THE GENETICS OF MUSCLE GROWTH IN CHICKENS AND MICE

ALAN E. TINCH

Ph.D

University of Edinburgh

1990

I declare that:

i this thesis was composed by me

ii I carried out the work described in this thesis

ABSTRACT

The development and growth of skeletal muscle is discussed in terms of its structure and synthetic processes. Available evidence on the effects of selection for improved growth is reviewed. Experiments are described which determine the effects on muscle structure and metabolism of selection in chicken and mouse strains.

The pectoralis and gastrocnemius muscles in strains of chicken differing in growth rate are compared in two experiments, the first examining broilers and layers from hatch to 10 weeks of age and the second using seven strains at 7 weeks of age. Selection for improved growth has increased the number and size of nuclear units, the number of nuclei being affected to a greater extent. Satellite cells are therefore implicated in having a major role in increasing muscle growth. The amount of RNA per gram of muscle and the RNA:DNA ratio are also slightly increased showing that selection has increased the protein synthetic capacity of muscle.

Comparison of strains on the basis of muscle weight shows that selection has altered the rate of growth of the number and size of nuclear units more than the relationship between these components.

The number and size of nuclear units is measured in muscles of 10 week old mice selected for lean body mass (P line) and percent fat (F line). Muscle weight in the P lines is modified by changes in the number and size of nuclear units, the number of nuclei being altered more. The F lines do not vary in muscle weight or in muscle composition. Satellite cells (or myoblasts) are again implicated in changing muscle weight.

Muscle fibre type, number and diameter of in growing broiler and layer muscle are compared from hatch to 15

weeks. No difference in the proportion of type I or type II fibres is seen between strains. Fibre number does not increase with age and broilers have more muscle fibres than layers. Broilers have larger fibre diameters than layers after 7 days of age and because of the volumetric relationship between fibre diameter and muscle weight, the increase in fibre diameter is concluded to be more important than fibre number in improving muscle weight of broilers. The role of satellite cells in altering fibre number and diameter during growth is discussed.

No variation in fibre diameter is seen in the P and F lines of mice when fibres are classified according to type. Fibre number could not be measured in this experiment. A mechanism whereby change in fibre type would alter mean fibre diameter and therefore muscle weight without changing fibre number is suggested.

The rate of autolysis is rejected as a potential method of estimating the rate of muscle protein degradation in mice and chickens.

Injection of the protease inhibitors leupeptin and pepstatin into growing mice is shown to be ineffective in increasing muscle mass. Protease inhibitors are suggested to affect growth via their action on hormone receptors.

ACKNOWLEDGEMENTS

I am indebted to the following people who carried out some of the experimental analysis described in this thesis: Laura Dick and Irene Anderson who carried out sectioning and histochemistry of muscle; Bob Paton who carried out cathepsin assays and some protease inhibitor injections.

I would like to thank Dr. Jim McKay for his enthusiasm and encouragement throughout the past five years. I am also grateful to Prof. W.G. Hill and Dr. Grahame Bulfield for their helpful suggestions and support during the revision of this thesis.

I gratefully acknowledge the support of Ross Breeders Ltd who allowed me use of 'out of hours' computing facilities during the past nine months.

I would like to take this opportunity to thank my mother and father for their encouragement and support, financial and otherwise, throughout my time at School and University. My father also deserves thanks for proof reading the text.

Finally, thanks are due to my wife, Gina, who, for the past 10 years, has been there whenever I needed her.

CONTENTS

CHAPTER 1. INTRODUCTION

1.1 MAN AND MEAT PRODUCTION

Improving the efficiency of meat production is the aim of animal breeders. One of the ways of improving efficiency is to increase the rate of growth. Modern breeds of chicken (*Gallus domesticus*) grow faster and to a larger size than their ancestors. A consequence of these increases is the faster growth of muscle, but little is known of how increase occurs at the level of the muscle cell.

Such knowledge is needed for several reasons. The attention of large poultry processors and breeders is moving away from fast growth to market weight towards the yield of meat and efficiency of growth. Knowledge of the effects of selection on muscle growth are important when considering selection for improved meat yield and efficiency of muscle growth. Improving meat quality is also one of the aims of the large breeding companies. The physiology and biochemistry of muscle cells are important with respect to the eating quality of meat (Greaser, 1986). A greater understanding of muscle physiology and biochemistry will help in this regard.

New technology in genetics has led to the possibility of improving production traits by injection and incorporation of novel genes into the zygotes of farm animals (Pursel *et al.*, 1987) and these techniques might be used to improve meat production (Palmiter *et al.*, 1982; Pursel *et al.*, 1987), but until the genes which control muscle growth are identified, manipulation of this kind can not be attempted. Further, until the processes underlying muscle growth are better understood, 'trait' genes will be hard to identify.

This thesis will attempt to draw conclusions concerning the control of muscle growth by examining the muscle growth of strains of chicken and mice (*Mus domesticus*) which differ in rates of muscle growth as a result of selection.

1.2 MUSCLE STRUCTURE

Meat is the skeletal muscle of meat-producing animals. Any review of the structure of muscle must consider its function in the live animal which is mainly to produce movement . Muscle takes the energy stored in the chemical bonds of ATP and transforms it into the kinetic energy of contraction. Each component of muscle has a function in this process

There are many good reviews of muscle structure. The following is a summary of muscle structure including aspects important to experiments carried out in this thesis. Similar reviews of muscle structure can be found by Swatland (1984), Waterlow *et al.* (1978), Goldspink (1977), Dutson and Carter (1985) and in text books such as Stryer (1981).

Muscle is composed of many muscle cells held together in bundles (fasiculi) by successive layers of connective tissue. The connective tissue which transmits the forces of contraction generated by the muscle cells, via tendons, to the skeleton (or skin) is made up of two proteins: collagen and elastin.

Muscle cells are relatively unusual cells. They are extremely elongated (up to 2 cm in length) and contain several hundred nuclei per cell which has resulted in their being called muscle fibres or myofibres. Muscle fibres are bound by plasma membrane and a basement membrane composed of collagen and a group of cells known as satellite cells are found between these two membranes.

These cells provide the new nuclei necessary for muscle growth and regeneration (Swatland, 1984).

Within the muscle fibre are the myofibrils which constitute more than half of the cellular protein. It is the myofibrils which generate the striated pattern characteristic of skeletal muscle when viewed under the microscope. This striated pattern is caused by the arrangement of the myofilaments within the myofibril. There are two types of myofilament: thick and thin; the thick myofilaments consist mainly of the protein myosin but contain other proteins (e.g. myomesin, creatine kinase, I protein, C protein, titin). Thin filaments consist mainly of actin but other proteins are again present (tropononin, tropomyosin, actinin). The filaments interdigitate with the different areas of overlap causing the striated pattern mentioned above. Interdigitation allows the fibrils to contract by the concerted movement of filaments relative to each other. During contraction, ATP is hydrolysed by myosin in the thick filament causing movement of a portion of the myosin molecule which is bound to actin in the thin filament. When this happens *en masse*, the filaments slide relative to each other, shortening the myofibril and therefore the muscle.

