, OFDA, WRONZ Medullameter and NIRA ($p<0.001$ for each). Fibre density was negatively correlated with the percent medullation by volume measured using these methods ($p<0.001$ for each). The relationships between projection microscope, OFDA, WRONZ Medullameter and NIRA measurements of medullation (percent by volume) have been established by Justine Lee (*pers. comm.*).

Predicted medullation was significantly correlated with all the methods of measuring percent medullation by volume mentioned ($p<0.001$). These relationships were stronger than those of the direct volume measurement were with measurements of medullation, but not as strong as the relationships between the projection microscope, OFDA, WRONZ Medullameter or NIRA methods of measuring medullation.

Table 6.1 Correlation coefficients (r) between fibre volume and fibre density measurements and predicted percent medullation by volume (predicted med.), or medullation (% med. vol.) measured using projection microscope (PM), OFDA, WRONZ Medullameter (WRONZ MM) or NIRA, for fifty three wools from commercial scours.

				Medulla content measured by -		
	fibre volume (cm^3)	fibre density (g/cm^3)	predicted med. (% vol.)	PM (%medvol)	OFDA (%medvol)	WRONZ MM (%medvol)
density (g/cm^3)	-0.986					
predicted med. (% vol.)	0.991	-0.996				
PM (% med. vol.)	0.570	-0.627	0.607			
OFDA (% med. vol.)	0.513	-0.578	0.553	0.949		
WRONZ MM (% med. vol.)	0.478	-0.560	0.530	0.891	0.940	
NIRA (% med. vol.)	0.526	-0.586	0.561	0.934	0.948	0.925

6.4 Discussion

The relationship between fibre volume and the level of medullation was more relevant than the relationship between fibre density and the level of medullation. Fibre density was calculated from the helium pycnometer measurement of fibre volume, and revealed little additional information than the raw volume values. The relationship between fibre density and fibre volume with measurements of percent medullation by volume were similar in magnitude but, logically, opposite in sign (Table 6.1).

The difference between measured and predicted volume for highly medullated wool reflects the effect of medullation on fibre volume. The vacuolated cells of the medullae contribute less mass per unit volume than keratin filled cells in other parts of the fibre.

Matoltsy (1953) reported the iso-electric point of medullary keratin as similar to that of wool keratin, and predicted the density of these keratins to be similar. Based on this, differences in fibre density between medullated and non-medullated wool would be expected to be due only to the air present in the medulla cells. The similar spread of fibre volume measurements, observed in this experiment, for wool with markedly different levels of medullation, indicates that factors additional to medullation contributed to the differences between the measured and predicted volumes of samples. Therefore, the potential for differences in the densities of the keratins which comprise wool fibres cannot be discounted.

Previous investigations into the density of wool keratin support the idea of variation in keratin density. By calculating density from measurements of fibre mass and fibre volume, Cassie (1942) suggested that the density of wool keratin could be as high as 1.35 g/cm^3. Further indications in support of differences in keratin density were perceived where there was only a 2.4 % difference in volume, at the same weight, in the experiment reported in this chapter but a 25 % difference in volume, at the same weight, in a previous experiment (Merrick and Scobie, 1997), between wools of similarly high and low levels of medullation. These volumes translated to densities of 1.264 g/cm^3 and 1.035 g/cm^3 for the highly medullated wool from the respective experiments. Similar types of wool were measured and the highly medullated wool had a medulla content of approximately 20 % by volume in both experiments. Thus, relatively large differences in volume and density were observed for wools that differed only marginally in medullation content. Small differences in the density of keratin have been measured for non-medullated wool (King, 1926; van Wyk and Nel, 1940; Connell and Andrews 1974) and assumed for medullated wool (Matoltsy, 1953; WIRA, 1973). Further investigation into the differences in the density of wool keratin was beyond the scope of this thesis.

The strong correlations between measured fibre volume and density, measured volume and predicted medullation, and measured density and predicted medullation (Table 6.1) were to be expected as measured volume was used to calculate both density and predicted medullation. An increase in volume when wool fibres were medullated produced a positive correlation between volume and medullation. The negative correlations between fibre density and predicted medullation, and fibre density and the other methods of measuring medullation, reflected a reduction in fibre density when medullation was present.

Reasons for the moderate relationships between fibre volume and medullation, and fibre density and medullation, other than differences in keratin density, are unclear. A possible reason for the difference in the density of the keratin may have been a difference in the hardness of the cortical cell types. Paracortical cells appear to be more heavily keratinised than orthocortical cells (Fraser and Short, 1960) and are possibly more dense. Changes in the proportions of the cortical cell types would then create differences in the overall density of fibres. Variation in the density of keratin within cortical cell types was a further potential cause of variation in fibre density but there exists no reported evidence of this source of variation.

Medullation contributed to, but did not account for the whole of, the difference between measured fibre volume and predicted volume. The relationships between the projection microscope, OFDA, WRONZ Medullameter or the NIRA methods of measuring medulla content were all strongly positive. All these relationships were appreciably stronger than those between measured volume, or density, and medullation measured by any of the methods examined. Predicted medullation showed little improvement to the relationship between measured volume or density and measured medullation. Rather than continuing to pursue a method of measuring medullation using the helium pycnometer, medullation for subsequent experiments was measured using an OFDA.

7. Seasonality of fibre volume, fibre density and medulla content

7.1 Introduction

Fibre diameter and fibre length growth in sheep vary seasonally. They display maximum production in summer and minimum production in winter (Reis and Downes, 1971; Reis *et al.*, 1973; Allden, 1979; Hawker and Crosbie, 1985). Medulla content has also been shown to vary with season in the Romney and Cheviot breeds of sheep (Rudall, 1934; Skårman and Nõmmera, 1954; Scobie *et al.*, 1993), although the production of maximum and minimum medullation occurs later in the season than maximum and minimum fibre diameter and fibre length. Logically, fibre volume and fibre density will also vary seasonally, in a similar way to medullation, out of phase with fibre diameter and fibre length.

The aim of this experiment was to study seasonal changes in medullation, fibre volume and fibre density over winter, spring and summer for four breeds of sheep, representing a range of medulla content and fibre growth rates. The hypothesis tested was that the medulla content (percent by volume) showed no seasonal variation. Fibre volume and fibre density, as determined by the helium pycnometer, were compared with the medulla content (percent by volume) of the wool. Comparisons of the fibre characteristics were focused on the winter minimum and summer maximum rates of fibre growth.

7.2 Materials and Methods

Wool was sourced from an AgResearch flock of sheep in each of two consecutive years (1992/93 and 1993/94). The four breeds used in this study were a subset of a larger flock, which was managed according to conventional farm practices, except all ewes were non-pregnant. Wool was harvested from a midside site every twenty eight days, over a period of nine months. The same site was harvested each month, but it was necessary to re-establish the site between years, for animals common to both years. In the first year wool was harvested from thirty two-year-old ewes (ten Merino ewes, ten Romney ewes and ten Drysdale ewes). In the second year, five Merino and five Drysdale ewes were retained and a further nineteen two-year-old ewes were selected for sampling (ten Romney ewes and nine Cheviot ewes). The Merino,

Romney, Drysdale and Cheviot breeds were chosen for the range of fibre characteristics they produce, and because the fibre growth rates were likely to produce sufficient fibre, within the twenty eight day sampling cycle, to meet the length and mass requirements of the helium pycnometer. These breeds were also common to previous experiments in this thesis.

Greasy samples were detergent scoured (Section 4.2) and conditioned in a standard atmosphere for 24 hours. Fibre volume and fibre density of the entire quantity of wool harvested from the midside site were measured using a helium pycnometer, using the individual fourth sequential measurement. Sample mass ranged from 1.98 g to 7.17 g. Fibre length was not measured, but was visually estimated to be greater than the minimum 10 mm constraint of the helium pycnometer. Fibre diameter and medulla content (percent by volume) were measured using an OFDA.

Analysis of variance was used to identify seasonal differences in fibre mass, fibre volume, fibre density, medulla content and mean fibre diameter. Fibre mass and fibre volume measurements were adjusted to a standard harvest area of 100 cm^2. Regression analysis was used to determine the relationship between fibre density and medulla content.

7.3 Results

Seasonal variation in the mean mass and the mean volume of wool fibre grown over two years of sampling, by the four sheep breeds used in this study are presented in Figure 7.1 and Figure 7.2, respectively. The mass of wool grown per 100 cm^2 area of skin was approximately three times greater in January than July for Merino, Romney and Drysdale ewes in the first year, ($p<0.001$ for each). The volume of fibre produced was 2.5 times greater for Merino ($p<0.001$), 3.0 times greater for Romney ($p<0.001$) and 3.5 times greater for Drysdale ($p<0.001$), between the same months. In the second year of sampling, the mass of wool produced did not differ significantly in January compared to July for either Merino ($p=0.060$) or Drysdale ewes ($p=0.115$). The volume of fibre produced did not differ significantly for Merino ($p=0.135$) between these same months, while the difference in fibre volume produced by Drysdale ewes approached significance ($p=0.053$). The mass of wool produced by Romney ewes was twice as high ($p<0.001$) and the volume of wool produced was 1.5 times greater ($p<0.001$) in January as in July, in the second year of sampling. Cheviot wool showed similar seasonal differences to Romney wool, in the second year of sampling,

producing 1.5 times more fibre mass ($p<0.001$) in January than July, and twice the volume of fibre ($p<0.001$).

Figure 7.1 Fibre mass of wool, harvested at twenty eight day intervals, from four breeds of sheep in two consecutive years (error bars represent the standard error of the difference of the mean between June and January, for each breed within each year).

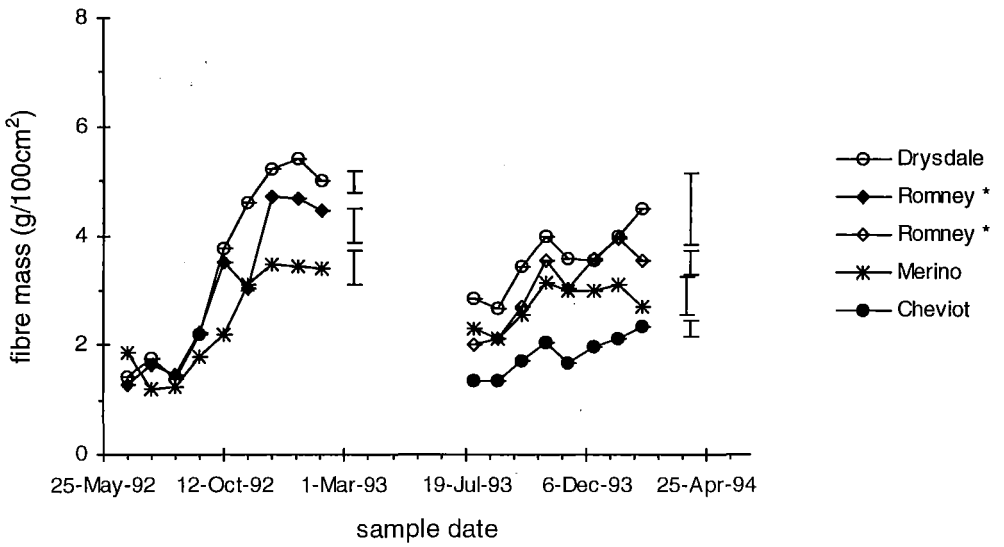
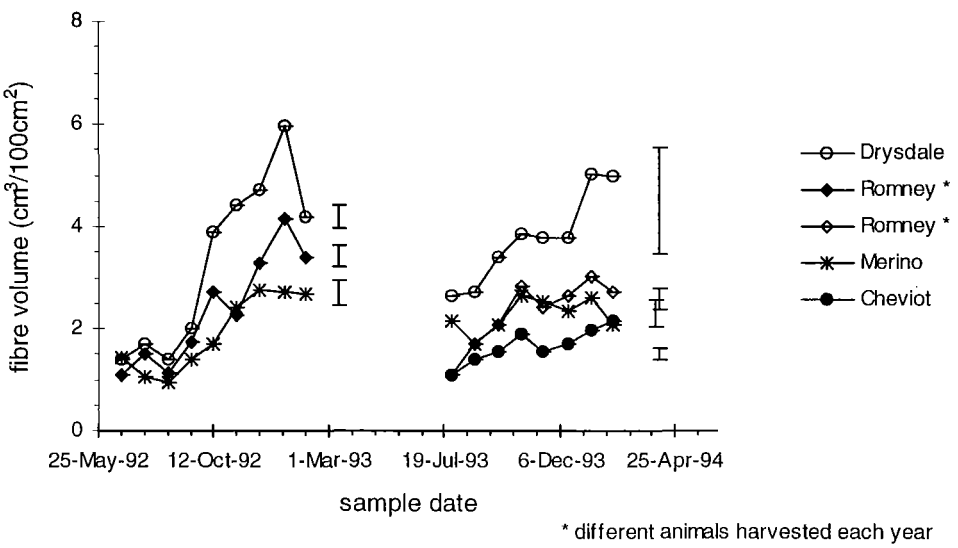
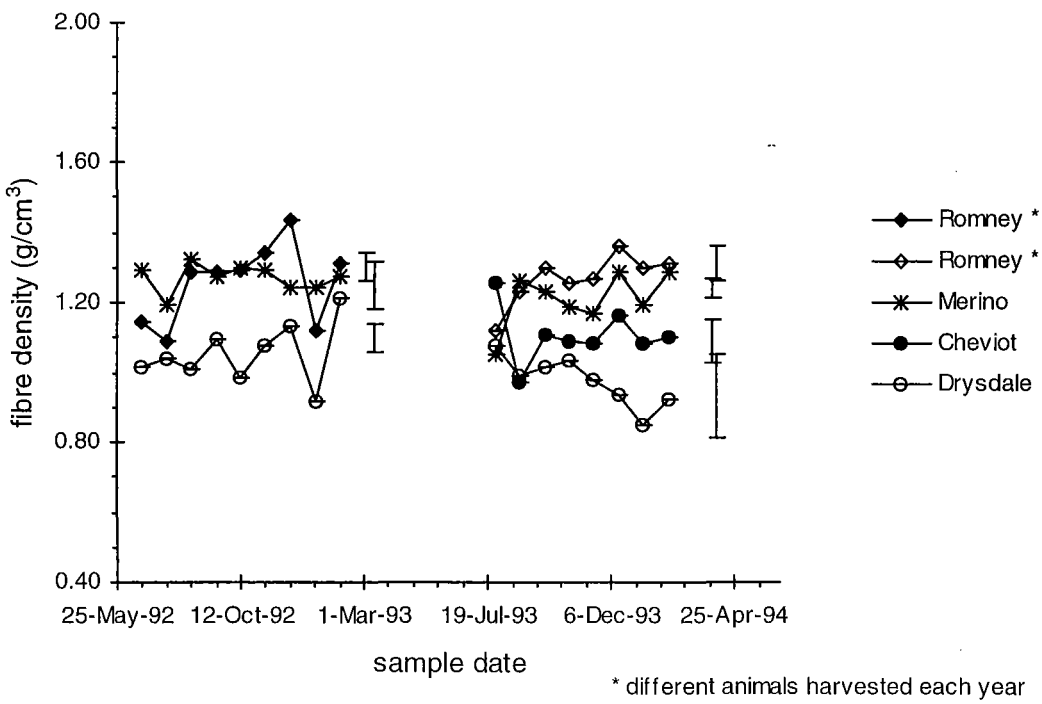


