THE EFFECTS OF LONG-TERM INFUSION OF LONG-R3-IGF-I ON WOOL GROWTH RATE AND WOOL FOLLICLE CHARACTERISTICS IN ROMNEY SHEEP

A thesis submitted in partial of fulfilment of the requirements for

the degree of Master of Agricultural Science

in Animal Science of

Massey University

SAMERA KASEM KHALAF

1995

This work dedicated to my parents who encouraged and supported me in my study and they always stand by me even during my absence from home (IRAQ)

TABLE OF CONTENTS

Acknowledgements

I owe a special debt to my supervisors, Professor S.N. McCutcheon, Associate Professor, P.M. Harris, Associate Professor G. Wickham and Dr. J.E. Hocking Edwards, for their sound guidance, constant encouragement, enthusiasm and assistance throughout the course of this study. Their contributions to this project has been immeasurable

I wish to extend my sincere gratitude to Dr. P.M.L. Morel and Mr. R. Fletcher, for their invaluable advice and criticism during the statistical analyses of the data.

The technical guidance received from Dr. J.E. Hocking Edwards, Mr. R.I. Sparksman and Mr. M.J. Birtles in acquainting myself with histological techniques are very much appreciated.

Special thanks to Associate Professor P.M. Harris, Mr. D. Shelton and Mrs. S. Waterman for their long hours in surgery. Also I would like to acknowledge Mr. M. Harcombe for the many hours spent caring for the animals.

The staff of the Physiology and Anatomy Laboratory who each provided invaluable technical assistance in histological evaluation are also gratefully acknowledged.

I am grateful to the Animal Science Department staff and postgraduate students for their friendship and assistance during the period of my study. Thanks to Miss. M. Scott for expert assistance in analysis of the hormones. Also I appreciate the help of Miss. Y. Cottam with many aspects of computer software. Special thanks to Mrs. B. Purchas for the friendship and her advice in different aspects.

Special thanks are also extended to the staff of the AgResearch, Grasslands, Palmerston North. I am grateful to Dr. J. Lee and Mr. B.R. Sinclair for their help and assistance with the laboratory works.

Sincere appreciation and gratitude is due to my husband, Dr. Isam Kadim, and also my children, Hamsah, Yasser and Shereen for the gift of their enthusiasm and discipline throughout this study without which I could not achieved my goal.

The project was supported by grants from Wools of New Zealand and the New Zealand Public Good Science Fund.

List of Abbreviations

A1	iliac artery
A2	saphenous artery
BF	blood flow
BSA	bovine serum albumin
BrdU	5-bromodeoxyuridine
CBR	cell birth rate
CDR	cell division rate
CI	control infusate
Cu	copper
des(1-3)IGF-I	des(1-3) insulin-like growth factor-I
EC	external control
EGF	epidermal growth factor
FBS	feotal bovine serum
FGF	fibroblast growth factor
GH	growth hormone
GRF	growth hormone releasing factor
h	hour
HS	high sulfur protein
IF	intermediate filament
IFAP	intermediate filament associated protein
IC	internal control
ICC	immunocytochemistry
IGFBPs	insulin-like growth factor binding proteins
IGFBP-1	insulin-like growth factor binding protein-1
IGFBP-2	insulin-like growth factor binding protein-2
IGFBP-3	insulin-like growth factor binding protein-3
IGFs	insulin-like growth factors
IGF-I	insulin-like growth factor-I
IGF-II	insulin-like growth factor-II
IRS	inner root sheath
KAP	keratin associated proteins
LA1	left artery 1
LG3IGF-I	long-G3-insulin-like growth factor-I

LIGF-I	long-insulin-like growth factor-I
LR3IGF-I	long-R3-insulin-like growth factor-I
LS	low sulfur protein
LV	left vein
oPRL	ovine prolactin
oRS	outer root sheath
P (solution)	control solution (refer to Appendix 1)
PAH	para-aminohippuric acid
RA1	right artery 1
RIA	radioimmunoassay
R3IGF-I	R3-insulin-like growth factor-I
RV	right vein
S	sulfur
S (solution)	LR3IGF-I stock solution
SmC	somatomedin C
TCA	trichloroacetic acid
TGFα	transforming growth factor-α
UTS	ultra-high sulfur protein
V	vein
Zn	zinc
min	minute
g	gram
kg	kilogram
µg	microgram
ng	nanogram
L	litre
µL	microlitre
ml	millitre
mM	millimole

Significant levels for statiatical tests

0.1%	P<0.001	***
1.0%	P<0.01	**
5.0%	P<0.05	*
>5.0%	P>0.05	NS†

† non-significant

List of Figures and Plates

Figure

Plate

List of Tables

Table

CHAPTER 1

INTRODUCTION

CHAPTER 1

INTRODUCTION

1 Skin Morphology In Relation To Wool Growth

This introduction gives a brief description of the morphology of wool-growing skin and the follicle in general. Textbook descriptions are available from sources such as Fraser and Short (1960), Ryder and Stephenson (1968) and Orwin (1979).

1.1 Morphology of The Skin

The skin of all mammals is structurally similar, consisting of two major components the epidermis and dermis (Ryder, 1973; Reis, 1982; Williams, 1991).

The epidermis is a stratified, squamous epithelium structure. The epidermis consists of two principal cellular layers, the outermost nucleate cornified cells (*Stratum corneum*) and a living inner layer (*Stratum Malpighii*), from which the surface cells arise by differentiation. The *Stratum Malpighii* is conventionally subdivided into: a basal layer one cell deep (*Stratum basale* or *Stratum germinativum*) which forms tight connections with the dermis; the *Stratum spinosum*, which comprises several layers of polyhedral cells lying above the germinal layer, and is usually thin in heavily haired skin; the *Stratum granulosum*, which is a layer of flattened nucleated cells containing distinctive cytoplasmic inclusions and keratohyalin granules; and the *Stratum lucidum*, which is a hyalin layer of variable thickness, usually present in areas where the epidermis is very thick.

The dermis, or corium, lies under the epidermis and consists basically of a matrix of loose connective tissue composed of the fibrous proteins (collagen, elastin, and reticulin) embedded in an amorphous ground substance. In addition, the dermis contains a few cells such as mast cells, histiocytes, melanocytes, and leukocytes. The dermis is composed of two major connective tissue layers: the papillary layer, underlying the basal membrane, and the reticular layer. Hairy skin is usually not papillated to a great extent. At the base of the dermis many mammals have a layer of fatty tissue. This is found in rats and mice, and is well developed in man and cattle, but is usually absent in sheep.

Hair or wool follicles and glands, which are in fact epidermal structures produced as downgrowths from the epidermis, are embedded in the dermis together with blood and lymph vessels and nerves.

There are three networks of blood vessels within the skin parallel to its surface. Arteries paired with veins enter the lowest, the dermal network, at the base of the dermis. Vertical vessels rise from this to the horizontal mid-dermal and sub-dermal nets. The mid dermal net feeds the follicles, while the sub-dermal net supplies the epidermis, and also functions in heat regulation (Ryder, 1973).

1.2 The Follicle

1.2.1 Follicle Morphology and Fibre Growth

All wool and hair follicles that are actively producing a fibre have the same basic structure. The final structure of the mature wool follicle (Fig. 1.1) and the process of wool growth are reviewed by Ryder and Stephenson (1968), Chapman and Ward (1979) and Orwin (1979).

The follicle is an epithelium-derived tubular downgrowth into the dermis of the skin. At the base of each follicle there is a bulb enclosing the dermal papilla, the cells of which are rarely divided. The dermal papilla is primarily composed of special fibroblasts with associated collagen and ground substance (Orwin, 1989). The cells present in the bulb divide rapidly and are moved up the follicle shaft as the fibre is formed. The wall of the follicle is divided into two distinct layers, the outer and the inner root sheaths. The outermost epidermal layer adjacent to the dermal connective tissue sheath is the outer root sheath (ORS). The inner root sheath (IRS) surrounds the wool fibre. The IRS consists of three distinct layers. The inner one is a thin cuticle which interlocks with the fibre cuticle, in the middle is the Huxley's layer, and on the outside the Henle's layer. The IRS is not continuous with the epidermis or the fibre. It does not extend beyond the opening of the sebaceous gland into the fibre canal. The innermost structure of the follicle, the fibre, consists of peripheral cuticle, followed by the cortex and sometimes a central medulla. In the upper part of the follicle there are sebaceous glands, which open through the neck of the follicle into the pilary canal. Sudiferous glands (sweat glands) are usually associated with primary follicles (Hardy and Lyne, 1956), and also open into the pilary canal just below skin level. Attached to the mid-follicle wall of primary follicles is the arrector pili muscle (Auber, 1952).

In sheep, most follicles are arranged in groups according to their time of development in the fetal skin. A group normally consists of three primary follicles (a central follicle forming first, followed by two lateral follicles) and a variable number (12-60) of

secondary follicles which develop later. Some secondary follicles may form as outgrowths from other secondaries (Hardy and Lyne, 1956). Primary follicles are usually larger than secondary follicles and produce coarser fibres (Ryder and Stephenson, 1968).

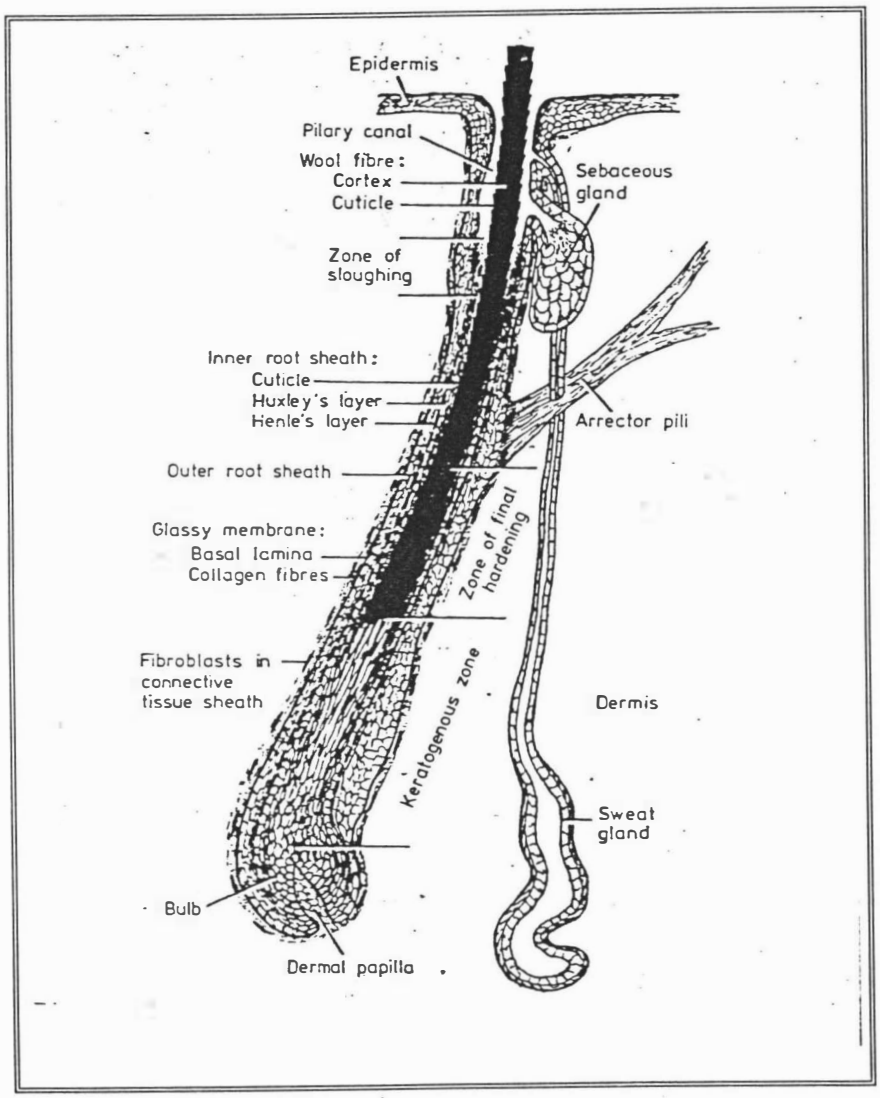

Figure 1.1 Diagrammatic representation of the anatomy of a primary follicle (after Chapman and Ward, 1979). This figure demonstrates the final structure of the mature wool follicle.

Chapman and Ward (1979) divided the follicle into 5 zones according to the cell function. Orwin (1979) divided the follicle into 7 zones. The system of Chapman and Ward (1979), as shown in Fig. 1.1, will be used as a reference guide in this review.

The bulb, which is the zone of active cell division, extends to the tip of the dermal papilla. Within the keratogenous zone, which extends from near the tip of the dermal papilla to about two-fifths up the follicle, there is active protein synthesis (Rogers, 1959), and elongation of cells. There is also sequential hardening of Henle's layer, the IRS cuticle, the fibre cuticle and Huxley's layer. In the zone of final hardening, which extends to a level about three-fifths of the distance up the follicle, final hardening of the fibre occurs and degradation of the IRS begins. The zone of sloughing, just below the level of the sebaceous gland duct, is where degraded IRS cells, and some ORS cells, are sloughed into the pilary canal. Finally, there is the pilary canal, which extends from the zone of sloughing to the skin surface.

The Hair or Wool Fibre

The wool fibre is the keratinized end-product of the follicle. It typically consists of a cuticle and cortex and, if thicker than about 40 μm diameter, a central medulla.

Fibre Cuticle

The cuticle forms a single layer of flattened cells (0.3-0.5 μm thick) around the periphery of the fibre. These cells are known as "scales" because they overlap one another like the scales of a fish, the free edges of the cells pointing towards the fibre's apex. Wool fibres are usually surrounded by one cell layer of cuticle with only a small degree of overlapping of the cell edges (Rogers, 1959). In human hair, on the other hand, the cuticle is several scales thick (Ryder, 1973). Cuticular cells vary in size and shape between species. The overlapping of cuticle cells exhibits a specific pattern on their surface which can vary not only between species but also along the length of individual fibres. The degree of overlapping can be used to identify fibres from different species and breeds (Williams, 1991).

The cuticle cells make up approximately 10% of the fibre weight. Each cutical cell consists of an outer keratinous layer, the exocutical, and an inner nonkeratinous layer, the endocutical (Rogers, 1959).

Cortex

The cortex contains 2-3 cell types in varying proportions and spatial distribution. Cortical cell variation is particularly noticeable in wool fibres from breeds of sheep that produce wool of different diameter (Orwin *et al.*, 1984). In the wool fibre, the cortical cells are about 95-102 μm long and 3-7 μm wide (Rogers, 1959; White and Henderson, 1973). The long axis of the cells lies parallel to that of the fibre (Auber, 1952), and their ends are tapered, often with fingerlike projections to neighbouring cells (Rogers, 1959). In medullated fibres these projections can be seen branching abaxially, as well as entering in between the cells of the medulla (Parakkal, 1969).

In wool fibres, the bulk of the fibre and the protein content of the wool is derived from the cortical cells. The intermediate filament keratin proteins (IF) in the cortical cells are assembled into a filamentous scaffold that becomes embedded in a matrix of the keratin associated proteins (KAP).

There are two major cortical cell types, the paracortical and the orthocortical cells (Fraser and Rogers, 1953) and their bilateral segmentation in fine wool fibres may be responsible for the crimp, or curliness of the fibre. A third type of cell, the mesocortical cell, appears to be intermediate in characteristics (Orwin, 1979; Chapman, 1986).

Study of the ultrastructure of orthocortical and paracortical cells shows that they are organized differently and appear to contain different proportions of the keratin proteins, with the orthocortical cells having a higher IF:matrix ratio. The IF are more densely packed in orthocortical cells than in paracortical cells, and arranged in a whorl-like pattern. The IF are arranged in an hexagonal array, while the matrix is heavily cross-linked with disulfide bonds (Orwin *et al.*, 1984; Powell and Rogers, 1986; Fratini *et al.*, 1994). As the proteins of the IF and matrix represent largely the low-and high-sulfur proteins respectively, the paracortical cells have higher matrix and sulfur contents.

Medulla

In coarse fibres and quills, the medulla consists of vacuolated cells in the centre of the fibre (Auber, 1952). Along the length of a fibre, the medulla can be discontinuous, being interrupted in thinner fibres and continuous in thicker fibres.

The differentiation into medulla is determined by the relation between the critical level in a zone where no new cells are being formed. As the cells move up from the germinative region there is a lack of cells above the papilla. Thus, perhaps due to a physiological necessity to fill in this space above the papilla, the cells differentiating nearest the papilla expand largely by increasing the amount of water in the cells. During

keratinization the protein of the medulla becomes concentrated around the outer wall of the cells where it hardens. The cell water left in the centre of the cells eventually dries out to give the normal spongy air-filled structure of the medulla.

1.2.2 Follicle Blood Supply

One of the main functions of the blood stream is to transport nutrients and other substances such as hormones to the tissues (Ryder and Stephenson, 1968). In the skin it carries nutrients to the epidermis. These nutrients then diffuse to the differentiated tissues, and nourish the wool follicles and their glands.

The three networks of vessels parallel to the skin surface have been mentioned previously. The immediate blood supply to the follicle consists of a basket-like network of capillaries around the lower third of the follicle, and branches lead from this net into the papilla (Ryder, 1956). The vessels surrounding the follicle do not extend above the point at which the fibre becomes keratinized.

The vessels of the papilla supply substances needed for cell division. The larger the papilla, the more vessels it contains, and, as has already been indicated, a large papilla is associated with a coarse fibre. Large follicles also have a well developed capillary network surrounding the follicle. These vessels become reduced in number during the resting stage of the wool growth cycle. Furthermore, Hardy (1952) stated that in the mouse no blood vessels are seen entering the dermal papilla at any stage of the growth cycle.

2 Follicle Growth

The wool or hair follicle is a fascinating cutaneous structure. During the growing phase (anagen) matrix keratinocytes located in the bulb region of the follicle rapidly proliferate (Van Scott *et al.*, 1963), providing cells that differentiate into the medulla, cortex, and cuticle of the wool fibre.

2.1 Cell Production

The rate of formation of a wool fibre is determined by the proliferation of cells within the follicle bulb and by subsequent growth and migration of the cells. Various studies have been made of the effects of plane of nutrition on cell proliferation in wool follicles (Schinckel, 1962; Fraser, 1965; Short *et al.*, 1965; Wilson and Short, 1979), but there is

relatively little information on the effects of plane of nutrition on cell migration (Chapman, 1971) or keratinization in wool fibres (Marston, 1946). Also there is a scarcity of information on the effects of hormones on proliferation of cells within the follicle bulb.

Cell production occurs in the follicle bulb and in the outer root sheath along the length of the follicle. Early workers attempted to divide the follicle bulb into various regions according to the occurrence of cell proliferation, morphological cell changes or ultimate cell function.

The exact boundary of the germinative cell population is debated. For instance, an imaginary line drawn by Auber (1952) across the widest part of the dermal papilla and dividing the bulb into two distinct regions has been called "the critical level". The part below this level consists mostly of rapidly dividing undifferentiated cells. Epstein and Maibach (1969) observed that, in mice, DNA is synthesised in cells which are distributed throughout the hair bulb, in which case there would be no well-defined basal layer along the papilla. DNA synthesising cells were also observed above Auber's critical line (Montagna, 1956). This implies that all bulb cells can undergo division (Bullough and Laurence, 1958; Schinckel, 1961; Willson and Short, 1979; Williams and Winston, 1987; Hynd, 1989a). Chapman and Ward (1979) divided the follicle into five zones according to the different functions of the cells that each contained. They generally referred to the bulb area as the "mitotically active zone". This zone coincides with the definition given by Fraser (1965) and Short *et al.* (1965) who stated that in the bulb the mitotic activity is confined to a region around the dermal papilla, and has a cone-shaped upper limit extending to the tip of the dermal papilla. According to Short *et al.* (1965) and Holle (1992) the germinative region is positioned one cell layer above the apex of the dermal papilla. They based the definition on the position of distal mitotic nuclei in the bulb, changes in cell size and the level at which differentiation of the IRS and cuticle cells becomes apparent. This definition results in a curved boundary line around the papilla. It differs from Fraser's (1965) definition of a straight line across the first cell layer on top of the dermal papilla. Fraser's definition includes mitotic as well as differentiating cells.

The growth of the wool fibre depends on continuous division of cells within the bulb of the follicle. As well as the rate of production of cells capable of mitotic activity and the average turnover time of the population of cells, the volumetric rate of fibre production from each follicle also depends on the size of the cells in the bulb and the changes in size during keratinization, and the proportion of the dividing bulb cells that eventually enter the cuticle and cortex of the fibre (Williams and Winston, 1987). For the Merino, Short *et al.* (1965) calculated that only a small proportion of dividing bulb cells finally enter the fibre as

compared to the proportion migrating to form the IRS, suggesting that fibre production is a relatively inefficient process. It is, as yet, unclear whether the proportion of dividing cells entering the fibre cortex is a genetically-determined constant or is influenced by nutrition. The results of Wilson and Short (1979) and Hynd (1989a) suggested that it is genetically determined and that nutrition has little effect on the proportion of dividing cells forming the fibre cortex (Wilson and Short, 1979).

Cells produced in the bulb and migrating out of the bulb may be pluripotential and move randomly into one or other of the various layers of the follicle (Montagna and Parakkal, 1974). Alternatively, and more likely, the cells may follow strict paths (Epstein and Maibach, 1969; Chapman and Gammell, 1971) or cell streams (Bullough and Laurence, 1958), with their differentiation into specific cell types being predetermined according to their position in the bulb (Chapman and Gammell, 1971).

Cell migration in wool follicles is a well ordered procedure. Cells destined to enter the IRS and fibre are produced in the follicle bulb, while ORS cells proliferate along the length of the follicle. Cells produced by mitosis in the bulb move upward in single rows to the upper bulb where they increase in volume and become elongated vertically (Montagna, 1956). Cells destined to form the IRS move out faster and undergo differentiation and maturation earlier and faster than fibre cells (Downes *et al.*, 1966; Epstein and Maibach, 1969; Chapman and Ward, 1979). Studies by Epstein and Maibach (1969), in the human scalp, and Chapman (1971) reveal that IRS cells move out of the bulb and up the proximal half of the follicle faster than contemporary cells formed closer to the dermal papilla apex. The different parts of the IRS and the fibre are believed to arise as separate streams of cells in the lower follicle bulb (Ryder, 1956).

The results of Chapman *et al.* (1980) suggested that the cells in the ORS behave as two different populations, namely: the proximal and the distal. The former population forms the outer sheath of the lower half of the follicle and undergoes renewal in an enlongation region extending from around the bulb to half-way up the follicle, where the greatest proliferation occurs in the ORS. The latter population comprises the outer layers of the ORS above that previously mentioned, and the epithelium around the follicle lumen. Cells produced in the enlarged part of the outer root sheath in the lower half of the follicle move initially towards the IRS. Subsequently cells apposing cells of the ORS and the IRS develop into the single cell layers. In this region the rates of movement of the outer and inner root sheath cells are similar. When the migrated ORS cells reach the zone of sloughing, they cornify, degrade and slough into the follicle lumen just above where the IRS sloughs (Chapman and Gammell, 1971).

2.2 Estimation of Cell Proliferation

The rate of wool fibre growth is determined largely by the proliferation of undifferentiated mitotically active cells in the follicle bulb and their subsequent hypertrophy, differentiation and sequential migration (Black, 1987). Measurement of mitotic activity becomes a most useful test system for the study of factors controlling fibre growth. Cell kinetic parameters and their relationships have been investigated by the use of stathmokinetic agents like colchicine and colcemid as metaphase arrest agents which allow counting of the number of arrested nuclei in a histological section (Schinckel, 1961, 1962; Fraser, 1963, 1965; Short *et al.*, 1965; Wilson and Short, 1979; Hynd *et al.*, 1986; Williams and Winston, 1987; Hynd and Everett, 1990; Hocking Edwards, 1993). It is possible that these agents may retard or stimulate cell proliferation. Colchicine will bind tightly to one tubulin diamer and thereby prevent its polymerization. The addition of colchicine to a dividing cell therefore causes the disappearance of the mitotic spindle and blocks cells in mitosis. For accurate estimation of cell proliferation with colchicine, it is important to establish a dose-response curve and a linear collection of metaphases after injection (Tannock, 1965). The dosage level of colchicine required for maximum metaphase stasis resulted in toxicity and sheep fatalities (Schinckel, 1961, 1962; Short *et al.*, 1965; Wilson and Short, 1979), and it has been observed that the dose margin between full metaphase arrest and death may be narrow (Schinckel, 1961 and Fraser, 1963). The linear accumulation of metaphase cells has been established in studies of follicle bulb cells (Schinckel, 1961; Wilson and Short, 1979; Hynd *et al.*, 1986; Williams and Winston, 1987). Autoradiography, based on the application of radioactive labelled DNA-precursors like ^{3}H-thymidine and examining either plucked fibre or histological sections, has also been used (Chapman, 1971; Chapman *et al.*, 1980). Disadvantages of autoradiography are that the technique is both time- consuming and expensive.

5-Bromodeoxyuridine (BrdU), a thymidine analogue, is incorporated into nuclear DNA during cell cycle S-phase and can be visualized with immunocytochemical techniques using a monoclonal antibody (Gratzner, 1982; Dean *et al.*, 1984). For this reason BrdU can be used to study the cell kinetics of different tissues (Harms *et al.*, 1986; Harms *et al.*, 1987; Gratzner, 1982). These investigations prove that BrdU labelling of S-phase cells can be a reliable, repeatable, rapid, and non-radioactive alternative method to ^{3}H-thymidine labelling of proliferating cells. The use of BrdU as a labelling agent of DNA in proliferating wool follicle bulb cells and its immunocytochemical detection utilizing a monoclonal antibody has been previously reported (Holle and Birtles, 1990; Hynd and Everett, 1990; Adelson *et al.*, 1991; Hocking Edwards, 1993; Holle and Harris, 1992; Holle *et al.*, 1994).