Closely associated with the myofibrils is the sarcoplasmic reticulum which is derived from the smooth endoplasmic reticulum of the muscle fibre. The sarcoplasmic reticulum functions in the control of muscle contraction by releasing calcium in response to a nerve impulse. The increase in concentration of calcium causes conformational changes in the thin filament allowing interaction of actin and myosin.

A population of cells known as satellite cells are intimately associated with the muscle fibres. These cells are important in muscle growth and repair of muscle after injury and are found between the plasma and basement

membranes of muscle fibres. This distinguishes them from true muscle nuclei (myonuclei) which are found beneath the plasma membrane within the muscle cytoplasm (Swatland, 1984). Because of their roles in growth and repair, the suggestion has been made that satellite cells are myoblasts that have been trapped beneath the basement membrane but have not fused with the fibre (Swatland, 1984; Allen, 1987). During muscle growth, mitosis occurs in the satellite cells and one or both of the daughter nuclei are passed into the muscle fibre. Mitosis in satellite cells has been observed by electron microscopy and autoradiography (Swatland, 1984). After muscle injury, satellite cells have been observed to move to the site of injury and go through a similar developmental pattern as is observed in normal muscle growth (Swatland, 1984; Salminen, 1985).

Other cell types are also found in muscle including adipocytes, fibroblasts and endothelial cells associated with capillaries. These cell types are readily identified under the light microscope (Swatland, 1984).

1.3 MUSCLE DEVELOPMENT

Muscle develops as a result of several processes occurring during embryonic and post-natal development. These are shown in diagrammatic form in Figure 1.1. Cells (pre-myoblasts) derived from the mesoderm migrate to the site at which the muscle will form and go through several rounds of cell division. The myoblasts formed in this way align and fuse to form multinucleate structures called myotubes (Allen *et al.*, 1979). The control of the processes of migration, division and fusion is not completely understood, however, theories involving fixed numbers of cell divisions of myoblasts before fusion have been proposed (Quinn, 1985). Two waves of migration, division and fusion take place (Miller and Stockdale, 1986a and b; Weydert *et al.*, 1987) forming two

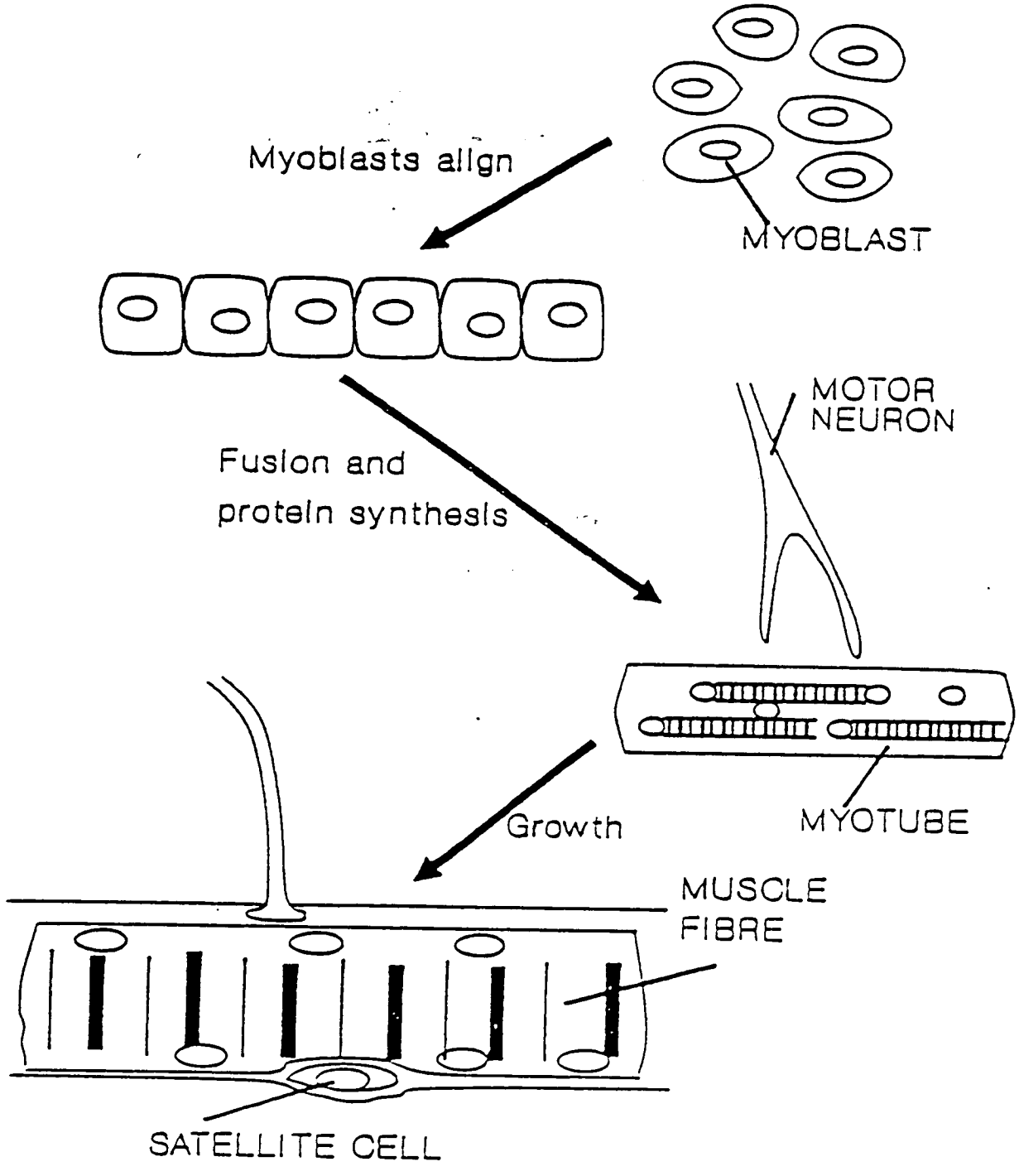


Figure 1.1. Diagramatic representation of muscle fibre development. After Goldspink (1977).

populations of myotubes, primary and secondary, which have different developmental fates with respect to muscle fibre type (see Section 1.4.1.2).

After fusion takes place, no further nuclear division occurs within the myotube (Goldspink, 1977; Swatland, 1984).

At a point coinciding with the second wave of myotube formation, motor neurons growing out from the central nervous system (CNS) establish contacts with the myotubes. These contacts may be essential for the formation of secondary myotubes (Weydert *et al.*, 1987; Harris, 1981). Neural contact is also important with respect to the determination of fibre type through causing variation in the types of contractile protein synthesized during growth of myotube (Goldspink, 1977).

At around the time of fusion, perhaps as a consequence of the withdrawal of nuclei from the cell cycle (Allen *et al.*, 1979), the synthesis of myofibrillar proteins begins (Goldspink, 1977; Swatland, 1984). Accretion of myofibrillar and other proteins within the myotube causes growth and maturation of the myotube into a muscle fibre. Although new myotubes are formed throughout embryonic development (Ontell and Kozeka, 1984) it is accepted that the number of muscle fibres in a muscle is fixed at birth or hatch (Goldspink, 1977; Swatland, 1984). All subsequent growth of a muscle is due to increase in the size of muscle fibres (hypertrophy).