Figure 7.2 Fibre volume of wool, harvested at twenty eight day intervals, from four breeds of sheep in two consecutive years (error bars represent the standard error of the difference of the mean between June and January, for each breed within each year).



The density of the fibre produced by the four breeds of sheep, and the seasonal variations in fibre density are presented in Figure 7.3. The density of Merino and Romney wool did not differ significantly in July compared to January in the first year of sampling, as two year old ewes ($p=0.506$ and $p=0.502$, for Merino and Romney, respectively). There was a significant difference in the fibre density of both Merino ($p<0.010$) and Romney ($p<0.010$) wool in the second year of sampling, between the same months. The Merino ewes were three years old and the Romney ewes two years old, in the second year of sampling. The density of Drysdale wool dropped from 1.039 g/cm^3 in July to 0.919 g/cm^3 in January ($p<0.010$) in the first year, and from 1.079 g/cm^3 in July to 0.848 g/cm^3 in January ($p=0.117$) in the second year. The density of Cheviot wool in January reduced to 90% of that measured in July ($p<0.010$), in the second and only year the Cheviot ewes were sampled.

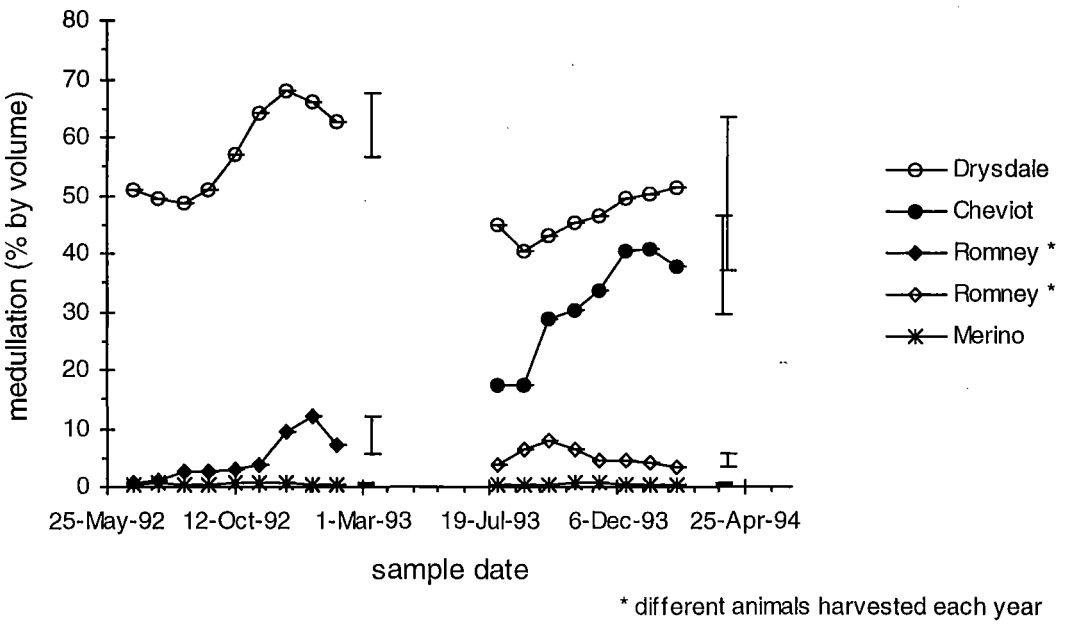
Figure 7.3 Fibre density (g/cm^3) of wool, harvested at twenty eight day intervals, from four breeds of sheep in two consecutive years (error bars represent the standard error of the difference of the mean between June and January, for each breed within each year).



The seasonal variations in the medulla content of the wool produced by the four breeds of sheep, used in this study, are presented in Figure 7.4. Drysdale wool was 40 % more medullated in January than July in both sampling years. This difference was significant in the first year ($p<0.010$), but not in the second ($p=0.236$). Cheviot

wool was 2.5 times more medullated in January compared to July ($p<0.050$). Although the Cheviot ewes had a relatively high proportion of medullation, they produced a smaller volume of wool than any of the other breeds (Figure 7.2). The medulla content of wool harvested from Romney ewes, sampled in the first year, increased from 2 % by volume in July to 12 % by volume in January ($p<0.010$). In the second year, when different Romney ewes were sampled, there was no significant difference in medulla content between July and January ($p=0.817$). Regression analysis showed that over the two years of the study, only 17 % of the variation in density could be accounted for by changes in medullation.

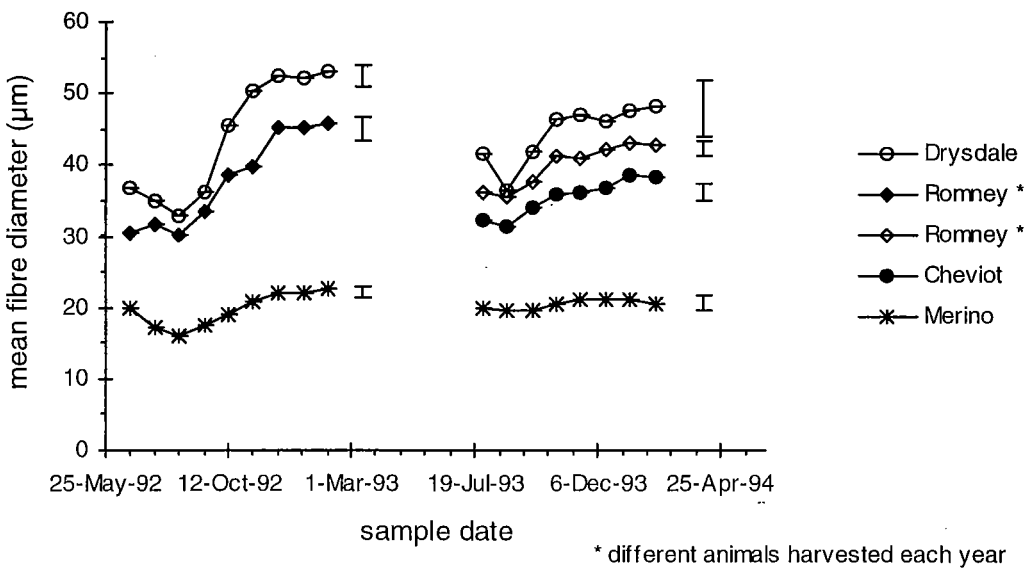
Figure 7.4 Medulla content (percent by volume) of wool harvested at twenty eight day intervals, from four breeds of sheep in two consecutive years (error bars represent the standard error of the difference of the mean between June and January, for each breed within each year).



The mean fibre diameter, averaged over all sampling periods within each year, did not differ significantly for either Merino (20 µm) or Drysdale (44 µm) ewes between years. The Romney ewes sampled in the first year had a mean fibre diameter of 38 µm, while those sampled in the second year were coarser (40 µm), ($p<0.010$). The mean fibre diameter of the Cheviot ewes was 36 µm. Mean fibre diameter varied seasonally for the four sheep breeds used, and over the two years of this study (Figure 7.5). Rankings of the mean fibre diameter of the breeds remained unchanged with seasonal variation, within and between years. Merino wool had a mean fibre diameter which was 30 % higher in January than July, in the first year ($p<0.001$), and was not significantly different between the same months in the second year ($p=0.329$). Romney wool was

40 % coarser in January than July in the first year ($p<0.001$) and 20 % coarser in the second year, between the same months ($p<0.001$). The mean fibre diameter of Drysdale wool increased by 50 % in the first year ($p<0.001$), but was not significantly different in January, compared to July, in the second year ($p=0.176$). The mean fibre diameter of Cheviot wool was 10 % higher in January than July ($p<0.001$).

Figure 7.5 Mean fibre diameter (µm) of wool harvested at twenty eight day intervals, from four breeds of sheep in two consecutive years (error bars represent the standard error of the difference of the mean between June and January, for each breed within each year).



The variability of the wool production characteristics, presented in Figures 7.1 to 7.5, are tabulated below as the mean and standard deviation of production levels, for the first and second year of sampling (Table 7.1 and Table 7.2, respectively). Fibre mass and fibre volume varied by 15 % to 20 % of the mean for each breed in each year, except in the second year where the fibre volume of Drysdale wool varied by almost 30 %. Medulla content showed the greatest fluctuations, varying by 20 % for Drysdale wool in the first year, and by 50% for both Drysdale and Cheviot wool in the second year. Fibre density was much less variable within years, changing 12 % for heavily medullated breeds and half that for breeds with little or no medullation. Fibre diameter varied by less than 10 % for each breed, within years.

Table 7.1 The mean and standard deviation of mass, volume, density and diameter of fibres and percent medullation by volume for Merino, Romney and Drysdale ewes in the first year of measurement (1992/93).

	Merino mean ± sd	Romney mean ± sd	Drysdale mean ± sd
mass (g)	2.63 ± 0.52	3.01 ± 0.69	3.53 ± 0.54
volume (cm^3)	2.07 ± 0.39	2.38 ± 0.50	3.37 ± 0.68
density (g/cm^3)	1.269 ± 0.069	1.257 ± 0.069	1.060 ± 0.128
medullation (percent volume)	0 ± 0	5 ± 5	58 ± 13
mean fibre diameter (μm)	20 ± 1	38 ± 3	44 ± 3

Table 7.2 The mean and standard deviation of mass, volume, density and diameter of fibres and percent medullation by volume for Merino, Romney, Cheviot and Drysdale ewes in the second year of measurement (1993/94).

	Merino mean ± sd	Romney mean ± sd	Cheviot mean ± sd	Drysdale mean ± sd
mass (g)	2.76 ± 0.54	3.07 ± 0.43	1.85 ± 0.42	3.51 ± 0.67
volume (cm^3)	2.29 ± 0.41	2.41 ± 0.37	1.68 ± 0.32	3.64 ± 0.97
density (g/cm^3)	1.209 ± 0.055	1.268 ± 0.081	1.108 ± 0.139	0.992 ± 0.119
medullation (% volume)	0 ± 0	5 ± 4	31 ± 17	46 ± 20
mean fibre diameter (μm)	24 ± 1	40 ± 2	36 ± 2	44 ± 5

7.4 Discussion

The choice of breeds appears to have satisfied the fibre length and sample mass constraints of the helium pycnometer. The mass of fibre harvested from the midside patch exceeded the minimum requirement of one gram, for all breeds in each sampling period, over the two years of the study. Variation in the mass of fibre produced, on a 100 cm^2 patch was unlikely to have affected measurements of fibre volume.

Merrick *et al.* (in press) reported a linear relationship between fibre mass and fibre volume for sample masses between one and ten grams. Although fibre length was not measured, there was no evidence of a recurrence of the blockage that occurred within the helium pycnometer during the length experiment (Section 4.4). This would suggest that fibre length exceeded the 10 mm minimum fibre length requirement.