BrdU has been found to affect cellular differentiation *in vivo* and *in vitro* (Abbott and Holtzer, 1968; Coleman *et al.*, 1970; Silagi and Bruce, 1970; Miura and Wilt, 1971; Turkington *et al.*, 1971; Adelson *et al.*, 1991), but BrdU has no effect on cell proliferation rates or the cell cycle (Miura and Wilt, 1971; Holle and Harris, 1992). Adelson *et al.* (1991) suggested that it may be possible to minimize the inhibitory effect on differentiation by using lower doses of BrdU.

2.3 Protein Deposition

The wool fibre is basically protein, which is called "keratin". The proteins which comprise the structural component of wool and hair fibre were reviewed by Gillespie (1983) and Powell and Rogers (1990). These proteins form two groups, approximately equally abundant in most types of wool. They are named according to their structural features and combinative role as the intermediate filament proteins (IF) and the intermediate filament-associated, or matrix, proteins (IFAP). The IF group comprises low-sulfur proteins, whereas the IFAP group is subdivided into the high-sulfur and high-glycine/tyrosine-rich proteins (Powell and Rogers, 1990).

These three groups of proteins (low-sulfur, high-sulfur and high glycine/tyrosine) have been identified in the soluble proteins extracted from wool after oxidation or reduction to break disulphide bonds.

2.3.1 Low-Sulfur Proteins (Intermediate Filament Proteins)

These have lower cysteine content than those of the high sulfur proteins, make up about 60-70% of the total protein and are the main component of the IF proteins. Qualitative studies of wool proteins have shown that there are eight low-sulfur proteins belonging to two families (Dowling *et al.*, 1979). These proteins have molecular weights in the range 45 000-58 000 (Woods, 1979). Proteins extracted from a variety of wool and hair of several species have the same general pattern of amino acid composition (Gillespie, 1983; Yn *et al.*, 1993); they contain all the methionine and most of the lysine in wool.

2.3.2 High-Sulfur Proteins (Matrix Proteins)

As matrix or interfilamentous components, this group, together with the high-glycine/tyrosine proteins (discussed later), could be referred to as filament-associated proteins, part of the matrix of the cortical cells (Powell and Rogers, 1986), and are especially rich in cysteine, proline and serine (Reis, 1982). Proteins with higher cysteine

content make up 20-40% of the total protein. These proteins are more numerous and more heterogeneous. There are at least 35 high-sulfur proteins classified into five families according to their molecular weights which range from 10 000 to 30 000. Within this group a sub-group termed the ultra-high-sulfur proteins has been identified, which have about 30% of their amino acid residues as cysteine. The proportion of these proteins in wool can vary from virtually zero to 10%. Variations in the cysteine content of wool can be accounted for by variations in the proportions of components of the high-sulfur proteins (Gillespie, 1965; Reis, 1979).

The proteins of the cuticle are generally similar to the high-sulfur proteins of the cortex.

2.3.3 High-Glycine/Tyrosine Proteins (Matrix Proteins)

Proteins with higher tyrosine, glycine and aromatic amino acid contents, these make up 1-12% of the total protein and form part of the matrix of the cortical cells although small amounts are associated with cell membranes. The high-glycine/tyrosine proteins contain at least 30 components (Gillespie, 1983). They have molecular weights below 10 000 and may be divided into two types, referred to as type I and type II, distinguished on the basis of their amino acid composition and solubility properties.

There is an extraordinarily high variability in the content of the high-glycine/tyrosine proteins across species, being as low as 1% for Lincoln wool and around 20% for mouse hair. There are genetic and physiological circumstances which also lead to changes in the amount of these proteins in wool or hair. The feeding of sheep with diets low in aromatic amino acids can produce a marked decrease in high-glycine/tyrosine protein in the wool (Reis, 1979).

3 Factors Influencing Wool Growth

Wool production differs between breeds (Daly and Carter, 1955) and strains (Dunlop *et al.*, 1966) of sheep under similar environmental conditions and feeding regimens. Factors such as hormones (Wallace, 1979; Williams *et al.*, 1986; Williams *et al.*, 1990) and feed intake (Williams, 1979) have been found to influence wool growth.

The rate of wool growth in sheep is governed by the interaction between nutrition, genotype, and photoperiod which alter both the rate of cell division in the germinative region of the wool follicle bulb and the rate of protein synthesis in the keratogenous zone of

the follicle (Schinckel, 1961, 1962; Black and Reis, 1979).

It is well recognized that wool or hair follicles are inherently programmed but can also be influenced by systemic factors. Although these factors have yet to be completely identified, several hormones have been shown to have profound effects on wool growth.

3.1 Nutrition

Many of the factors controlling the rate of wool growth are genetically determined, namely:

i) the number of wool follicles.

ii) the maximum number of bulb cells in each follicle.

iii) the proportion of cells migrating from the follicle bulb that enter the fibre.

iv) the maximum size of bulb and fibre cells.

This potential can be manipulated by level of nutrition (Black and Reis, 1979). The rate of wool growth can be increased or decreased several fold by extremes of feeding (Coop. 1953). Black and Reis (1979) concluded that the major influence of nutrition is likely to be on the rate of division of follicle bulb cells and on the final size of cells in the follicle bulb and in the fibre cortex. Short *et al.* (1965) examined the relationship between wool production and the mitotic rate of bulb matrix tissue of only one Merino sheep at two levels of nutrition using intravenous injection of colchicine. They found that the number of mitoses in the bulb matrix as well as the germinative cell population increased with the higher nutritional plane. Also Wilson and Short (1979) noted that changes in wool growth in response to nutritional change are accompanied by changes in the size of the follicle bulb produced by an increase in cell number rather than in cell size. A major reason for the reduction in wool growth following a restriction in nutrient availability appears to be a decline in the rate of bulb cell division (Fraser, 1965; Short *et al.*, 1965; Hynd *et al.*, 1986). Coincident with the change in cell turnover time, the number of bulb cells in each follicle normally declines (Short *et al.*, 1965). Schinckel (1962) studied changes in fibre and bulb diameter, as well as mitotic rate, caused by dietary intake in Merinos. Because cell number increase did not account fully for higher fibre output at higher levels of nutrition, he suggested that there might be an increase in fibre cortical cell size. This suggestion could be only partly supported by Williams and Winston (1987). Hynd and Everett (1990) compared the effects of high and low levels of nutrition on strongwool Merino sheep, measuring cell birth rate (CBR) in the follicle bulb. They concluded that CBR determines the rate of fibre growth. CBR was dependent on nutrition and closely related to fibre growth. According to

Black (1987) there is some evidence that the proportion of migrating bulb cells entering the fibre declines with reduced nutrient supply. Similarly, there is evidence indicating that nutrition has a substantial effect on differentiation of cortical cells, but few animals have been examined and the results are inconsistent. Black (1987) concluded that the significance for wool growth of changes in either the proportion of bulb cells entering the fibre or their differentiation following a change in nutrition is unclear. However, nutrient supply can have a small effect on cortical cell size. In the same way, Short *et al.* (1965) estimated that cortical cell volume increased by at least 20% in sheep following a substantial rise in plane of nutrition.

Feed intake in general, and the supply of the sulfur amino acids (S-amino acids) and lysine in particular, directly influences fibre production, largely via effects on the rate of bulb cell division and to a lesser extent on the size of the cortical cells (Hynd, 1989a; 1989b). Reis (1979) suggested that some effects of amino acids on wool growth may be mediated via the endocrine system, but clear evidence is lacking. The protein composition of wool can be altered by genetic, physiological and nutritional factors (as reviewed by Gillespie, 1990). As reviewed by Reis (1979) the abomasal or intravenous infusion of cysteine or methionine into sheep on low-quality roughage diets increases the growth rate and sulfur content of the wool as well as the fibre diameter. As was mentioned by Fratini *et al.* (1994) the increase in sulfur content occurs as a result of the increased synthesis of cysteine-rich proteins of the wool fibre. In this respect, unpublished work by Hynd (cited by Hynd *et al.*, 1986) revealed that cysteine increases wool output not only by providing substrate for keratin synthesis, but also by stimulating the rate of follicle bulb cell division. Hynd (1989a) found that the increase in wool growth with the high-protein diet was accompanied by an increase in both the average volume of the germinative region of the follicle bulb, and the total number of mitotic cells present in the bulb. This indicates that both wool growth and mitotic rate are high in sheep fed a high-protein ration. The proportion of high-tyrosine proteins in wool is also influenced by various nutritional treatments, but a control mechanism has not been identified (Reis, 1982). However, another possible effect of S-amino acids may be a stimulation of mitotic rate in the follicle bulb, with a resultant increase in the rate of synthesis of all protein fractions (Reis and Schinckel, 1964; Hynd, 1989b).

Levels of many minerals in the diet can influence wool growth, but these effects are largely non-specific through their effect on feed intake and/or rumen function, which influence nutrient supply. Copper and zinc function directly in the process of wool growth, copper being involved in keratinization. Zinc is largely associated with and necessary for cell replication, and a deficiency of zinc could reduce the rate of cell division in the follicle bulb

(Williams, 1991). Therefore, it is specifically required by rapidly proliferating tissues (Reis, 1989; Williams, 1991).

3.2 Blood Flow

Blood flow (BF) is important in determining delivery of metabolites to any tissue, including the wool follicle. High levels of wool production may be related to a greater partitioning of nutrients to the skin and its follicles or to the ability of some follicles to draw substances from the blood stream and utilize then more effectively.

The importance of blood supply for wool growth has been indicated by a number of observations, but this has largely arisen from indirect evidence. For example, in studies on hair growth in the rat, Durward and Rudall (1949) found that the density of the blood vessels associated with hair follicles varied according to the stage of hair growth cycle. At about the same time, Ferguson (1949) observed an increase in wool growth after unilateral skin sympathectomy. The increase in weight of wool produced was attributed to an increase in BF due to dilation of the blood vessels, which resulted from interruption of their nerve supply. Nay (1966) obtained histological evidence suggestive of a greater blood supply to the wool follicles in higher-producing sheep. Exposure of a section of skin to cold conditions substantially reduced wool growth (Doney and Griffiths, 1967; Lyne *et al.*, 1970), and BF to the skin of sheep suffering acute cold stress was reduced (Hales *et al.*, 1976). Furthermore, Cockrem (1962) used skin temperature as measure of BF and found variations in wool growth over the body similar to those found by Henderson (1953), 50% of which could be attributed to differences in BF. This is further supported by a computer simulation model which has shown theoretically that there would be a 50% increase in wool growth by doubling skin blood flow (Black and Reis, 1979; Table 1.1). The theoretical increase in wool production was associated with an increased proportion of high-sulfur and ultra-high-sulfur protein in wool.

In considering the relationship between blood supply and wool growth it is important to distinguish between the supply to the skin as a whole, and the immediate supply to the individual follicle. The differences in the type and the amount of wool produced in different parts of the body have led to suggestions that there are differences in nutrient supply to different regions of the skin (Henderson, 1953). In this respect, Setchell and Waites (1965) developed a radioactive method to study the variation in BF in different areas of the skin of adult Merinos (n=7) and adult Southdowns (n=4). They found that BF through the skin, expressed as a fraction of cardiac output, was not significantly different

between breeds. Also they demonstrated a greater flow in the fleece area than in the legs. This is in agreement with the findings of Hocking Edwards (1993) who found a difference in BF to the skin of the midside and abdominal regions. However, measurements of skin BF rates in sheep selected for high and low wool growth rate showed no significant difference (J.R.S. Hales unpublished; cited by Williams, 1987).

Recently, Hales and Fawcett (1993) made quantitative measurements of BF to the skin, in 9 "Fleece plus" (genetically high for fleece weight) and 6 "Fleece minus" (genetically low for fleece weight) lines of Merino ewes, using radioactive microspheres. They found that the BF rate per unit area of wool-bearing skin was significantly greater in the Fleece plus than in the Fleece minus group. Similarly, in two strains of Merinos (Finewool, n=4 and Strongwool, n=4), Hocking Edwards (1993) found that strongwool Merinos had a greater rate of blood flow through the skin than Finewool Merinos, and this was positively associated with both wool production per unit area of skin and the total volume of germinative tissue in the skin. The results obtained indicated that a 100% increase in BF through the skin was associated with a 150% increase in wool growth.

There are likely to be physiological differences, that are not apparent in the anatomy of the blood supply, which induce differences in skin BF. It is possible that at least part of the control of wool growth exerted by hormones is brought about through the blood-stream by effects such as constriction and dilation of blood vessels. For instance, some hormones of the adrenal cortex that depress wool growth are known also to decrease capillary permeability (Ryder and Stephenson, 1968).

Table 1.1 Predicted effects of changes in the rate of blood flow to the skin of sheep on the utilization of sulfur-amino acids (after Black and Reis, 1979).

Blood flow rate to skin (ml/min)	Wool growth rate (g/day)	Wool protein type synthesised* LS HS UHS (% of wool weight)			Irreversible loss of sulfur-amino acids (m moles/day)	Plasma concentration of sulfur-amino acids (mmoles/L)	Follicle bulb cell turnover time (hours)
29	3.0	81.6	16.4	2.0	25.8	0.098	98.1
58	5.1	80.5	17.4	2.3	24.1	0.087	57.8
116	7.8	78.4	18.9	2.7	21.5	0.073	37.8
175	9.5	77.1	19.8	3.1	19.9	0.063	31.2
234	10.5	76.0	20.5	3.5	18.7	0.059	28.1
349	11.7	75.0	21.1	3.9	17.1	0.053	25.1

* LS: low sulfur protein; HS: high sulfur protein; UHS ultra-high sulfur protein.

3.3 Hormones

Fibre growth is markedly hormone dependent, and experimental manipulation of hormone status causes large changes in the rate of wool growth. Hormone regulation of wool growth was reviewed by Ferguson (1965). Several hormones have been shown to have profound effects on wool growth. The local application of hormones to the skin has been used in several species to study hormonal effects on wool or hair growth (Wallace, 1979), but the majority of experiments have used injections to raise circulating levels of hormones. Relationships between plasma concentrations of a number of hormones and wool growth have studied by many investigators. For several hormones no relationship has been found, e.g. cortisol (WiIlliams *et al.*, 1986), prolactin (McCloghory *et al.*, 1992) and placental lactogen (Chan *et al.*, 1976). There was, however, a positive relationship between plasma insulin and hair growth (Mohn, 1958), and a negative relationship between plasma growth hormone and wool growth (Ferguson *et al.*, 1965; Wheatley *et al.*, 1966). Conversely, Hough *et al.* (1988) measured levels of hormones in the fleece-plus and fleece-minus ewes and found no difference in plasma growth hormone or plasma insulin levels.

Large, prolonged doses of cortisol and cortisol analogues depress the mitotic activity and size of follicle bulb cells (Chapman *et al.*, 1982), but little work has been conducted with lower levels of cortisol. Downes and Wallace (1965) observed increases in the rate of wool growth, measured as weight produced per unit area, when low doses of cortisol were injected intradermally. This was supported by Chapman and Bassett (1970) who found that cortisol injection enhanced wool growth, and they attributed that to the associated increase in feed intake. Hynd (1989b) concluded that cortisol has little effect on the normal processes of bulb cell division, except when present at very high concentrations. This was supported by P.I. Hynd and B.K. Applebee (unpublished work cited by Hynd, 1989a) who showed that cortisol has little effect on ^{3}H-thymidine uptake by the skin both *in vivo* and *in vitro*.

Thyroxine has long been considered to exert its effect through protein synthesis and cell size. Rougeot (1965) concluded that thyroid hormones act only on the size of the cells and probably have little or no effect on the mitotic activity of follicle bulb cells. However, there is evidence from other tissues that thyroxine does affect mitotic rate (Smith, 1951; Smelser and Ozanics, 1954). The results obtained by Hynd (1989b) suggested a significant depression in the rate of bulb cell division in the fully thyroidectomised sheep. Cortical cell size, on the other hand, was little affected by thyroid status, a result in accord with the findings of Rougeot (1965). Other investigators have found that only 15% to 30% of normal circulating levels of thyroxine are needed to maintain wool growth at original rates

in thyroidectomised sheep (Ferguson *et al.*, 1965; Maddocks *et al.*, 1985, Hynd, 1989b). Hynd (1989b) attributed the inability of thyroxine to stimulate wool growth above "normal" levels to thyroxine having a facilitatory role rather than a regulatory role in wool production.

In animals with defined hair growth cycles, there is a marked decline in mitotic activity in follicle bulb cells at the start of the catagen phase of the cycle. This is associated with a substantial change in follicle bulb and dermal papilla dimensions, fibre shape and cell type, and in the vascularity of the follicle (Priestly, 1967; Panaretto, 1979). It is unclear whether the changes in the rate of bulb cell division are due to a direct effect of pineal indoles, such as melatonin, or are an indirect effect of reduced blood flow and nutrient supply to the follicle (Panaretto, 1979). In fine wool Merino wethers, Foldes *et al.* (1990) observed that subcutaneous melatonin treatment did not influence wool growth.

Adrenaline appears to play small part in the regulation of mitosis in the follicle bulb (Bullough, 1962), and is unlikely to directly affect wool production, since adrenalectomy did not alter wool growth in sheep (Ferguson *et al.*, 1965). Noradrenaline, on the other hand, does depress fibre growth (Cunningham *et al.*, 1979) and this may account for the increase in wool growth following sympathectomy (Ferguson, 1949).

3.4 Growth Factors

The growth of a wool fibre is dependent on the proliferation of cells in the follicle bulb. There is little information available about the control of cell division in the wool follicle. Sutton *et al.* (1991) hypothesised that the proliferation of the follicle bulb cells is caused by local production of growth factors, which induce the expression of several oncogenes including c-myc or N-myc, which then enable the cells to divide. There are several pieces of indirect evidence for this hypothesis. First, several growth factors, including transforming growth factor-$\propto$ (TGFα), fibroblast growth factor (FGF), epidermal growth factor (EGF) and insulin-like growth factor-I (IGF-I, will be considered in more detail in section 4) are found in the skin and can stimulate the proliferation of keratinocytes *in vitro* and *in vivo* (Moore, 1989). EGF was the first peptide growth factor reported to enhance cell proliferation. It operates through a transmembrane glycoprotein receptor (EGFR) on basal epidermal cells and several epithelial tissues (Hardy, 1992). It was noted by Wynn *et al.* (1989) that TGFα binds to EGFR and also retards hair growth in mice. An *in vitro* study by Moore *et al.* (1991) found that the growth factors EGF and FGF had mitogenic effects, although the extent of the response varied with tissues. FGF was found to

be more potent than EGF. Moore *et al.* (1991) concluded that, in general, cell proliferation was promoted by foetal bovine serum (FBS), EGF and FGF.

4 Insulin-Like Growth Factor-I (IGF-I)

Somatomedin C (SmC) or insulin-like growth factor-I (IGF-I) is widely recognised as the mediator of many, but not all, actions of growth hormone (GH) on growth (Daughaday, 1981).

This section will consider the structure, role and biological function of IGF-I and its variants in domestic animals, with particular emphasis on how IGF-I may regulate wool growth.

4.1 Native IGF-I

4.1.1 Structure and Function of IGF-I

IGF-I is a single-chain molecule consisting of 70 amino acids, and with three intrachain disulfide bridges (Rinderkencht and Humbel, 1978). It has a molecular weight of 7649 daltons and exhibits extensive homology with insulin.

Bovine, porcine, and human IGF-I are identical, whereas rat and mouse IGF-I differ from human IGF-I by three and four amino acid residues, respectively. Only one amino acid residue difference is observed between rat and mouse IGF-I (McBride *et al.*, 1988; Sara and Hall, 1990).

The actions of IGF-I *in vitro* and *in vivo* have been extensively described and reviewed by Sara and Hall (1990). *In vitro*, IGF-I was shown to stimulate DNA synthesis and cell proliferation in a variety of cells, including human skin fibroblasts, embryonic chicken fibroblasts and rat myoblasts (Scanes *et al.*, 1986; Francis *et al.*, 1988). *In vivo*, studies showed that administration of IGF-I can alter muscle protein turnover in sheep, pigs, cattle and rats (Douglas *et al.*, 1991; Oddy *et al.*, 1991; Tomas *et al.*, 1993; Owens, *et al.* 1994; Walton *et al.*, 1994). The biological actions of IGF-I are initiated by its binding to specific cell surface receptors. Many tissues contain receptors for IGF-I (LeRoith and Roberts, 1991).

4.1.2 IGF-I Secretion and Control

Circulating concentrations of IGF-I are dependent upon the rate of secretion and of clearance, the latter being affected by the concentration of carrier-proteins (Scances *et al.*, 1986). A great deal is now known of the hormonal control of secretion of IGF-I in domestic animals. Indirect evidence on control of IGF-I secretion can be inferred from circulating concentrations.

Considerable evidence indicates that circulating concentrations of IGF-I are at least partially dependent on GH status. For example, in dogs, circulating concentrations of IGF-I are low in GH-deficient dwarfs (Eigenmann *et al.*, 1984a), and high in dogs in which GH levels are elevated, including those showing acromegaly (Eignmann *et al.*, 1984b). In sheep, circulating concentrations of IGF-I are reduced by hypophysectomy and increased in acromegalics (Salmon and Daughaday, 1957). Chronic administration of GH increased circulating concentrations of IGF-I in intact pigs (Chung *et al.*, 1985), but not in intact sheep (Wien *et al.*, 1983).

On the basis of the negative feedback effect of IGF-I, direct evidence has shown that IGF-I inhibits GH release by affecting both the hypothalamus (increasing release of somatostatin) and pituitary gland (decreasing GH synthesis and growth hormone releasing factor (GRF)-induced GH release) (Abe *et al.*, 1983; Tannenbaum *et al.*, 1983). Others have found that purified IGF-I can stimulate somatostatin release from the hypothalamus (Berelowitz *et al.*, 1981). Such negative feedback may be blunted by IGF-I inhibitors, which appear to be elevated in *diabetes mennitus* (Phillips, 1986).

The levels of circulating IGF-I are also regulated by somatomedin inhibitors, present in normal serum (Phillips, 1986). Somatomedin inhibitors are peptides (molecular weight 20 000 to 30 000) which can blunt both IGF-I and insulin action on cartilage, fat and muscle (Phillips and Saholz, 1982).

Production of IGF-I by the liver (the major source of circulating IGF-I, D'Erocle *et al.*, 1984) is dependent upon GH and influenced by other factors such as circulating insulin concentrations and nutrition, which can directly affect IGF-I generation or influence GH-induced IGF-I secretion (Phillips and Vassilopoulou-Sellin, 1980; Phillips, 1986). In recent years it has become apparent that a large number of non-hepatic tissues also synthesise IGF-I, and this may be related to the para/autocrine actions of IGF-I (Breier *et al.*, 1991).

4.1.3 IGF-I Binding Proteins (IGFBPs)

Insulin-like growth factor-I, unlike other peptide hormones, is transported in blood in association with specific high-affinity proteins (Hintz, 1986; Sara and Hall, 1990). Six of

these, named IGFBP-1 to -6, have been isolated and characterised. They are known to affect the distribution, action and metabolism of the IGFs (Binoux *et al.*, 1991). At present, only the roles of the IGFBP-1 and IGFBP-3 are well understood. These binding proteins can be classified broadly on the basis of molecular weight (MW) into two distinct groups, the first a protein complex of about 150 000 daltons (this complex is under growth hormone control), and the second group of proteins ranging in MW from approximately 25 000 to 50 000 daltons (Hintz, 1986). In adult sheep, IGF-I circulates primarily in a high MW complex, and only a small proportion of IGF-I is carried as part of a low MW complex. Very small amount of unbound IGF is found in circulation (Gluckman *et al.*, 1987). Several investigators have found that IGFBPs increase the half-life of circulating IGF-I, e.g. IGFBPs prolong the half-life of IGF-I from 10 minutes, when free in the plasma, to 3 hours in bound form (Wallis, 1988). IGFBPs are therefore considered to possibly act as a storage pool since no tissue appears to store IGF-I (Zapf *et al.*, 1984).

The function of these binding proteins is not fully understood. They may be involved with restricting, controlling or facilitating an endocrine role for IGF-I or may simply serve to expedite excretion of IGF-I secreted in excess of that required to elicit paracrine or autocrine activity (Davis *et al.*, 1989). The IGFBPs inhibit IGF-I activity in some cell types (Ritvos *et al.*, 1988; Lewitt *et al.*, 1993; Hopkins *et al.*, 1994) and enhance its activity in others, e.g. mammary tissue has been shown to release IGFBPs which provide the potential for local regulation (Campell *et al.*, 1991). It has become apparent that the IGFBPs secreted by many cell types have the potential to modulate the biological actions of IGF-I.

Another potential role of the IGF-I:IGFBPs complex is the possibility that only certain tissues would be able to remove the peptide from the binding protein and thus have biologically potent free IGF-I available to their receptors sites (Hintz, 1986). Also the bound form of IGF-I does not diffuse readily across capillary walls.

The biological role of the IGFBPs presumably is to assure a continuous supply of IGF-I to target cells. The anabolic effect of the IGFs on the target tissues depends on the equilibrium between binding to the carrier proteins and to the cell receptors (Hall and Sara, 1983).