This is not to say that nuclear division plays no part in muscle growth after embryonic differentiation. Satellite cells (see Section 1.2) retain the ability to go through mitosis and donate daughter nuclei to the growing muscle fibre (Goldspink, 1977; Allen *et al.*, 1979; Swatland, 1984).

Considering the structure of muscle and the processes occurring during its development, it can be seen that there are two cellular units of muscle: the muscle fibre and the muscle nucleus. Figure 1.2 outlines muscle cell growth in terms of the growth of these units. It shows that the growth of a muscle fibre can be partitioned into two processes: hyperplasia (increase in number of nuclei) and hypertrophy (increase in muscle fibre size). Since protein is the major component of muscle (apart from water), protein accretion accounts for the largest part of muscle fibre growth.

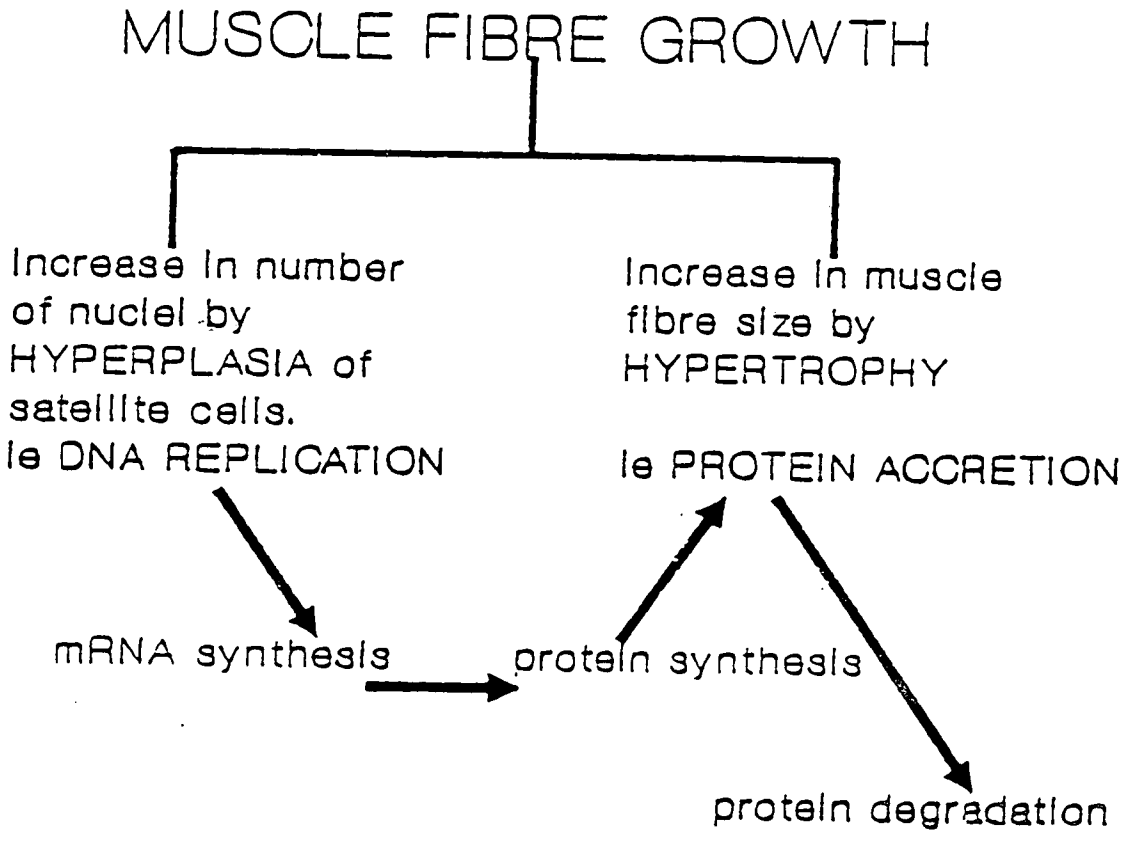
The inter-relation of hyperplasia and hypertrophy is also seen from Figure 1.2. Protein accretion occurs as a net result of two opposing pathways: protein synthesis and protein degradation. Protein synthesis in the cytosol is directed by molecules of messenger RNA (mRNA) in a process known as translation. mRNA is synthesized from a template of DNA in the nucleus in a process known as transcription. Hyperplasia and hypertrophy are therefore linked by the processes of transcription and translation. Any study of muscle growth must take all these processes into consideration.

The rates of protein accretion, protein synthesis and protein degradation are linked by the equation:

Accretion = Synthesis - Degradation

For muscle growth to occur, the rate of protein synthesis must exceed the rate of protein degradation (providing the amount of protein per gram wet weight of muscle stays constant). Early studies of protein accretion during growth concentrated on protein synthesis, but the high rates of protein degradation associated with growth, particularly of muscle, has meant that more recent studies of growth have examined both processes (Waterlow

Figure 1.2. Relationships between components of muscle growth.



et al., 1978). This research is discussed in Section 1.4.1.

Muscle size can therefore be described in terms of two units: nuclei and muscle fibres. In addition, the growth of individual muscles, or the entire musculature can be described. Muscle growth can be considered in terms of the growth of these units and the rates of processes under-lying this growth, i.e. DNA replication, protein synthesis (transcription and translation) and protein degradation. The experiments described in this thesis are concerned with how selection has altered these processes to increase muscle growth.

1.4 VARIATION IN MUSCLE GROWTH RATE

The literature on variation in the rate of muscle growth can be divided into that which deals with changes brought about genetically (usually by selection) and that brought about by environmental manipulation. There are many examples of the former since selection for almost any character of importance to meat production will alter the rate of body and/or muscle growth. In the latter, I shall include experiments which alter muscle growth by changes in nutrition (for example: comparison of the effects of normal and protein deficient diets - MacDonald and Swick, 1981), forced changes in muscle activity (for example: by immobilisation - Goldspink, 1977a and b) and alteration of endocrine status (for example: by hypophysectomy - Cheek *et al.*, 1971). Both approaches give valuable insight into how muscle growth may be manipulated and I shall discuss each separately.

1.4.1 The effect of selection on muscle growth

The effects of selection on muscle growth have been examined in several species. The experiments include farm animals such as cattle, pigs and chickens which are

important in commercial meat production and species such as mice and quail which have been selected experimentally in attempts to model commercial species. In many of the commercial species, e.g. cattle, the exact history of the selection is not known. Breed differences can only be observed and assumptions made about the nature of selection. In recent decades commercial selection in pigs and poultry has become more intensive making it possible to carry out strain comparisons where the objectives of selection are known (or can be worked out).

The literature on genetic effects on muscle growth will be described under three headings: the nuclear unit, the muscle fibre and whole muscle protein accretion. This is in accordance with the different levels at which muscle growth has been analysed and described in Section 1.3.