Mass, volume, density, medulla content and mean diameter of the fibre produced, varied between harvest periods in each year. The point of maximum production of these characteristics occurred in different harvest periods, and this also differed between breeds. However all points of maximum production, for all characteristics, did occur within the summer months. Similarly, all points of minimum production, for all characteristics measured, occurred in the winter months. Expression of the maximum fibre mass and the maximum mean fibre diameter in summer, and the minimum of both in winter, was consistent with seasonal trends previously reported for Romney (Goot, 1945; Tibbits, 1959; Reis and Downes, 1971; Allden, 1979; Hawker and Crosbie, 1985; Scobie *et al.*,1993) and Merino sheep (Robards, 1979). The lag in the occurrence of maximum and minimum medulla content, relative to mean fibre diameter, has previously been reported in Romney (Goot 1945; Tibbits 1959; Scobie *et al.,* 1993) and Cheviot breeds (Skårman and Nõmmera, 1954), and in the present study has been shown to occur in the Drysdale breed. Seasonal trends in fibre volume and fibre density have not previously been reported.

The extent to which fibre volume and fibre density were affected by the presence of medullation was unclear. Fitting medulla content as a covariate to fibre density, to determine whether fibre density varied independently of medulla content, was not practical for this experiment. The four breeds and two sample dates used, were selected for their wide variation in medulla content, and resulted in skewed distributions for fibre volume, fibre density and medulla content. Consequently, applying covariate analysis between breeds and/or sample dates produced unrealistic changes in the mean density of breeds with little or no medullation. The small number of animals measured meant it was unwise to restrict the covariate analysis to within breed within sample date. Through regression analysis, only 17 % of the variation in fibre density could be accounted for by medulla content, in this experiment. This would again seem to indicate that some of the variation in fibre density could have been due to factors other than medullation. Such factors could include keratin densities which may have differed from the widely accepted value of 1.31 g/cm^3 (WIRA, 1973). The possibility of differing keratin density has already been raised (Section 6.4).

The effects of season can not be separated from the effects of nutrition in this study. Hawker and Crosbie (1985) showed that, although twice as much wool was grown in summer as winter, the relative effects of nutrition within these seasons were similar, for Romney and Perendale ewes. Greater wool growth in summer, compared to winter, appeared to be due to different partitioning of nutrients to wool growth, rather than variation in feed intake. Levels of nutrition were not measured as part of this study, but would have been the same within years for all animals and breeds, which were run as a single mob throughout each year of sampling.

The January and July harvest periods were chosen to represent points of maximum and minimum production at which the levels of fibre mass, fibre volume, fibre density, medulla content and mean fibre diameter could be compared between breeds. The choice of January as the month of maximum production could be questioned. The values for fibre volume and fibre density were markedly lower in January than in either December or February, for Drysdale and Romney ewes (Figure 7.3). The occurrence of this trend for Romney wool in the first year, and Drysdale wool in both years indicated that this occurrence was unlikely to have been due to measurement anomalies. The trend may not have occurred to the same extent in the second year for Romney wool, because the Romney ewes sampled in the second year were different animals to those sampled in the first year, and they exhibited little seasonal variation in medullation content. While use of the month either side of January would produce different results for fibre volume and fibre density, the majority of the production characteristics measured were at or near their maximum production in January, for most of the breeds studied.

There was a large increase in the mass, volume, medulla content and mean diameter of fibre produced by Drysdale ewes, in January compared to July, in the first year of sampling. The expected decrease in fibre density was small. In the second year, the increase in fibre mass and fibre volume, between July and January, was less than for the first year, but the increase in medulla content and decrease in fibre density were similar to the first year. Mean fibre diameter did not differ significantly between years for the Drysdale ewes. The similar magnitude of change in medulla content and fibre density in the Drysdale ewes in both years is difficult to explain, given the differences in fibre volume between years. The variation in the proportion of medullation has been shown to be independent of mean fibre diameter, such that fibres can be medullated at one point and not at another without a change in fibre diameter (Wildman, 1954; Tibbits, 1959; Ryder and Stephenson, 1968; Scobie *et al.*, 1998). Medullae cells are essentially air-filled so they contribute little to the mass of wool grown. An increase in

medullation without a change in fibre mass would necessitate an increase in the volume of fibre produced, whether through an increase in fibre length, fibre diameter or both. Where the difference in fibre mass and fibre volume exceeded the difference in medullation e.g. in July compared to January, there was a difference in fibre diameter. The small differences in fibre density seemed disproportionate to the differences in medullation, but without measurements of fibre length it is impossible to determine if there were differences in keratin density.

Romney and Merino wools expressed a three-fold difference in the mass and volume of fibre produced in July compared to January, in the first year of measurement. There was no measurable difference in fibre density but small changes in both medulla content and mean fibre diameter were observed. In this situation, the difference in mass and volume can conceivably be accounted for by changes in either fibre diameter, fibre length or both. In the second year of harvest, Merino wool expressed a small difference in mass but no difference occurred in either mean fibre diameter or fibre density, in July compared to January. These differences in mass and volume may have been largely accounted for by a difference in the length of fibres grown, as the Merino wool contained no medullation. However, no fibre length measurements were available to confirm this explanation.

The same Merino and Drysdale ewes were used throughout the two years of sampling, being two years old in the first year and three years old in the second year. Two year old Romney ewes were used in each year. The Cheviot ewes, only present in the second year, were also two years old. There was a significant increase in the mass of wool produced and the mean fibre diameter in Merino wool between the two sampling years, which was consistent with increasing animal age, as reported by Corbett (1979). The same increase was not evident in the Drysdale wool, but a decrease in medullation with an increase in age was evident. This has previously been reported by Rudall (1935), Goot (1945) and Tibbits (1959), for heavily medullated sheep breeds. The significant decrease in fibre density of Merino and Drysdale wool, observed as age increased (Table 7.1 and 7.2) will not be discussed here. Effects of age on fibre volume, fibre density, medulla content and mean fibre diameter are examined in more depth in the next chapter (Chapter 8).

8. Fibre density and medulla content of lambswool

8.1 Introduction

The term 'lambswool' describes the wool from lambs first shorn before seven months of age, and includes the birthcoat at the tip of the staples. The birthcoat manifests itself as characteristic spiral fibre tips (Ryder, 1956; Ryder and Stephenson, 1968).

A large number of the follicles in the skin of the lamb begin growing wool before the lamb is born. A notable feature of the well developed birthcoat is the variation in coverage and coarseness of the fibres (Fraser and Short, 1960; Ryder and Stephenson, 1968). Ryder (1956) identified two main pre-natal fibre types; pre-curly tip and curly-tip fibres. Both fibre types exhibit coarse, medullated regions and fine, crimped regions. A third fibre type, histerotrich fibres, appear post-nataly. These are the shortest and finest fibres in the birthcoat.

Rendel (1954) noted that the Romney, strong-wool Merino and Welsh Mountain breeds have similar birthcoats, but markedly different adult fleeces. Ryder and Stephenson (1968) also noted a variation with genotype in the pattern of birthcoat shedding and replacement of fibres. Medullated fibres, present in the birthcoat of the Romney, usually cease growing and are shed by the time the lamb is two to three months old, but medullation may persist in the fibres which regrow (Dry, 1933a,b; Rudall, 1933). In contrast, the birthcoat of Merino lambs exhibit a wide variation in medullation, which disappears in the first few weeks of life, as non-medullated fibres replace the shed fibres (Lockart, 1956).

Aside from differences in medullation, van Luijk (1984) suggested a difference in specific gravity between lambswool and that of the adult ewe. To date this difference has not been substantiated. The hypothesis being examined was that the density of lambswool is less than the density of the adult fleece.

8.2 Materials and methods

Midside wool samples were collected from the same Perendale sheep at four ages, for each of two cohorts. Wool was harvested immediately prior to shearing, at approximately 3, 12, 22 and 34 months of age (lamb, hogget, ewe and ewe, respectively), within each cohort. Wool was harvested at all four ages from thirty two animals born in 1990, and thirty four animals born in 1991.

Fibre volume and fibre density, of a 4.00 ± 0.05 g sub-sample, were measured using the helium pycnometer. Sufficient wool for only one specimen from each sample was available for measurement. Sample mass was recorded immediately prior to placement in, and on removal from the measurement chamber of the helium pycnometer. An OFDA was used to measure the medulla content (percent by volume) and mean fibre diameter of the samples.

Fibre length was not measured but would have varied according to the three, nine, ten and twelve month sampling intervals. The samples had been detergent scoured (Section 4.2) prior to receipt for measurement. Estimates of staple length were made, based on an average 120 mm fleece length grown by Perendale sheep (NZWB, 1982). Staple lengths of between 30 mm and 120 mm were estimated for the sampling intervals used, all of which would have exceeded the minimum length requirements for the helium pycnometer.

Samples were measured in a randomised block design. Individual animals were randomised within each cohort and age randomised within animal. Measurements were evenly spread over four days for each cohort. Analysis of variance was used to identify differences in fibre density and medullation, between cohorts at all four ages, and between animals of the same age in different years. Wool from the animals was categorised as having low (<10 % by volume) or high medullation (>10 % by volume) at each age of shearing. The division of medullation categories was arbitrary but consistent with previous experiments (Chapter 5, Chapter 6, Merrick and Scobie, 1997). The extent to which differences in density were due to differences in medulla content were examined using covariate analysis.

8.3 Results

Measurements of fibre density and percent medullation by volume, for wool shorn from two cohorts at three, twelve, twenty two and thirty four months of age, are presented in Table 8.1 and Table 8.2. Wool shorn from animals at three months of age was significantly more medullated than wool shorn at all other ages, within each cohort ($p<0.001$, for each cohort). There was a reduction of approximately 70 % in the mean medulla content between three and twelve months of age, for both years of birth. The fibre density of wool shorn at three months of age tended to be lower than the fibre density of wool shorn at twelve, twenty two or thirty four months of age, for animals born in 1990, but the difference was not significant ($p=0.082$). The tendency for fibre density to be lower at three months of age was largely removed ($p=0.774$) when

analysis of covariance was used to adjust for medulla content ($p<0.010$). Fibre density did not differ between animals of any age, within the second cohort ($p=0.785$).

The mean fibre density of wool shorn at three months of age was significantly lower for animals born in 1990, than for animals born in 1991 ($p<0.010$). Fibre density was similar between cohorts at all other ages. Medulla content was greater at each age, for animals born in 1990 than animals born in 1991, but not significantly so ($p=0.223$).

The high variability (Table 8.2) of the medulla content of wool shorn at three months of age, relative to shearing at older ages, was reflected in both the range of medullation expressed, and the number of animals within the medullation range. Twelve of the thirty two animals born in 1990 expressed between fourteen and fifty three percent medullation by volume. This compared to nine animals born in 1991, with medulla contents ranging between eleven and thirty eight percent by volume. All other animals expressed less than ten percent medullation by volume at each age of sampling, for both years of birth. The number of animals and the upper limit of the medullation range decreased rapidly with increasing age, for both years of birth. Two of the animals born in 1990 remained highly medullated (19 % and 29 % by volume), at thirty four months of age, while none of the animals born in 1991 expressed more than ten percent medullation by volume at either twenty two or thirty four months of age.

Table 8.1 Mean and variation of fibre density of a 4.00 g sample of wool shorn at three, twelve, twenty two and thirty four months of age.

	Fibre density (g/cm^3)			
Age (months)	born 1990 (n=32) mean	sd	born 1991 (n=34) mean	sd
3	1.259	± 0.037	1.289	± 0.033
12	1.271	± 0.019	1.288	± 0.028
22	1.274	± 0.021	1.294	± 0.037
34	1.273	± 0.028	1.288	± 0.032
$lsd_{0.05}$	0.013		0.012	

Table 8.2 Mean and standard deviation of percent medullation by volume for wool shorn from the same animals at three, twelve, twenty two and thirty four months of age, for animals born in 1990 and 1991.

Age (months)	Medulla content (% by volume) born 1990 mean	sd	born 1991 mean	sd
3	13.43	± 16.14	9.04	± 10.29
12	4.51	± 7.53	2.58	± 3.54
22	3.62	± 5.87	1.70	± 1.43
34	3.78	± 5.92	2.04	± 1.66
lsd $_{0.05}$	4.79		2.63	

The increases in mean fibre diameter with increasing age are presented in Table 8.3. The mean fibre diameter of wool shorn at three and twelve months of age was significantly lower than at twenty two and thirty four months of age ($p<0.001$), for both years of birth. Mean fibre diameter at three months of age was also significantly lower than at twelve months of age ($p<0.001$), for both years of birth. Mean fibre diameter differed significantly between years at twelve ($p<0.001$) and twenty two ($p<0.050$) months of age, but was not significantly different between years at three ($p=0.280$) and thirty four ($p=0.828$) months of age.

Table 8.3 Mean fibre diameter (µm) of wool shorn from animals at three, twelve, twenty two and thirty four months of age, for thirty two animals born in 1990 and thirty four animals born in 1991.