There is evidence that the IGFBPs may also play a functional role in the autocrine or paracrine actions of IGF-I, a situation in which IGF-I is retained close to the cell secreting it and exerts its actions locally.

4.1.4 IGF-I Receptors

The biological actions of IGF-I are initiated by their binding to specific cell surface receptors (Breier *et al.*, 1991). There are at least two types of IGF-I receptors, located in the plasma membrane, type I and II. IGF-I binds to the type I receptor, which exhibits homology with the insulin receptor (Daughaday, 1982; Rechler and Nissley, 1985). The IGF-I receptor is structurally similar to the insulin receptor and binds IGF-I with higher affinity than IGF-II (and insulin only in high concentrations). The IGF-II receptor binds IGF-II with much higher affinity than IGF-I and dose not crossreact with insulin. The cellular effects of IGF-I and IGF-II seem to be mediated by the IGF-I receptor (Sara and Hall., 1990; Gammeltoft *et al.* 1994). The IGF-I receptors have been found in cartilage, muscle, placenta, liver, fibroblasts and monocytes (Daughaday, 1982). The number of IGF-I receptors appears to be modulated by nutrition. Fasting is reported to increase type I receptors in most tissues except the brain.

Studies with monoclonal antibodies to the human type I IGF receptor have shown that IGF-I acts via this receptor to stimulate DNA synthesis and amino acid transport (Van Wyk *et al.*, 1985). Although there is some evidence that there are heterogeneous type I receptors (Etherton, 1991), the implications of this are not clear.

4.1.5 The Mode of Action of IGF-I at Target Cells

In general terms the IGFs may be considered to be anabolic hormones that are active throughout life. The end result of their action will depend on the responsiveness of their target cells. If the cells are in the hyperplastic phase (increase in the number of cells), then IGF-I stimulates proliferation. However, if the cells are undergoing hypertrophic growth (increase in cell size), or are mature non-dividing cells such as neurones, then other anabolic process, such as protein synthesis, are stimulated instead (Spencer, 1985; Sara and Hall, 1990; Jennische *et al.*, 1992).

In tissue culture, IGF-I stimulates DNA synthesis, cell multiplication and mitotic division in oocytes (Phillips, 1986; Sara and Hall., 1990), and also stimulates the replication of cells of the osteoblast lineage, presumably preosteoblastic cells, which can differentiate into osteoblasts (Canalis, 1994). It has been suggested that IGF-I acts as a progression factor to stimulate cells through the DNA synthesis phase of the cell cycle.

IGF-I has been demonstrated to induce additional anabolic responses in target cells. For example, the uptake of amino acids and protein synthesis has been shown to be estimulated by IGF-I in rat cartilage and human skin fibroblast. In target organs for insulin, especially adipose tissue and muscle, IGF-I displays potent insulin-like activity, stimulating

glucose transport and metabolism. In addition, IGF-I has a much greater potency in cartilage than in other insulin targets (Phillips and Vassilopulou-Sellin, 1980).

Muscle is an important target tissue for IGF-I action. The expression of IGF peptides, receptors and binding proteins by muscle suggests that a significant component of IGF-I action in this tissue is mediated through autocrine/paracrine mechanisms (Rosenthal *et al.*, 1994). Metabolic of IGF-I effects *in vitro* occur at much lower concentrations in muscle than in adipose tissue (Zapf *et al.*, 1978; Poggi *et al.*, 1979).

In rat skeletal muscle, Ewton *et al.*(1994) investigated the roles of IGF-I, IGF-II (insulin-like growth factor-II) and IGFBPs in the regulation of L6 muscle cell differentiation in cultures. They found that IGF-I acts as a mitogen while IGF-II causes the cells to differentiate sooner.

In vitro studies of fetal rat bones indicate a direct effect of IGF-I on bone formation (Canalis *et al.*, 1977; Canalis, 1980). IGF-I increased collagen synthesis and DNA content in cultured fetal calvariae (Canalis, 1980). Therefore, Hock *et al.*(1988) examined the effects of IGF-I on bone matrix formation and cell replication. They showed that the stimulatory effect of IGF-I on collagen synthesis in bone organ cultures results in an increase in matrix production. In addition, IGF-I stimulated DNA synthesis and nuclear labelling with [^{3}H]thymidine, confirming previous observations that IGF-I increases cell replication in calvarial cultures (Canalis, 1980). This finding led Hock and his colleagues to suggest that the stimulatory effect of IGF-I on bone matrix synthesis appears to be consequence of at least two regulatory signals; the first is a direct effect on the differentiated function of the osteoblast (i.e. enhanced bone collagen production), while the second is an increase in osteoprogenitor cell replication, resulting in a large number of functional osteoblasts.

IGF-I is also a potent mitogen and metabolic regulator of mammary tissue from a variety of species (Baumrucker, 1986a,b; Imagawa *et al.*, 1986; Prosser *et al.*, 1987; Shamay *et al.*, 1988; Campbell *et al.*, 1991).

4.1.6 The Effect of IGF-I on Metabolism and Growth

The responses produced following IGF-I binding differ between cell types, but include "metabolic" effects such as stimulation of glucose uptake, amino acid uptake, glycogen deposition and lipogenesis as well as inhibition of glycoeogesis and lipolysis. Longer term "growth" responses are stimulation of protein and DNA synthesis, an inhibition of protein breakdown and differentiation functions such as the induction of specific enzymes (Ballard *et al.*, 1994).

The original somatomedin hypothesis postulated that growth hormone did not act directly to stimulate growth, but that its effects were mediated by a serum factor termed somatomedin (now called IGF-I) which is synthesised and secreted by the liver under the influence of growth hormone (Daughaday *et al.*, 1972; Froesch *et al.*, 1985).

It was originally believed that IGF-I was produced exclusively in the liver and acted on target tissues in the endocrine (via the blood stream) manner. It is now known that IGF-I is synthesised in many, if not all, tissues (D'Ercole *et al.*, 1984), and so may act in a paracrine or autocrine manner (influencing growth and metabolism close to the site of synthesis).

It is now generally accepted that the growth promoting and anabolic actions of growth hormone are mediated in part by IGF-I acting in both an endocrine and paracrine manner. The relative importance of endocrine versus paracrine actions of IGF-I is unclear as yet. The investigation of the biological activity of IGF-I has been restrained by the scarcity of purified material derived from serum. However, the advances in DNA technology have increased the potential for more definitive studies on the effects of IGF-I *in vivo* (Bang and Hall, 1992).

In vivo studies demonstrate that IGF-I has both an acute insulin-like action as well as a chronic growth-promoting effect. As Zapf *et al.* (1984) pointed out, the effects of IGF-I depend on their mode of administration, with intravenous bolus injections causing acute insulin-like effects and with long-term subcutaneous administration inducing growth. They suggested that this because of a difference in the availability of IGF-I to the target cells. The acute metabolic actions of IGF-I were reported by Zapf *et al.* (1986). Using both normal and hypophysectomised rats, they demonstrated that the intravenous bolus injection of IGF-I induced hypoglycaemia (a decline in plasma glucose concentration) and enhanced glucose uptake from serum and incorporation into glycogen. Only a minor stimulation of lipid synthesis was observed in normal rats. Acute hypoglycaemia has also been reported after bolus infusion of recombinant IGF-I to pigs (Zenobi *et al.*, 1988). Similarly, Jacob *et al.* (1989) reported acute hypoglycaemia after continuous infusion of recombinant human IGF-I into fasted rats for 90 minute . Their results also showed that IGF-I administration exerted potent effects on protein metabolism, inhibiting proteolysis, which may be useful in protein-wasting states (e.g. as those associated with severe burns or trauma).

Somatomedins influence growth by affecting mitosis. For instance, IGF-I stimulates [^{3}H]-thymidine incorporation into chick embryo fibroblasts (Haselbacher *et al.*, 1980).

Exogenous IGF-I has been shown to promote growth in hypophysectomised animals (mainly rodents) or strains that are genetically deficient in growth hormone (Russell and

Spencer, 1985; Guler *et al.*, 1988; Skottner *et al.*, 1989; Pell and Bates, 1992; Butler *et al.*, 1994). Russel and Spencer (1985) injected IGF-I and GH directly into the tibial epiphysial plate of hypophysectomised rats, which caused significant cartilage growth. This demonstrated that IGF-I and GH have direct growth-promoting effects on cartilage *in vivo*. In study of Guler *et al.* (1988), IGF-I or GH were infused into hypophysectomised rats for 18 days. They noted that kidney, spleen and thymus were more sensitive to IGF-I, whereas skeletal muscle was more sensitive to the effects of GH. This suggests that these peptides act independently in different tissues, perhaps on different cellular targets within these tissues, as has been suggested for longitudinal bone growth (Isaksson *et al.*, 1987).

The effect of frequency of subcutaneous administration of IGF-I in growth hormone-deficient mice was studied by Woodall *et al.* (1991). Frequent injections of IGF-I significantly increased body weight gain, but the same dose administered in one daily injection generated no response in weight gain and the mice became severely hypoglycaemic.

In intact animals, Hizuka *et al.*, (1986) infused recombinant IGF-I for 7 days into normal growing rats. Plasma IGF-I levels were significantly higher in treated than in control rats. Body weight and length, and tibial epiphysial width, were increased in treated rats. Hizuka *et al.* (1986) concluded that IGF-I stimulated growth in normal growing rats *in vivo*, and might be useful in the treatment of growth retardation. The recent study by Cottam *et al.* (1992) showed that administration of IGF-I over an 8 weeks period had virtually no growth-promoting effects in well fed sheep. Similar results were obtained by Min *et al.* (1995) who concluded that IGF-I does not have marked growth-promoting effects in sheep fed a near-maintenance diet. However, Schalch *et al.*, (1989) reported that infusion of IGF-I for 7 days had growth-promoting effects in energy-restricted normal rats, but not *ad libitum* fed rats. In another study with rats, stimulation of growth and nitrogen retention with IGF-I administration was greater during malnutrition (Yang *et al.*, 1990)

4.1.7 Effects of IGF-I on Wool Growth

It is now generally accepted that the growth-promoting effects of pituitary growth hormone are mediated in part by IGF-I acting in both an endocrine and paracrine manner (Frosech *et al.*, 1985). However, the role of IGF-I in the wool or hair follicle is not known.

There is only limited data on the regulatory effects (if any) of IGF-I on wool growth. A study involving administration of 50 µg/kg body weight/8 hours of IGF-I for 56 days to 8 male sheep by Cottam *et al.* (1992) found that there was no significant effect of IGF-I administration on wool growth rate, measured as rate of wool growth per unit area of

skin. Similar results were reported by Min *et al.* (1995).

Imunohistochemistry has shown that IGF-I is expressed in rat hair follicles, and dermal papilla fibroblasts, which are essential for normal hair growth *in vivo* (Hansson *et al.*, 1988). Philpott *et al.* (1994) studied the effects of IGF-I on cultured human hair follicles. Their results suggested that, *in vitro,* IGF-I may be an important physiologic regulator of hair growth and possibly the hair growth cycle.

Short-term infusion of IGF-I variants (LR3IGF-I; see section 4.2) directly into a patch of skin of 6 sheep for 4 hours resulted in no significant effects on wool growth rate or replicating cell numbers in the bulbs of wool follicles (Harris *et al.*, 1993). This was supported by the recent study of Hocking Edwards *et al.* (1995), who observed no stimulatory effect of LR3IGF-I infusion (for 21 days) on wool production, and no change in either follicle dimensions or cell replication rate.

4.2 IGF-I Analogues

A corollary of these findings has been a search for analogues of IGF-I that bind less well than IGF-I to the insulin receptors, on the assumption that such analogues will produce a lesser degree of hypoglycaemia than IGF-I and, hence, will have greater therapeutic potential (Ballard *et al.*, 1994).

Three classes of IGF-I analogues have been identified and constructed using DNA technology at both protein and gene level, with the purpose of enhancing biological activity. They are:

i) deletion of the N-terminal tripeptide, i.e. des(1-3)IGF-I.

ii) subsitution of the Glu residue of position 3 with Arg or Gly, i.e. R3IGF-I and G3IGF-I, and

iii) addition of a 13-residue N-terminal extention peptide either with or without the substitution at position 3, i.e. LongIGF-I, LongR3IGF-I (LR3IGF-I which is used in present study), and LongG3IGF-I. (Francis *et al.*, 1986; Francis *et al.*, 1988; Sara and Hall, 1990; Walton *et al.*, 1990; Francis *et al.*, 1991).

Walton *et al.* (1990) and Francis *et al.* (1991) measured the biological activities of these analogues compared with IGF-I. All IGF analogues were more potent than IGF-I in stimulating protein and DNA synthesis in cultured L6 myoblasts. The order of potency was: LR3IGF-I = des(1-3)IGF-I > R3IGF-I > G3IGF-I > LG3IGF-I > LIGF-I > IGF-I. However, one of the most potent peptides in stimulating growth, LR3IGF-I, was 3 to 4-fold less potent than IGF-I in the receptor binding assay.

IGF-I analogues bind weakly to IGF-binding proteins (IGFBPs) and produce more potent anabolic responses than native IGF-I in cultured cells despite showing reduced binding to the type I receptor (Francis *et al.*, 1988; Walton *et al.*, 1990; Francis *et al.*, 1991;Tomas *et al.*, 1992; Tomas *et al.*, 1993). These modifications dramatically reduce the ability of these analogues to bind to IGFBPs although they remain active at the IGF-I receptor (Flint *et al.*, 1994). The increased bioactivity of IGF-I analogues appears to be the result of reduced affinity for IGFBPs secreted into the extracellular medium (Francis *et al.*, 1994).

In comparisons of the effects of IGF-I and IGF-I analogues on rat skeletal muscle cell proliferation and differentiation, Ewton *et al.* (1994) found that IGF-I analogues with reduced affinity for IGFBPs, such as LR3IGF-I, were about 10 times more potent than native IGF-I in stimulating L6 myoblast cell proliferation, and about 100 times more potent than native IGF-I in stimulating differentiation. This finding provide indirect evidence that IGFBPs inhibit differentiation induced by IGF-I (Ewton *et al.*, 1994; Rosenthal *et al.*, 1994).

The study of McGrath *et al.*(1991) demonstrated that proliferation of normal epithelial cells isolated from the mammary glands of non-lactating Holstein heifers is stimulated by the addition of IGF-I or the truncated form of IGF-I (des(1-3)IGF-I). Their results shown that des(1-3)IGF-I was more potent in stimulating thymidine incorporation into mammary cells. Compared to IGF-I, des(1-3)IGF-I was approximately 4-5 times more potent in stimulating bovine mammary cell proliferation. This result agrees with reports stating that the truncated molecule is more potent than native IGF-I in stimulating myoblast proliferation and protein accretion (Francis *et al.*, 1986), and demonstrated that the difference in proliferative activity between full-length and truncated IGF-I was due to affinity for IGFBPs (McGrath *et al.*, 1991; Sliverman *et al.*, 1995). As indicated by Forbes *et al.* (1988) and Silverman *et al.* (1995), IGF-I analogues, such as des(1-3)IGF-I bind weakly to IGFBP-3, the major form circulating in blood, and very poorly, compared to that for native IGF-I, to IGFBP-1 and IGFBP-2. Moreover, des(1-3)IGF-I is more potent than IGF-I with cells that release these binding proteins (Ross *et al.*, 1989).

5 Summary

It is evident that detailed knowledge is still lacking in areas related to cell proliferation, growth and migration in wool cell follicle bulb. There is uncertainty regarding the relative importance of the modes of action of IGF-I and its variants, and the role of IGF-I as an anabolic or somatogenic agent is still not fully understood. Finally, there is lack of information on the effects of IGF-I on the functional unit of wool growth -the follicle.

There is only one study, by Harris *et al.* (1993), on the effect of infusion of LR3IGF-I into sheep skin (for 4 hours) on wool production. The result from this study led the authors to conclude that short-term infusion of LR3IGF-I has no effects on wool follicle bulb cell proliferation. Therefore, this present study was undertaken to investigate the effect of long-term infusion of LR3IGF-I (21 days) directly into a patch of skin, on wool production and any associated changes in wool follicle characteristics such as bulb cell replication rate.

CHAPTER 2

EXPERIMENTAL

CHAPTER 2

EXPERIMENTAL

ABSTRACT

The effects of long-term (21 days) local infusion of a variant of insulin-like growth factor-I (Long-R3-IGF-I; LR3IGF-I) on wool follicle characteristics and wool production in a defined patch of skin were investigated in well-fed castrated Romney sheep. During treatment, control sheep were pair-fed to the *ad libitum* intake of the LR3IGF-I treated sheep.

A bilateral arterio-venous preparation was used to infuse LR3IGF-I into one abdominal flank and control infusate (CI) into the other abdominal flank of 6 sheep (LR3IGF-I sheep); a further 6 sheep had a single flank infused with CI (control sheep; C).

Measurements of blood flow and oxygen uptake were made on both sides of the LR3IGF-I sheep and one side of the control sheep 1 hour prior to, and 4, 24, and 48 hours after, LR3IGF-I or CI infusion.

Circulating levels of endogenous IGF-I, insulin and prolactin were measured on blood samples from all 12 sheep taken prior to, and 24 hours, 7, 14 and 21 days after, the start of infusion. Follicle characteristics and wool production were examined at the end of the treatment period.

There was a significant decrease in circulating endogenous IGF-I levels ($P<0.05$) of the LR3IGF-I sheep by day 7 of treatment compared to control sheep, followed by a significant increase between day 7 and day 21 of treatment in LR3IGF-I sheep. Plasma insulin levels followed a similar pattern. LR3IGF-I caused a significant increase in the skin blood flow ($P<0.05$) and oxygen uptake ($P<0.05$). After 48 hours of treatment oxygen uptake by the skin had returned to pre-infusion levels in LR3IGF-I sheep.

After 21 days of infusion, there was no effect of LR3IGF-I on the amount of wool produced, wool follicle bulb diameter, bulb area, bulb cell proliferation or wool composition. However, the LR3IGF-I treated side tended to have a lower number of BrdU-labelled wool follicle cells ($P=0.055$) and to produce less wool than the internal control side (IC) and the external control side (EC).

This study therefore found no effects of LR3IGF-I infusion, for 21 days, on bulb cell replication (estimated by colchicine or BrdU) or wool production, although LR3IGF-I treated sheep tend to produce less wool than control sheep.

INTRODUCTION

It is generally accepted that the growth promoting and anabolic actions of growth hormone (GH) are mediated in part by insulin like growth factor-I (IGF-I) acting in both an endocrine and paracrine manner. However, earlier *in vivo* studies have been restricted to small animals, and few direct studies have been undertaken in large animals because of the scarcity of pure material and the expense of IGF-I. Advances in DNA technology have increased the potential for more definitive studies on the effects of IGF-I (Bang and Hall, 1992).

Insulin-like growth factors act as mitogens on various cell types via activation of the IGF-I receptor. *In vitro* studies have shown that the IGFs stimulate DNA synthesis and cell proliferation (Ballard *et al.*, 1986; Ewton *et al.*, 1987; McGrathy *et al.*, 1989; McGrath *et al.*, 1991; Ohlsson *et al.*, 1992; Gammeltoft *et al.*, 1994) in various cell types, and cause differentiation (Ewton *et al.*, 1987) in cultured myogenic cells. IGF-I has been demonstrated to induce additional anabolic responses in target cells, for example the uptake of amino acids (Hill *et al.*, 1986; Ewton *et al.*, 1987). In target organs for insulin, especially adipose tissue and muscle, IGF-I displays potent insulin-like activity, stimulating glucose transport and metabolism (Sara and Hall, 1990).

It has been found by Francis *et al.* (1991) that variants of IGF-I such as LR3IGF-I and des(1-3)IGF-I are more potent than the native IGF-I in stimulating protein and DNA synthesis in rat L6 myoblast cultures and in bovine mammary cell (Francis *et al.*, 1991; McGrath *et al.*, 1991). It has been reported that IGF-I has a potent effect in stimulating proliferation and differentiation of keratinocytes in mouse (Ristow and Messmer, 1988) and human (Neely *et al.*, 1991) skin. Suttie *et al.* (1985, 1989) and Sadighi *et al.* (1994) noted that IGF-I also can stimulate the proliferation of undifferentiated cells of red deer antlers. Recently, Philpott *et al.* (1994) suggested that IGF-I may be an important physiologic regulator of hair growth *in vitro* and possibly the hair growth cycle.

In vivo studies have indicated that increased circulating levels of IGF-I restrict weight loss during starvation in rats (O'Sullivan *et al.*, 1989) and reduce protein loss in lambs systemically infused with IGF-I (Douglas *et al.*, 1991). Similarly, Oddy *et al.* (1991) found that infusion of IGF-I into the hind-limb of sheep resulted in a decrease in protein degradation associated with a smaller concomitant decrease in protein synthesis. They also found that local infusion of IGF-I into the hind-limb had no effect on oxygen utilization or blood flow. In direct contrast to previously cited results, Prosser *et al.* (1990) found that local infusion of IGF-I into the mammary gland of goats resulted in an increase in blood flow and milk yield, and these responses were attributed to a direct effect of IGF-I. Short-

term infusion of an IGF-1 analogue, LR3IGF-I, directly into skin of sheep resulted in an increase in skin blood flow, oxygen uptake and the uptake of amino acids by the skin for protein synthesis, but had no effect on cell replication rate in the wool follicle bulb (Harris *et al.*, 1993). Long-term (56 days) of IGF-I treatment of well-fed, growing, sheep resulted in no change in wool production (Cottam *et al.*, 1992). Recently, in nitrogen-restricted sheep, Min *et al.* (1995) found that IGF-I treatment for 56 or 84 days had no effect on wool growth.

This study was designed to provide more information on the effects of IGF-I on wool growth and wool follicle characteristics. Therefore, LR3IGF-I was infused into the skin of sheep for 21 days to allow measurement of wool production and any associated changes in wool follicle characteristics such as bulb cell replication rate, bulb diameter, and bulb area.

During the first 48 hours of LR3IGF-I infusion, detailed measurements of skin blood flow, and oxygen and glucose uptake by the skin were also undertaken.

MATERIALS AND METHODS

1 Animals, Diet and Surgical Preparation

Twelve, one year old Romney wethers of 30-35 kg body weight, were housed indoors in metabolism crates and assigned to pairs by body weight. Chaffed and pelleted lucerne hay were fed *ad libitum* at hourly intervals from overhead constant feeders, before and immediately after surgery. Water was available *ad libitum* at all times.

At weekly intervals, after 7-10 days of adaptation to the indoor environment, pairs of animals were anaesthetised with halothane (fluothane; ICI Pharmaceuticals, Macclesfield, Cheshire, England) and had fine bore polyvinylchloride (PVC; Dural Plastics and Engineering, N.S.W., Australia) catheters implanted into the descending lateral branches of the deep circumflex iliac artery (***A1***) and vein (***V***) of both the left (***LA1*** and ***LV***) and right (***RA1*** and ***RV***) abdominal flanks, as described in detail by Harris *et al.* (1989). This surgical preparation allows infusion into, and collection of the venous drainage from, a defined patch of skin on both abdominal flanks of each sheep.

A fifth catheter was implanted in the saphenous artery (***A2***) to allow sampling of arterial blood and another catheter was placed in the jugular vein. Apart from the jugular catheter, catheters were maintained by a continuous infusion of sterile heparinized saline, at 150 ml/day (0.9% sodium chloride, Baxter Healthcare Pty Ltd, Toongabbie, NSW, Australia; NZP Heparin Sodium, 30 iu/ml, N.Z. Pharmaceuticals Ltd, Palmerston North, New Zealand). The jugular catheter was flushed daily with 30 iu/ml sterile heparinized saline.

Two to three days after surgery, the infused area was defined for later biopsy purposes by slow injection of 3 ml Patent Blue V (Patent Blue V Sodium Salt 0.1% (w/v) in physiological saline; Laboratory Supplies, Poole, England; Lot 3811010M) into ***LA1*** and ***RA1***. The outline of the perfused area of skin was marked by a permanent marker.

Before any experimental work was undertaken, assessment of blood flow in the infused area was made in both sides of each animal by infusion of para-aminohippuric acid (PAH) into ***LA1*** and ***RA1*** catheters (Harris *et al.*, 1989). Then, for each pair, one sheep with two patent sides (*i.e.*,the arteriovenous preparation was supplying into, and draining from, only the defined skin patch) ***(LR3IGF-I sheep)***, had Long-R3-insulin-like growth factor-I (LR3IGF-I; Gropep Pty Limited, Adelaide, Australia; Lot No. LJB-A10) infused into one side (***LR3IGF-I side***) and control infusate (***CI***; as described in section 2.3) into the other (***internal control side, IC***). The remaining sheep from each pair (***control sheep, C***) was infused with ***CI*** into one side only ***(external control side, EC),*** allowing an

inadequately prepared side to be discarded.

Wool was clipped by small animal clippers with No. 40 blades (Oster® "Golden A5" professional clipper, Sunbeam Corp., U.S.A) from each infusion area close to the skin before the start of the LR3IGF-I infusion.

Feed intake returned to pre-surgery levels within 24 h in all animals. Experimental work commenced 4 days after surgery, and the control animals were then pair-fed to the *ad libitum* intakes of the LR3IGF-I infused sheep.

2 Experimental

2.1 Summary of Daily Experimental Events

Details of infusion, sampling and analytical procedures are given in sections 2.2.2-2.2.6. Either LR3IGF-I or CI was infused into the LR3IGF-I side or IC and EC sides, respectively, for 21 days (as in Fig. 2.1). Detailed metabolic measurements were made in the skin of the LR3IGF-I side and the IC side during the first 3-4 days of infusion as part of a larger trial and are not presented here.