1.4.1.1 The nuclear unit

This approach considers muscle growth and development in terms of changes in the number of nuclei and the size of the nuclear unit (nucleus plus associated cytoplasm). The number of nuclei is estimated by measuring the total amount of DNA in a muscle. Cheek *et al.* (1971) and Cheek (1985) have defined the DNA unit as the ratio of protein to DNA in a muscle. They consider this a more convenient unit than the reciprocal of the DNA concentration (µg/g wet weight) since it reflects the protein metabolism of muscle and because during normal, post-natal muscle growth (i.e. with no nutritional or hormonal imbalances) there is a constant ratio of cell protein to water in the muscle fibre (Cheek *et al.*, 1971). Since 1971 the DNA unit has gained acceptance as a logical approach to the analysis of muscle growth and metabolism. However, since several authors pre-1971 used slightly different approaches to estimate the size of the nucleus-plus-cytoplasm unit the term 'nuclear-unit' will be used as a general term when discussing their analyses.

Of the commercial species examined, the chicken has received most attention (Moss *et al.*, 1964; Mizuno and Hikami, 1971; MacDonald and Swick, 1981; Henteges *et al.*, 1983; Kang *et al.*, 1985a; Jones *et al.*, 1986a and b). The consensus of results from these studies comparing heavy breeds with lighter breeds is that increased muscle growth is associated with an increase in the number of nuclear units and a small or no increase in the size of the nuclear unit. However, the validity of comparing these studies might be questioned since different authors compare different muscles from different strains using different methods. There are no published data which allow comparison of the number of nuclei and size of nuclear units between modern fast and slow growing strains on the basis of age or muscle weight. The latter comparison is important since it allows us to investigate whether selection has fundamentally altered the way (as opposed to the rate) in which a muscle grows.

Muscle growth in the pig has been investigated with respect to number and size of nuclear units (Ezekwe and Martin, 1975; Powell and Aberle, 1975; Harbison *et al.*, 1976; Hausman and Campion, 1986). Again, larger muscles are associated with increases in the number of nuclei and similar, or slightly smaller DNA units. Several of these authors were, however, concerned primarily with the effects of obesity on muscle growth (Harbison *et al.*, 1976; Hausman and Campion, 1986) so comparison of results not be justified.

Breeds of cattle have been compared for these components. Trenkle *et al.* (1978) compared steers from Charolais and Angus bulls at the same live weights. These two groups had similar muscle weights, total DNA and DNA unit size. Solomon *et al.* (1986) compared purebred Angus and Brahman bulls at percentages of the average dam weight for respective breeds. Brahman cattle have larger mature size

so in this analysis animals of different size are compared. Brahman bulls had more nuclei per muscle but similar DNA unit size. Direct comparison of animals at the same age or muscle weight is not possible from these studies.

Three species have been used as models of muscle growth in farm animals: quail, mice and rats.

Quail selected for high four week body weight for over 60 generations have been used as a model for long term selection in meat type poultry (Marks, 1983). These lines (high body weight and control) were compared for number of nuclei and DNA unit size by Fowler *et al.* (1980) and Campion *et al.* (1982b). Both these papers show that increased muscle size in the selected strain is associated with an increase in total DNA. DNA unit size was also increased, but this difference was insignificant.

Several authors have investigated the number of nuclei in strains of mice selected for high (and low) growth rate (Robinson and Bradford; 1969; Ezekwe and Martin, 1975 and Aberle and Doolittle, 1976). In all three studies, increased muscle growth was associated with increased number of nuclei. Only Robinson and Bradford (1969) estimate nuclear unit size. They show a slight fall in nuclear unit size in their high growth line. They suggest that their selection regime (high post- weaning gain) has acted to decrease the size of the DNA unit.

The other model species which has been used is the rat. The strains used are not selected lines but may be considered in the same way as breed differences in cattle and pigs described above. Waterlow *et al.* (1978) describe a comparison of the hooded (slow growing) and CFY albino (fast growing) strains of rat. In this case, faster muscle growth is associated with increased total DNA but

little change in DNA unit size. Durschlag and Layman (1983) compare lean and obese Zucker rats. Although results from different muscles vary slightly, the smaller muscle mass of the obese rats is associated with a smaller number of nuclei but similar DNA unit size.

Over all the studies, it seems that increased growth of muscle is associated with a proportionate increase in the number of nuclei and a small or no increase in the size of the nuclear unit. In none of the species investigated, are the data clear cut. Among other reasons this is due to the comparison of inappropriate strains or incomplete analysis. The data for chickens are largely out of date. The greatly improved muscle growth of the modern broiler is an obvious place to investigate how selection alters muscle growth at the level of the nucleus.

1.4.1.2 The muscle fibre

The post-natal growth of the muscle fibre is well reviewed by Swatland (1984). I shall review the main observations and conclusions discussed in this text and add some observations of my own.

Three variables are important with respect to muscle fibre growth. These are: muscle fibre type, muscle fibre size and muscle fibre number. These variables act and interact to change the size of muscles during growth.

The literature on muscle fibre type is complicated. There are several reasons for this, the major one being that muscle fibre type is the result of several different biological processes. It depends on the activity of several metabolic enzymes, the type of contraction and the type of innervation received by the muscle fibre. These factors may alter during the growth and development of a muscle fibre.

The most relevant work to this study was published in 1971 by Ashmore and Doerr. These authors investigated the postnatal development of fibre type in the chick and compared this to the development in other species (mouse, cattle and pig) (Ashmore and Doerr, 1971a and b). They used several histochemical stains to define the major fibre types but also showed how these fibre types could be identified using one (or two) staining methods on single muscle sections. Although Ashmore and Doerr's nomenclature has been slightly altered since 1971, their analysis is still the basis of accepted methods of fibre typing (Barnard *et al.*, 1982). The importance of their analysis is in the acceptance of the important roles of enzyme activity, rate of contraction and innervation in the production of an individual fibre type. Previous classifications had attempted to use only one of these factors to specify fibre type.

The different fibre types, their nomenclature and physiological activities are shown in table 1.1. This table follows the classification of Barnard *et al.* (1982) but the nomenclature used by Ashmore and Doerr (1971a and b) is also given. The table describes the properties of chicken muscle fibres which differ in several ways from that of mammals, notably in the innervation of type I fibres and the presence of type III fibres in some muscles. Such differences are not of great relevance to this analysis. Alternative patterns of innervation reflect the different evolutionary history of birds and mammals, and are not likely to cause fundamental variation in growth of muscles within species. Type III fibres are tonic, contracting over long periods to, for example, hold the wings in the folded position. Tonic contraction is carried out by type I fibres in mammals, but again, this is not likely to cause variation in growth rate within species.

Table 1.1. Summary of the nomenclature and major physiological properties of different fibre types in the chicken.