Age (months)	Mean fibre diameter (µm) born 1990	born 1991	lsd $_{0.05}$
3	30.0	30.6	1.1
12	32.8	34.8	1.0
22	37.0	38.4	1.3
34	38.7	38.8	1.3
lsd $_{0.05}$	1.2	1.2	

Measurements of sample mass, prior to placement in and following removal from the helium pycnometer are presented in Table 8.4. The decrease in fibre mass during measurement of volume and density was significant within each age class ($p<0.001$, for all ages), for both years of birth. Samples harvested at thirty four months of age lost

significantly more mass during measurement (0.004 g, $p<0.010$) than those harvested at twelve months of age, within the first cohort.

The decrease in mass during measurement of volume and density was significantly higher for the first cohort than for the second cohort, at three months ($p<0.001$), twenty two months ($p<0.010$) and thirty four months of age ($p<0.010$), but not at twelve months of age ($p=0.280$). The decrease in mass during measurement was similar for all ages, within the second cohort ($p=0.144$).

Table 8.4 Mean mass of wool samples immediately prior to (before) and following (after) measurement of volume and density, and the loss of fibre mass (difference) during measurement of wool shorn from animals at three, twelve, twenty two and thirty four months of age, for animals born in 1990 and 1991.

Year of birth		Age at shearing (months) three	twelve	twenty two	thirty four	lsd $_{0.05}$
1990	before	4.004	4.003	4.002	4.002	0.001
	after	3.992	3.993	3.991	3.989	0.002
	difference	0.011	0.010	0.012	0.013	0.002
	lsd $_{0.05}$	0.004	0.004	0.004	0.005	
1991	before	4.002	4.003	4.002	4.003	0.001
	after	3.994	3.994	3.993	3.993	0.001
	diff	0.008	0.009	0.010	0.010	0.002
	lsd $_{0.05}$	0.002	0.004	0.003	0.004	

8.4 Discussion

The density of lambswool, shorn from Perendale lambs at three months of age, did not differ from the density of wool shorn at twelve, twenty two or thirty four months of age, once the effect of medullation was accounted for. The ability of the animal to produce keratin has been found to improve after the lamb stage (Rudall, 1935), and had previously been observed in Romney sheep. Dry (1933a and 1933b) and Rudall (1933) reported that medullated fibres, present in the birthcoat of the Romney, were shed by the time lambs were two to three months old. In contrast to this, Goot (1945) and Tibbits (1959) found that peak medullation occurred in the hogget fleece.

The high between animal variability of the medulla content of lambswool is consistent with previous studies of variation in medullation. Goot (1945) reported that for Romney sheep, fifty percent of the variation in mean medulla content was due to variation between individual animals and seventeen percent was due to age. Such variation between individual animals was clearly illustrated in the experiment described in this chapter. While medullation decreased with age, two animals within the first cohort retained high levels of medullation at all sampling ages. In contrast, the medulla content of all animals was less than ten percent by volume for the samples taken at twenty two months of age, for the second cohort.

The medullation data was analysed using analyses of variance and covariance. The results of the analyses presented above were those of the raw data values. A logarithmic transformation was applied to the medullation values to normalise the data. Transforming the data did not greatly affect the direction or magnitude of the reported differences.

The difference in medulla content between cohorts, at the same age, was possibly due to differences between individual animals and differing seasonal conditions. It was not possible to separate these effects in the current experiment. However, Goot (1945) estimated that only four percent of the variation in mean medulla content was due to environmental differences. The differences between animals of the same age between years in the current study were therefore probably only marginally due to different environmental conditions.

The increase in mean fibre diameter over the four ages was consistent with known changes in fleece characteristics over the lifetime of sheep. Corbett (1979) reported that fibre diameter increased for the first three or four years of age, followed by a gradual decline in older sheep. As a consequence, fibres from lambswool would gradually increase in diameter from tip to butt (Morton and Hearle, 1962). Van Luijk (1984) postulated that for lambswool, differences in fibre shape, differences in specific gravity and different scale structures were possible reasons for airflow measurements of mean fibre diameter being finer than projection microscope measurements of mean fibre diameter. These differences were suggested in addition to differences in medulla content.

Published measurements of specific gravity were most probably measurements of keratin density rather than fibre density, due to the non-medullated wool types

measured. Now that quantitative measurements of fibre density and medulla content have been made, it would appear that the suggested differences in specific gravity of lambswool (van Luijk, 1984) could be attributed largely to differences in medullation, for the wools measured here.

There was a small but significant decrease in mass between cohorts, during measurement at three, twenty two and thirty four months of age. The difference was most likely explained by significant differences in mass prior to measurement at three months of age, and following measurement at twenty two and thirty four months of age. No explanation was forthcoming for the small but significantly greater decrease in mass at twelve months of age compared to thirty four months of age, within the first cohort. Potentially, more moisture was removed (Section 5.4) from the shorter fibre lengths measured at twelve months of age than from the longer fibre lengths measured at thirty four months of age. This was unlikely though, as the decrease in mass during measurement did not differ with age in the second cohort.

Differences in the specific gravity of keratin between lambswool and that of the adult fleece, suggested by van Luijk (1984), were not detected in the Perendale wool samples measured in this chapter. Any measurable differences in fibre density were accounted for by differences in medulla content, and fibre density appeared to be significantly reduced only when the medulla content exceeded ten percent by volume.

9. General Discussion

Fibre density and specific gravity are numerically equivalent, and the terms are often used interchangeably. The work of King (1926) and van Wyk and Nel (1940) measured specific gravity, while that of Connell and Andrews (1974) and the work presented in this thesis measured density.

Helium pycnometer measurements of fibre volume include the volume of keratin and the volume of medullae, if present. The volume and density of keratin in the fibres would be measured by the helium pycnometer when no medullae were present. Fibres cut to short lengths (2 mm and 5 mm) in an effort to remove the effect of medullation on fibre volume, caused blockages within the helium pycnometer. Thus, for medullated wools, the helium pycnometer was capable of measuring the total density and volume of fibres, but not the density and volume of the keratin component of fibres.

Changes in sample mass during measurement of fibre volume and fibre density were measured for the precision (Chapter 5) and lambswool (Chapter 8) experiments. There was a significant reduction in mass during measurement for both experiments. In the precision experiment it was proposed that the reduction in mass could have been due to fibre loss while transferring the sample to and from the helium pycnometer, moisture loss during measurement, or both. The suggestion was made that fibre loss during handling could be minimised by measuring longer fibre lengths. Long fibres were measured in Chapter 8. The decrease in mass during measurement was of a similar magnitude to that reported in Chapter 5, and short lengths of fibre were not observed clinging to the balance nor the measurement cup, as in Chapter 5. Measurement of longer fibres did not appear to reduce the decrease in mass during measurement of fibre volume and fibre density, indicating that the decrease in sample mass was due largely to moisture loss. Values of sample mass measured prior to the helium pycnometer measurement of volume were used for the calculation of density. Fibre density calculated using the reduced post-measurement sample mass would have been lower than the fibre density presented in the relevant chapters. However, a difference would only be noted in the third decimal place, for a four gram mass of sample measured.

The coefficient of variation (CV) for a single fibre volume measurement was 3.2 %, and the coefficient of variation for fibre density was 2.9 %. The helium pycnometer method of measuring density was less precise than either the benzene pycnometer (0.7 % CV, van Wyk and Nel, 1940) or density gradient column methods (1.0 % CV, Connell and Andrews, 1974). The mean fibre density of wool measured using the helium

pycnometer was lower than the specific gravity and density measured by other methods, but not markedly so. This was presumably due to medullated wool samples being included in the helium pycnometer measurements, while wools measured using the benzene pycnometer or density gradient column were free from medullation, as Merino wool was measured using the latter methods. The precision of the helium pycnometer measurement of fibre density was unaffected by the level of medullation in a wool sample. Effects of medullation on benzene pycnometer and density gradient column measurements of density are unknown.

The amount of medullation in a wool sample can be expressed as percent by number, percent by volume or both, depending on the method of measurement. Percent volume medullation was thought to be more relevant for the studies in this thesis, as fibre density is calculated from fibre volume. Also, a small number of heavily medullated fibres and a large number of fibres with little medullation would differ in percent medullation by number, but could potentially have the same percent medullation by volume. Logically, the total volume of medulla would affect fibre density to a greater extent than the number of medullated fibres.

An estimate of percent medullation by volume was derived from helium pycnometer measurements of fibre volume. While there was a significant relationship between the predicted percent medullation by volume and projection microscope measurements of percent medullation by volume, the relationship was not as strong as those between the OFDA, WRONZ Medullameter, or NIRA and the projection microscope methods of measuring medullation (Lee, unpublished). It was therefore not practical to pursue a measurement of medullation based on helium pycnometer measurements of fibre volume or fibre density. Subsequently, percent medullation by volume was measured using an OFDA, as the OFDA was capable of rapidly measuring the large numbers of samples requiring measurement.

However, Maher *et al.* (in press) highlighted inaccuracies in the OFDA software, which concern the calculation of percent medullation by volume. An exponential correction for the raw data has been proposed, but not yet implemented. The correction reduces the slope of the regression of OFDA versus projection microscope estimates of percent medullation by volume closer to unity, and the intercept closer to zero, than is currently the case. The percent medullation by volume for samples with a high medulla content would currently have been under-estimated, while that of samples with low medullation would have been over-estimated. The impact of the proposed correction on the work presented in this thesis is unclear, but would probably have been minimal, since the precision of the OFDA method was found to be good (Maher *et al.*, in press). The

medulla content of the Drysdale breed, examined in the seasonality experiment (Chapter 7), was probably under-estimated, while the medulla content of the Romney wool (4.8 %) may have been falsely inflated. The medulla content of the non-medullated Merino wool was unlikely to have been affected. There would probably be minimal difference in medullation values within the lambswool experiment (Chapter 8), as very few of the Perendale animals expressed excessively high or low levels of medullation.

Van Wyk and Nel (1940) suggested that a difference in the third decimal place represented an important difference in specific gravity. Their work was based on a small number of samples from various strains of Merino sheep. The work presented in this thesis encompassed breeds with a wide range of fleece characteristics, for which large numbers of samples were measured. The average fibre density of wool with less than five percent medullation by volume varied between 1.238 g/cm^3, 1.287 g/cm^3, 1.256 g/cm^3 and 1.290 g/cm^3, for Chapters 4, 5, 7 and 8, respectively. Allowing for the precision of the helium pycnometer, the magnitude of these differences were not important, in practical terms.

Investigations into the effect of medullation on density indicated that a medulla content of less than ten percent by volume did not significantly affect fibre density. The fibre density of wool with greater than ten percent medullation was significantly less than the fibre density of wool with little or no medullation, for the experiments reported. Such findings confirm the presumed lower specific gravity of medullated wools (WIRA, 1973; van Luijk, 1984). The variation in fibre density between medullated and non-medullated wools was only partially explained by the medulla content of the wool. Thirty eight, seventeen and twenty three percent of the variation in fibre density was explained by medulla content for the experiments reported in Chapters 6, 7 and 8, respectively. The low proportion of variation in fibre density which is explained by medullation strongly suggests that factors other than medullation may be responsible for differences in fibre density, and raises the possibility of differences in keratin density. This is an area of work which requires further investigation. Until the possibility of variable keratin density is confirmed there is no reason to use a value for the density of wool fibre which differs from that published for non-medullated wool. Accepted values for the fibre density of non-medullated wool range from 1.304 g/cm^3 (van Wyk and Nel, 1940) to 1.312 g/cm^3 (WIRA, 1973).

The helium pycnometer provided a rapid method for the measurement of fibre volume and fibre density, for both medullated and non-medullated wool types, provided sample constraints, summarised in the conclusion (Chapter 10), were adhered to.

10. Conclusions

The objectives of this research were to:

(i) complete the development of a rapid method to measure the density of wool, using a helium pycnometer

(ii) examine and quantify the relationship between fibre volume, fibre density and the medulla content of wool

(iii) examine and quantify seasonal differences in fibre density and the medulla content of wool and

(iv) substantiate differences in fibre density between lambswool and that of the adult ewe

- The recommendation that only fibre lengths greater than or equal to ten millimetres be measured in the helium pycnometer completed the development of a rapid method for measuring the fibre density of wool.

  In conjunction with previously published measurement constraints for the helium pycnometer (Merrick *et al.*, in press), the recommended standardised method for the rapid measurement of volume and density of wool fibre is to use:
  - standard conditions of temperature (20°C ± 2°C) and relative humidity (65% ± 2%) for measurement
  - a sample mass between one and ten grams
  - fibre lengths greater than or equal to ten millimetres
  - the single fourth sequential measurement of fibre volume for the calculation of fibre density

- There was a moderate relationship between medulla content (percent by volume) and fibre density, suggesting that medullation is an important source of variation in the density of wool but factors additional to medullation may also contribute to differences in fibre density.

- Fibre volume, fibre density and medulla content varied seasonally. The maximum fibre volume and highest levels of medullation (percent by volume) were produced in the summer months. Minimum fibre volume and medullation occurred during the winter months. Fibre density varied inversely to fibre volume and medulla content.

- Significant differences in wool density with age were detected. However, there was no significant difference between the density of lambswool and that of the adult ewe, once the effect of medullation was accounted for.

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References

Abbott, N.J. and Goodings, A.C. 1949. Moisture absorption, density and swelling properties of nylon filaments. *Journal of the Textile Institute* **40:** T232-246.