On day one, a preliminary assessment over a 1 h period was made of blood flow, glucose uptake and oxygen utilization by the skin during infusion of ***solution P*** containing PAH (Appendix 1) as described in section 2.2.2. A 21 day skin infusion was then started with LR3IGF-I infused into the treatment side ***(LR3IGF-I)*** and CI into the other side ***(IC)*** of the LR3IGF-I sheep and CI alone infused into control sheep ***(EC side)***. After 3 h of infusion, measurements of blood flow, glucose and oxygen uptake in the skin were again made during infusion of solution ***P*** plus LR3IGF-I (***solution PI***; LR3IGF-I side) or solution ***P*** (IC and EC sides) directly into the skin patch.

After a further 24 and 48 h of infusion, simultaneous measurements of blood flow, glucose uptake, and oxygen utilization were again made for each skin patch.

Blood samples for hormone measurement were taken from catheter ***A2*** before, and after 2, 7, 15 and 21 days of, continuous LR3IGF-I or CI infusion.

After 21 days of infusion of LR3IGF-I or CI, wool was clipped close to the skin within each patch defined by Patent Blue V. The wool was stored in paper bags for later measurement and the outline of the clipped area was immediately traced onto an acetate sheet.

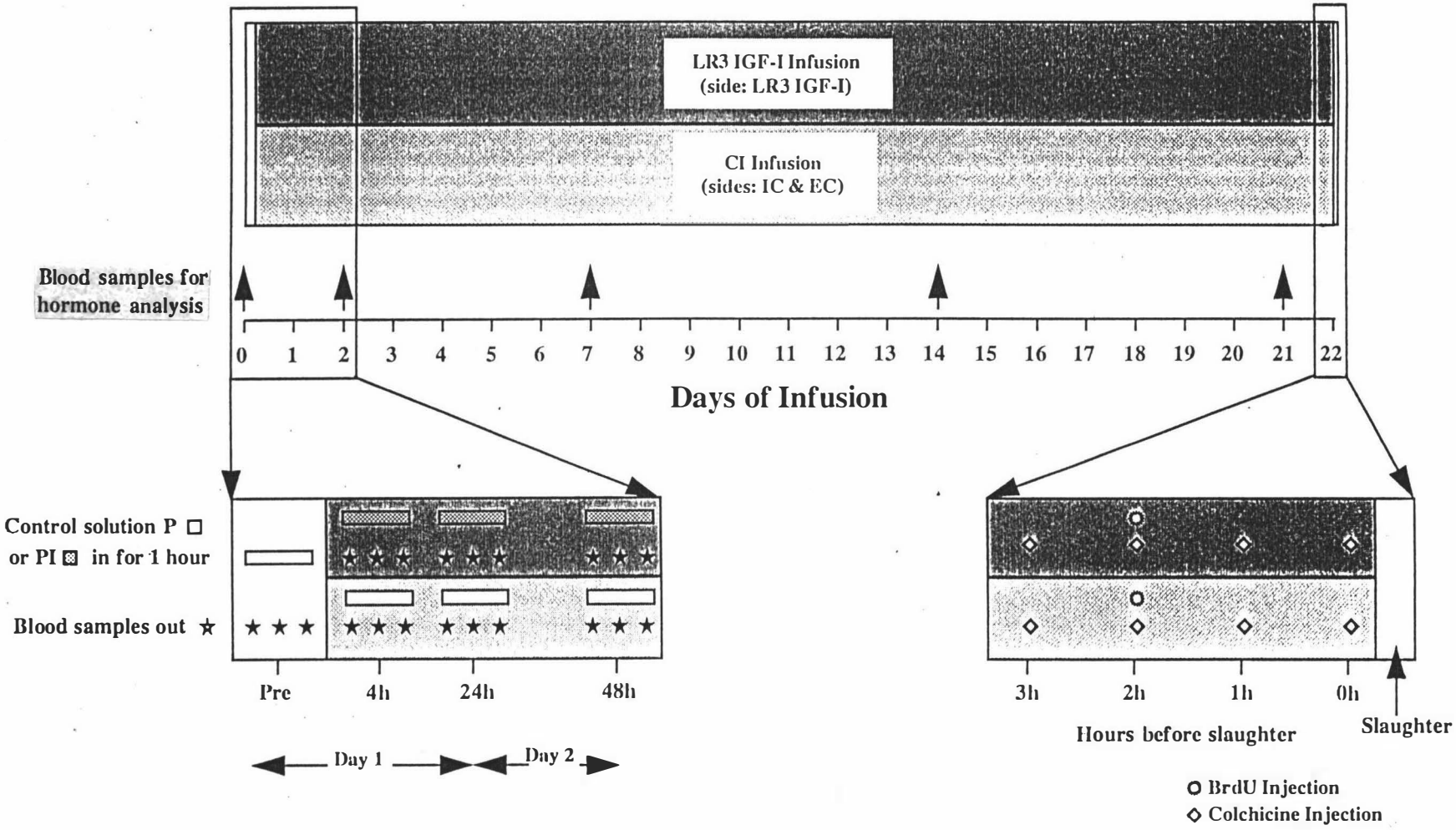

Fig. 2.1 The protocol of daily experimental events

Four sites on the skin patch of the sheep were marked. Three sites were injected intradermally with 0.1 ml colchicine (500 µg/ml; Sigma Chemical Co, St. Louis, Mo 63178, USA) in saline at 3, 2 and 1 h before slaughter. The fourth site was injected with 0.1 ml 5-Bromo 2'-deoxyuridine (BrdU; 3 mg/ml; Sigma Chemical Co, St. Louis, Mo 63178, USA; Lot No. 119F0959) 2 h before slaughter. Sheep were anaesthetised with sodium pentobarbitone (Pentobarb 500, South Island Chemicals Limited, Christchurch) 1 h after the last colchicine injection, and a skin biopsy was removed from the infused patches as well as biopsy samples from each site of the colchicine and BrdU injections. Biopsies were taken with a 1 cm diameter trephine and the samples were directly placed into Bouin's fluid fixative where they remained for 6-7 h before being transferred to 70% ethanol. Each sheep was then killed with an overdose of barbiturate through the jugular catheter.

2.2 Pre-Infusion Measurements

A control solution (***solution P***), was infused into catheter ***A1*** of ***C*** sheep and into ***LA1*** and ***RA1*** of ***LR3IGF-I*** sheep at 0.36 g/min. After 10 min of infusion of solution ***P***, blood samples were taken continuously by peristaltic pump at a rate of 0.36 g/min over each sampling period from catheters ***V*** and ***A2*** of the ***EC*** patch and ***LV, RV*** and ***A2*** of ***IC*** and ***LR3IGF-I*** patches, via PVC tubing and collected into plastic tubes on ice containing 0.1 ml heparinized (1000IU/ml) saline. Blood samples were taken simultaneously from catheters ***V, LV, RV*** and ***A2*** over three consecutive 20 min periods for measurements of blood flow and glucose uptake by the skin. During each 20-min collection period, the collection lines were removed briefly (about 15 sec.) from the collection tubes and samples of arterial and venous blood were collected into 1 ml eppendorf vials on ice containing 10 µl heparinized saline and overlaid with about 0.5 ml liquid paraffin. These samples were used immediately for measurement of blood oxygen in each arteriovenous pair.

Immediately on completion of each 20-min sampling period, the main blood samples were subsampled for immediate measurement of PAH content and a further subsample was taken to provide plasma for subsequent glucose analysis. Plasma for endogenous IGF-I, prolactin and insulin assays was obtained from blood centrifuged at 4000 RPM at 4 °C for 20 min. All blood and plasma samples were stored at -85 °C until analysis was undertaken.

2.3 LR3IGF-I Infusion and Measurements

IGF-I stock ***solution (S)*** consisting of 100 mg LR3-IGF-I, 10 g bovine serum albumin (BSA; Fraction V, United States Biochemical Corporation, Cleveland,. Ohio 44128; Lot No. 42989) and 1L of sterile 100 mM sodium acetate was made up in a sterile siliconized bottle (Appendix 2). Each LR3IGF-I infusate (***I***) was then made by transferring 50 ml of solution ***S*** through a sterile 0.2 μm Millipore filter (Millipore Corporation, Bedford, MA, USA) into 1L bags of sterile saline. A second solution (***PI***) was then prepared in a sterile siliconized bottle containing 1.9 g solution ***S*** and 98.1 g solution *P* for measurement of blood flow and metabolite uptake while continuing infusion of LR3IGF-I at a constant rate. A control stock ***solution (C)*** containing 10 g BSA and 1L of sterile 100 mM sodium acetate was prepared in a sterile siliconized bottle. The control infusate (***CI***) for the ***IC*** and ***EC*** patches was then made by transferring 50 ml of solution ***C*** through a sterile 0.2 μm Millipore filter into 1L bags of sterile saline.

On completion of the period of pre-infusion measurements, a continuous infusion of solution ***I*** at 0.13 g/min (about 950 μg LR3IGF-I/sheep/day) was started into the LR3IGF-I treated side, via either catheter ***LA1*** or ***RA1*** of the LR3IGF-I sheep. At the same time, continuous infusions of solution ***CI*** were started into the arterial catheter of the IC patch of the LR3IGF-I sheep and the patent ***A1*** catheter of the EC patch, at 0.13 g/min. These infusions continued for the next 21 days, interrupted only for measurements of blood flow and metabolite uptake. After four hours of infusion, solution ***I*** was changed to solution ***PI*** and solution ***CI*** changed to solution ***P***, both flowing at 0.35 g/min and three, 20 minute, incremental blood samples were taken simultaneously from catheters ***LV, RV*** and ***A2*** as described for the pre-infusion measurements. On completion of the measurements. infusates were changed back to solutions ***I*** and ***CI*** at 0.13 g/min. This sequence was repeated at 24 hours and 48 hours after the start of the LR3IGF-I infusion. Collection of samples for measurement of blood oxygen, and blood sample handling, were as for the pre-infusion measurements.

2.4 Analytical Methods

2.4.1 Blood

Blood oxygen content was measured in triplicate on 5 μl blood with a galvanic oxygen cell (Grubb & Mills, 1981) using 5 μl air samples to calibrate the system.

Blood samples for determination of PAH were precipitated in 1:2 ratio with 10% trichloroacetic acid (TCA; BDH Laboratory Supplies, Poole, BH15 1TD, England) and

then centrifuged for 10 min. The supernatant was mixed with an equal quantity of a 1% solution of 4-dimethylaminobenzaldehyde (Peking Reagent, Peking Chemical Works, Peking, China) in ethanol (99.7-100% v/v; Alcol ethilico (BDH) AnalaR, Pro. 10107, Lot No. L229102), and the developed colour was read against standard samples at 470 nm (Cecil Instruments).

Samples for the determination of glucose were mixed in a 1:2 ratio with 0.6 M perchloric acid ($HClO_4$), centrifuged, and the supernatant was neutralized with 3 M KOH. Glucose was determined enzymatically with glucose oxidase, together with *o*-dianisidine as described previously by Harris *et al.* (1989) (Sigma Diagnostic Kit 510, Sigma Chemicals, U.S.A.). The developed colour was read at 450 nm (GBC UV/VIS 918).

2.4.2 Plasma

2.4.2.1 IGF-I Assay

The concentrations of endogenous IGF-I in plasma were measured as described by Prosser *et al.* (1990) using a specific radioimmunoassay (RIA; Furlanetto *et al.*, 1977) except that the plasma was extracted according to the methods of Bruce *et al.* (1991). The antiserum to IGF-I was supplied by Drs L.E. Underwood and J.J. Van Wyk of the University of North Carolina, distributed by the National Hormone and Pituitary Program, National Institutes of Health, Bethesda, MD, U.S.A. Intra- and inter-assay coefficients of variation for IGF-I RIA were 4.7% and 10.7%, respectively.

2.4.2.2 Prolactin Assay

Concentrations of prolactin in plasma were measured by homologous double-antibody RIA based on the method of Van Landeghem and Van de Weil (1978). The protocol utilised was derived from Kirkwood *et al.* (1984) as described by Peterson *et al.* (1994). The first antibody, NIADDK-anti-oPRL-1 (AFP-973269) (rabbit-anti-oPRL) (donated by the National Hormone and Pituitary Program, University of Maryland School of Medicine, Baltimore, Maryland, U.S.A.) was used at a working dilution of 1:50,000. The second antibody, donkey anti- rabbit serum (IDS, Washinton, Tyne and Wear, England, Code APPT 1, Lot 11656), was used at a working dilution of 1:40. Intra- and inter- assay coefficients of variation for prolactin RIA were 9.6 and 15.7% respectively.

2.4.2.3 Insulin Assay

The concentration of insulin was measured by a heterologous double-antibody RIA developed by Flux *et al.* (1984) based on the method of Hales & Randle (1963). Bovine insulin (Catalogue No. 1 -1550, Lot No. 55F- 0536, 23.4 IU/mg, Sigma Chemical Company) was used for iodination and as the reference standard. Intra- and inter- assay coefficients of variation for insulin RIA were 8.2 and 12.4% respectively.

2.5 Histological Techniques

2.5.1 Tissues Processing and Microtomy

All samples were processed for paraffin wax embedding. Tissues were dehydrated, cleared in chloroform and two changes of xylene, and infiltrated with paraffin wax at 56 °C according to a standard schedule on an automatic tissue processer (Shandon Elloit, London), as shown in Appendix 3.1.

The samples were embedded on a Tissue-Tek embedding console (Tissue-Tek, Miles Scientific) and then sectioned on a rotary microtome. The sections were floated into a water bath (46 °C), attached to microscope slides and oven-dried (60 °C) overnight.

Tissues from colchicine and BrdU injection sites were sectioned at 5 μm longitudinal to the plane of the follicle. Every 7th section was retained to ensure the same bulb was not counted twice.

2.5.2 Bulb Cell Kinetics

2.5.2.1 Metaphase Arrest

Sections were stained with Haematoxylin and Eosin as described in Appendix 3.2. Under 10X magnification, a minimum of 200 bulbs/sample were randomly selected (sectioned through the base of the bulb and close to the midline) as described by Schinckel (1961).

The frequency of follicles having 0, 1, 2, 3, 4, ...n mitotic nuclei per bulb section was determined, and the median number of mitotic nuclei/bulb was estimated for each treatment since the frequency distribution of cell counts was not always normal.

2.5.2.2 Immunocytochemistry Techniques (ICC)

BrdU was detected after deparaffinisation and rehydration by employing ICC as described in Appendix 3.3. The sections were counterstained with Eosin as described in Appendix 3.4. The follicles were localized under 40X magnification, and the number of cells

labelled with BrdU was counted in a minimum of 100 bulbs/sample, sectioned through the base of the bulb close to the midline, by the Sigma-Scan measurement program (version 3.90; Glen Albinger 1988).

The diameter of the follicle bulb and the area of germinative tissue in the bulb were measured by the Sigma-Scan program in follicles sectioned through the midline, based on the presence of a full dermal papilla.

2.6 Wool Growth and Composition

The wool samples were placed in pre-weighed nylon bags (after conditioning overnight), washed in 0.2% Triton (a total of 15 min, shaking every 5 min), rinsed with reverse osmosis (RO) water (10 min. x4), to remove grease and suint respectively, and then oven-dried overnight at 60 °C. The samples were left overnight, and weighed, in a constant conditioning room (50% ± 3% relative humidity and temperature of 22 ± 0.5 °C)

Wool S, Zn and Cu content was measured by plasma emission spectrometry (Model 34000, Applied Research Laboratories, Sunland, L.A., CA), as described by Antram *et al.* (1991).

Wool production (mg/cm^2/day) in each skin patch was assessed by dividing the weight of the clean wool (mg) grown during 21 days by the traced area (cm^2) of the acetate sheet. The area was estimated from the weight of the acetate sheet calibrated to a known weight of a standard area of an acetate sheet.

Mineral output (S, Zn and Cu) was calculated from the product of wool growth rate and mineral concentrations of wool.

3 Calculations

3.1 Oxygen Uptake by Skin

$$\text{oxygen concentration (ml } O_2 \text{ /ml blood)} = \frac{\text{value measured}}{\text{mean air measurement}} \times 20.94$$

[Equation 2.1]

oxygen uptake (ml/min) =

[mean arterial concentration (A2) - mean venous concentration (V)] x blood flow

[Equation 2.2]

3.2 Blood Flow

Blood flow (ml blood/rnin) to the defined patch of skin was calculated by infusion of para aminohippuric acid (PAH) as:

$$\text{Blood flow} = \frac{\text{weight of PAH infused/min}}{[(\text{weight of PAH})/(\text{ml of blood at V})] - [(\text{weight of PAH})/(\text{ml of blood at A2})]}$$

[Equation 2.3]

4 Statistical Analysis

Data arising from single-time measurements on animals (wool growth rate, bulb diameter, bulb area, cell division rate, cell proliferation, the concentrations of zinc, sulfur and copper in the wool and the mineral output) were subjected to Student's t-test to test the effects of LR3IGF-I treatment after 21 days. The blood flow and oxygen uptake data were subjected to two-way analysis of variance for the variation between LR3IGF-I and internal and external control sides. Two-way analysis of variance statistics were used to test the effect of LR3IGF-I or CI infusion over time on the plasma concentrations of endogenous IGF-I, insulin and prolactin and the animal by time interaction (SAS, 1985). Differences between means were examined using Student's t-test. The data are presented as least square means±SEM. Results were considered significantly different when $P<0.05$.

RESULTS

Voluntary food intake of both LR3IGF-I and C groups of sheep declined immediately after surgery but recovered to 1000 to 1100g/day of lucerne chaff before the start of LR3IGF-I treatment (Fig. 2.2). There was a trend for voluntary food intake of the LR3IGF-I treated group to decline about 5-7 days after the start of LR3IGF-I treatment (C sheep were paired to the intakes of the LR3IGF-I sheep) but in most cases there was a recovery back to intakes of 900-1000g/day chaff by 10 days of treatment. This level of intake is approximately 1.2 X maintenance in sheep weighing 30-35kg.

There was no difference in mean plasma endogenous IGF-I concentrations between control and LR3IGF-I sheep (83.8±6.7 vs 79.0±6.5 ng/ml) over the whole period of 21 days of infusion with LR3IGF-I or CI. However, there was a significant change in plasma IGF-I concentrations with time in LR3IGF-I sheep ($P<0.05$). Plasma endogenous IGF-I levels were similar in the IGF-I and control sheep prior to treatment. (Fig. 2.3a) However, by day 7 of long-R3IGF-I infusion, the endogenous IGF-I levels had significantly decreased compared with the control sheep ($P<0.05$). Plasma endogenous IGF-I levels increased significantly by day 21 of treatment in LR3IGF-I infused sheep compared with both the control sheep ($P=0.02$) and their own levels earlier on infusion (day 7) ($P=0.0001$).

Mean plasma insulin concentration was not significantly different between the control and LR3IGF-I sheep over a period of 21 days of LR3IGF-I or CI infusion (429.5±64.7, 382.8±64.7 pg/ml). There was a significant ($P<0.05$) interaction between treatments and time (Fig. 2.3b). Plasma insulin levels on day 21 of treatment were significantly higher than these pre-treatment or on day 2 and day 7 of treatment in LR3IGF-I sheep ($P<0.05$). By day 14 of treatment, plasma insulin levels of the control sheep were significantly lower than pre-treatment levels ($P<0.05$).

As shown in Fig.2.4 there was no significant difference in mean plasma prolactin levels between the control and LR3IGF-I sheep (210.0±20.4, 218.0±20.4 ng/ml) over the infusion period. There was no interaction between treatment and time in plasma prolactin levels, but there were significant differences in plasma prolactin concentrations between times within treatment ($P=0.047$). Plasma prolactin levels on day 21 of treatment were significantly higher than pre-treatment and on day 2 of treatment in LR3IGF-I sheep ($P<0.05$). Control sheep had significantly lower plasma prolactin levels on day 7 compared with day 21 of treatment ($P<0.05$).

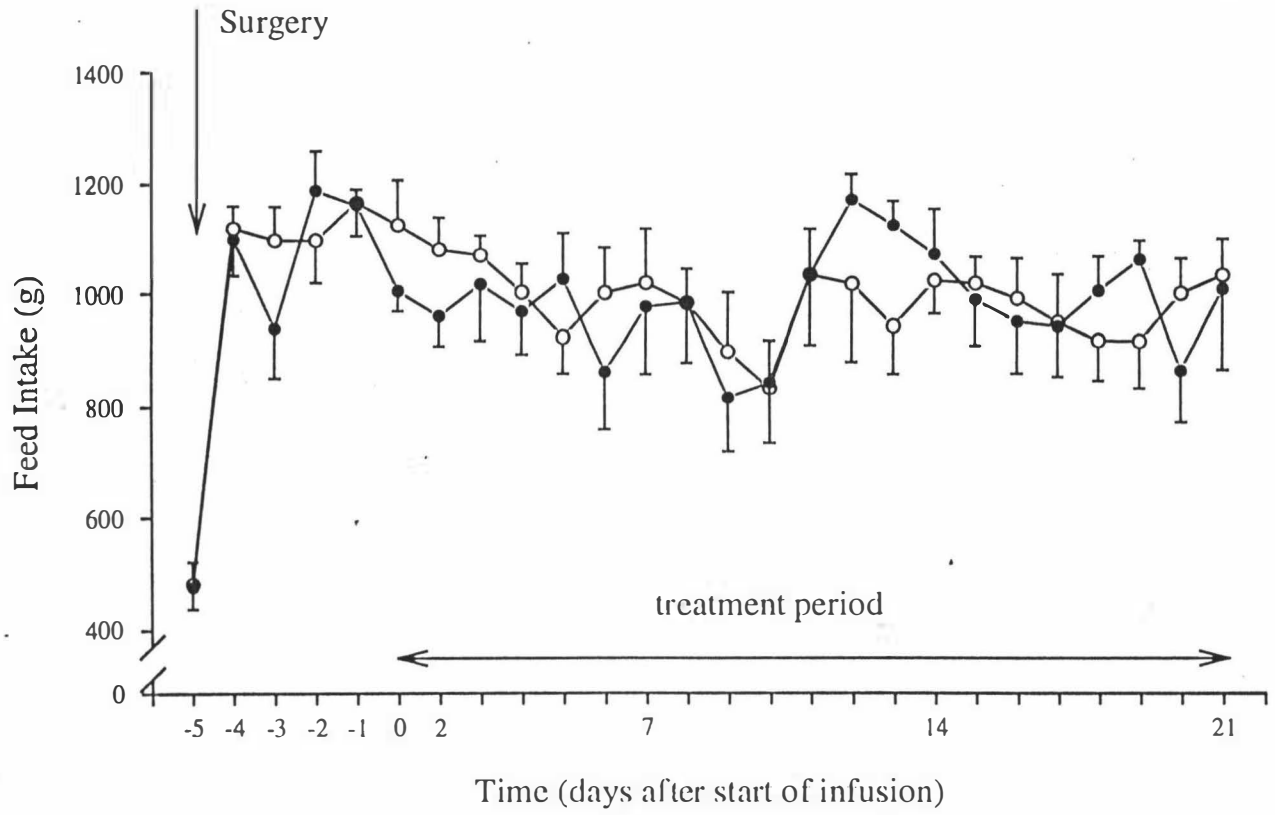

Fig. 2.2 Daily feed intake of Romney sheep infused with LR3IGF-I (•) or control infusate (o) for 21 days. LR3IGF-I sheep were allowed *ad libitum* access to mixed lucerne chaff and pellets, while control sheep were pair-fed to the same diet at two intakes of LR3IGF-I sheep.

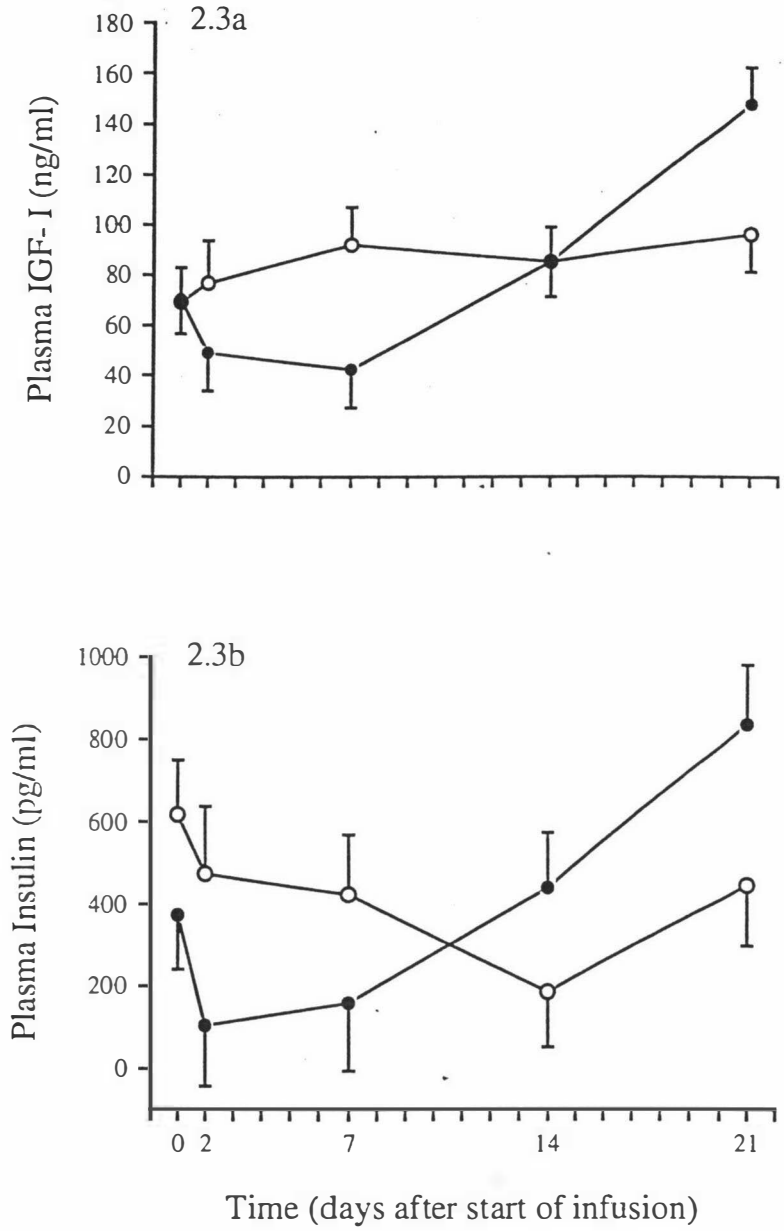

Fig. 2.3 Mean arterial plasma IGF- I (2.3a) and insulin (2.3b) concentration in Romney sheep locally infused with LR3IGF-I (•) or control infusate (o) for 21 days.