	FIBRE TYPE				
Barnard et al. (1982)	I	IIA	IIB	IIIA	IIIB
Ashmore & Doerr (1971)	β-red	α-red	α-white	α'	β'
Metabolism	red oxidative	red/white intermed.	white glycolytic	red/white intermed.	red/white intermed.
Contraction	slow twitch	fast twitch	fast twitch	tonic	tonic
Innervation	multiple	focal	focal	multiple	multiple
ATP-ase (pH 10)	weak	strong	strong	intermed.	strong
Size	small	medium	medium	large	medium
Lipid inclusions	no	yes	no	no	no

Table 1.1 describes the physiological state of the fibres in the adult. During development the state of individual fibres may change (Ashmore and Doerr, 1971b; Guth and Samantha, 1972; Barnard *et al.*, 1982). This is especially important in the chicken since at slaughter weight the chicken is still juvenile and the fibres of young animals have high aerobic activity (i.e. are classified as red, having a high concentration of oxidative enzymes). During subsequent growth, there is a gradual change from aerobic to anaerobic metabolism, (i.e. fibres become whiter, more glycolytic). The extent to which this anaerobic activity develops depends on the genetic constitution of the animal, the position of the muscle and the amount of activity the muscle is subjected to. Highly active muscles are more aerobic (red, oxidative) (Swatland, 1984).

Only the fibres derived from the second phase of myotube formation (secondary myotubes: type II fibres) appear to be able to alter their metabolism. Fibres derived from primary myotubes (type I fibres) seem incapable of changing fibre type (Swatland, 1984).

Selection for increased growth in pigs and chickens seems to have increased the anaerobic potential of muscles (Aberle *et al.*, 1979; Swatland, 1984). However, since many early authors were unaware of the plasticity of fibre types, work in other species (where it exists) is unreliable (Swatland, 1984).

Swatland (1984) reviews the literature on muscle fibre number and its effects on muscle size. Obviously animals with larger numbers of fibres will have larger muscles (provided fibre size is similar). Swatland (1984) also points out the problems in measuring fibre number and shows that many precautions must be taken when attempting to measure muscle fibre number in meat animals. Taking account of possible inadequacies in technique, several

studies have attempted to measure the effect of selection for increased growth on muscle fibre number. In cattle, sheep and pigs, selection has increased the number of fibres in individual muscles (Swatland, 1984). Similar analyses in the chicken have led to similar conclusions (Smith, 1963; Moss, 1968a; Mizuno and Hikami, 1971; Holcman and Zagozen, 1980), but this work is largely out of date as modern broiler chickens grow very much faster than the chickens used in these analyses. Current broilers reach weights of over 2 Kg at 40 days whereas at this age 1972 broilers would only weigh 1.3 Kg (Ross Breeders Ltd, personal communication).

A condition known as double muscling has occurred in cattle and sheep and is associated with changes in muscle fibre number (Swatland, 1984; Hanset, 1986). The syndrome is found at high frequency in the Belgian Blue breed of cattle and is due to a single, autosomal recessive gene. Double muscling is characterised by gross increase in size of several muscle groups caused primarily by increased numbers of fibres in affected muscles. This increase is shared disproportionately between fibre types, affected individuals having larger numbers of glycolytic (white) fibres (Swatland, 1984; Hanset, 1986). Double muscling may be caused by accelerated or increased persistency of mitosis in myoblasts during foetal development (Hanset, 1986).

The data for model species are considered by Swatland (1984) to be more accurate than comparable data in large animals since experimental analysis is easier in small muscles.This is because the number of fibres in a muscle can be counted in small animals, whereas fibre number can only be estimated in large animals.

In the quail, long term selection for increased growth has resulted in an increase in muscle fibre number (Fowler *et al.*, 1980). This increase was shared

disproportionately between fibre types with ß (type I) fibres increasing by 7% and α (type II) fibres increasing by 59%.

In the mouse there are several studies looking at effects of selection on muscle growth and its components (Luff and Goldspink, 1967, 1970; Byrne *et al.*, 1973, Hanrahan *et al.*, 1973, Ezekwe and Martin, 1975; Aberle and Doolittle, 1976). All these studies show a tendency for fibre number to increase with growth rate.

In the rat there are no studies which allow comparison of muscle fibre number between fast and slow growing strains (Swatland, 1984).

Swatland (1984) also reviews the effect of selection for increased growth on muscle fibre size. Although the muscle fibre is a three dimensional structure having length as well as cross sectional area (CSA), most authors regard fibre diameter or CSA as an adequate estimate of fibre size. Whether this is justifiable is the subject of debate (Moss, 1968a; Swatland, 1978 and 1984).

Postnatal growth of sheep, pigs and cattle is associated with increase in muscle fibre diameter. The data for strains or breeds of these species is somewhat inconclusive (Swatland, 1984). Breed differences considered on an age basis disappear when strains are compared at similar liveweights. There is some evidence to suggest that a similar situation exists in chickens (Smith, 1963). However, other workers can demonstrate no difference in fibre diameter between large and small strains of chicken whether considered on an age or muscle weight basis (Mizuno and Hikami, 1971). Overall, the data for farm animals are inconclusive.

In the quail selected for increased growth discussed above, analysis has shown that the diameter of type I (ß) fibres is increased by selection, whereas type II (α) fibres are unchanged (Fowler *et al.*, 1980). When considered in conjunction with fibre number, it was concluded that although both fibre number and diameter had changed during selection, the net contribution of **each fibre type to the size of the muscle was unchanged.** This analysis makes an important point. When considering strain differences in muscle growth it is important to consider all variables; muscle fibre type, number and size may all change to alter muscle size.

The possibility of selecting on fibre diameter or fibre number has been considered on several occasions (Stickland and Goldspink, 1973; McCarthy and Shiel, 1975; Holcman and Zagozen, 1980). Stickland and Goldspink (1973) developed the idea of using a small muscle in the foot of the pig as an indicator of fibre number in the rest of the musculature. Fibre number in this muscle was highly correlated with fibre number in major carcass muscles. Although this indicator muscle was subsequently used to analyse fibre number in nutritional comparisons (Stickland *et al.*, 1975) and to test breed differences associated with fat levels (Stickland and Goldspink, 1975), its use as a selection criterion has not been reported. Recently, this muscle has been used in pigs to indicate the post-natal growth performance of piglets (Handel and Stickland, 1988). McCarthy and Shiel (1975) report a small scale selection experiment in mice using fibre diameter as a character for selection. After four generations of selection, no difference in fibre diameter was observed. Holcman and Zagozen (1980) report the heritability of fibre diameter and number in broilers to be 0.13 $\pm$ 0.08 and 0.27 $\pm$ 0.08 respectively.

1.4.1.3 Muscle protein accretion

Section 1.2 describes the structure of muscle and the function of the proteins which make up much of the muscle. Skeletal muscle contains between 15 and 20% protein. This is the major, non-water component of muscle so an understanding of the processes underlying the deposition of protein is fundamental to a complete understanding of muscle growth.

Much of the relevant work on the growth of muscle protein is summarised and reviewed in Waterlow *et al.* (1978). Subsequent work has only underlined conclusions discussed in this text. I shall discuss the major observations of these authors but the reader should consult Waterlow *et al.* (1978) for a more complete review of this area.