Alexander, P. and Earland, C. 1950. The sub-cuticle membrane - a recently discovered morphological component of the wool fiber. *Textile Research Journal* **20:** 298-300.

Alexander, P. and Hudson, R.F. 1963. *Wool, it's chemistry and physics.* Chapman and Hall, London.

Allden, W.G. 1979. Feed intake, diet composition and wool growth. In *Physiological and Environmental Limitations to Wool Growth* (ed. J. L. Black and P. J. Reis), pp. 61-78. University of New England, Armidale.

Anderson, S.L. and Benson, F. 1953. Fibre ellipticity and its effect on fibre diameter measurement. *Journal of the Textile Institute* **44:** 98-104.

Anderson, S.L. and Palmer, R.C. 1951.The effect of non-circular cross-section on fibre diameter measurement of wool by the profile method. *Journal of the Textile Institute* **42:** P114-P116.

Arora, R.K. and Gupta, N.P. 1977. Medullated and non-medullated fibres. *The Indian Textile Journal* **32:** 121-124.

Auber, L. 1950. The anatomy of follicles producing wool fibres, with special reference to keratinization. *Transactions of the Royal Society of Edinburgh* **62:** 191-254.

Auber, L. and Ryder, M.L. 1956. Anomolies in structure and development of wool fibres. *Proceedings of the 1st International Wool Research Conference* **F:** 32-36.

Austin, J.C. and Roberts, J.S. 1956. A comparison of gradient and flotation methods for determining density of degraded cotton. *Textile Research Journal* **26:** 303-309.

Barker, S.G. 1931. Density of wool. In *Modern Methods and Developments of Fibre Measurement* pp. 116-120. His Majesty's Stationery Office, London.

Barker, S.G. and King, A.T. 1926. A comparison of measurements of diameters of wool fibres with the micro-balance and the projecting microscope, with applications to the determination of density and medulla (kemp) composition. *Journal of the Textile Institute* **17:** T68-T74.

Baxter, B.P. 1993. Influences on comparisons between the mean fibre diameter of wools measured by airflow and by projected image methods. *International Wool Textile Organisation Technical Committee* **Istanbul:** Report No. 13

Baxter, B.P., Brims, M.A. and Taylor, T.B. 1991. Description and performance of the Optical Fibre Diameter Analyser (OFDA). *International Wool Textile Organisation Technical Committee* **Nice:** Report No. 8

Baxter, P. in press. An initial investigation into the effects of medullation on comparisons between Airflow, OFDA and Laserscan. *IWTO Technology and Standards Committee* **Dresden:** Report No. 16.

Belin, R.E. and Goldstone, C.L. 1951. A wool medullameter. *Journal of the Textile Institute* **42:** 332-334.

Bendit, E.G. 1956. *A review of proposed molecular structures of keratin.* Wool Textile Research Laboratories, Melbourne.

Besançon, R.M. 1974. *The Encyclopaedia of Physics.* Van Nostrand Reinhold Company, New York.

Black, J.L. 1987. Mechanisms controlling the rate of growth, composition and morphology of wool. In *Merino Improvement Programs in Australia* (ed. B. J. McGuirk), pp. 457-480. Australian Wool Corporation, Melbourne.

Bradbury, J.H. 1973. The structure and chemistry of keratin fibers. *Advances in Protein Chemistry* **27:** 111-211.

Bradbury, J.H., Leeder, J.D. and Watt, I.C. 1971. The cell membrane complex of wool. *Applied Polymer Symposium* **18:** 227-236.

Bradbury, J.H. and O'Shea, J.M. 1969. Keratin fibres II. Separation and analysis of medullary cells. *Australian Journal of Biological Science* **22:** 1205-1215.

Brady, P.R. 1990. Penetration pathways of dyes into natural protein fibres. *Proceedings of the 8th International Wool Textile Research Conference* **IV:** 217-226.

Bray, R.J. 1942. Hairiness in wool and its measurement. *Wool Industries Research Association Bulletin* **9:** 76-79.

Brims, M.A. and Peterson, A.D. 1994. Measuring fibre opacity and medullation using OFDA: Theory and experimental results on mohair. *IWTO Technology and Standards Committee* **New Delhi:** Report No. 21.

Browne, N.McM. and Hindson, W.R. 1982.The effect of snippet length on the estimation of wool-fibre diameter by the projection-microscope method. *Journal of the Textile Institute* **73:** 196-199.

Brown, T.D. and Onions, W.J. 1960. Anomolies in the microscopic structure of some wools. *Nature* **186:** 93-94.

Cassie, A.B.D. 1942. The porous plug and fibre diameter measurement. 1. A practical method of wool fibre diameter measurement. *The Journal of the Textile Institute* **33:** T195-T204.

Chapman, R.E. 1971. Cell migration in wool follicles of sheep. *Journal of Cell Science* **9:** 791-803.

Chapman, R.E. 1976. Electron microscopic and histochemical features of the formation of the orthocortex and paracortex in wool. *Proceedings of the 5th International Wool Textile Research Conference* **II:** 152-161.

Chapman, R.E. and Gemmell, R.T. 1971. Stages in the formation and keratinization of the cortex of the wool fiber. *Journal of Ultrastructure Research* **36:** 342-354.

Chapman, R.E. and Ward, K.A. 1979. Histological and biochemical features of the wool fibre and follicle. In *Physiological and Environmental Limitations to Wool Growth* (ed. J. L. Black and P. J. Reis), pp. 193-208. University of New England, Armidale, New South Wales.

Collins Concise Dictionary and Thesaurus 1994. Harper Collins Publishers, Glasgow.

Connell, J.P. and Andrews, M.W. 1974. 1. The variability of the specific gravity of Australian wools and the implications for the air-flow measurement of diameter. *Journal of the Textile Institute* **65:** 1-5.

Corbett, J.L. 1979. Variation in wool growth with physiological state. In *Physiological and Environmental Limitations to Wool Growth* (ed. J. L. Black and P. J. Reis), pp. 79-98. University of New England, Armidale, New South Wales.

Davidson, G.F. 1927. The specific volume of cotton cellulose. *Journal of the Textile Institute* **18:** T175-T186.

Doney, J.M. and Smith, W.F. 1962. Multiple medullae in wool fibres. *Nature* **195:** 723.

Dry, F.W. 1933a. Halo-hairs in the Romney lamb. Breeding experiments up to 1933 season. *New Zealand Journal of Agriculture* **46:** 289-296.

Dry, F.W. 1933b. Types of hairy fibres in the fleece of the Romney lamb, their identification and importance. *New Zealand Journal of Agriculture* **46:** 39-42.

Duerden, J.E. 1926. Kemp fibres in the Merino. In *Kemp* (ed. H. J. W. Bliss, J. E. Duerden, F. J. A. Roberts, J. S. S. Blyth, H. R. Hirsch and A. T. King), pp. 5-10. British Association for the Woollen and Worsted Industries, Leeds.

Earland, C., Blakey, P. and Stell, J.G. 1962. Studies on the structure of keratin IV. *Biochimica et Biophysica Acta* **56:** 268-274.

Edmunds, A.R. 1992a. Effects of snippet length on profile fibre diameter measurements. *IWTO Technology and Standards Committee* **Nice:** Report No. 14.

Edmunds, A.R. 1992b. Image processing in wool fibre diameter measurement. *Proceedings of the New Zealand Image Processing Workshop* **7:** 227-232.

Elphick, B.L. 1932. The detection and estimation of medullated fibre in New Zealand Romney fleeces. *Journal of the Textile Institute* **23:** T367-T385.

Fraser, A.S. and Short, B.F. 1960. *The Biology of the Fleece.* Commonwealth Scientific and Industrial Research Organization, Australia, Melbourne.

Fraser, I.E.B. 1965. Cellular proliferation in the wool follicle bulb. In *Biology of the Skin and Hair Growth* (ed. A. G. Lyne and B. F. Short), pp. 427-445. Angus and Robertson, Sydney.

Fraser, R.D.B., Gillespie, J.M. and MacRae, T.P. 1973. Tyrosine-rich proteins in keratins. *Comparative Biochemistry and Physiology* **44B:** 943-947.

Fraser, R.D.B. and MacRae, T.P. 1957. Molecular organization in keratins. Part II: The densities of native keratins. *Textile Research Journal* **27:** 384-390.

Fraser, R.D.B., MacRae, T.P. and Rogers, G.E. 1972. Keratins - their composition, structure and biosynthesis. Springfield, New York.

Gemmell, R.T. and Chapman, R.E. 1971. Formation and breakdown of the inner root sheath and features of the pilary canal epithelium in the wool follicle. *Journal of Ultrastructure Research* **36:** 355-366.

Goot, H. 1945. Hairiness in wool. Part II. Yearly changes in hairiness in New Zealand Romney Marsh sheep. *New Zealand Journal of Science and Technology* **27:** 173-187.

Gourdie, R.G. 1989. The relationship between wool fibre intrinsic material strength variation and staple strength in a flock of New Zealand Romney ewes. Ph.D. Thesis, Lincoln College, University of Canterbury, Christchurch.

Hammersley, M.J., Townsend, P.E., Grayston, G.F. and Ranford, S.L. 1995. Visible/near infrared spectroscopy of scoured wool. *Textile Research Journal* **65:** 241-246.

Hansford, K.A., Emery, G.B. and Teasdale, D.C. 1985. A method to measure the diameter profile of wool staples. *Wool Technology and Sheep Breeding* **33:** 25-27.

Hawker, H. and Crosbie, S.F. 1985. Effects of level of nutrition in winter and summer on the wool growth of Romney and Perendale ewes with a history of high or low winter wool growth. *Proceedings of the New Zealand Society of Animal Production* **45:** 225-228.

Horio, M. and Kondo, T. 1953. Crimping of wool fibers. *Textile Research Journal* **23:** 373-387.

Ince, J. 1977. The relationship between fibre and yarn properties and the performance of tufted carpets. PhD Dissertation, University of Leeds.

Ince, J. and Ryder, M.L. 1984. The evaluation of carpets made from experimental wools. *Journal of the Textile Institute* **75:** 47-59.

ISO 1992. Coating powders - Part 2. Determination of density by gas comparison pyknometer (referee method). *International Organization for Standardization* **ISO 8130-2:1992(E)**

Ito, H., Miyamoto, T. and Inagaki, H. 1987. States of water sorbed on wool as studied by differential scanning calorimetry. *Textile Research Journal* **57:** 66-72.

IWTO 1982. Determination by the airflow method of the mean fibre diameter of core samples of raw wool. **IWTO-28-82** *International Wool Textile Organisation.*

IWTO 1989. Method of determining fibre diameter and percentage of medullated fibres in wool and other animal fibres by the projection microscope. **IWTO-8-89(E)** *International Wool Textile Organisation.*

IWTO 1995. Measurement of the mean and distribution of fibre diameter of wool using an Optical Fibre Diameter Analyser (OFDA). **IWTO-47-95** *International Wool Textile Organisation.*

IWTO 1996. Determination of objectionable medullated fibre content of wool and mohair samples by opacity measurements using an OFDA. **IWTO Draft TM-57-96** *International Wool Textile Organisation.*

James, J.F.P. and David, H.G. 1968. The air-flow diameter of wool tops: The effect of coefficient of variation. *Journal of the Textile Institute* **59:** 585-592.

Kaplin, I.J. and Whiteley, K.J. 1978. An electron microscope study of fibril: matrix arrangements in high- and low-crimp wool fibres. *Australian Journal of Biological Science* **31:** 231-240.

Keogh, H.J. and Haylett, T. 1973. Observations on the morphology and chemical analysis of medullary granules in Chinchilla hair. *South African Journal of Science* **79:** 65-67.

King, A.T. 1926. V. The specific gravity of wool and its relation to swelling and sorption in water and other liquids. *Journal of the Textile Institute* **17:** T53-T67.

Kulkarni, V.G., Robson, R.M. and Robson, A. 1971. Studies on the orthocortex and paracortex of Merino wool. *Applied Polymer Symposium* **18:** 127-146.

Lang, W.R. 1947. The comparative thickness of medullated and non-medullated fibres in Corriedale wool. *Journal of the Australian Institute of Agricultural Science* **13:** 69-70.

Lappage, J. and Bedford, J. 1983. *The WRONZ Medullameter* No. R107). Wool Research Organisation of New Zealand (Inc.).

Lee, J.A., Maher, A.P., Frampton, C.M.A. and Ranford, S.L. 1996. Comparisons of medullation measurements on the same fibre sites using OFDA and projection microscope. *International Wool Textile Organisation Technology and Standards Committee* **Capetown:** Report No.14

Leeder, J.D., Holt, L.A., Rippon, J.A. and Stapleton, I.W. 1990. Diffusion of dyes and other reagents into the wool fibre. *Proceedings of the 8th International Wool Textile Research Conference* **IV:** 227 - 237.

Lewis, D.M. 1990. Dyeing and wet processing of wool. *Proceedings of the 8th International Wool Textile Research Conference* **IV:** 1-49.

Lockart, L.W. 1956. Birthcoat of lambs and adult fibre diameter. *Australian Journal of Agricultural Research* **7:** 152-157.