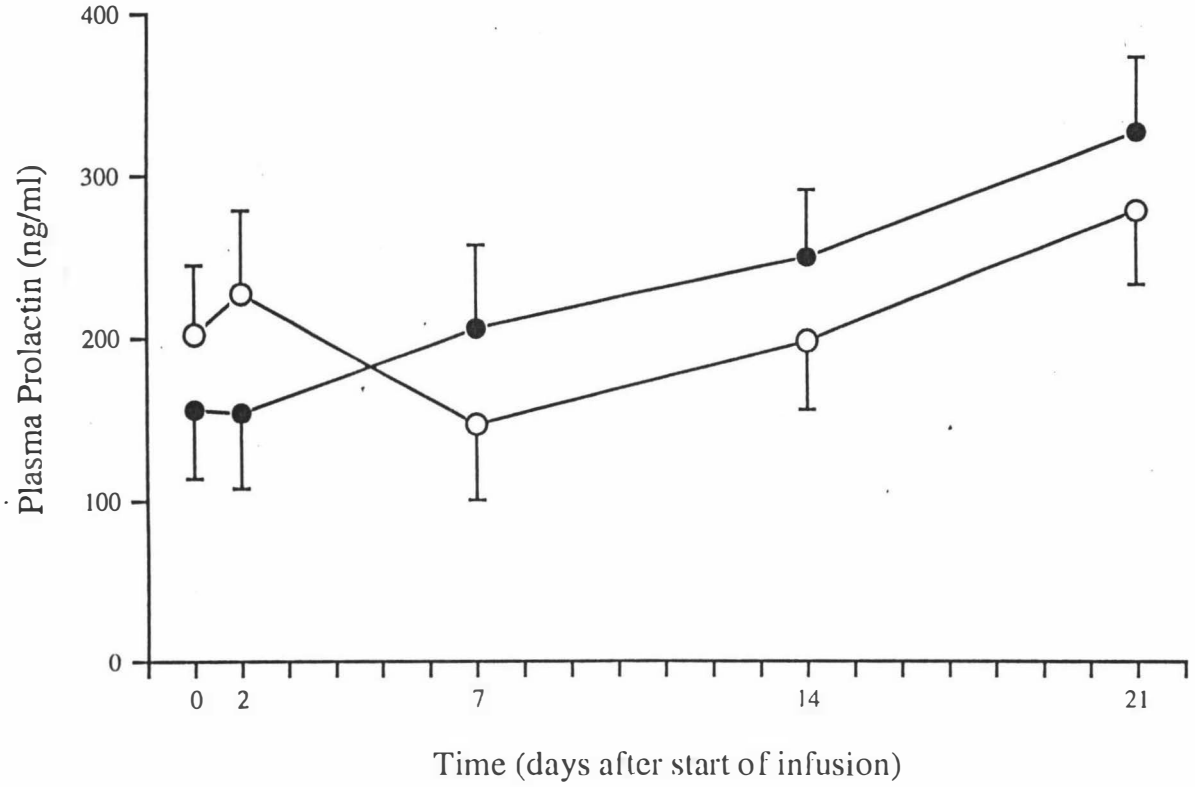

Fig. 2.4 Mean arterial plasma prolactin concentration in Romney sheep locally infused with LR3IGF-I (●) or control infusate (○) for 21 days.

Measurements of the rate of blood flow (g/min) through the skin patches and the oxygen uptake by the skin in the LR3IGF-I or CI infused control (IC and EC) sides prior to the start of infusion, and 4, 24 and 48 hours after the start of treatment are shown in Table 2.1.

Measurements were not able to be made when the tip of the catheter was blocked. Therefore, missing values were estimated in the analysis of the data. Comparisons of the rate of blood flow between the CI infused control sides (IC and EC), as well as, between the LR3IGF-I infused side and IC control side were made within each time (pre, 4, 24 and 48 hours) and presented in Fig. 2.5 and Table 2.1. The analysis of variance revealed that there were no significant differences between the two control sides. Blood flow was significantly greater at 24 hours ($P<0.05$) after the start of the LR3IGF-I infusion than immediately prior to the infusion starting (Fig.2.6; Table 2.1). There was a trend for blood flow to the LR3IGF-I treated side to be greater overall than that of the CI infused side (IC side).

There were no significant differences in oxygen uptake between the two control sides (IC and EC), nor between time effects or treatment X time interactions, but the comparison between the LR3IGF-I infused side and IC side showed significant treatment effects (Table 2.1). Moreover, there was no significant time effects or treatment X time interactions. However, after 24 hours of LR3IGF-I infusion there was a reduction in level of oxygen uptake. For the LR3IGF-I treated side, oxygen uptake increased again after 48 hours of the LR3IGF-I infusion, while the lower level of oxygen uptake was still evident in control sides (IC and EC). Moreover, the EC side shows a significant decline in oxygen uptake at 48 hours after CI infusion compared with 4 and 24 hours after the treatment.

Table. 2.1. Blood flow and net uptake of oxygen by the skin patches of sheep infused either with LR3IGF-1 (LR3IGF-1 side) or with control infusate (internal control side, IC; and external control side, EC) for 21 days (mean±SEM).

	LR3IGF-I infused side				Internal control side (IC)				External control side (EC)				Significance of effect (P)		
Time	pre	4 h	24 h	48 h*	pre	4 h	24 h	48 h	pre	4 h	24 h	48 h	Treat	Time	Treat x Time
Blood flow (g/min)	6.0^{a}	12.5^{ab}	17.3^{b}	14.4^{b}	5.6	6.9	7.3	10.6	9.1	14.9	15.0	10.1	NS	NS	NS
Oxygen uptake (ml/min)	0.27	0.41	0.13	0.35	0.25	0.27	0.14	0.15	0.26^{ab}	0.36^{a}	0.32^{a}	0.19^{b}	NS	NS	NS

* pre, prior to and 4 h, 24 h and 48 h after the start of infusion with LR3IGF-I or control infusate (CI).

ab Means in the same row within a treatment without a common superscript letter differ significantly ($P<0.05$).

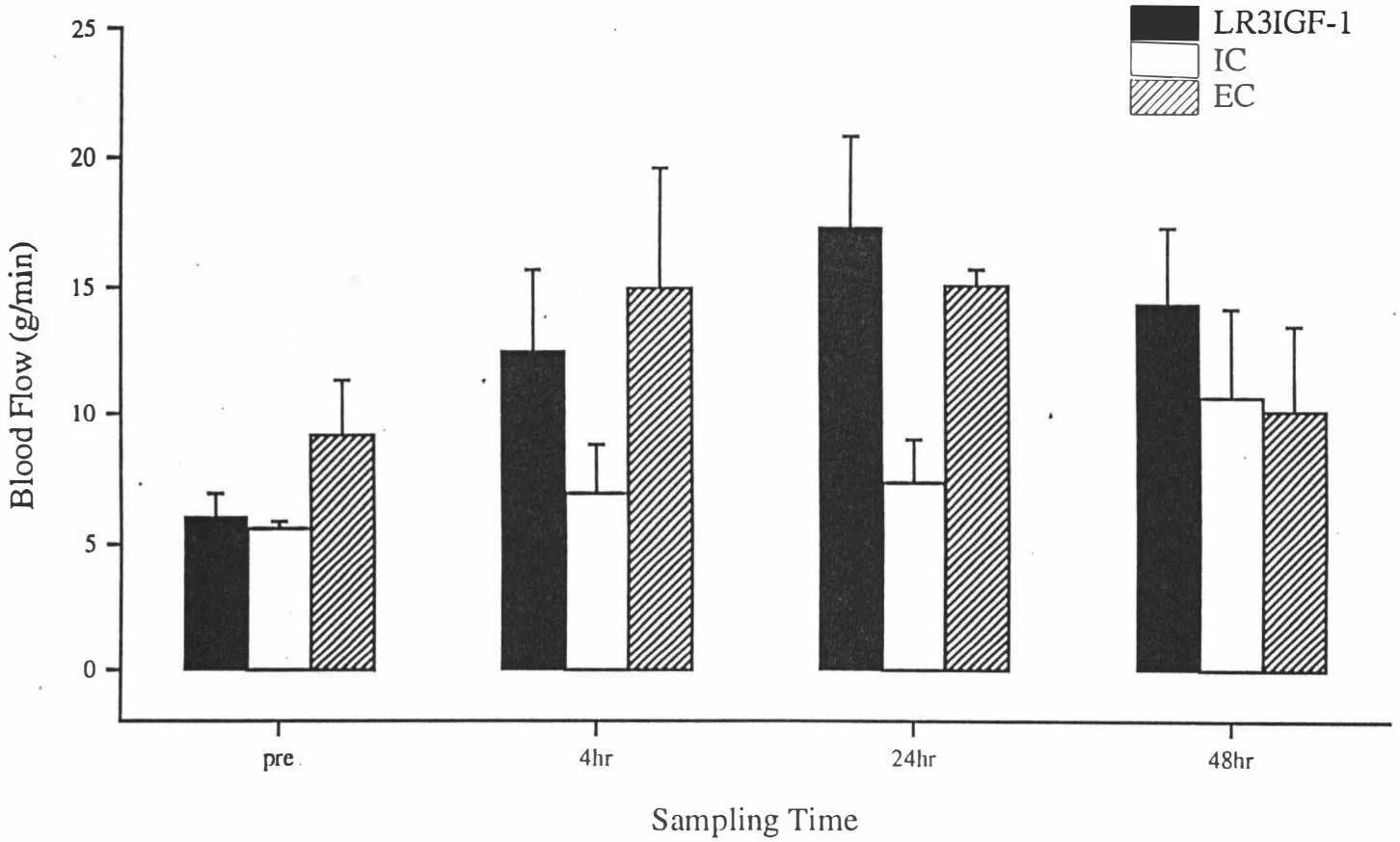

Fig. 2.5 Differences in blood flow between the LR3IGF-I infused side and the control infusate control sides (IC and EC) during pre-infusion and 4, 24 and 48 hours after the infusion.

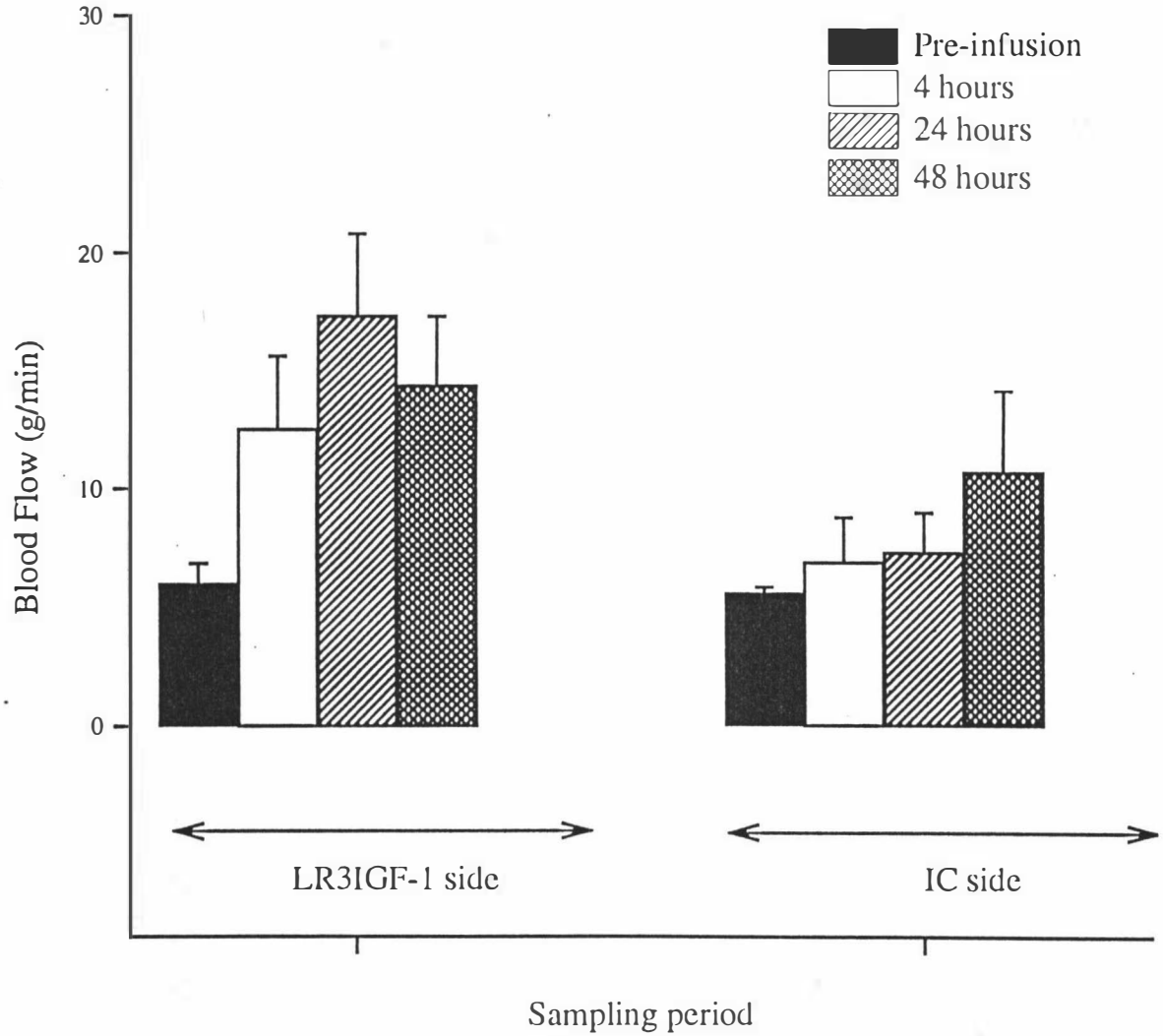

Fig 2.6 Differences in blood flow during pre-infusion and 4, 24 and 48 hours after the LR3IGF-I infusion or control infusate into LR3IGF-I side and the internal control side (IC).

The effect of infusion of LR3IGF-I or CI on wool production, bulb diameter and bulb area, cell division rate, cell replication rate, number of labelled cells and the output and production of S, Zn and Cu in wool are given in Table 2.2. Although wool production was decreased to a small extent by infusion of LR3IGF-I, there were no significant differences between the treated side (LR3IGF-I) and internal or external control sides.

The mean diameter of follicle bulbs of the LR3IGF-I, IC and EC sides, measured in those bulbs exhibiting a dermal papilla in cross section, was 119±5.9 124±10.6 and 123±3.4 μm, respectively. The analysis showed a trend for the LR3IGF-I treated side to have smaller bulb diameter, but the value did not reach significance. Also there was no significant difference in the bulb area between the three sides (P=0.06). Similarly, the LR3IGF-I treated side tended to have a smaller germinative bulb area compared with the two control sides (LR3IGF-I side, 8831.3±779.6 μm^2; IC side, 9621.5±704.5 μm^2; EC side, 9573.0±205.6 μm^2).

Cell replication in the bulb of wool follicles from skin sampled from the LR3IGF-I treated side, IC and EC control sides was assessed by labelling with BrdU. There was no effect of LR3IGF-I treatment on the cell replication rate, although, there was a trend for the LR3IGF-I treated side to have a lower number of labelled cells.

The accumulation of cells undergoing metaphase was linear (r=0.95) with time after colchicine injection (Fig. 2.7). Analysis of variance indicated that there was a significant difference between the IC and EC control sides in cell division rate (P<0.05), but neither was significantly different from the LR3IGF-1 infused side (Table 2.2).

LR3IGF-I infusion into the skin of sheep had no significant effects on the concentration or output of S, Zn and Cu in the wool (Table 2.2; P>0.05), although the output of S, Zn and Cu in the wool of LR3IGF-I sheep was reduced compared to that in wool from the C sheep (a reflection of the level wool growth rates in sheep infused with LR3IGF-I).

Table 2. 2 . Wool-related charechterastics in the LR3IGF-1 infused side (LR3IGF-1) and control sides (internal control, IC; and external control, EC) after 21 days of infusion of LR3IGF-1 or control infusate. (Mean±SEM)

Item	*LR3IGF-1 side*	*Internal control (IC) side*	*External control (EC) side*
Wool production (mg/cm^2 /d)	0.8±0.10	1.0±0.31	1.1±0.22
Bulb diameter (μm)	119.2±5.89	123.5±10.58	123±3.39
Bulb area (μm^2)	8831.3±779.6	9621.5±704.5	9573.0±205.6
CDR* (No. mitotics/bulb/h/5μm section)	2.2±0.21^{ab}	1.9±0.15^{b}	2.6±0.31^{a}
Cell rep. (No. of cells in S-phase/μm section)	0.0047±0.0003	0.0039±0.0003	0.0047±0.0003
No. of BrdU-labelled cells	40.6±2.44	42.82±3.37	48.02±
The output of:			
S (μg/cm^2/d)	24.0±0.34	26.0±3.7	29.0±3.7
Zn (ng/cm^2/d)	70.0±8.9	74.0±9.8	82.0±9.8
Cu (ng/cm^2/d)	3.2±0.56	3.6±0.62	3.8±0.62
The concentration of:			
S (mg/g wool)	29.7±0.74	28.9±0.66	29.8±0.26
Zn (μg/g wool)	85.8±1.45	84.0±2.30	88.4±2.92
Cu (μg/g wool)	3.8±0.31	4.0±0.00	3.6±0.25

^{ab} Means in the same row without a common superscript letter differ significantly (P<0.05).

*CDR= cell division rate

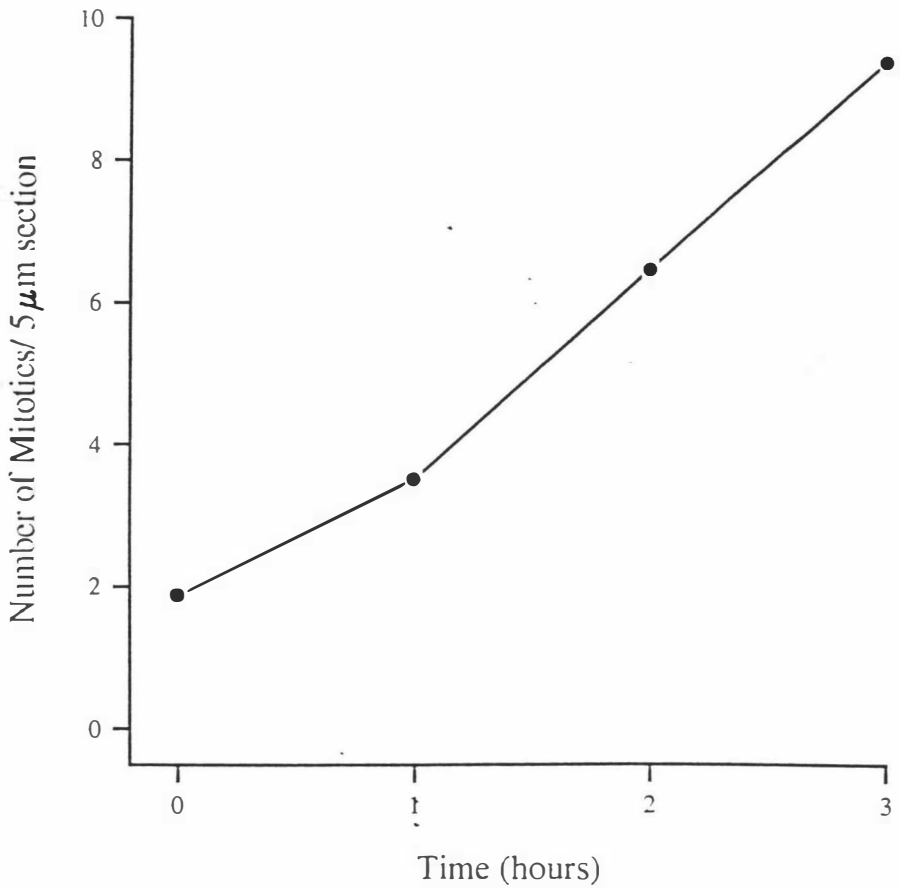

Fig. 2.7 The mean number of cells arrested in metaphase in the follicle bulb of Romney sheep at time 0, 1, 2 and 3 hours after the colchicine injection.

DISCUSSION

Insulin-like growth factor-I shares a high degree of structural and functional homology with insulin (Humbel, 1990) and, *in vitro*, is a potent mitogene for a large number of tissues and cell types (Daughaday *et al.*, 1989; Daughaday, 1990). However, the role of IGF-I in the regulation of wool or hair follicle growth and development is not known. Therefore, this current study was designed to measure the effects of direct LR3IGF-I infusion into the skin of the abdominal flank of 6 sheep for 21 days. Measurements were made of changes in wool growth per unit area of skin, and of changes in the germinative cell production in the follicle bulb. This current study was a part of a larger study, the results of which were outlined by Hocking Edwards *et al.* (1995) and Lee *et al.* (1995).

1 Whole-Body Effects

A number of studies in sheep *in vivo* have shown that recombinant IGF-I has no effect on feed intake, either when infused directly into the ventricle for 6 days (Foster *et al.*, 1991) or when delivered by subcutaneous injection for 56 days (Cottam *et al.*, 1992). The present study showed that although there are no significant variation in feed intake, compared with intakes at the start of infusion there was a non-significant decline in voluntary feed intake during the first week of the LR3IGF-I infusion. Feed intake recovered to pre-infusion intake after 6-8 days of treatment. Walton *et al.* (1994) studied the effects of infusion of IGF-I and the N-terminally modified IGF-I variants, R3IGF-I and LR3IGF-I, for 90 hours on feed intake of pigs. They found that native IGF-I had no effect on feed intake, but that both R3IGF-I and LR3IGF-I variants resulted in a 24% decrease in voluntary feed intake. Compared with the trend in the present sheep study, there was a higher and faster response to LR3IGF-I in the pig intakes. This may reflect either differences in species sensitivity, or differences in dose rate, because the pigs received 7μg/kg/hour compared with the 1.2μg/kg/hour used in the present sheep study.

The chronic infusion of LR3IGF-I (for 21 days) did not produce any significant changes in the overall mean plasma concentration of endogenous IGF-I of treated sheep compared with CI-infused control sheep. However, there was a reduction in endogenous IGF-I (and insulin) concentration during the first 7 days of the LR3IGF-I infusion. This was not related to the decline in voluntary feed intake because the control sheep were pair-fed to the intakes of the LR3IGF-I treated sheep. The decline in plasma IGF-I concentration of the treated sheep was comparable but, as with feed intakes, smaller than that observed in the pigs treated with LR3IGF-I for 90 hours (Walton *et al.*, 1994). This was probably due to suppression of endogenous IGF-I production resulting from the increase in total free IGF-I (LR3IGF-I + endogenous) which may have acted as a feedback mechanism to limit the

synthesis of IGF-I by liver and other tissues. After 14 days, plasma endogenous IGF-I concentrations had increased in the LR3IGF-I infused sheep to reach a significant difference at 21 days compared with day 7 and with CI infused control sheep. This increase could be expected to be associated with induction of IGFBPs, especially IGFBP-3. It has been demonstrated that LR3IGF-I binds weakly to IGFBPs compared with IGF-I and even more weakly than other IGF-I variants such as des(1-3)IGF-I (Walton *et al.*, 1990; Francis *et al.*, 1991; Tomas *et al.*, 1993; Zhao *et al.*, 1995, B.W. McBride personal communcation). *In vivo,* Lord *et al.* (1994) showed that in sheep plasma, there was a significant association of LR3IGF-I with the 150 kDa complex which indicates the potential for IGFBP induction. Induction of IGFBPs has been documented previously for the rat and human (Zapf *et al.*, 1989; 1990) and in lactating goats treated with either IGF-I or des(1-3)IGF-I (Prosser *et al.*, 1995). Moreover, *in vitro*, McGrath *et al.* (1991) observed the induction of IGFBP-3 which is stimulated by des(1-3)IGF-I. In an extension of the present study, plasma samples from two of the LR3IGF-I treated sheep were analyzed using the ligand blotting technique (Hocking Edwards *et al.*, 1995). No changes were revealed in any of the IGFBPs, suggesting that induction of the IGFBPs was not responsible for the longer term increase in endogenous IGF-I. Therefore, this increase must be solely a consequence of an increase in IGF-I secretion. This may be related to the particular species used in the study. In rat, the infusion of LR3IGF-I for 14 days did not produce any changes in the plasma IGF-I concentration measured on the 14th day of treatment (Tomes *et al.*, 1993). However, in the rat trial, only a single sample was obtained and this might mean that any response to LR3IGF-I in plasma IGF-I concentration could have been missed, as the increase in plasma IGF-I in the present study was not greater than the control sheep values until after the 14th day of treatment.