As is discussed in Section 1.3, the rate of protein accretion in muscle depends on the rate of protein synthesis and the rate of protein degradation. These processes are related by the equation:

accretion = synthesis - degradation

If muscle growth rate is to be increased (for example: by selection) the rate of protein accretion must also be increased, provided the concentration of muscle protein remains the same. An increase in protein accretion may come about via an increase in synthesis, a decrease in degradation or some combination of these two. Research in this area has attempted to establish which option occurs.

In order to discuss changes in protein accretion, the major problems in measurement of protein synthesis and degradation need to be stressed. To measure protein synthesis or degradation the system is usually perturbed in some way (for example: by injection of labelled amino acids). The experimenter cannot be sure that the measured rate is identical to the rate prior to disturbance.

Another problem concerns independent measurement of the rates of synthesis, degradation and accretion which are linked within the animal. An estimate of synthesis, for example, may affect the rate of degradation and *vice versa*. As such, independent measurements may not give a complete view of the system.

Protein synthesis is usually measured by supplying the animal or tissue with a radioactive precursor (amino acid) and then measuring the rate of incorporation into protein (Zak *et al.*, 1979; Waterlow *et al.*, 1978). Aside from various technical problems involving pools of precursors, the major problem with such measurements is one of expense. Protein synthesis estimates need 'flooding' doses of precursor which are prohibitively expensive in large animals. Much of the work examining rates of protein synthesis involves the rat (Waterlow *et al.*, 1978) or *in vitro* studies using isolated tissues. However, even in the rat, the expense in terms of money and time is large meaning that few studies have examined enough individuals to make meaningful conclusions concerning genetic effects on protein synthesis. Because of the aforementioned problems, the effects of selection on protein synthesis are not completely understood.

Protein degradation can be measured in four ways (Ballard, 1977):

1. rate of protein loss or amino acid production after insertion of labelled proteins.

2. rate of protein loss or amino acid production after labelling protein with radioactive precursors.

3. rate of production of non-metabolisable product from protein of interest.

4. indirect calculation of degradation rate from rates of protein synthesis and accretion.

Of these methods, the latter three are the methods usually used in studying growth. The first method is more suited to the breakdown of specific types of protein (Ballard, 1977). A major problem with measurements of protein degradation is re-incorporation of newly liberated amino acids. This problem is not seen in method 3 where the rate of production of non-metabolisable product is measured. This stratagem is best exemplified by studies which use the excretion rate of 3-methyl histidine (3 MeHis) as an index of protein degradation (Ballard, 1977; Saunderson and Leslie 1983; Harris and Milne 1981a and b). These experiments use the fact that histidine residues in actin and myosin of skeletal muscle are methylated and upon degradation of these proteins, the 3 MeHis is excreted. Although this method has been validated in cattle and pigs (Harris and Milne 1981a and b) measurements in sheep are inaccurate (Harris and Milne, 1980). In chickens, its use is the subject of some controversy because of the purported existence of a pool of labile 3 MeHis in adults (Harris *et al.*, 1987). However, several sets of authors have used this method in growing birds (Saunderson and Leslie, 1983; Maeda *et al.*, 1984; Hayashi *et al.*, 1985; Jones *et al.*, 1986a). Method 4 is the method most commonly used for growth studies (Waterlow *et al.*, 1978) but its use can be criticised because of the problems inherent in measuring protein synthesis and because of the confounding effects of errors in measurement of synthesis and accretion.

Published levels of protein synthesis in rats and chickens are shown in Table 1.2 to indicate 'typical' levels and to make several important points. Muscle protein synthesis, degradation and accretion are high in young animals, and fall with increasing age. Protein degradation falls less than protein synthesis causing the developmental decline in accretion and resulting in a zero rate of accretion at maturity, although synthesis

Table 1.2. Fractional rates of protein synthesis, degradation and accretion (% per day) in fast and slow growing strains of rat and chicken.

Species	Strain	Age (days)	Synthesis (%/day)	Degradation (%/day)	Accretion (%/day)
Rat (1)	Hooded (fast)	23	28.6	22.5	6.1
		330	4.9	4.9	0
(1)	CFY (slow)	25	15.6	9.8	5.8
		320	4.5	4.5	0
Chicken(2)	White	7	42	26	16
	Leghorn	42	17	14	3
(3)	Broiler	7	48	16	33
		42	16	12	4

(1) Bates and Millward (1981)
(2) MacDonald and Swick (1981)
(3) Kang et al. (1985)

and degradation still occur. Different rates of synthesis and degradation can result in similar rates of accretion. Table 1.2 shows that hooded and CFY rats have similar rates of accretion but the former has approximately double the rates of synthesis and degradation.

A developmental decline in synthesis, degradation and accretion has been observed in all species studied (Waterlow *et al.*, 1978; MacDonald and Swick 1981; Davies *et al.*, 1981; Henteges *et al.*, 1983; Lauretio *et al.*, 1983; McCarthy *et al.*, 1983; Kang *et al.*, 1985a and b; Mulvany *et al.*, 1985; Jones *et al.*, 1986a and b; Maeda *et al.*, 1984)..

The studies summarised in Table 1.2 conclude that the primary cause of increased muscle protein accretion in faster growing strains is reduced rates of protein degradation. This has been reported on several occasions in the rat (Waterlow *et al.*, 1978) and in the chicken (Maruyama *et al.*, 1978; Saunderson and Leslie 1983; Maeda *et al.*, 1984; Saunderson, 1984 , Bryan *et al.*, 1985a and b; Hayashi *et al.*, 1985; Kang *et al.*, 1985a; Klasing *et al.*, 1987; Jones *et al.*, 1985a and b). Studies in large animals have been prevented due to the reasons discussed earlier. One study in cattle using 3 MeHis has shown no difference between breeds with different frame size in synthesis, degradation and accretion rates (McCarthy *et al.*, 1983). Most of the evidence therefore points to changes in the rate of protein degradation as the factor causing increases in muscle growth rate due to selection.

This observation should not be surprising from a genetic point of view. Natural selection would be expected to maximise the efficiency of an important physiological activity such as protein synthesis or degradation. Natural selection would also tend to reduce the genetic variation in such an important physiological process. Any mutation occurring would be more likely to have negative

effects on the rate of a process rather than causing increases. Considering a compound process like protein accretion made up of two opposing processes, selection for increased protein accretion is more likely to 'see' variation which reduces protein degradation rather than increases protein synthesis.

The importance of protein degradation in determining the rate of protein accretion has lead to the investigation of the pathways of protein degradation and their possible roles in increasing growth rate. It is generally accepted that cellular components are broken down in the cellular bodies called lysosomes (Stryer, 1981). Lysosomes contain many degradative enzymes including proteolytic enzymes and because of their presence it has been suggested that this system is important in determining the rate of protein degradation (Waterlow *et al.*, 1978; Lindsay, 1983). Ballard (1977) suggests that there are at least two possible routes for protein degradation: one via the lysosome and the other by a substrate dependent protease. Although there have been many reports describing proteolytic enzymes which may be active in muscle (for review see Bond and Butler, 1987) the respective roles of protease systems during normal growth or in strains of animal which differ in growth rate have not been investigated.

In summary, the increased muscle protein accretion brought about by selection is primarily caused by a decreased rate of protein degradation but the method by which this decrease occurs has not been identified.