Lofts, P.F. and Truter, E.V. 1969. The constitution of the epicuticle of wool. *The Journal of the Textile Institute* **60:** 46-56.

MacKinnon, P.J., Powell, B.C. and Rogers, G.E. 1990. Structure and expression of genes for a class of cysteine-rich proteins of the cuticle layers of differentiating wool and hair follicles. *The Journal of Cell Biology* **111:** 2587-2600.

Maher, A.P., Cassidy, B.D. and Lee, J.A. in press. The measurement of medullation percent by volume of wool using an OFDA. *International Wool Textile Organisation Technology and Standards Committee* **Dresden:** Report No. 19.

Marshall, R.C. and Gillespie, J.M. 1988. Variations in the proteins of wool and hair. In *The Biology of Wool and Hair* (ed. G. E. Rogers, P. J. Reis, K. A. Ward and R. C. Marshall), pp. 117-125. Chapman & Hall, London & New York.

Matoltsy, A.G. 1953. A study of the medullary cells of the hair. *Experimental Cell Research* **5:** 98-110.

McMahon, P.R. 1937. Methods for the estimation of medullation in wool samples. *Journal of the Textile Institute* **28:** 349-360.

Mercer, E.M. 1953. The heterogeneity of the keratin fibers. *Textile Research Journal* **23:** 388-397.

Mercer, E.H. 1954. The relation between external shape and internal structure of wool fibers. *Textile Research Journal* **24:** 39-43.

Mercer, E.H., Golden, R.L. and Jefferies, E.B. 1954. Distribution of cystine in the cortex of wool. *Textile Research Journal* **24:** 615-618.

Merrick, N.C. and Scobie, D.R. 1997. Volume measurement of wool samples. *Proceedings of the New Zealand Society of Animal Production* **57:** 57-60.

Merrick, N.C., Scobie, D.R. and Baird, D.B. in press. A rapid method for measurement of wool volume and density. *Journal of the Textile Institute*

Morton, W.E. and Hearle, J.W.S. 1962. *Physical Properties of Textile Fibres.* Butterworth & Co. Ltd. and the Textile Institute, Manchester and London.

Nagorcka, B.N. 1979. The effect of photoperiod on wool growth. In *Physiological and Environmental Limitations to Wool Growth* (ed. J. L. Black and P. J. Reis), pp. 127-137. University of New England, Armidale, New South Wales.

Nissen-Wooller, B., Baird, K. and Marler, J.W. 1994. Mean fibre diameter measurement of coarse tops using the projection microscope method - comparison of two snippet lengths. *IWTO Technology and Standards Committee* **Nice:** Report No. 8.

Nott, J.A. and Sikorski, J. 1965. The fine structure and external morphology of birthcoat fibres from sheep. *Proceedings of the 3rd International Wool Textile Research Conference* **I:** 197-208.

NZWB 1982. *New Zealand Sheep and their Wool.* Grower Services Division, New Zealand Wool Board, Wellington

Orwin, D.F.G. 1979. Cytology and cytochemistry of the wool follicle. *International Review of Cytology* **60:** 331-374.

Orwin, D.F.G., Woods, J.L. and Elliot, K.H. 1980. Composition of the cortex of sound and tender wools. *Proceedings of the 6th International Wool Textile Research Conference* **II:** 193-205.

Orwin, D.F.G. 1988. Variations in wool follicle morphology. In *The Biology of Wool and Hair* (ed. G. E. Rogers, P. J. Reis, K. A. Ward and R. C. Marshall), pp. 227-241. Chapman & Hall, London & New York.

Orwin, D.F.G. and Bailey, D.G. 1988. Measurement of wool cortical cell proportions by image processing. In *The Application of Mathematics and Physics in the Wool Industry.* (ed. G. A. Carnaby, E. J. Wood and L. F. Story), pp. 330. WRONZ Special Publication, Christchurch.

Orwin, D.F.G., Woods, J.L. and Ranford, S.L. 1984. Cortical cell types and their distribution in wool fibres. *Australian Journal of Biological Science* **37:** 237-255.

Perkin, M.E.A. and Appleyard, H.M. 1964. Abnormalities in wool and some other animal fibres. *Journal of the Textile Institute* **55:** T396-T400.

Peryman, R.V. 1952. Significance of hairiness (medullation) to the textile industry. Part II. Corriedale hogget wools. *New Zealand Journal of Science and Technology* **34:** 47-58.

Peters, D.E. and Bradbury, J.H. 1976. The chemical composition of wool. XV. The cell membrane complex. *Australian Journal of Biological Science* **29:** 43-55.

Pierce, F.T. 1929. A two-phase theory of the absorption of water vapour by cotton cellulose. *Journal of the Textile Institute* **20:** T133-T150.

Powell, B., Kuczek, E., Crocker, L., O'Donnell, M. and Rogers, G. 1988. Keratin gene expression in wool fibre development. In *The Biology of Wool and Hair* (ed. G. E. Rogers, P. J. Reis, K. A. Ward and R. C. Marshall), pp. 325. Chapman & Hall, London & New York.

Powell, B.C. and Rogers, G.E. 1990. Hard keratin IF and associated protein. In *Cellular and Molecular Biology of Intermediate Filaments* (ed. R. D. Goldman and P. M. Steinert), pp. 267-300. Plenum Press, New York.

Preston, J.M. and Nimkar, M.V. 1950. The use of density gradient tubes in fibre studies and their application to the analysis of fibre mixtures. *Journal of the Textile Institute* **41:** T446-T454.

Rama Rao, D. and Chopra, S.K. 1987. The effect of the medulla on the stress/strain properties of wool fibres. *Journal of the Textile Institute* **78:** 306-308.

Ranford, S.L., Hammersley, M.J., Acker, V.C. and Glassey, F.L. 1990. The measurement of medullation using Near Infrared Analysis. *Proceedings of the 8th International Wool Textile Research Conference* **II:** 94-103.

Reis, P.J. 1979. Amino acids and wool growth. In *Physiological and Environmental Limitations to Wool Growth* (ed. J. L. Black and P. J. Reis), pp. 223-242. University of New England, Armidale, New South Wales.

Reis, P.J. and Downes, A.M. 1971. The rate of response of wool growth to abomasal supplements of casein. *Journal of Agricultural Science* **76:** 173-176.

Reis, P.J., Tunks, D.A. and Downes, A.M. 1973. The influence of abomasal and intravenous supplements of sulphur-containing amino acids on wool growth rate. *Australian Journal of Biological Science* **26:** 249-258.

Rendel, J.M. 1954. Inheritance of birthcoat in a flock of improved Welsh Mountain sheep. *Australian Journal of Agricultural Research* **5:** 297-304.

Robards, G.E. 1979. Regional and seasonal variation in wool growth throughout Australia. In *Physiological and Environmental Limitations to Wool Growth* (ed. J. L. Black and P. J. Reis), pp. 1-42. University of New England, Armidale, New South Wales.

Roberts, J.A.F. 1930. Fleece analysis for biological and agricultural purposes. I - The average fineness of a sample of wool. *Journal of the Textile Institute* **21:** 1-38.

Rogers, G.E. 1959. Electron microscope studies of hair and wool. *Annals of the New York Academy of Science* **83:** 378-399.

Rogers, G.E. 1962. Occurence of citrulline in proteins. *Nature* **194:** 1149-1151.

Ross, D.A. 1988. *Wool Manual.* Lincoln University, Christchurch, New Zealand.

Ross, D.A. 1990. Fibre diameter, medulla diameter and fibre tenacity. *Proceedings of the 8th International Wool Textile Research Conference* **I:** 569-579.

Ross, D.A. 1991. *Wool characteristics and processing.* Lincoln University, Lincoln.

Rudall, K.M. 1933. Effect of shearing on hairiness in the fleece of the Romney lamb. *New Zealand Journal of Agriculture* **47:** 20-28.

Rudall, K.M. 1934. Pulling out wool fibres and its effect on hairiness. *New Zealand Journal of Agriculture* **48:** 3-11.

Rudall, K.M. 1935. Growth changes in wool fibres; experiments on removing the heat conserving fleece. I. *Journal of the Textile Institute* **26:** T359-T370.

Ryder, M.L. 1956. The pre-natal development of follicle populations in the Romney lamb. *Journal of Agricultural Science* **47:** 6-11.

Ryder, M.L. 1966. Coat structure and seasonal shedding in the goat. *Animal Production* **8:** 289-302.

Ryder, M.L. and Stephenson, S.K. 1968. *Wool Growth.* Academic Press, London.

Schåfer, K. 1994. Microscopical investigation of the diffusion of dyestuffs into keratin fibres. *Wool Technology and Sheep Breeding* **42:** 59-82.

Scobie, D.R., Bray, A.R. and Merrick, N.C. 1998. Medullation and average fibre diameter vary independently in the wool of Romney sheep. *New Zealand Journal of Agricultural Research* **41:** 101-110.

Scobie, D.R. and Woods, J.L. 1992. A review implicating a two compartment model for the processes of cell division and differentiation in the wool follicle. *Proceedings of the New Zealand Society of Animal Production* **52:** 265-271

Scobie, D.R., Woods, J.L. and Baird, D.B. 1993. Seasonal and between sheep differences in medullation of wool fibres. *Proceedings of the New Zealand Society of Animal Production* **53:** 319-322.

Skårman, S. and Nõmmera, E. 1954. Seasonal variations of fineness and medullation in Cheviot wool. *Annals of the Royal Agricultural College of Sweden* **21:** 367-384.

Speakman, J.B. 1944. An analysis of the water adsorption isotherm of wool. *Transactions of the Faraday Society* **40:** 6-10.

Stec Inc. 1992. Instruction Manual, Gas Pycnometer VM-100. *Head Office: No.2, Miyanohigashimachi, Kisshoin Minami-Ku, Kyoto, Japan*

Steinert, P.M., Harding, H.J.W. and Rogers, G.E. 1969. The characterisation of protein-bound citrulline. *Biochimica et Biophysica Acta* **175:** 1-9.

Stock, C.R. and Scofield, E.R. 1951. Application of the specific gradient column to the quantitative determination of additives in a base material. *Textile Research Journal* **21:** 521-526.

Story, L.F. 1978. Carpet wools - carpet manufacture. *WRONZ miscellaneous publication*

Story, L.F. and Ross, D.A. 1960. Effect of shearing time on wool. VI. The rate of growth of wool and its relation to time of shearing. *New Zealand Journal of Agricultural Research* **3:** 113-124.

Swift, J.A. 1992. Swelling of human hair by water. *Proceedings of the 8th International Hair Science Symposium* **8:** 123.

Tibbits, J.P. 1959. Post-natal growth changes in the fibre cortex and medulla of the New Zealand Romney and N-type sheep. *Journal of Agricultural Science* **53:** 358-368.

Turpie, D.W.F. and Steenkamp, C.H. 1995. Objective measurement of 'objectionable' medullated fibres in commercial mohair tops using an Optical Fibre Diameter Analyser (OFDA) - An introductory study. *International Wool Textile Organisation Technical Committee* **Harrogate:** Report No. 1

Van Luijk, C.J. 1984. *The measurement of wool fibre diameter* Report No.C96. Wool Research Organisation of New Zealand (Inc.).

Van Wyk, C.M. and Nel, H.A.M. 1940. Studies on the basic characteristics of South African Merino wool. II. Specific gravity. *Onderstepoort Journal of Vetrinary Science and Animal Industry* **15:** 325-332.

Warburton, F.L. 1947. A direct measurement of the transverse swelling of wool fibres in water vapour. *Journal of the Textile Institute* **38:** T65-T72.

Watt, I.C., Kennett, R.H. and James, J.F.P. 1959. The dry weight of wool. *Textile Research Journal* **29:** 975-981.

White, D.H., Nagorcka, B.N. and Birrell, H.A. 1979. Predicting wool growth of sheep under field conditions. In *Physiological and Environmental Limitations to Wool Growth* (ed. J. L. Black and P. J. Reis), pp. 139-161. University of New England, Armidale, New South Wales.

Wildman, A.B. 1954. *The microscopy of animal textile fibres.* Wool Industries Research Association, Leeds.

WIRA 1973. *The Wool Industries Research Association Textile Data Book.* Thornton & Pearson (Printers) Ltd., Bradford.

WRONZ Internal Method. 01.20 1989. Preparation of sample and calibration of medullameter for the measurement of medullation.

Wortmann, F.-J. and de Jong, S. 1985. Analysis of the humidity-time superposition for wool fibers. *Textile Research Journal* **55:** 750-756.

Appendices

Appendix I

A Rapid Method for the Measurement of Wool Volume and Density

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The volume of wool was measured with a gas pycnometer, and its density was calculated. Samples with a high degree of medullation, low degree of medullation, and no medullation were measured at five relative humidities and over a range of masses. The procedure adopted required a sample mass of between 2 and 10 g and used the fourth sequential measurement of volume to calculate density at 65% relative humidity and 20°C. The total measurement time per sample was 10 minutes. Density decreased as the degree of medullation increased. Non-medullated wools had a density of 1.307 g/cm^3.