In current study, the response of plasma insulin concentrations to LR3IGF-I infusion followed a similar trend to the endogenous IGF-I concentration. However, there was a significant interaction between treatment with time. During the first 24 hours of LR3IGF-I treatment there was, however, a proportionally greater and possibly more rapid decline in plasma insulin concentration than in endogenous IGF-I concentration. These linked changes in whole body plasma IGF-I and insulin concentrations in response to the LR3IGF-I treatment have been ascribed (Hocking Edwards *et al.*, 1995) to an acute effect of LR3IGF-I on insulin, with concomitant changes to endogenous IGF-I, with these changes in IGF-I itself over-riding those of the LR3IGF-I treatment in later periods.

2 Skin Effects

A particular focus of the present study was the local effect of LR3IGF-I infusion on skin blood flow and oxygen uptake.

The intention when originally designing the trial was to compare the IC and EC sides to test for whole body effects of the LR3IGF-I infusion, and then to compare within animal effects of the infusion. When making both the IC versus EC comparison and the IC versus LR3IGF-I comparison there were no significant effects found for treatment, time or the interaction of treatment X time (Table 2.1 and Fig. 2.5). The data is difficult to interpret from the ANOVA comparison, the EC side started with a higher blood flow prior to the LR3IGF-I infusion and generally showed a larger variance.

In addition, within only the LR3IGF-I treated side there were significant changes in blood flow with time (see Table 2.1 and Fig. 2.6). But these results must be treated with caution, from the combined data it can't be decided if the LR3IGF-I truly directly enhanced blood flow in the LR3IGF-I treated side or, alternatively suppressed blood flow in the IC side through some whole body effect.

If it is accepted there was an increase in local blood flow with LR3IGF-I treatment it is similar to the response in blood flow observed after 4 hours of LR3IGF-I infusion and reported by Harris *et al.* (1993). In the current study, the increase in blood flow was sustained for 48 hours of LR3IGF-I infusion. In lactating goats, short-term (2-6 hours) infusion of IGF-I into the pudic artery of the mammary gland resulted in an increase in mammary blood flow (Prosser *et al.*, 1990). Fleet *et al.* (1992) suggested that the mechanism of this effect may involve direct stimulation of the vascular system, as it is notable that type-I IGF-I receptors are present on bovine capillary cells (Bar and Boes, 1984).

Measured changes in uptake of oxygen and glucose by the skin in response to LR3IGF-I were initially (4hours) proportional to the changes in blood flow. However, blood flow to the skin was still enhanced after 24 hours of LR3IGF-I infusion, while the stimulation of oxygen and glucose uptake by skin was not sustained and returned to pre-treatment levels. In an extension of this study Hocking Edwards *et al.* (1995) found that the amount of lactate produced by anaerobic oxidation was still enhanced after 24 hours infusion, suggesting that the changes in glucose and oxygen uptake were not entirely synchronised and may be controlled independently.

The most important result of the present study was that, although blood flow and hence nutrient flux was enhanced, direct LR3IGF-I infusion into the skin had no significant effect on any morphological features or rate processes measured, including the amount of wool produced, wool follicle bulb diameter, bulb area, bulb cell proliferation or wool

composition (Table 2.2).

The present observations on cell proliferation in the follicle bulb were undertaken to examine the effect of infusion of LR3IGF-I on the mitotic rate of the matrix tissue of the wool follicle bulb. The proliferative activity of the follicle matrix cells was assessed from nuclear population counts in appropriate histological sections of colchicine-or BrdU-treated material. No correlation was observed between the estimates provided by the two techniques (r=0.13), probably because the two techniques are measuring different parameters: the colchicine technique arrested and measured bulb cell nuclei in metaphase, whereas the BrdU is incorporated and counted in nuclear DNA during S-phase. However, the results obtained from both techniques indicated that treatment with LR3IGF-I has no significant effect on cell production rate. This result supports, but does not add to, the previous results of Harris *et al.* (1993), who concluded that short-term treatment with LR3IGF-I resulted in no effect on cell replication rate in the wool follicle bulb.

Injection of colchicine arrested bulb cell nuclei in metaphase as expected (Plate 2.1), The increase in the number of arrested cells was linear with time (r=0.95 from 0 to 4 hours when data were pooled for three animals (Fig. 2.7) and r= 0.95, 0.97 and 0.98 for the three animals separately. However, there have been reports that bulbs exhibit asymmetrical mitotic activity associated with bilaterally segmented fibres (Fraser, 1964; Adelson *et al.*, 1991). Observations in the present experiment did not indicate any asymmetry in the accumulation of nuclei in metaphase when the colchicine technique was used (Plate 2.1), nor was there any asymmetry in cells labelled with BrdU (Plate 2.2), which is in agreement with observations by others (Williams and Winston, 1987; Hynd, 1989a; Holle, 1992; Hocking Edwards, 1993).

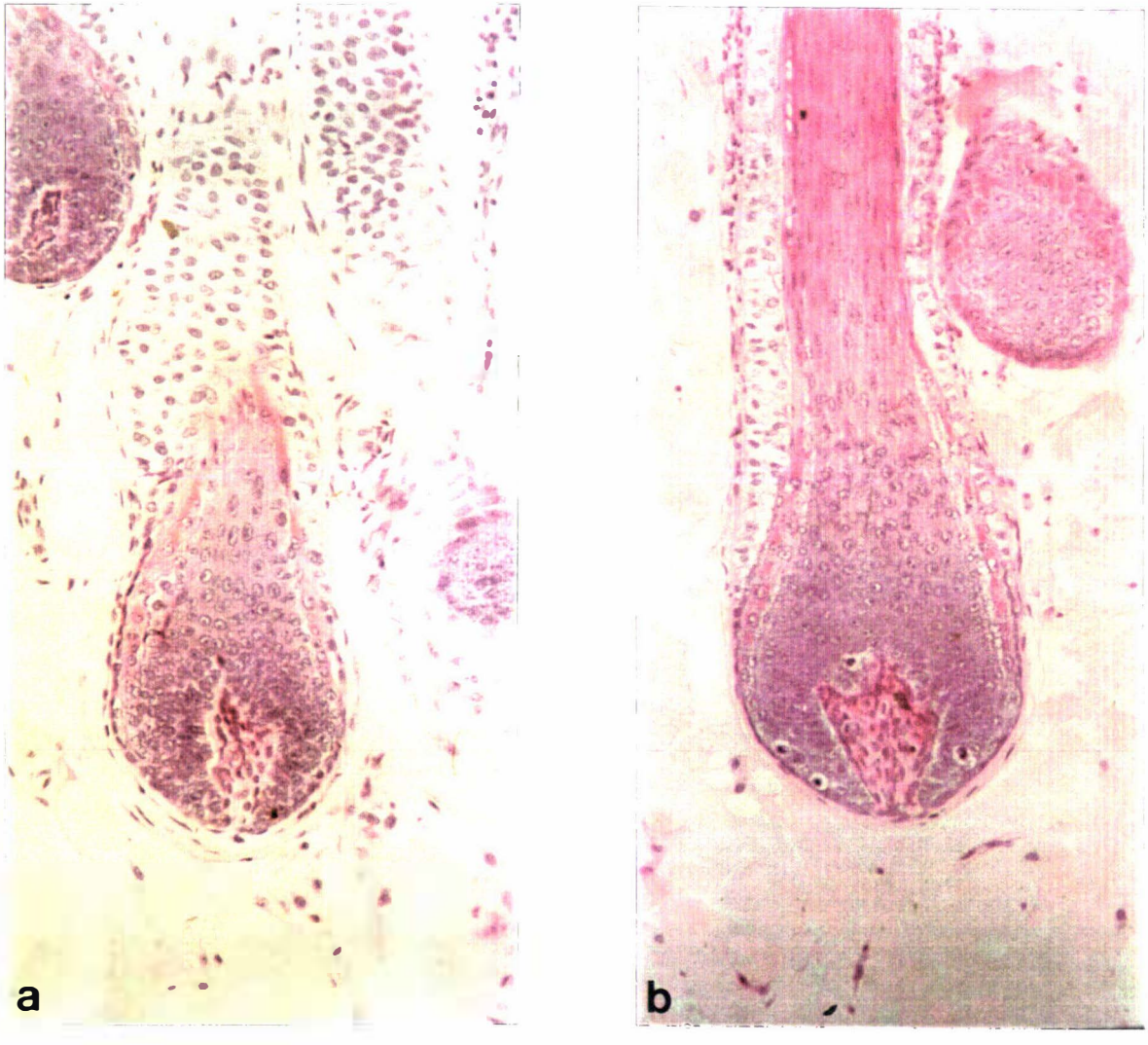

Plate 2.1 Longitudinal sections (5 µm thick) through the base of the wool follicle bulb and close to its midline, of skin sampled (a) 0 h and (b) 2 h after the colchicine injection. Counterstained with Haematoxyline and Eosin. The even distribution of darkly stained mitotic nuclei is apparent.

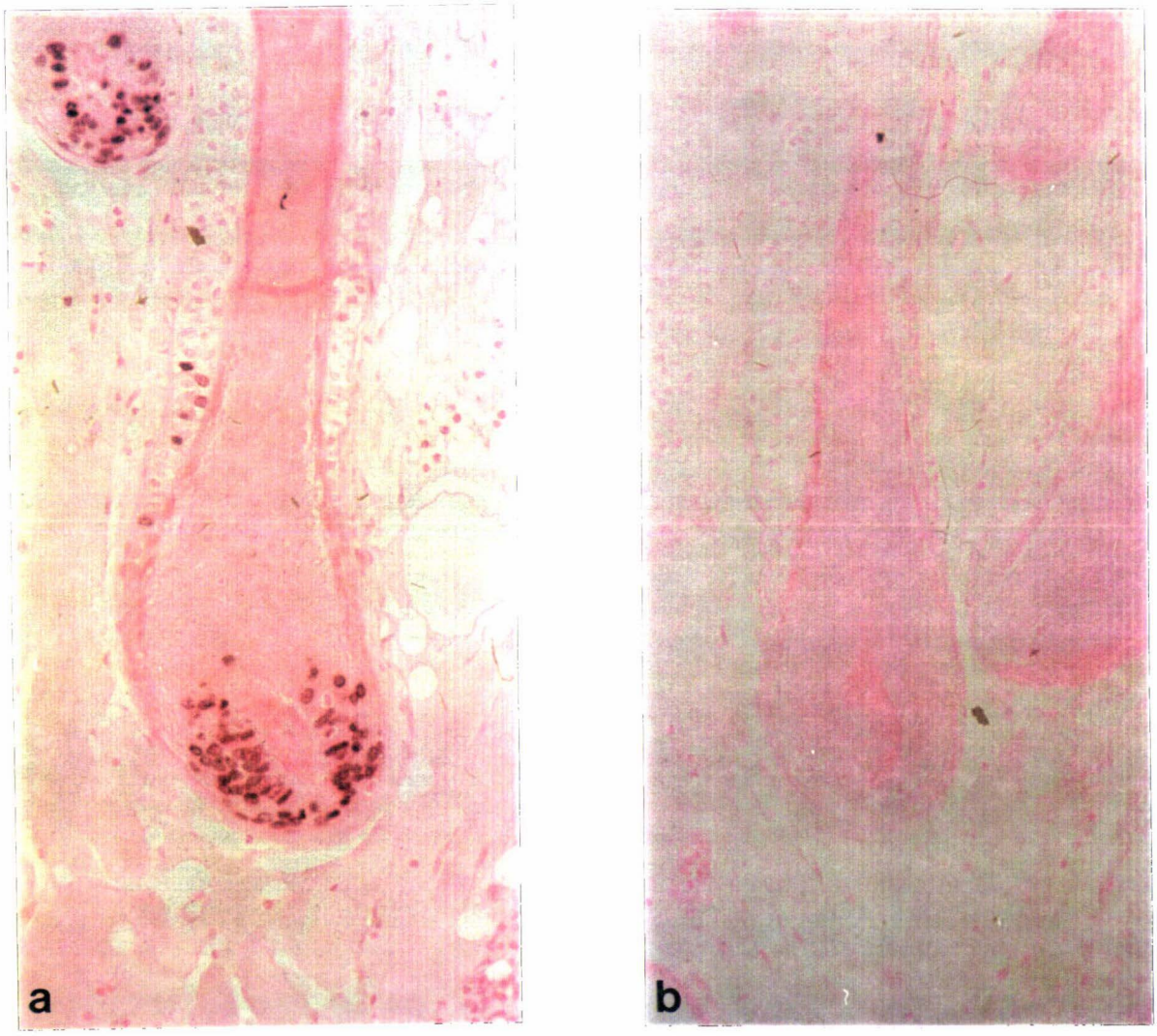

Plate 2.2 Longitudinal sections (5 μm thick) through the base of the wool follicle bulb and close to its midline, of skin sampled 2 h after the BrdU injection:
(a) Immunostained with the biotin-streptavidin method for BrdU incorporation into cellular DNA.
(b) Example of a negative control, omission of anti-BrdU antibody, showing absence of immunoreactivity.

Holle and Harris (1992) noted that the use of BrdU to label cells in the S-phase of replication in the replication zone within the bulb of wool follicles is an indicative measure of overall production. It has been shown that IGF-I and its variants (des(1-3)IGF-I and LR3IGF-I) stimulate DNA synthesis in bovine mammary tissue grown *in vitro* (Baumrucker *et al.*, 1989; McGrath *et al.*, 1991), as well as rat intestinal epithelium (Corps and Brown, 1987). Moreover, in normal rats and those with a gut disorder, IGF-I or LR3IGF-I have been found to stimulate intestinal growth by increasing the total number and the size of epithelial cells in the intestine (Read *et al.*, 1994). However, the present results showed no significant difference in numbers of BrdU-labelled wool follicle cells, if any thing, there was a decline in the total number of BrdU-labelled wool follicle cells (Table 2.2) in the LR3IGF-I treated side (P=0.056), despite the significant increase in plasma IGF-I levels during the last week of the trial when compared with samples taken prior to LR3IGF-I treatment and from the control sheep. This is in accord with the results of Cottam *et al.* (1992), who showed no effect on wool production after 56 days whole body administration of IGF-I. Both these results are still perhaps surprising as IGF-I receptors have been detected in the skin and in the epidermal components of wool follicles particularly the ORS and corticle matrix (Tavakkal *et al.*, 1992; Little *et al.*, 1994). However J.E. Hocking Edwards (Unpublished data), detected the presence of the IGF-I peptide only in the arrector pili muscle of the follicle. More work is needed in this field to clarify the location and function of IGF-I binding in the follicle.

As indicated in Table 2.2, LR3IGF-I treatment had no effect on either the output, or the concentrations of S, Zn and Cu in wool produced over 21 days. As part of the larger study, Lee *et al.*(1995) studied the effect of continuous infusion of LR3IGF-I for 21 days into the skin of 6 sheep (the same sheep were used in current study) on plasma concentrations of macro-elements (Ca, Mg, Na, P and S), and the trace elements (Cu, Fe, Mn and Zn). Their data showed no change in plasma concentrations of the macro-elements over the treatment period and there were no significant differences between the groups in levels of macro-elements in the plasma. However, at day 7 of LR3IGF-I treatment there was a significant increase in Cu concentrations and a decrease in Zn concentrations compared with both levels prior to LR3IGF-I treatment and in the control sheep. These differences continued over the remainder of the treatment period. Lee *et al.* (1995) concluded that it is unclear whether the continuous infusion of LR3IGF-I has a direct and independent effect on plasma Cu and Zn concentrations or whether the measured effects are a consequence of the resulting changes to endogenous plasma IGF-I and/or insulin, or to other unmeasured hormonal changes.

In conclusion, long-term infusion of LR3IGF-I caused a significant increase in skin

blood flow. It had marked endocrine effects, including suppression of plasma IGF-I and insulin concentrations, during the first 24 hours of LR3IGF-I infusion, followed by significant increased in plasma IGF-I and insulin at the end of treatment period.

This study confirmed previous findings that IGF-I had no effect on the amount of wool produced or cell replication rate in the wool follicle bulb. Therefore, it is unlikely that IGF-I regulate wool growth or has any direct effect on cell division rate in the wool follicle bulb.

APPENDIX

1 Control Solution (Solution P)

Conteins the following reagents made up in 0.9% NaCl (w/v) sterile saline:

1. [2,6-^{3}H] phenylalanine (NEN Research Products), 1mCi/L .
2. PAH, 250 mg/L .
3. Heparin, 100 000 IU/L.
4. Bovine serum albumin (BSA; fraction V, United States Biochemical orporation Cleveland. Ohio 44128, Lot No. 42989), 10 g/L
5. Sodium acetate (Analar), 100 mmol/L.

2 Siliconizing Glassware and Plasticware

Each container to be siliconised was filled with solution of 2%-5% Diclorodimethylisilane in $CHCO_3L$. Then the container was rinsed thoroughly with water and baked at 180 °C for 2 hours.

3 Histology

3.1 Tissue Processing

1. Dehydration	95% Ethyl Alcohol	1 hour
	100% Ethyl Alcohol	1 hour
	100% Ethyl Alcohol	3 hour
	100% Ethyl Alcohol	1 hour
	100% Ethyl Alcohol	2 hour
2. Clearing	Chloroform	1 hour
	Xylene	1 hour
	Xylene	1 hour
3. Impregnation	Paraffin Wax 56 °C MP	1 hour
	Paraffin Wax 56 °C MP	2 hour

3.2 Staining With Haematoxylin and Eosin Stain

This method stains nuclei purple-blue and the remaining tissues various shades of pink.

1. Xylene	5min
2. Xylene	5min
3. Absolute Alcohol	2min
4. 70% Alcoho	2 min
5. Tap water	2 min
6. Mayers Haemalum .	5 min
7. Running water	2 min
8. Scotts' tap water	2 min
9. Eosin 10 sec	
10. Quick rinse in water	
11. 70% Alcohol	2 sec
12. Absolute Alcohol	2 min
13. Absolute Alcohol	2 min
14. Xylene	2 min
15. Xylene	2 min
16. Mount with DePeX and cover slip.	

3.3 Immunocytochemistry (ICC)

1. Xylene	2 min
2. Xylene	2 min
3. Absolute Alcohol	2 min
4. 70% Alcohol	2 min
5. Tap water	2 min
6. 0.01M phosphate buffered saline (PBS)	5 min
7. 1% bovine serum albumin (BSA)[1] in PBS[2]	5 min
8. Mouse anti-BrdU antibody[3]	60 min
9. Rinse in PBS three time	1 min each
10. Biotinylated goat anti-mouse (IgG)[4]	30 min
11. Rinse in PBS three times	1 min each
12. Preformed Streptavidin biotinylated Peroxidase complex[5]	15 min
13. Rinse in PBS three times	1 min each
14. 0.005% 3,3'-diaminobenzidin solution (DAB)[6]	4 min[7]

1. BSA (Sigma Chemical Co. St. Louis, Mo 63178 USA).

2. Steps 7, 8, 10, and 12 were incubated at room temperature in a humidified chamber.

3. Mouse anti-BrdU antibody (RPN 202. Amersham Life Science) diluted 1:100 in 1% BSA in PBS.

4. IgG (PRN 1177. Amersham. Life Science) diluted 1:200 in 1% BSA in PBS.

5. Streptavidin biotinylated proxidase complex (RPN 1051. Amersham. Life Sceinse) diluted 1:200 in 1% BSA in PBS.

6. 5 mg DAB (97% F.W. 396.15. Aldrich Chemical Co., Inc).

10 ml PBS

12 µ l H_2O_2

1 drop of Cobalt- Nickel (1% Co+Ni solution).

7. Incubation period was determined by the presence of the black-brown stains at sites of BrdU incorporation in the tissue section.

3.4 Eosin Stain

This stain gives a pink background colour to the tissues

1. Rinse in PBS	
2. Rinse in tap water	
3. Eosin	20 sec
4. Quick rinse in tap water	
5. 70% Alcohol	2 sec
6. Absolute Alcohol	2 min
7. Absolute Alcohol	2 min
8. Xylene	2 min
9. Xylene	2 min
10. Mount with DePeX and cover slip.	

BIBLIOGRAPHY

Abbott, J. & Holtzer, H. (1968) The loss of phenotypic traits by differentiated cells. The effect of 5-bromodeoxyuridine on cloned chondrocytes. *Proceedings of the National Academy of Sciences,* USA. **59,** 1144-1151.

Abe, H., Molitch, M.E., Van Wyk, J.J. & Underwood, L.E. (1983) Human growth hormone and somatomedin C suppress the spontaneous release of growth hormone in unanesthetised rats. *Endocrinology.* **113**, 1319-1324.

Adelson, D.L., Munro, S.G. & Tunks, D.A. (1991) Perturbation of wool fibre cell differentiation by a mixture of bromodeoxyuridine and fluorodeoxyuridine. *Australian journal of agricultural research.* **42**, 1293-1300.

Antram, R.J., McCutcheon, S.N., Blair, H.T., Lee, J. & McClelland, L.A. (1991) Wool sulfur concentrations and output in fleeceweight-selected and control Romney rams. *Australian journal of agricultural research.* **42,** 269-277.

Auber, L. (1952) The anatomy of follicles producing wool-fibre, with special reference to keratinization. *Transcripts of the Royal Society of Edinburgh.* **62,** 191-254.

Ballard, F.J., Read, L.C., Francis, G.L., Bagley, C.J. & Wallace, J.C. (1986) Binding proteins and biological potencies of insulin-like growth factors in L6 myoblasts. *Biochemical journal.* **233**, 223-230.

Ballard, F.J., Walton, P.E., Dunshea, F.R., Francis, G.L. & Tomas, F.M. (1994) Does IGF-I ever act through the insulin receptor. in: *The insulin-like growth factors and their regulatory proteins. Proceedings of the Third International Symposium on Insulin-like Growth Factors,* pp. 131-139. Editors. R.C. Baxter, P.D. Gluckman & R.G. Rosenfeld. Sydney: Excerpta Medica.

Bang, O. & Hall, K. (1992) Insulin-like growth factors as endocrine and paracrine hormones. in: *The insulin-like growth factors: structure and biological function,* pp. 151-177. Editor. P.N. Schofield. Oxford, New York: Oxford University Press.

Oxford New York. **Bar, R.S. & Boes, M. (1984)** Distinct receptors for IGF-I, IGF-II and insulin are present on bovine capillary cells. *Biochimica et biophysica research communications.* **124,** 203-207.

Baumrucker, C.R. (1986a) Insulin-like growth factor-I (IGF-I) and insulin stimulate lactating bovine mammary tissue DNA synthesis and milk production *in vitro. Journal of dairy science* (Suppl. 1). **69,** 120 (abstract).

Baumrucker, C.R. (1986b) Insulin-like growth factor-I (IGF-I) and insulin stimulates DNA synthesis in bovine mammary tissue explants obtained from pregnant cows. *Journal of dairy science* (Suppl.1). **69,** 120 (abstract).

Baumrucker, C.R. & Stemberger, B.H. (1989) Insulin and insulin-like growth factor-I

stimulate DNA synthesis in bovine mammary tissue *in vitro*. *Journal of animal science*. **67,** 3503-3514.

Berelowitz, M., Szabo, M., Frohman, L.A., Firestne, S., Chu, L. & Hintz, R.L. (1981) Somatomedin-C mediates growth hormone negative feedback by effects on both the hypothalamus and the pituitary. *Science*. **212,** 1279-1281.

Binoux, M., Hossenlopp, L., Lessarre, C. & Segovia, B. (1991) Degradation of IGF binding protein-3 by protease: physiological implications. in: *Modern concepts of insulin-like growth factors,* pp. 329-336. Editor. E.M. Spencer. New York: Elsevier Science Publishing Company, Inc.

Black, J.L. (1987) Mechanisms controlling the rate of growth, composition and morphology of wool. in: *Merino improvement programs in Australia,* pp. 457-480. Editor. B.J. McGuirck. Melborne, Australia: Australian Wool Corporation.

Black, J.L. & Reis, P.G. (1979) Speculation on the control of nutrient partition between wool growth and other body functions. in: *Physiological and environmental limitations to wool growth,* pp. 269-293. Editors. J.L. Black & P.G. Reis. Armidale, Australia: The University of New England Publishing Unit.

Breier, B.H., Gallaher, B.W. & Gluckman, P.D. (1991) Radioimmunoassay for insulin-like growth factor-I: solutions to some potential problems and pitfalls. *Journal of endocrinology*. **128,** 347-357.

Bruce, L.A., Atkinson, T., Hutchinson, J.S.M., Shakespear, R.A. & McRae, J.C. (1991) The measurment of insulin-like growth factor-I in sheep plasma. *Journal of endocrinology*. **128,** R1-R4.

Bullough, W.S. (1962) Growth control in mammalian skin. *Nature*. **193,** 520-523.

Bullough, W.S. & Laurence, E.B. (1958) The mitotic activity of the follicle. in: *The biology of hair growth,* pp. 171-187. Editors. W. Montagna & R.A. Ellis. New York: Academic Press Inc.

Butler, A.A., Ambler, G.R., Breier, B.H., LeRoith, D., Roberts, C.T. & Gluckman, P.D. (1994) Growth hormone (GH) and insulin-like growth factor-I (IGF-I) treatment of the GH deficient dwarf rat: differential effects on IGF-I transcription start site expression in hepatic and extrahepatic tissues and lack of effect on type II IGF receptor mRNA. *Molecular and cellular endocrinology*. **101,** 321-330.