1.4.2 Alteration of muscle growth by non-genetic means

Muscle growth can be modified by changes in activity, nutrition and hormonal status. At the gross level, increase in activity increases muscle size whereas decrease in activity (for example: by immobilisation in a

shortened position) reduces muscle size. Similarly a reduction in the level of nutrition decreases muscle growth. Change in the levels of various hormones modifies muscle growth. These effects have been used by man to his advantage (for example: weight training by athletes, modification of growth pattern of muscle in male lambs via reduced testosterone levels after castration).

There are too many published reports describing effects of these changes to discuss in this thesis. The reader is referred to Waterlow *et al.* (1978) and Swatland (1984) for more complete discussions. This review is restricted to the general effects of the above changes on the growth of muscle and its components.

1.4.2.1 The nuclear unit

Change in activity pattern causes atrophy or hypertrophy of muscle depending on the type of change. Immobilisation of a muscle in the shortened position causes atrophy, whereas immobilisation in the lengthened position causes hypertrophy. Overall, reduction in activity of a muscle will cause atrophy, whereas increased activity will cause hypertrophy (Goldspink, 1981). A general observation is that where changes in activity cause increase in muscle mass, the size of the nuclear unit is maintained and the number of nuclear units is increased (Waterlow *et al.*, 1978; Goldspink, 1981).

Waterlow *et al.* (1978) discuss effects of malnutrition on the nuclear unit. The extent of malnutrition and the stage of the life cycle at which malnutrition occurs both affect the extent to which the nuclear unit is changed. Marginal malnourishment during the growth of the rat causes reduction in the rate of DNA accumulation (i.e. number of nuclei) but not in the size of the DNA unit. Similar malnourishment in the adult rat causes net loss of protein and reduction in the size of the DNA unit but

not the number of nuclei. More severe malnourishment, completely suppressing growth, causes reductions in both number of nuclei and the size of the DNA unit through reduction in the rate of DNA accumulation and loss of protein from the muscle.

The behaviour of muscle during malnutrition reflects a biological role of muscle which is often overlooked. In periods of malnutrition, skeletal muscle acts as a source of substrates for protein synthesis and energy metabolism in other tissues (Waterlow *et al.*, 1978). This role must be kept in mind when discussing the response of muscle to malnutrition.

Cheek (1985) discusses the effects of hormones on the DNA unit. He considers growth hormone (GH) as the hormone which controls the number of nuclei through controlling the rate of cell division in muscle. Insulin is considered to control cytoplasmic growth and therefore the size of the DNA unit. Sex hormones are considered to mediate the actions of GH and insulin. Glucocorticoids are also known to decrease muscle growth through decreases in total DNA (Waterlow *et al.*, 1978). The somatomedins or insulin-like growth factors (IGF) are thought to mediate many of the above processes but no conclusions as to their direct effects are available as yet (Waterlow *et al.*, 1978; Cheek, 1985).

1.4.2.2 The muscle fibre

Muscle fibre number is fixed at birth or hatch (Swatland, 1984). Changes in activity, nutrition or hormonal status which alter post-natal muscle size must act without changing fibre number and therefore changes in muscle fibre size should reflect changes in muscle size. This prediction is borne out for changes in activity (Goldspink and Ward, 1979; Goldspink, 1980; Swatland, 1984) and undernutrition (Goldspink and Ward, 1979;

Timpson, 1982). This type of analysis has not been extensively carried out for changes in hormonal status. Androgens have been shown to increase muscle size by increasing fibre size in Fallow Deer (Field *et al.*, 1985) and in the temporal muscles of some rodents (Swatland, 1984). There is a suggestion, however, that this effect may be a secondary result of the androgen on activity (Swatland, 1984).

A large amount of work has been published on the effect of exercise on muscle fibre type and this is well reviewed by Goldspink, (1980) and Swatland (1984). The effect of training varies with type, for example: endurance running increases the aerobic capacity of muscles (IIA fibres - red), whereas weight lifting increases glycolytic potential (IIB fibres - white). These changes involve transition in fibre type (Swatland, 1984) and selective hypertrophy of relevant fibre types (Goldspink, 1980). Although there is a genetic contribution to fibre type, the above results suggest that the fibre type of a muscle can be regarded as being fluid within the lifetime of an individual. Muscle fibre type reflects the recent activity history of the individual animal (Swatland, 1984; Perry, 1985).

Goldspink and Ward (1979) describe the effects of undernutrition on fibre type in hamsters and mice. There was no effect on the proportions of different fibre types. All fibre types were reduced in size, but type II fibres were affected to a greater extent than type I.

There are few studies looking at the effect of hormonal status on fibre type except those mentioned above looking at the effect of androgens which seem to cause an increase in aerobic capacity of muscles (IIA - red) (Swatland, 1984; Field *et al.*, 1985). This result may however be a secondary consequence of the effect of androgens on activity.

1.4.2.3 Protein accretion

Muscle may respond to changes in activity by accreting or excreting protein (amino acids). Goldspink (1980) comprehensively reviews the response of muscle in terms of protein synthesis and degradation to changes in activity. Both synthesis and degradation are modulated by changes in activity, meaning that no consistent conclusions concerning the control of muscle growth in these situations may be drawn. Waterlow *et al.* (1978) note, however, that in situations of rapid growth caused by changes in activity, rates of both protein synthesis and degradation are increased. The results reviewed by Goldspink (1980) do not contradict this.

The response of muscle protein synthesis and degradation to malnutrition must be viewed in terms of the role of muscle as a store of amino acids and can be divided into three stages (Waterlow *et al.*, 1978). Initially there is a fall in the rate of protein synthesis with no changes in the rate of degradation. This is followed by a further fall in synthesis accompanied by an increase in degradation when a net loss of protein from muscle may occur. The third stage involves a large increase in degradation and a large net loss of protein from muscle.

The effects of hormonal status on muscle protein accretion are reviewed by Waterlow *et al.* (1978), Goldspink (1980) and Lindsay (1983). Insulin has an anabolic effect on muscle; increased levels increasing protein synthesis and decreasing degradation. Glucocorticoids are considered to be antagonistic to the effects of insulin and this reflects their role as mediators of the body's response to trauma. GH is considered to have effects on protein accretion because hypophysectomy reduces protein synthesis, this activity being restored by administration of GH. The direct effects of GH are hard to analyse in this model since

hypophysectomy reduces the level of other hormones in addition to GH. Cheek (1985) suggests that GH acts on protein accretion via its effects on DNA accumulation. Thus, there may be two sets of receptors in muscle: one controlling protein accretion and responding to insulin, the other controlling DNA synthesis and responding to GH via the IGFs (Waterlow *et al.*, 1978; Cheek, 1985).

Other hormones such as thyroid hormones and sex steroids are considered in these three reviews to have less direct affects on muscle protein accretion. Thyroid hormones are suggested to modulate the action of GH (Lindsay, 1983), sex steroids may also act on protein synthesis and degradation through their effects on secretion of other hormones (Lindsay, 1983). These suggestions may, however, be the result of the lack of data on the effect of these hormones on protein synthesis and degradation.