1. INTRODUCTION

Previous methods of measuring the specific gravity and volume of wool samples involved tedious liquid-displacement techniques. The benzene-pycnometer method used by King (1926) and van Wyk and Nel (1940) to derive specific gravity by weight difference was slow. Using density-gradient columns, Connell and Andrews (1974) reduced the measurement time to around 16 h per sample. Both these methods use liquid as the displacement medium, which introduces a potential problem of absorption of the liquid by the fibre.

Accurate estimates of specific gravity or volume will only be obtained if the displacement medium is not absorbed by the wool. Potentially, liquid could also penetrate the airspace of medullated fibres. Non-medullated merino wool from Australian and South African sheep was used in early attempts to determine specific gravity. For the benzene-pycnometer method, King (1926) found that absorption of the liquid by the fibre was minimised by using liquids such as benzene, toluene, nitrobenzene, oleic acid, or olive oil. Similarly, in the density-gradient columns, Connell and Andrews (1974) used liquids with bulky molecules (t-butanol and tetrachloroethene) to minimise the problem. With these methods, measured differences in specific gravity between wool samples from different strains of merino sheep were small (van Wyk and Nel, 1940; Connell and Andrews, 1974).

Both the benzene pycnometer and gradient columns measure specific gravity, from which wool volume can be calculated. A gas pycnometer has the ability to measure the volume of a solid directly. From this and the mass of the solid, the density can be calculated. The pycnometer uses gas rather than liquid as the displacement medium, thus avoiding liquid-absorption problems. The volume measurement of a wool sample, carried out by using a gas pycnometer, is likely to be affected by the moisture content, degree of medullation, and mass of the sample. The series of three experiments described in this paper was designed to develop a procedure to measure the volume of wool samples, with the gas pycnometer, and for the calculation of density by using that volume and mass.

2. MATERIALS AND METHODS

2.1 Principle of the Gas Pycnometer

The gas pycnometer consists of two chambers connected by a series of valves. The system is purged to atmospheric pressure (P_a) before placing the sample, of unknown volume (V_x), in the measurement chamber, of known volume (V_1). Helium gas is pumped into the measurement chamber and the pressure (P_1) measured. The gas is then released to the second chamber, of known volume (V_2), where it expands to fill the chamber. The resultant pressure (P_2) is measured. The sample volume (V_x) is calculated from volumes V_1 and V_2 and the ratio of pressures P_1:P_2, as described by the following equation:

$$V_x = V_1 + \frac{1}{1 - \dfrac{P_1 - P_a}{P_2 - P_a}} V_2$$

A helium pycnometer, manufactured by Stec Incorporated (No. 2, Miyanohigashimachi, Kisshoin, Minami-Ku, Kyoto, Japan) was used in this study. Each time the pycnometer was switched on, it was calibrated at the zero limit and upper limits, the empty sample cup and a stainless-steel ball of known volume, respectively, being used. Four sequential measurements were made at each limit. Air enters the system when the measurement chamber is loaded. Several complete measurement cycles are required for the helium to displace all the air in the chambers. Individual measurement cycles take 99 s. The full calibration requires around 15 min.

2.2 Sequential Measurement

In the first experiment, 64 midside wool samples were harvested from eight sheep breeds (halfbred (English Leicester × merino), merino, Poll Dorset, Romney, English Leicester, Drysdale, Suffolk, and Lincoln), after 28 days' growth in winter. The animals and breeds were selected to represent a range of wool types, varying in fibre diameter, medullation, and fibre-growth rate. Greasy samples were scoured prior to measurement with a 3-min wash in a warm aqueous solution of Teric GN9 (0.15%) followed by two warm-water rinses. The samples were then oven-dried (45°C) overnight and left to condition for 24 h at 65% relative humidity and 20°C. Five sequential measurements of volume were made on each conditioned sample.

The ability of individual or combinations of measurements to predict volume was investigated by regression analysis (GENSTAT5, Rothamsted Agricultural Research Station) of the five sequential volume measurements.

To establish when the measured volume had reached a plateau, a rate of decay (R) with sequential measurement was calculated, by fitting an exponential function between the first and fifth measurements, according to the equation:

$$V_\infty = V_F + (V_0 - V_F) * e^{-Rt}$$

where V_∞ = predicted volume;
V_0 = first volume;
V_F = fifth volume;
e = the exponential function; and
t = sequential-measurement number.

2.3 Effect of Humidity on Wool Volume

As a continuation of the first experiment, the same 64 wool samples were measured at five relative humidities (80, 65, 40, 20, and 0% r.h.). The temperature remained constant at 20°C.

A humidity-controlled room was set to 80, 65, 40, or 20% r.h. and left to equilibrate for at least 48 h. The gas pycnometer and wool samples were placed in the conditioned room 24 h before five sequential volume measurements were made on each sample. At each humidity, the mass of the wool sample was recorded immediately prior to placement in the pycnometer. To simulate conditions of zero humidity, samples were oven-dried (45°C) overnight and then placed in an airtight desiccator for 24 h before being measured, in a conditioned room set at 20% r.h. and 20°C. The transfer time between desiccator and pycnometer was minimised to keep samples as close to 0% r.h. as practicable.

2.4 Effect of Mass on Wool Volume

In the second experiment, the volume of full-length fleece samples for four animals from each of thirteen sheep breeds (halfbred, merino, Poll Dorset, Romney, English Leicester, Drysdale, Suffolk, Lincoln, Polwarth, Shropshire, Wiltshire, Cheviot, and Arapawa (feral merino)) was measured at up to nine masses (0.2, 0.4, 0.6, 0.8, 1–3, 6, and 10 g).

Samples were scoured and conditioned as for the first experiment. Volume was measured at 65% r.h. and 20°C, starting with 10 g and then taking a subsample of 6 g. A 3-g subsample of the 6 g was next measured and so on. At each mass, the relative variability, expressed as the coefficient of variation (CV%), was calculated from the standard deviation of the mean of all samples.

An additional 2.5-g fleece sample from each of the 52 animals was measured for medullation (% by volume), by using the WRONZ Medullameter (Lappage and Bedford, 1983). From these results, samples were divided into non-medullated (<2%), low-medullation (2–10%), and high-medullation (>10%) categories.

2.5 Density

In the third experiment, midside wool samples were collected monthly from the same 100-cm^2 site through winter, spring, and summer for two years (June–February in 1992/93 and 1993/94) from the same thirteen breeds used in the second experiment. Between two and ten animals represented each breed at each sampling. Volume was determined at 65% r.h. and 20°C, the fourth sequential measurement being used, on samples with a clean-wool mass greater than 2 g. Density was calculated from mass and volume.

These wool samples were required for further measurements and could not be measured for medulla content with the medullameter, a destructive technique. Instead, samples were subjectively divided, on the basis of breed, into non-medullated (merino, Polwarth, halfbred), low-medullation (Romney, Poll Dorset, Lincoln, English Leicester, Shropshire), and high-medullation (Drysdale, Cheviot) categories.

Analysis of variance and regression analysis, by GENSTAT5, were used to determine the effects of humidity, mass, and density.

3. RESULTS

3.1 Sequential Measurement

With each sequential measurement, the volume of midside wool samples decreased ($p < 0.001$). The largest decline was between the first and second measurements, with only small changes between the fourth and fifth measurements. There was a corresponding exponential increase in density ($p < 0.001$), (Fig. 1).

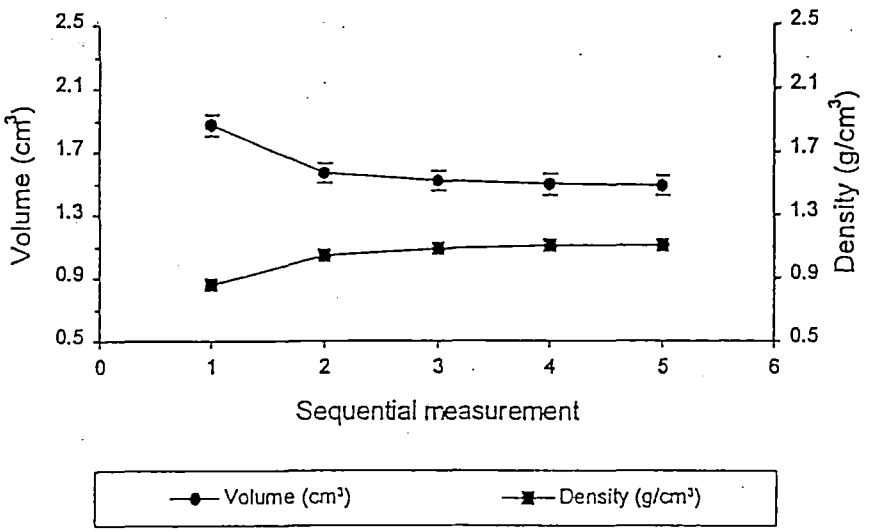

Fig. 1 Reduction in mean volume (error bars indicate the 95% confidence interval) and corresponding increase in mean density over five sequential measurements (the 64 midside wool samples were from merino, halfbred (English Leicester × merino), Romney, Poll Dorset, Suffolk, English Leicester, Lincoln, and Drysdale breeds)

All volume measurements obtained from individual and combined values were highly correlated with the final-volume estimates from all five measurements ($r^2 = 0.94$–1.00). The third, fourth, and fifth individual measurements ($r^2 = 1.00$ for each) were as good as any combinations. Use of the fourth volume measurement reduced the residual variance of the regression on final volume, relative to the third measurement (0.0006 vs 0.0003 cm³), and reduced measurement time, relative to use of the fifth measurement. The fifth measurement did not further reduce the residual variance. Hence the fourth sequential measurement was used when examining subsequent effects of mass and density.

3.2 Effect of Humidity on Volume

Measured volume was greatest at 40% r.h. ($p < 0.001$), reduced at 0, 20, and 80% r.h., and least at 65% r.h. ($p < 0.001$) (Table I). At 65 and 80% r.h., volume measurements decayed at a slower rate ($p = 0.015$) than at the lower humidities.

There was nothing to indicate that the alternative humidity levels were better than the standard 65% used for other wool measurements. For this reason, all subsequent samples were measured at 65% r.h. The measured volume of 1.75 g of highly medullated wool was 49% greater than that of a similar mass of non-medullated wools ($p < 0.001$). Wools low in medullation were intermediate (Table II). The rate of decay, however, was similar for all three wool types ($p = 0.403$).

Table I
Effects of Relative Humidity, at 20°C, on Volume of 64 Midside Wool Samples from Eight Sheep Breeds (Merino, Halfbred, Romney, Poll Dorset, Suffolk, English Leicester, Lincoln, and Drysdale), and the Rate of Decay during Five Sequential Measurements

Relative Humidity	Volume Measurement (cm^3)		Decay Rate
	One	Five	
0%	1.95	1.49	0.25
20%	1.90	1.49	0.24
40%	1.94	1.61	0.25
65%	1.73	1.38	0.21
80%	1.84	1.44	0.19
$lsd_{0.05}$	0.08	0.07	0.04

Table II
Effect of Medullation Category on Volume Measurement and Decay Rate, at 65% r.h., over Five Sequential Measurements, for Midside Wool Samples from Eight Sheep Breeds with Varying Medulla Content*

	Volume Measurement (cm^3)		Decay Rate
	One	Five	
Non-medullated	1.63	1.23	0.22
Low medullation	1.93	1.56	0.24
High medullation	2.25	1.83	0.22
$lsd_{0.05}$	0.26	0.25	0.04

*Non-medullated (<2%): merino, halfbred, Suffolk; low medullation: (2–10%) Romney, Poll Dorset, English Leicester, Lincoln; high medullation: (>10%) Drysdale.

3.3 Effect of Mass on Volume Measurement

When the mass was greater than 1 g, there was a linear relationship with volume for all breeds. However, below 1 g, the relationship was non-linear, indicating measurement error (Fig. 2). The relative variability of volume measurements decreased exponentially with increasing mass, from 180% coefficient of variation at 0.2 g to 19% coefficient of variation at 10 g ($p < 0.001$). The coefficient of variation was 50% at a sample mass of 2 g and was 25% at 6 g. Sample masses of at least 6 g are recommended for routine measurement. The size of the measurement chamber physically limited measurement of masses greater than 10 g.

3.4 Density Measurement

Density was greatest for non-medullated wools and least for highly medullated wools ($p < 0.001$) (Table III). Breeds with highly medullated wools generally grew 25% more wool in terms of volume than non-medullated breeds but only 1% more in terms of mass.