Campbell, P.G., Skaar, T.C., Vega, J.R. & Baumrucker, C.R. (1991) Secretion of insulin-like growth factor-I (IGF-I) and IGF-binding proteins from bovine mammary tissue *in vitro*. *Journal of endocrinology*. **128,** 219-228.

Canalis, E. (1980) Effect of insulin-like growth factor-I on DNA and protein synthesis in cultured rat calvaria. *Journal of clinical investigation*. **66,** 709-719.

Canalis, E. (1994) Insulin-like growth factor, an autocrine regulator of skeletal cells. in: *The insulin-like growth factors and their regulatory proteins. Proceedings of the Third International Symposium on Insulin-like Growth Factors,* pp. 307-314 Editors. R.C. Baxter, P.D. Gluckman & R.G. Rosenfeld. Sydney: Excerpta Medica.

Canalis, E., Dietrich, J.W., Maina, D.M. & Raisz, L.G. (1977) Hormonal in wool follicles. *Journal of investigative dermatology.* **57,** 377-381.

Chan, J.C.D., Robertson, H.A. & Friesen, H.G. (1976) The purification and characterisation of ovine placental lactogen. *Endocrinology.* **98,** 65-76.

Chapman, R.E. (1986) Hair, wool, quill, nail, claw, hoof and horn. in: *Biology of the integument, Vol.2. Vertebrates,* pp. 293-317. Editors. J. Bereiter-Hahn, A.G. Matoltsy & K.S. Richards. New York, Tokyo: Speinger-Aerlag, Berlin, Heidelberg,.

Chapman, R.E. & Ward, K.A. (1979) Histological and biochemical features of the wool fibre and follicle. in: *Physiological and environmental limitations to wool growth,* pp. 193-208. Editors. J.L Black & P.J. Reis. Armidale, Australia: The University of New England Publishing Unit.

Chapman, R.E., Downes, A.M. & Wilson, P.A. (1980) Migration and keratinization of cells in wool follicles. *Australian journal of biological science.* **33,** 587-603.

Chapman, R.E., Panaretto, A.M. & Frith, P.A. (1982) Changes in wool follicles of sheep following administration of dexamethasone trimethylacetate. *Journal of the cell science.* **53,** 323-335.

Chung, C.S., Etherton, T.D. & Wiggins, J.P. (1985) Stimulation of swine growth by porcine growth hormone. *Journal of animal science.* **60,** 118-130.

Cockrem, F. (1962) A relationship between skin temperature and amount of wool grown on various positions on the New Zealand Romney Marsh sheep. *Nature.* **196,** 560-561.

Coleman, A.W., Coleman, J.R., Kankel, D. & Werner, I. (1970) The reversible control of animal cell differentiation by the thymidine analog, 5-bromodeoxyuridine. *Experimental cell research.* **59,** 319-328.

Coop, I.E. (1953) Wool growth as affected by nutritional and climatic factors. *Journal of agricultural science, Cambridge.* **43,** 456-472.

Corps, A.N. & Brown, K.D. (1987) Stimulation of intestinal epithelial cell proliferation in culture by growth factors in human and ruminant mammary secretions. *Journal of endocrinology.* **113,** 285-290.

Cottam, Y.H., Blair, H.T., Gallaher, B.W., Purchas, R.W., Breier, B.H., McCutcheon, S.N. & Gluckman, P.D. (1992) Body growth, carcass composition, and endocrine changes in lambs chronically treated with recombinantly derived insulin-like growth

factor-I. *Endocrinology.* **130,** 2924-2930.

Cunningham, A., Lambert, G.A. & Hales, J.R.S. (1979) Wool growth and adrenergic mechanisms in skin. *Proceedings of the Australian Physiological and Pharmacological Society.* **10,** 157P.

Daly, R.A. & Carter, H.B. (1955) The fleece growth of young Lincoln, Corriedale, Polwarth and Fine Merino maiden ewes under housed conditions and unrestricted and progressively restricted feeding on a standard diet. *Australian journal of agricultural research.* **6,** 476-513.

Daughaday, W.H. (1981) Growth hormone and the somatomedins. in: *Endocrine control of growth,* pp. 1-24. Editor. W.H. Daughaday. New York: Elsevier.

Daughaday, W.H. (1982) Divergence of binding sites, *in vitro* action, and secretory regulation of the somatomedin peptides, IGF-I and IGF-II. *Proceedings of the Society for Experimental Biology and Medicine.* **170,** 257-263.

Daughaday, W.H. (1990) The possible autocrine/paracrine and endocrine roles of insulin-like growth factors of human tissues. *Endocrinology.* **127,** 1-4.

Daughaday, W.H. & Rotwein, P. (1989) Insulin-like growth factors I and II peptide, messenger ribonucleic acid and gene structure, serum and tissue concentrations. *Endocrine reviews.* **10,** 68-91.

Daughaday, W.H., Hall, K., Raben, M., Salmon, W.D., Jr., Van den Brabde, J.L. & Van Wyk, J.L. (1972) Somatomedins: Proposed designation for sulfation factor. *Nature.* **235,** 107-108.

Davis, S.R., Hodgkinson, S.C., Moore, L.G. & Gluckman, P.D. (1989) Improved estimates of clearance of ^{131}I-labelled insulin-like growth factor-I carrier protein complexes from blood plasma of sheep. *Journal of endocrinology.* **123,** 469-475.

Dean, P.N., Dolbeare, F., Gratzner, H., Rice, G.C. & Gray, J.W. (1984) Cell-cycle analysis using a monoclonal antibody to BrdU. *Cell and tissue kinetics.* **17,** 427-436.

D'Ercole, A.J., Stiles, A.D. & Underwood, L.E. (1984) Tissue concentrations of somatomedin C: further evidence for multiple sites of synthesis and paracrine or autocrine mechanisms of action. *Proceedings of the National Academy of Sciences,* USA. **81,** 935-939.

Doney, L.M. & Griffiths, T.G. (1967) Wool growth regulation by local skin cooling. *Animal production.* **9,** 393-397.

Douglas, R.G., Gluckman, P.D., Ball, K., Breier, B.H. & Shaw, J.H.F. (1991) The effects of infusion of insulin-like growth factor (IGF)-I, IGF-II and insulin on glucose and protein metabolism in fasted lambs. *Journal of clinical investigation.* **88,** 614-622.

Dowling, L.M., Gough, K.H., Inglis, A.S. & Sparrow, L.G. (1979) Composition of some microfibrillar proteins from wool. *Australian journal of biological science.* **32,** 437-442.

Downes, A.M. & Wallace, A.L.C. (1965) Local effects on wool growth of intradermal injections of hormones. in: *Biology of the skin and hair growth,* pp. 679-704. Editors. A.G. Lyne & B.F. Short. Sydney: Angus & Roberson.

Downes, A.M., Chapman, R.E., Till, A.R. & Wilson, P.A. (1966) Proliferative cycle and fate of cell nuclei in wool follicles. *Nature.* London. **212,** 477-479.

Dunlop, A.A., Dollinger, C.H.S. & Carpenters, M.T. (1966) Efficiency of conversion of food to wool at two nutritional levels by three Merino strains. *Australian journal of agricultural research.* **17,** 81-89.

Durward, A. & Rudall, K.M. (1949) Studies on hair growth in the rat. *Journal of anatomy.* **83,** 325-353.

Eigenmann, J.E., Zanesco, S., Arnold & Ftoesch, E.R. (1984a) Growth hormone and insulin-like growth factor-I in German Shepherd dwarf dogs. *Acta endocrinologica (Copenh).* **105,** 289-294.

Eigenmann, J.E., Patterson, D.F., Zapf, J. & Frosech, E.R. (1984b) Insulin-like growth factor-I in the dog: a study in different dog breeds and in dogs with growth hormone elevation. *Acta endocrinologica (Copenh).* **105,** 294-301.

Epstein, W.L. & Maibach, H.I. (1969) Cell proliferation and movement in human hair bulbs. in: *Advances in biology of skin.* **Vol. 9,** pp. 83-97. Editors. W. Montagna & R.L. Dobson. Oxford: Pergamon Press.

Etherton, T.D. (1991) The role of insulin-like growth factors (IGFs) and the IGF-binding proteins in growth and metabolism. in: *Growth regulation in farm animals:* advances in meat research. **Vol.7,** pp. 343-372. Editors. A.M. Pearson & T.R. Dutson. London and New York: Elsevier Applied Science.

Ewton, D.Z., Falen, S.L. & Florini, J.R. (1987) The type II insulin-like growth factor (IGF) receptor has low affinity for IGF-I analogs: pleiotypic actions of IGFs on myoblasts are apparently mediated by the type I receptor. *Endocrinology.* **120,** 115-123.

Ewton, D.Z., Magri, K.A. & Florini, J.K. (1994) The roles of IGF-1, IGF-II and IGFBPs in myogenesis. in: *The insulin-like growth factors and their regulatory proteins. Proceedings of the Third International Symposium on Insulin-like Growth Factors,* pp. 283-290. Editors R.C. Baxter, P.D. Gluckman & K.G. Rosenfeld. Sydney: Excerpta Medica .

Ferguson, K.A. (1949) The effect of sympathectomy on wool growth. *Australian journal*

of scientific research. **B2,** 438-443.

Ferguson, K.A., Wallace, A.L.C. & Lindner, H.R. (1965) Hormonal regulation of wool growth. in: *Biology of the skin and hair growth,* pp. 655-678. Editors. A.G. Lyne & B.F. Short. Sydney: Angus & Roberson.

Fleet, I.R., Davis, A., Richardson, M. & Heap, R.B. (1992) The effects of recombinantly-derived insulin-like growth factor-I and insulin-like growth factor-II on mammary vasodilation in the goat. *Journal of physiology. Proceedings of the Physiological Society.* **446,** 69P.

Flint, D.J, Tonner, E., Beattie, J. & Gardner, M. (1994) Several insulin-like growth factor-I and-II with complexes of insulin-like growth factor-binding protein-3 fail to mimic the effect of growth hormone upon lactation in the rat. *Journal of endocrinology.* **140,** 211-216.

Flux, D.S., MacKenzie, D.D.S. & Wilson, G.F.(1984) Plasma metabolism and hormone concentration in Friesian cows of differing genetic merit measured at two feeding levels. *Animal production.* **38,** 377-384.

Foldes, A., Maxwell, C.A, Rintoul, A.J., Scaramuzzi, R.J. & Baker, P. (1990) Effects of melatonin implants on selected hormonal profiles and wool growth in Merino wethers. *Proceedings of Australian Society of Animal Production.* **18,** 208-215.

Forbes, B., Szabo, L., Baxter, R.C., Ballard, F.J. & Wallace, J.C. (1988) Classification of the insulin-like growth factor binding proteins into three distinct categories according to their binding specificities. *Biochemical and biophysical research communications.* **157,** 196-202.

Foster, L.A., Ames, N.K. & Emery, R.S. (1991) Food intake and serum insulin responses to intraventicular infusions of insulin and IGF-I. *Physiology and behaviour.* **50,** 745-749.

Francis, G.L., Read, L.C., Ballard, F.J., Bagley, C.J., Upton, F.M., Gravestock, P.M. & Wallace, J.C. (1986) Purification and partial sequence analysis of insulin-like growth factor-I from bovine colostrum. *Biochemical journal.* **233,** 207-213.

Francis, G.L., Upton, F.M., Ballard, F.J., McNeil, K.A. & Wallace, J.C. (1988) Insulin-like growth factors I and II in bovine colostrum, sequences and biological activities compared with those of a potent truncated form. *Biochemical journal.* **251,** 95-103.

Francis, G.L., Ross, M., Brazier, J.A., Walton, P.E., Wallace, J.C. & Ballard, F.J. (1991) Novel IGF-I analogs display enhanced anabolic actions in cultured cells. *2nd International Symposium on Insulin-like Growth Factors/Somatomedins.* San Francisco, California.

Francis, G.L., Aplin, S.E., NcNeil, K.A., Milner, S.J., Forbes, B.E., Ross, M., Ballard, F.J. & Wallace, J.C. (1994) Molecular interactions of IGF-I and IGF-II with their binding proteins and receptor. in: *The insulin-like growth factors and their regulatory proteins. Proceedings of the Third Iinternational Symposium on Insulin-like Growth Factors,* pp. 57-66. Editors. R.C. Baxter, P.D. Gluckman & R.G. Rosenfeld. Sydney: Excerpta Medica

Fraser, I.E.B. (1963) The use of colchicine and colcemid for metaphase stasis in the matrix cells of wool follicles. *Australian journal of biological science.* **16,** 211-217.

Fraser, I.E.B. (1964) Studies on the follicle bulb of fibres. 1. Mitotic and cellular segmentation in the wool follicle with reference to ortho-and parasegmentation. *Australian journal of biological science.* **17,** 521-531.

Fraser, P.G. (1965) Cellular proliferation in the wool follicle bulb. in: *Biology of the skin and hair growth,* pp. 427-446. Editors. A.G. Lyne & B.F. Short. Sydney: Angus & Robertson.

Fraser, R.D.B. & Rogers, G.E. (1953) Microscopic observation of the alkaline thioglycollate extraction of wool. *Biochimica et biophysica acta.* **12,** 484-485.

Fraser, A.S. & Short, B.F. (1960) The biology of the fleece. Melbourne, Australian: Animal Research Laboratories technical paper. No. 3; Commonwealth Scientific and Industrial Research Organisation.

Fratini, A., Powell, B.C., Hynd, P.I., Keough, R.A. & Rogers, G.E. (1994) Dietary cysteine regulates the levels of mRNAs encoding a family of cysteine-rich proteins of hair. *Journal of investigative dermatology.* **102,** 178-185.

Froesch, E.R., Schmid, Ch., Schwander, J. & Zapf, J. (1985) Actions of insulin-like-growth factors. *Annual review of physiology.* **47,** 443-467.

Furlandetto, R.W., Underwood, L.E., Van Wyk, J.J. & D'Ercole, A.J. (1977) Estimation of somatomedin-C levels in normals and patients with pituitary disease by radiommunoassay. *Journal of clinical investigation.* **60,** 648-657.

Gammeltoft, S., Danielsen, A., Frödin, M., GrØnborg, M., Hansen, L.B.H., Holm, N.R., Drakenbery, K., Sara, V.R., Hultberg, B. & Nillson, C. (1994) Insulin-like growth factors in the nervous system: evolution, fetal development, maintenance and tumour formation. in: *The insulin-like growth factors and their regulatory proteins. Proceedings of the Third International Symposium on Insulin-like Growth Factors,* pp. 295-306. Editors R.C. Baxter, P.D., Gluckman & R.G. Rosenfeld. Sydney: Excerpta Medica.

Gillespie, J.M. (1965) The high-sulfur proteins of normal and aberrant keratins. in: *Biology of the skin and hair growth.* pp. 377-398. Editors A.G. Lyne & B.F. Short. Sydney:

Angus & Robertson.

Gillespie, J.M. (1983) The structural proteins of hair: isolation characterisation and regulation of biosynthesis. in: *Biochemistry and physiology of the skin. **Vol. 1,*** pp. 475-510. Editors. A. Powell & M.D. Goldsmith. Oxford: Oxford University Press.

Gillespie, J.M. (1990) The protein of hair and other hard α-keratins. in: *Cellular and molecular biology of intermediate filaments,* pp. 95-128. Editors. R.D. Golgman & P.M. Steinert. New York and London: Plenum Press.

Gluckman, P.D., Breier, B.H. & Davis, S.R. (1987) Physiology of the somatotropic axis with particular reference to the ruminant. *Journal of dairy science.* **70,** 442-466.

Gratzner, H.G. (1982) Monoclonal antibody to 5-bromo and 5-iododeoxyuridine: a new reagent for detection of DNA relocation. *Science.* **218,** 474-477.

Grubb, B.R. & Mills, L.D. (1981) Blood oxygen content in microliter samples using an easy to build galvanic oxygen cell. *Journal of applied physiology: respiration, environment and exercise physiology.* **50,** 456-464.

Guler, H.P., Zapf, J., Scheiwiller, E. & Froesch, E.R. (1988) Recombinant human insulin-like growth factor-I stimulates growth and distinct effects on organ size in hypophysectomised rats. P*roceedings of the National Academy of Sciences*. USA. **85,** 4889-4893.

Hales, C.N. & Randle, P.J. (1963) Immunoassay of insulin with insulin-antibody precipitate. *Biochemical journal.* **88,** 137-146.

Hales, J.R.S. & Fawcett, A.A. (1993) Wool production and blood supply to skin and other tissues in sheep. *Journal of animal science.* **71,** 492-498.

Hales, J.R.S., Bennett, J.W. & Fawcett, A.A. (1976) Effects of acute cold exposure on the distribution of cardiac output in the sheep. *Pfluegers archive.* **366,** 153-157.

Hall, K. & Sara, V.R. (1983) Growth and somatomedins. V*itamins and hormones.* **40,** 175-233.

Hansson, H.A., Nilsson, A., Isgaard, J., Billing, H., Isaksson, O., Skottner, A., Anderson, I.B. & Rozell, B. (1988) Immunohistochemical localisation of insulin-like growth factor-I in the adult rat. *Immunohistochemistry.* **89,** 403-410.

Hardy, M.H. (1952) The histochemistry of hair follicles in the mouse. *American journal of anatomy.* **90,** 285-329.

Hardy, M.H. (1992) The secret life of the hair follicle. *Trends in genetics.* **8,** 55-61.

Hardy, M.H. & Lyne, A.G. (1956) The pre-natal development of wool follicles in Merino sheep. *Australian journal of biological science.* **9,** 423-441.

Harms, G., Van Goor, H., Koudstaal, J., de Ley, L. & Hardon, K.M.J. (1986) Immunohistochemical demonstration of DNA-incorporated 5-bromode-oxyuridine

in frozen and plastic embedded sections. *Histochemistry.* **85,** 139-143.

Harms, G., Van Goor, H., Koudstaal, J., de Ley, L. & Hardon, K.M.J. (1987) Simultaneous immunohistochemical demonstration of antigen expression and 5-bromodeoxyuridine incorporation in plastic embedded sections. *Histochemistry.* **86,** 393-397.

Harris, P.M., Dellow, D.W. & Sinclair, B.R. (1989) Preliminary *"in vivo"* measurements of protein and energy metabolism in the skin of sheep. *Australian journal of agricultural research.* **40,** 879-888.

Harris, P.M., McBride, B.W., Gurnsey, M.P., Sinclair, B.R. & Lee, J. (1993) Direct infusion of a variant of insulin-like growth factor-I into the skin of sheep and effects on local blood flow, amino acid utilisation and cell replication. *Journal of endocrinology.* **139,** 463-472.

Haselbacher, G.K., Andres, R.Y. & Humbel, R.E. (1980) Evidence for the synthesis of somatomedin similar to insulin-like growth factor-I by chick embryo liver cells. *Euorpean journal of biochemistry.* **111,** 245-250.

Henderson, A.E. (1953) Fleece development and wool growth in the Romney lamb. *Journal of agricultural science.* **43,** 12-53.

Hill, J.M., Lesniak, M.A., Pert, C.B. & Roth, J. (1986) Autoradiographic localization of insulin receptors in rat brain: prominence in olfactory and limbic areas. *Neuroscience.* **17,** 1127-1138.

Hintz, R.L. (1986) The somatomedin binding proteins. in: *Human growth hormone,* pp. 553-562. Editors S. Raiti & R.A. Tolman. New York: Plenum Publishing Company.

Hizuka, N., Takano, K., Shizume, K., Hsakawa, K. Miyakawa, M., Tanaka, I. & Horikawa, R. (1986) Insulin-like growth factor-I stimulates growth in normal growing rats. *European journal of pharmacology.* **125,** 143-146.

Hock, J.M., Centrella, M. & Canalis, E. (1988) Insulin-like growth factor-I has independent effects on bone matrix formation and cell replication. *Endocrinology.* **122,** 254-260.

Hocking Edwards, J.E. (1993) The physiology of skin and wool follicle of finewool and strongwool Merinos. *PhD Thesis.* The University of Adelaide, Australia.

Hocking Edwards, J.E., Khalaf, S.K., Sinclair, B.R., Lee, J., Prosser, C.G. and Harris, P.M. (1995) Metabolic response of sheep skin to a chronic infusion of a variant of insulin-like growth factor-I. *Biochemical journal.* **308,** 411-418.

Holle, S.A. (1992) Aspects of wool follicle morphology and cell proliferation in Romney sheep selected for high fleece production. *PhD Thesis.* Massey University, Palmarstone North.

Holle, S.A. & Birtles, M.J. (1990) An immunocytochemical method for study patterns of cell proliferation in the wool follicle. *New Zealand veterinary journal.* **38,** 89-93.

Holle, S.A. & Harris, P.M. (1992) Studies of kinetics of *in vivo* labelling of proliferating wool follicle bulb cell with 5-Bromo-2'-deoxyuridine (BrdU): intracutaneous labelling with BrdU and pharmokinetics of free BrdU in the skin tissue of sheep. *Australian journal of agricultural research.* **43,** 183-184.

Holle, S.A., Harris, P.M., Davis, A.S. & Birtles, M.J. (1994) Wool follicle morphology and cell proliferation in New Zealand Romney sheep, a seasonal comparison of fleece weight-selected and control rams. *Australian journal of agricultural research.* **45,** 769-782.

Hopkins, K.D., Lehmann, E.D., Parker, J.R. & Gosling, R.G. (1994) Insulin-like growth factor-binding protein-1 is correlated with low density lipoprotein cholesterol in normal subjects. *Journal of endocrinology.* **140,** 521-524.

Hough, G.M., Williams, A.L.C., McDowell, G.H. & Annison, E.F. (1988) Blood metabolites in ewes selectively breed for high or low clean fleece weight possible use for selection of superior animals. *Proceedings of the Australian Society of Animal Production.* **17,** 422.

Humbel, R.E. (1990) Insulin-like growth factors-I and II. *European journal of biochemistry.* **190**, 445-462.

Hynd, P.I (1989a) Effects of nutrition on wool follicle cell kinetics in sheep differing in efficiency of wool production. *Australian journal of agricultural research.* **40,** 409-417.

Hynd, P.I. (1989b) Factors influencing cellular events in the wool follicle. in: *The biology of wool and hair,* pp. 169-184. Editors G.E. Rogers, P.J. Reis, K.A. Ward & R.C. Marshall. New York: Chapman & Hall .

Hynd, P.I. & Everett, B.K. (1990) Estimation of cell birth rate in the wool follicle bulb using Colchicine metaphase arrest or DNA labelling with Bromodeoxyuridine. *Australian journal of agricultural research.* **41,** 741-749.

Hynd, P.I., Schlink, A.C., Phillips, P.M. & Scobie, D.R. (1986) Mitotic activity in cells of the wool follicle bulb. *Australian journal of biological science.* **39,** 329-339.

Imagawa, W., Spencer, E.M., Larson, L. & Nandi, S. (1986) Somatonedin C substitutes for insulin for the growth of mammary epithelial cells from normal virgin mice in serum-free collagen gell cell culture. *Endocrinology.* **119,** 2695-2700.

Isaksson, O.G.P., Lindahl, A., Nilsson, A. & Isggard, J. (1987) Mechanism of the stimulatory effect of growth hormone on longitudinal bone growth. *Endocrine review.* **8,** 426-438.

Jacob, R., Barrett, E., Plewe, G., Fagin, K.D. & Sherwin, R.S. (1989) Acute effects of insulin-like growth factor-I on glucose and amino acid metabolism in the a wake fasted rat. *Journal of clinical investigation.* **83,** 1717-1723.

Jennische, E., Isgaard, J. & Isaksson, O.G.P. (1992) Local expression of insulin-like growth factors during tissue growth and regeneration. in: *The insulin-like growth factors: structure and biological functions*, pp. 221-239. Editor P.N. Schofeld. Oxford, New York: Oxford University Press.

Kirkwood, R.N., Lapwood, K.R., Smith, W.C. & Anderson, I.L. (1984) Plasma concentrations of LH, Prolactin, oestradiol-17β and progesterone in sows weaned after lactation for 10 or 35 days. *Journal of reproduction and fertility.* **70**, 95-102.

Lee, J., Rounce, J.R., Hocking Edwards, J.E. & Harris, P.M. (1995) Response to trace element concentrations in plasma of sheep to chronic infusion of a recombinant variant of IGF-I. in: *The Proceedings of the New Zealand Society of Animal Production.* **Vol. 55**. (In press).

LeRoith, D. & Roberts, C.T. (1991) Insulin-like growth factor-I (IGF-I): a molecular basis for endocrine versus local action. *Molecular and cellular endocrinology.* **77,** C57-C61.

Lewitt, M.S., Saunders, H. Cooney, G.J. & Baxter, R.C. (1993) Effect of human insulin-like growth factor-binding protein-1 on the half-life and action of administered insulin-like growth factor-I in rats. *Journal of endocrinology.* **136,** 253-260.

Little, J.C., Reedwood, K.R., Stone, A.J., Gibson, W.T. & Granger, S.P. (1994) The insulin-like growth factor-I receptor is important in controlling the hair growth cycle. *The journal of investigative dermatology.* **102 (4),** 533 (abstract).

Lord, A.P.D., Bastian, S.E.P., Read, L.C., Walton, P.E. & Ballard, F.J. (1994) Differences in the association of insulin-like growth factor-I and IGF-I variants with rat, sheep, pig, human and chicken plasma binding proteins. *Journal of endocrinology.* **140,** 475-482.

Lyne, A.G., Jolly, M. & Hollis, D.E. (1970) Effects of experimentally produced local subdermal temperature changes on skin temperature on wool growth in sheep. *Journal of agricultural science, Cambridge.* **74,** 83-90.

Maddocks, S., Chandrasekhar, Y. & Setchell, B.P. (1985) Effect on wool growth of thyroxin replacement in thyroidectomized Merino rams. *Australian journal of biological science.* **38,** 405-410

Marston, H.R. (1946) Nutrition and wool production. in: *Fibrous productions: a symposium*, pp. 207-214. Society of Dyers & Colourists, Leeds.

McBride, B.W., Burton, J.L. & Burton, J.H. (1988) The influence of bovine growth hormone (somatotropin) on animals and their products. *Research and development in agriculture.* **Vol. 5 (1)**, 1-21.

McCloghory, C.E., Foldes, A., Rintoul, A.J., Hollis, D.E., Baker, P.J., Maxwell, C.A., Kennedy, J.P. & Wynn, P.C. (1992) Effects of suppressed prolactin levels on wool growth in Romney ewes. in: *The Proceedings of the New Zealand Society of Animal Production.* **Vol. 52,** 179-181.

McGrathy, T.L., Centrella, M. & Canalis, E. (1989) Regulatory effects of insulin-like growth factors I and II on bone collagen synthesis in rat calvarial cultures. *Endocrinology.* **124**, 301-309.

McGrath, M.F., Collier, P.J. Clemmons, D.R., Busby, W.H., Sweeny, C.A. & Krivi, G.G. (1991) The direct *in vitro* effect of insulin-like growth factors (IGFs) on normal bovine mammary cell proliferation and production of IGF binding proteins. *Endocrinology.* **129**, 671-678.

Min, S.H., Mackenzie, D.D.S., Breier, B.H., McCutcheon, S.N. & Gluckman, P.D. (1995) metabolic and endocrine effects of chronically administered IGF-I in young energy restricted sheep. *Endocrinology (submitted).*

Miura, Y. & Wilt, F.H. (1971) The effects of 5-bromodeoxyuridine on yolk sac erythropoieisis in the chick embryo. *Journal of cell biology.* **48,** 523-532.

Mohn, M.P. (1958) The effects of different hormonal states on the growth of hair in rats. in: *The biology of hair growth,* pp 335-398. Editors W. Montagna & R.A. Ellis. New York: Academic Press Inc.

Montagna, W. (1956) The structure and function of skin. New York: Academic Press.

Montagna, W. & Parakkal, P.F. (1974) The structure and function of skin. 3rd edition. New York: Academic Press Inc.

Moore, G.P.M. (1989) Growth factors, cell-cell and cell-matrix interactions in skin during follicle development and growth. in: *The biology of wool and hair,* pp. 351-364. Editors. G.E. Rogers, P.J. Reis, K.A. Ward & R.C. Marshall. New York: Chapman & Hall

Moore, G.P.M., Pisansarakit, P., du Cross, D.L. & Isaacs, K. (1991) The dermal papilla in follicle morphogenesis and function. in: *Wool biology,* pp. 33-41. Editor. P.H. Hynd. Melbourn, Australia: Australian Wool Corporation.

Nay, T. (1966) Wool follicle arrangement and vascular pattern in the Australian Merino. *Australian journal of agricultural research.* **17,** 797-805.

Neely, E.K., Morhenn, V.B., Hintze, R.L., Wilson, D.M. & Rosenfeld, R.G. (1991) Insulin-like growth factors are mitogenic for human keratinocytes and squamous cell

carcinoma. *Journal of investigative dermatology.* **96,** 104-110.

Oddy, V.H., Warren, H.M., Moyse, K.J. & Owens, P.C. (1991) IGF-I inhibits muscle protein breakdown in sheep. *Procedings of the 2nd International Symposium on Insulin-like Growth Factors/Somatomedins.* San Francisco, Calfornia.

Ohlsson, C., Nisson, A., Isaksson, O.G.P. & Lindahl, A. (1992) Effect of growth hormone and insulin-like growth factor-I on DNA synthesis and matrix production in rat epiphyseal chondrocytes in monolayer culture. *Journal of endocrinology.* **133,** 291-300.

Orwin, D.F.G. (1979) the cytology and cytochemistry of the wool follicle. *International review of cytology.* **60,** 331-374.

Orwin, D.F.G. (1989) Variations in wool follicle morphology. in: *The biology of wool and hair,* pp. 227-242. Editors. G.E. Rogers, P.J. Reis, K.A. Ward, & R.C. Marshall. New York: Chapman & Hall.

Orwin, D.F., Woods, J.L. & Ranford, S.L. (1984) Cortical cell types and their distribution in wool fibres. *Australian journal of biological science.* **37,** 237-255.

O'Sullivan, U., Gluckman, P.D., Breier, B.H., Woodall, S., Sidigui, R.A. & McCutcheon, S.N. (1989) Insulin-like growth factor-I (IGF-I) in mice reduce weight loss during starvation. *Endocrinology.* **125,** 2793-2795.

Owens, P.C., Lindsay, D.B. & Hunter, R.A. (1994) Long-R3-IGF-I inhibits catabolism of muscle protein in underfed steers. *Growth regulation.* **4S1,** 125.

Panaretto, B.A. (1979) Effects of light on cyclic activity of wool follicles and possible relationships to changes in the pelage of other mammals. in: *Physiological and environmental limitations to wool growth,* pp. 327-336. Editors. J.L Black & P.J. Reis. Armidale, Australia: The University of New England Publishing Unit.

Parakkal, P.F. (1969) The fine structure of anagen hair follicle of the mouse. in: *Advances in biology of skin. Vol.9 Hair growth,* pp. 441-469. Editors. W. Montagna & R.L. Dobson. Oxford: Pergamon Press.

Pell, J.M. & Bates, P.C. (1992) Differential actions of growth factor-I on tissue protein metabolism in dwarf mice. *Endocrinology.* **130,** 1942-1950.

Peterson, S.W., McKenzie, D.D.S., McCutcheon, S.N. & Lapwood, K.R. (1994) The effect of peripartum administration of ovine prolactin on lactogenesis in autumn-lambing ewes. *Proceedings of the New Zealand Society of Animal Production.* **Vol. 54.** 115-118.

Phillips, L.S. (1986) Nutrition, somatomedins and the brain. *Metabolism.* **35 (1),** 78-87.

Phillips, L.S. & Vassilopoulou-Sellin, R. (1980) Somatomedins. *The New England journal of medicine.* **302 (7),** 371-446.

Phillips, L.S. & Saholz,T.D. (1982) Nutrition and somatomedin. IX. Blunting of insulin-like activity by inhibitor in diabetic rat serum. *Diabetes.* **31,** 97-104.

Philpott, M.P., Sanders, D.A. & Kealey,T (1994) Effects of insulin and insulin like growth factors on cultural human hair follicles: IGF-I at physiological concentrations is an important regulator of hair follicle growth *in vitro. Journal of investigative dermatology.* **102,** 857-861.

Poggi, C., Le Marchand-Brustedl, Y., Zapf, J. & Froesch, E.R. (1979) Effects and binding of insulin-like growth factor-I in the isolated soleus muscle lean of obese mice: comparison with insulin. *Endocrinology.* **105,** 723-730.

Powell, B.C. & Rogers, G.E. (1986) Hair keratin: Composition, structure and biogenesis. in: *Biology of integument. Vol. 2. Vertebrates,* pp. 695-721. Editors. J. Breier-Hahn. A.G. Matoltsy & K.S. Richards. New York. Tokyo: Springer-Veriag. Berlin. Heidelerg.

Powell, B.C. & Rogers, G.E. (1990) Hard keratin IF and associated protein. in: cellular and molecular biology of intermediate filaments, pp. 267-300. Editors R. D. Goldman & P. M. Steinert. New York: Plenum Press.

Priestley, G.C. (1967) Seasonal changes in the inner root sheath of the primary follicles in Herwick sheep. *Journal of agricultural science.* **69,** 9-12.

Prosser, C.G., Sankaran, L., Henninghausen, L. & Topper, Y.J. (1987) Comparison of the roles of insulin-like growth factor-I in casein gene expression and in the development of α-lactoalbumin and glucose transport activities in the mouse mammary epithelial cell. *Endocrinology.* **120,** 1411-1416.

Prosser, C.G., Fleet, I.R., Corps, A.N., Froesh, E.R. & Heap, R.B. (1990) Increase in milk secretion and mammary blood flow by intra-arterial infusion of insulin-like growth factor-I into the mammary gland of the goat. *Journal of endocrinology.* **126,** 437-443.

Prosser, C.G., Davis, S.R., Hodglinson, S.C. & Mohler, M.A. (1995) Pharmacokinetic and bioactivetiy of intact versus truncated IGF-I during a 24-h infusion into lactating goats. *Journal of endocrinology.* **144,** 99-107.

Read, L.C., Howarth, G.S., Steeb, C-B. & Lemmey, A.B. (1994) *In vivo* effects of IGF-I on gut growth and function. in: *The insulin-like growth factors and their regulatory proteins. Proceedings of the Third International Symposium on Insulin-like Growth Factors,* pp. 404-416. Editors. R.C. Baxter, P.D. Gulckman & R.G. Rosenfeld. Sydney: Excerpta Medica.

Rechler, M.M. & Nissley, S.P. (1985) The nature and regulation of the receptors for insulin-like growth factors. *Annual reviews of physiology.* **47,** 425-442.

Reis, P.J. (1965) Variation in the sulfur content of wool. in: *Biology of the skin and hair growth,* pp. 365-376. Editors. A.G. Lyne & B.F. Short. Sydney: Angus & Roberson.

Reis, P.J. (1979) Effects of amino acids on the growth and properties of wool. in: *Physiological and environmental limitations to wool growth,* pp. 223-242. Editors. J.L. Black & P.J. Reis. Armidale, Australia: The University of New England Publishing Unit

Reis, P.J. (1982) Growth and characteristics of wool and hair. in: *Sheep and goat production.* From: *Word Animal Science Production-System. **Approach I**,* pp. 205-223. Editors. A. Nelmann, Sorensen, D.E., Tripe. Amsterdam-Oxford-New York: Elsevier Scientific Publishing Company.

Reis, P.J. (1989) The influence of absorbed nutrients on wool growth. in: *The biology of wool and hair,* pp. 185-203. Editors. G.E. Rogers, P.J. Reis, K.A. Ward & R.C. Marshall. New York: Chapman & Hall.

Reis, P.J. & Schinckel, P.G. (1964) The growth and composition of wool. The effect of casein, gelatine, and sulfur-containing amino acids given per abomasum. *Australian journal of biological science.* **17,** 532-547.

Rinderknecht,E. & Humbel ,R.E (1978) The amino-acid sequence of human insulin-like growth factor-1 and its structural homology with proinsulin. *Journal of biological chemistry.* **253,** 2769-2777.

Ristow, H.J. & Messmer, T.O. (1988) Basic fibroblast growth factor and insulin-like growth factor I are strong mitogens for cultured mouse keratinocytes. *Journal of cell physiology.* **137,** 277-284.

Ritvos, O., Ranta, T., Jalkanen, J., Suikkari, A.M., Voutilainen, R., Bohn, H. & Rutanan, E.M. (1988) Insulin-like growth factor-(IGF) binding protein from human decidua inhibits binding and biological action of IGF-I in cultured choriocarcinoma cells. *Endocrinology.* **122,** 2150-2157.

Rogers, G.E. (1959) Electron microscopy of wool. *Journal of ultrastructure research.* **2,** 309-330.

Rosenthal, S.M., Hasiao, D., Cheng, Z-Q & Silverman, L.A. (1994) Insulin-like growth factors and muscle cell differentiation. in: *The insulin-like growth factors and their regulatory proteins. Proceedings of the Third International Symposium on Insulin-like Growth Factors,* pp. 275-282. Editors. R.C. Baxter, P.D. Gluckman & R.G. Rosenfeld. Sydney: Exserpta Medica.

Ross, M., Francis, G.L., Szabo, L., Wallace, J.C. & Ballard, F.J. (1989) Insulin-like growth factor (IGF-I)-binding proteins inhibit the biological activities of IGF-I and IGF-II but not des-(1-3)IGF-I. *Biological journal.* **258,** 267-272.

Rougeot, J. (1965) The effect of thyroid hormones on the morphology of the wool cuticle. in: *Biology of the skin and hair growth,* pp. 625-640. Editors. A.G. Lyne & B.F. Short. Sydney: Angus & Robertson.

Russell, S.M. & Spencer, E.M. (1985) Local injections of human or rat growth hormone or of purified human somatomedin-C stimulate unilateral tibial epiphyseal growth in hypophysectomised rats. *Endocrinology.* **116,** 2563-2567.

Ryder, M.L. (1956) Use of radioisotopes in the study of wool growth and fibre composition. *Nature,* London. **178,** 1409-1410.

Ryder, M.L. (1973) Hair. London: Edward Armold.

Ryder, M.L. & Stephenson, S.K. (1968) *Wool growth.* London: Academic Press.

Sadighi, M., Haines. S.R., Skottner, A., Harris, A.J. & Sutti, J.M. (1994) Effects of insulin-like growth factor-I (IGF-I) and IGF-II on the growth of antler cell *in vitro. Journal of endocrinology.* **143,** 461-469.

Salmon, W.D. & Daughaday, W.H. (1957) A hormonally controlled serum factor which stimulates sulfate incorporation by caritlage in vitro. *Journal of laboratory and clinical medicine.* **49,** 825-836.

Sara, V.R. & hall, K. (1990) Insulin-like growth factors and their binding proteins. *Physiological reviews.* **Vol. 70 (3),** 591-614.

SAS **(1985)** "SAS User Guide Statistics". (SAS Institute Inc., Cory, NC.).

Scanes, C.G., Lauterio, T.J & Perez, F.M. (1986) Somatomedins in domestic animal. *IRCS medical science.* **14,** 515-518.

Schalch, D.S., Yang, H., Ney, D.M. & DiMarch, R.D. (1989) Infusion of human insulin-like growth factor-I (IGF-I) into malnourished rats reduces hepatic mRNA abundance. *Biochemical and biophysical research communications.* **160,** 795-800.

Schinkel, P.G. (1961) Mitotic activity in wool follicle bulbs. *Australian journal of biological science.* **14,** 659-675.

Schinkel, P.G. (1962) Variation in wool growth and mitotic activity in the follicle bulbs induced by nutritional changes. *Animal production.* **4,** 122-127.

Setchell, B.P. & Waties, G.M.H. (1965) Variations in blood flow in the skin. in: *Biology of skin and hair growth,* pp. 617-624. Editors. A.G. Lyne & B.F. Short. Sydney: Angus & Robertson.

Shamay, A., Cohen, N., Niwa, M. & Gertler, A. (1988) Effect of insulin-like growth factor-I on Deoxyribonucleic acid synthesis and galactopoiesis in bovine undifferentiated and lactating mammary tissue *in vitro. Endocrinology.* **123,** 804, 809.

Short, B.F., Wilson, P.A. & Schinckel, P.G. (1965) Proliferation of follicle matrix cells in

relation to wool growth. in: *Biology of skin and hair growth,* pp. 409-426. Editors. A.G. Lyne & B.F. Short. Sydney: Angus & Robertson.

Silagi, S. & Bruce. A. (1970) Suppression of malignancy and differentiation in melatonic melanoma cells. *Proceedings of the National Academy of Sciences,* USA. **66,** 72-78.

Silverman, L.A., Cheng, Z-Q, Hasiao, D. & Rosenthal, S.M. (1995) Skeletal muscle cell-derived insulin-like growth factor (IGF) binding proteins inhibit IGF-I induced myogenesis in rat L6E9 cells. *Endocrinology.* **136,** 720-726.

Skottner, A., Clark, R.G., Fryklund, L. & Robinson, I.C.A.F. (1989) Growth responses in the mutant dwarf rat to human growth hormone and reconbinant human insulin-like growth factor-I. *Endocrinology.* **124,** 2519-2526.

Smelser, G,K, & Ozanics, V. (1954) Effect of thyroid hormone on mitosis and regulation in corneal epithelium. *Journal of cell composition and physiology.* **43,** 107-117.

Smith (1951) The effect of thyroid hormone and radiation on the mitotic index of mouse epidermis. *Journal of cell composition and physiology.* **38,** 41-49.

Spencer, G.S.G. (1985) Hormonal systems regulating growth. *A review. Livestock production science.* **12,** 31-46. Amsterdam: Elsevier Science Publishers B.V.

Suttie, J.M., Gluckman, P.D., Buttler, J.H., Fennessy, P.F., Corson, I.D. & Lass, F.J. (1985) Insulin-like growth factor-I (IGF-I) antler stimulating hormone. *Endocrinology.* **116,** 846-848.

Suttie, J.M., Fennessy, P.F., Corson, I.D., Laas, F.J., Crosbie, S.F., Butler, J.H. & Gluckman, P.D. (1989) Pulstile growth hormone, insulin-like growth factor and antler development in red deer (Cervus elaphus Scoticus) stags. *Journal of endocrinology.* **121,** 351-360.

Sutton, R., Cam, G.R., Raphael, K.A. & Ward, K.A. (1991) Ontogenes, growth factors and follicle cell proliferation. in: *Wool biology,* pp. 69-74. Editor. P.H. Hynd. Melbourne, Australia: Australian Wool Corporation.

Tannenbaum, G.S., Guyda, H.J., & Posner, B.I. (1983) Insulin-like growth factors: a role in growth hormone negative feedback and body weight regulation via brain. *Science.* **220,** 77-79.

Tannock, I.F. (1965) A comparison of the relative efficiencies of various metaphase arrest agents. *Experimental cell research.* **47,** 345-357.

Tavakkal, A., Elder, J.T., Griffiths, C.E.M., Cooper, K.D., Tawar, H., Fisher, G.J., Keane, K.H., Foltin, S.K. & Vorhees, J.J. (1992) Exoression of growth hormone receptor, insulin-like growth factor-I (IGF-I) and IGF-I receptor mRNA and proteins in human skin. *Journal of investigative dermatology.* **99,** 343-349.

Tomas, F.M., Knowles, S.E., Owens, P.C., Chandler, C.S., Francis, G.L., Read, L.C.

& Ballard, F.J. (1992) Insulin-like growth factor-I (IGF-I) and especially IGF-I variants are anabolic in dexamethasone-treated rats. *Biochemical journal.* **282,** 91-97.

Tomes, F.M., Knowles, S.E., Chandler, C.S., Francis, G.L., Owens, P.C. & Ballard, F.J.(1993) Anabolic effects of insulin-like growth factor-I (IGF-I) and an IGF-I variant in normal female rats. *Journal of endocrinology.* **137,** 413-421.

Turkington, R.W., Majumder, G.C. & Riddle, M. (1971) Inhibition of mammary gland differentiation *in vitro* by 5-bromo-2′-deoxyuridine. *Journal of biological chemistry.* **246,** 1814-1819.

Van Landeghem, A.A.J. & Van de Weil, D.F.M. (1978) Radioimmunoassy for porcine prolactin: Plasma levels during lactation, suckling and weaning and after TRH administration. *Acta endocrinologica.* **88,** 653-667.

Van Scott, E.J. Ekel, T.M. & Auerbach, R. (1963) Determinations of rate and kinetics of cell division in scalp hair. *Journal of investigative dermatology.* **41,** 269-273.

Van Wyk, J.J., Graves, D.C., Casella, S.J. & Gacobs, S. (1985) Evidence from monoclonal antibody studies that insulin stimulated deoxyribonucleic acid synthesis through the type I somatomedin receptor. *Journal of clinical endocrinology and metabolism.* **61,** 639-643.

Wallace, A.L.C. (1979) The effect of hormones on wool growth. in: *Physiological and environmental limitations to wool growth,* pp. 257-268. Editors. J.L. Black & P.J. Reis. Armidale, Australia: The University of New England Publishing Unit.

Wallis, M. (1988) Mechanism of action of growth hormone. in: *Hormones and their actions. **Part 2***, pp. 264-295. Editors. B.A. Cooke, R.J.B. King & J.H. Van der Molen. Amsterdam: Elsevier Science Publishers.

Walton, P.E., Francis, G.L., Ross, M., Brazier, J.A., Wallace, J.C. & Ballard, F.J. (1990) Novel IGF analogues display enhanced biological activity in cultured cells. *Journal of cell biology.* **111,** 2645 (abstract).

Walton, P.E., Owens, P.C., Knowles, S.E., Dunaiski, V., Grant, P.A., Tomas, F.M. & Dunshea, F.R. (1994) Effects of IGF-I analogues on growth and metabolism in pigs. *Growth regulation.* **4S1,** 86.

Wheatley, I.S., Wallace, A.L.C. & Bassett, J.M. (1966) Metabolic effects of ovine growth hormone in sheep. *Journal of endocrinology.* **35,** 341-353.

White, D.H. & Henderson, A.E. (1973) Wool production of Romney ewes and its relationship to cortical cell size. *New Zealand journal of agricultural research.* **16,** 539-544.

Wien, S., Duguette, P.F., Rickes, E.L. & Muir, L.A. (1983) Concentrations of

somatomedin C and anterior pituitary hormones in plasma of wether lambs injected daily with DES or ovine GH for 8 weeks. *Journal of animal science.* **57** (Suppl.1), 210.

Williams, A.J. (1979) Speculation on the biological mechanisms responsible for genetic variation in the rate of wool growth. in: *Physiological and environmental limitations to wool growth,* pp. 337-354. Editors. J.L. Black & P.J. Reis. Armidale, Australia: The University of New England Publishing Unit.

Williams, A.J. (1987) Physiological consequences of selection for increased fleece weight. in: *Merino improvement programmes in Australia,* pp. 481-494. Editor. B.J. McGurick. Armidale, Australia: Australian Wool Corporation.

Williams, A.J. (1991) Wool growth. in: *Australian sheep and wool handbook,* pp. 224-252. Editor. D.J. Cottle. Melbourne, Australia: Inkata Press.

Williams, A.J. & Winston, R.J. (1987) A study of the characteristics of wool follicle and fibre in Merino sheep genetically different in wool production. *Australian journal of agricultural research.* **38,** 743-755.

Williams, A.J., Murison, R.D. & Cross, C.C. (1986) Reactions of cyst(e)ine concentration in plasma of Merino sheep from two genetic groups to fasting and ACTH injection. *Australian journal of agricultural research.* **37,** 657-663.

Williams, A.J., Thornberry, K.J. & Gilmour, A.R. (1990) The activity of the thyroid glands in relation to genetic differences in wool production. *Australian journal of agricultural research.* **41,** 573-581.

Wilson, P.A. & Short, B.F. (1979) Cell proliferation and cortical cell production in relation to wool growth. *Australian journal of biological science.* **32,** 317-327.

Woodall, S.M., Breier, B.H., O'Sullivan, U. & Gluckman, P.D. (1991) The effect of the frequency of subcutaneous insulin-like growth factor-I administration on weight gain in growth hormone deficient mice. *Hormone and metabolic research.* **23,** 581-584.

Woods, E.F. (1979) Microfibrillar proteins of wool: Partial specific volumes and molecular weights in denaturing solvents. *Australian journal of biological science.* **32,** 423-435.

Wynn, P.C., Maddocks, I.G., Moore, G.P.M., Panaretto, B.A., Djura, P. Ward, W.G., Fleck, E. & Chapman, R.E. (1989) Characterisation and localisation of receptors for epidermal growth factor in ovine skin. *Journal of endocrinology.* **121,** 81-90.

Yang, H., Schalch, D.S. & Ney, D.M. (1990) Anabolic effects of insulin-like growth factor-I in calorie restricted and *ad libtum* fed rats. *Nutrition research.* **10,** 1151-1160.

Yu, J., Yu, D.W., Checkla, D.M., Freedberg, I.M. & Bertolino, A.P. (1993) Human

hair keratins. *Journal of investigative dermatology.* **101,** 56S-59S.

Zaho, X., McBride, B.W. Trouten-Radford, L. & Burton, J.H. (1995) Effects of insulin-like growth factor-I and its analogs on bovine hydrogenperoxide release by neutrophils and blastogensis by mononuclear cells. (B. McBirde, Personal communication).

Zapf, J., Rinderknecht, E., Humbel, R.E. & Froesch, E.R. (1978) Nonsupperssible insulin-like activity (NSILA) from human serum: recent accomplishment and their physiologic implications. *Metabolism.* **27,** 1803-1828.

Zapf, J., Schmid, C.H. & Foresch, E.R. (1984) Biological and immunological properties of insulin-like growth factor-I and-II. *Clinical endocrinology and metabolism.* **13,** 3-30.

Zapf, J., Hauri, C., Waldvocgel, M. & Frosech, E.R. (1986) Acute metabolic effects and half-lives of intravenously administered insulin-like growth factors I and II in normal and hypohysectomised rats. *Journal of clinical investigation.* **77,** 1768-1775.

Zapf, J., Hauri, C., Waldvogel, M., Futo, E., Hasler, H., Binz, K., Guler, H.P., Schmid, C. & Froesch, E.R. (1989) Recombinant human insulin-like growth factor-I induces its own specific carrier protein in hypophysectomised and diabetic rats. *Proceedings of the National Academy of Sciences,* USA. **86,** 3813-3817.

Zapf, T., Schimd, C. & Guler, H.P. (1990) Regulation of binding proteins for insulin-like growth factors (IGF) in humans. Increased expression of IGF binding protein-2 during treatment of healthy adults and in patients with extrapancreatic tumour hypoglycaemia. *Journal of clinical investigation.* **86,** 952-961.

Zenobi, P.D., Guler, H.P., Zapf, J. & Frosech, E.R. (1988) Insulin-like growth factors in the Gottinger miniature-pig. *Acta endocrinogica.* **117,** 343-352.