4. DISCUSSION

The automated calibration sequence of the pycnometer ignores the first measurement and averages the second, third, and fourth sequential measurements. In our experience, the fourth individual measurement of a wool sample increased the accuracy of prediction of the final volume and decreased the relative variability (CV%) of the volume measurement relative to the calibration procedure. This is possibly due to moisture present in the wool samples. As dry gas is pumped into and flushed from the measurement chamber, moisture will be removed from between and within the fibres. Within the fibre, moisture is loosely

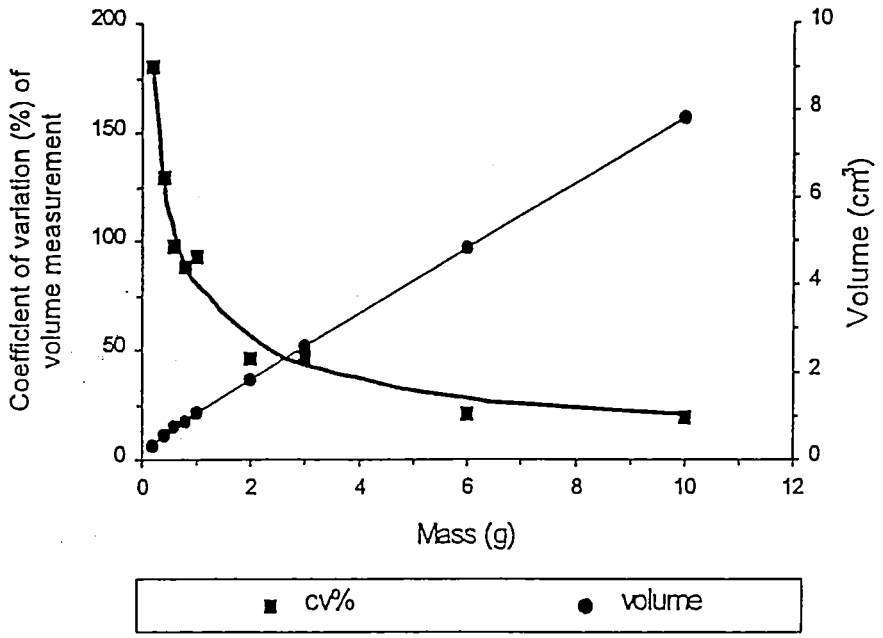

Fig. 2 Effect of sample mass on volume and relative variability(CV%) of the fifth volume measurement of wool samples from thirteen sheep breeds (merino, Arapawa (feral merino), Polwarth, halfbred, Romney, Poll Dorset, Suffolk, Shropshire, Wiltshire, English Leicester, Lincoln, Cheviot, and Drysdale)

Table III
Average Mass, Volume, and Calculated Density for Wool Samples with Varying Degrees of Medullation*

Degree of Medullation	Mass (g)	Volume (cm^3)	Density (g/cm^3)
Non-medullated	3.171	2.437	1.307
Low medullation	3.002	2.436	1.235
High medullation	3.193	3.049	1.076
$lsd_{0.05}$	0.238	0.207	0.023

*Non-medullated (<2%): merino, Polwarth, halfbred; low medullation (2–10%): Romney, Poll Dorset, English Leicester, Lincoln, Shropshire; high medullation (>10%): Drysdale, Cheviot. Samples were harvested at monthly intervals over two years.

attached to hydrophilic sites and strongly bound to specific sites on the keratinous material (Speakman, 1944). When moisture levels are low, water is attached to hydrophilic sites rather than bound to specific sites. Removal of the bound moisture would require temperatures of 140–160°C (Watt *et al.*, 1959). The faster rate of decay at low humidities, where regain was low, is likely to have reflected the removal of moisture from hydrophilic sites (Watt *et al.*, 1959). Conversely, the slower decay rates at higher humidities may suggest that more moisture was bound to specific sites within the fibre and so less readily available for removal.

The similar decay rate between medullation categories, at the different humidities, indicates that the release of moisture due to the flushing effect of sequential measurements was not affected by the presence or degree of medullation. The small increase in the

measured volume with increasing humidity is consistent with the behaviour of equilibrium weights reported by Watt and Kennett (1960), in whose work weights reached a maximum at 50% r.h. and declined at higher humidities. The order in which volumes were measured under different conditions (i.e. 65, 40, 20, 0, and 80% r.h.) was determined by the availability of conditioned rooms and ignored possible effects of hysteresis. This may account for volume measurements at 80% r.h. being greater than those at 65%. For conformity with other test methods, it is recommended that the pycnometer measurements be carried out at 65% r.h. and a temperature of 20°C.

The density of non-medullated wools, as measured by the gas pycnometer (1.307 g/cm^3) was comparable with specific gravities measured by using a benzene pycnometer (1.305 g/cm^3) (van Wyk and Nel, 1940) and density-gradient columns (1.304 g/cm^3) (Connell and Andrews, 1974). Wools with a low degree of medullation (2–10%) had a density of 1.235 g/cm^3, whereas highly medullated wools (>10%) were less dense (1.076 g/cm^3). It is acknowledged that considerable differences in the degree of medullation can occur between the seasons of the year, between individuals within a breed, and between the breeds studied (Skårman and Nõmmera, 1954; Story and Ross, 1960; Scobie *et al.*, 1993). It is expected that, with levels of medullation higher than those in the experimental categories, density values would be lower than 1.076 g/cm^3. The 'extreme' density values for Wensleydale (1.304 g/cm^3) and merino (1.309 g/cm^3), reported by Speakman *et al.* (1933), describe a range within non-medullated breeds and underestimate the range of values for all types of wool.

Wools as diverse as merino and Drysdale vary with respect to fibre diameter and variability of diameter. Coarse fibres tend to be comparatively irregular in shape and vary more along their length but generally retain a near-elliptical cross-section (Sidey, 1931; Lang, 1952; Anon., 1954; Orwin *et al.*, 1985; Woods and Orwin, 1988; Woods *et al.*, 1990). Lang (1947) found that, for Corriedale wool, medullated fibres were no more elliptical than non-medullated fibres. Pilkington and Purser (1958) found that the same applied to much coarser wool from Scottish Blackface sheep. It was only in kemp fibres that fibres deviated from elliptical, where an extremely coarse diameter was accompanied by large medullae, and concavity was observed (Lang, 1942; Pilkington and Purser, 1958; Anon., 1975). Fibre ellipticity was not measured as part of the experiments reported here. It is not expected that potential differences in ellipticity would have a large influence on the volume measurement. If, however, a significant number of fibres with collapsed medullae were present in the highly medullated samples, volume would be underestimated and density overestimated. The much lower density of the medullated wools measured in this experiment appears to indicate a low incidence of concavity.

REFERENCES

Anon., 1954. The Measurement of Wool Fibre Ellipticity. *Wool Sci. Rev.*, No. 12, 33–43.

Anon., 1975. *The Identification of Textile Materials*, The Textile Institute, Manchester, 7th edition.

Connell, J.P., and Andrews, M.W., 1974. The Variability of the Specific Gravity of Australian Wools and the Implications for the Air-flow Measurement of Diameter. *J. Text. Inst.*, 65, 1–5.

King, A.T., 1926. The Specific Gravity of Wool and its Relation to Swelling and Sorption in Water and Other Liquids. *J. Text. Inst.*, 17, T53–T67.

Lang, W.R., 1942. Some Aspects of the Occurrence and Significance of Hairy Fibres in Australian Wools. Gordon Institute of Technology, Publication No. 3.

Lang, W.R., 1947. The Comparative Thickness of Medullated and Non-medullated Fibres in Corriedale Wool. Gordon Institute of Technology, Publication No. 7.

Lang, W.R., 1952. Wool Fibre Contour: Its Significance to the Mechanical Properties of the Fibre. *J. Text. Inst.*, 43, P245–P251.

Lappage, J., and Bedford, J., 1983. The WRONZ Medullameter. Report No. R107, Wool Research Organisation of New Zealand, Christchurch, New Zealand.
Orwin, D.F.G., Woods, J.L., and Gourdie, R.G., 1985. Cortical Cell Type and Wool Strength. *Proc. Int. Wool Text. Res. Conf. Tokyo*, I-194–203.
Pilkington, J.M., and Purser, A.F., 1958. Fibre Medullation in Blackface Lambs and Hoggs. *J. Agr. Sci.*, 51, 257–264.
Scobie, D.R., Woods, J.L., and Baird, D.B., 1993. Seasonal and Between-sheep Differences in Medullation of Wool Fibres. *Proc. NZ Soc. Anim. Prod.*, 53, 319–322.
Sidey, D.J., 1931. Some Results of Cross-sectional Area and Contour Measurements of New Zealand Romney and Corriedale Wools. *J. Text. Inst.*, 22, 1–6.
Skårman, S., and Nömmera, E., 1954. Seasonal Variations of Fineness and Medullation in Cheviot Wool. *Ann. Roy. Agric. College, Sweden*, 21, 367–384.
Speakman, J.B., 1944. An Analysis of the Water Adsorption Isotherm of Wool. *Trans. Faraday Soc.*, 40, 6–10.
Speakman, J.B., Stott, E., and Chang, H., 1933. A Contribution to the Theory of Milling. Part II. *J. Text. Inst.*, 24, T273–T292.
Story, L.F., and Ross, D.A., 1960. Effect of Shearing Time on Wool. Part VI: The Rate of Growth of Wool and its Relation to Time of Shearing. *NZ J. Agric. Res.*, 3, 113–124.
van Wyk, C.M., and Nel, H.A.M., 1940. Studies on the Basic Characteristics of South African Merino Wool. Part II: Specific Gravity. *Onderstepoort J. Vet. Sci. Anim. Industr.*, 15, 325–332.
Watt, I.C., and Kennett, R.H., 1960. Variations in the Equilibrium Weight of Wool at Zero Humidity. *Text. Res. J.*, 30, 489–494.
Watt, I.C., Kennett, R.H., and James, J.F.P., 1959. The Dry Weight of Wool. *Text. Res. J.*, 29, 975–981.
Woods, J.L., and Orwin, D.F.G., 1988. Seasonal Variations in the Dimensions of Individual Romney Wool Fibres Determined by a Rapid Autoradiographic Technique. *NZ J. Agric. Res.*, 31, 311–323.
Woods, J.L., Orwin, D.F.G., and Nelson, W.G., 1990. Variation in the Breaking Stress of Romney Wool Fibres. *Proc. Int. Wool Text. Res. Conf, Christchurch*, I-557–568.

Appendix II Bray's formula (Bray, 1942), used to calculate percent medullation by volume (% med vol) from projection microscope measurements of percent medullation by number.

$$\% \text{ med vol} = \frac{Nm}{Nw}.100.\frac{\bar{d}^2 m + \sigma^2 m}{\bar{d}^2 w + \sigma^2 w}$$

where: Nw = total number of fibres measured

$\bar{d}^2 w$ = mean fibre diameter

$\sigma^2 w$ = standard deviation of fibre diameter

Nm = number of medullated fibres

$\bar{d}^2 m$ = mean medulla diameter

$\sigma^2 m$ = standard deviation of medulla diameter

Appendix III Derivation of the equation for the calculation of the volume of a wool sample measured using the gas pycnometer VM-100 (Stec Inc., 1992)

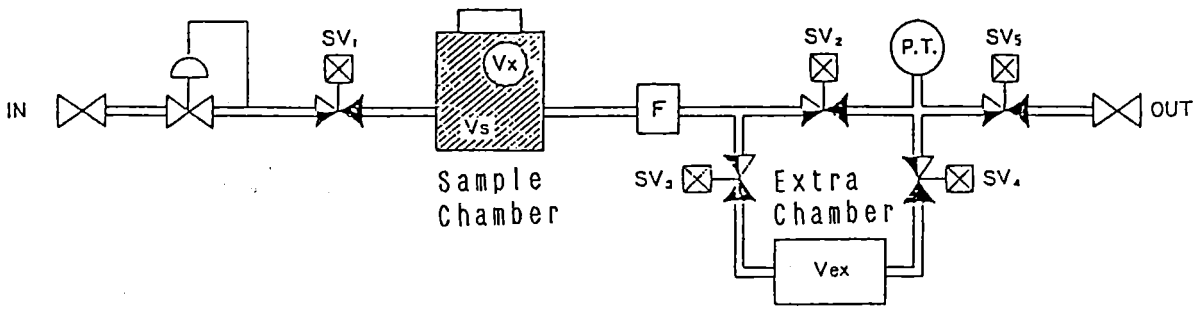

Fig. 1

In Fig. 1, provided that the volume of the Sample Chamber system be Vs (l), the Atmospheric Pressure, Pa (atm), the Air Temperature, Ta (°K), and the Mol Number, n (mol), the equation of state for an ideal gas is:

$$PaVs = nRTa.$$

Similarlly, the volume of the Extra Chamber system is Vex (l), the Atmospheric Pressure, Pa (atm), the Air Temperature, Ta (°K) and the Mol Number, m(mol), then we have:

$$PaVex = mRTa$$

When a sample with its volume Vx (l) weight W (g) is put into the Sample Chamber and increasing the Sample Chamber system pressure with Helium Gas up to the Pressure P_1, the following relationship is realized:

$$P_1 (Vs - Vx) = (n + \alpha) RTa$$

Later, when the gas is introduced into the Extra Chamber system to be expanded, we have:

$$P_2(Vs-Vx+Vex) = (n+\alpha+m)RTa = P_1(Vs-Vx)+PaVex$$

The above equation is simplified as:

$$Vx = Vs + \frac{1}{1 - \frac{P_1 - Pa}{P_2 - Pa}} Vex$$

Where P_1 and P_2 are in absolute pressure.

The density of the sample, Dx, can be expressed as:

$$Dx = \frac{W}{Vx}$$

Appendix IV Two millimetre guillotine, designed for preparation of fibre snippets for measurement of fibre diameter by a Laserscan instrument, and used for the preparation of 2 mm length treatments (Chapter 4).