Prostaglandins have been cited as having a role in controlling muscle protein and degradation. Prostaglandin F2α appears to control protein synthesis whereas prostaglandin E2 controls protein degradation (Reeds and Palmer, 1985).

1.4.2.4 Overall

The way in which the literature on muscle growth is discussed above may suggest that each of the factors mentioned acts independently on the individual components of muscle growth. It must be emphasised that changes in these factors act in concert to change the number or size of nuclear units or the number, size or type of muscle fibres or the rates of protein synthesis or degradation. In this way the muscular status of the animal is altered allowing the animal to cope with changes in its environment.

1.5 MUSCLE CELL CULTURE

Up to this point experiments which use whole animals or tissue derived directly from whole animals have been discussed. Over recent years, techniques for in vitro culture of skeletal muscle have been developed. Proliferating cells from muscle (myoblasts and/or satellite cells) are separated from other cell types and placed in culture medium where they go through a 'normal' sequence of development (Bischoff, 1974). This sequence includes all the events seen in vivo: division, alignment,fusion and growth (Allen, 1987). These techniques can be used to study fundamental aspects of the biology of muscle development.

Allen (1987) reviews the use of these techniques in the study of muscle growth. He cites three sets of events which in vitro culture can be used to study

1. Myogenic cell proliferation

2. Myogenic cell differentiation

3. Muscle-specific protein synthesis

These events can be studied and the influence of extrinsic factors such as hormones, growth factors or nutrients can be analysed. However, caution must be used in translating conclusions from this type of study directly to the development of the animal. In culture, development is independent of important inputs such as neural stimulation which in vivo determines subsequent development of a muscle fibre (Swatland, 1984). Another note of caution concerns the source of myogenic cells used in this type of study. Several muscle cell lines (for example: L6 rat myoblasts) are available which are more easily propagated than repeatedly using primary muscle cell cultures (i.e. myogenic cells isolated directly from growing muscle). These cell lines are, by

definition, deficient in some aspects of the control of cell proliferation. Conclusions regarding the control of cell proliferation using these cell lines must be regarded sceptically (Allen, 1987).

Despite these limitations, culture systems are being used to carry out valuable experimental work on the development of muscle. The effects of hormones, growth factors and nutrients on the proliferation and development of myoblasts are being assessed. New regulatory factors are being isolated (Allen, 1987). Muscle cell culture has been used to identify several sub-populations of myoblast which differ in developmental fate within the seemingly homogeneous population of myoblasts (Miller *et al.*, 1985; Miller and Stockdale, 1986a and b; Schafer *et al.*, 1987). These sub-populations are suggested to go on to form primary and secondary myotubes.

Muscle cell culture technique has also been used to investigate intrinsic differences in cellular differentiation between strains selected for increased growth and unselected strains. Orcutt and Young (1982) compared the development and differentiation of myogenic cells derived from 12 day layer and broiler embryos. Differentiation was qualitatively similar between strains but broilers showed a lower rate of fusion, less myonuclei per culture and accumulated more protein per nucleus than layers. This last difference was largely due to broilers having a lower protein degradation rate. Ridpath *et al.* (1984) report similar experiments but their results are slightly different. Their broiler cultures showed larger numbers of total nuclei, larger numbers of fused nuclei, accumulated more protein per nucleus and responded more to the addition of insulin (by increasing fusion rate and protein accretion) than layer cultures. The differences between these sets of results

may be due to variation in culture techniques or strains, however these findings suggest that intrinsic differences in the behaviour of myogenic cells may exist between strains. This type of study should lead to a greater understanding of how differences in muscle growth rate occur between strains.

1.6 QUESTIONS TO BE ADDRESSED

So far the literature on muscle growth has been reviewed concentrating on no species in particular. This is because valuable work on muscle growth has been carried out in several species and because the central processes operating during muscle growth do not differ qualitatively between species. The experimental work described later in this thesis uses the chicken and the mouse. Both species have strains which differ in muscle growth. In the chicken, there are strains of broiler and layer which differ greatly in the rate of body and muscle growth as a result of selection for increased growth. These strains are replicated in the form of stocks from different commercial breeders, although the extent of contamination between stocks is not known. In the mouse, replicated strains selected divergently for high and low appetite, fat percentage and lean body mass are available (G lines - Sharp *et al.*, 1984).

Results from these strains of mice and chickens have relevance to the agricultural industry because of the importance of the chicken as a meat producing animal and because of the suitability of the mouse and the chicken as model species.

Given the state of knowledge discussed so far and the strains of chicken and mouse at our disposal, several questions are addressed :

1. How are DNA unit size and number of nuclei altered by selection for growth in chickens and mice?

2. How are muscle fibre type, size and number affected by selection for growth in chickens and mice?

3. Can the rate of autolysis be used to estimate the rate of muscle protein degradation?

4. Can muscle growth be altered by injection of inhibitors of protease enzymes?

Questions 1 and 2 are important because the data for modern breeds of broiler chicken or the G lines of mice are not available. Questions 3 and 4 represent attempts to apply currently available knowledge to increase muscle growth.

CHAPTER 2. MATERIALS AND METHODS

2.1 ANIMALS

2.1.1 Chickens

Most of the strains used in these experiments were obtained from commercial breeding companies. Some of these strains were grandparent strains, others were production type stock. The selection history of these commercial stocks is not freely available but the type of selection used can be surmised. Broilers have been selected for increased weight at an age to increase the rate of growth. Other factors such as food efficiency and conformation have also been used to varying extents. Layers have been selected for increased egg production, and other factors important with respect to the efficiency of egg production. In the experiments described here layers are used as controls since their growth rates are low compared to broilers.

The housing and husbandry of birds was kept as similar as possible between strains. Birds were obtained on the day of hatch and brought to the Poultry Research Centre, Roslin (now the Institute of Animal Physiology and Genetics Research). On this day, birds were vaccinated against Mareks Disease and wingbanded. Chicks were housed in electrically heated brooders until a maximum of four weeks when they were transferred to 40 inch by 48 inch wire runs. Transfer to wire runs occurred at less than four weeks for some of the faster growing strains because of their faster growth. All birds were allowed *ad libitum* access to food and water. All strains were grown on standard PRC broiler rations. Birds were maintained on the standard lighting regime at PRC of 14h light:10h dark.

In one experiment (Chapter 4) as many strains as were available were compared at one age. In this case it was not possible to treat all birds in exactly the same way since several strains were grown as part of other scientists' experiments.

2.1.2 Mice

The selected strains of mice used in these experiments were from generations 21 to 30 of the P and F lines generated in a selection experiment run by Professor W.G. Hill at the University of Edinburgh. The exact details of the base population and selection procedures are described by Sharp *et al.* (1984). Both lines have three high, three low and three random bred control replicates.

The P lines (P=protein) were selected for lean body mass using the index body weight - (8 X gonadal fat pad weight) in 10 week old males. The gonadal fat pad (GFP) represents approximately one eighth of total body fat so this index is a phenotypic predictor of lean body mass. Selection was carried out within families. Bishop and Hill (1985) show that at generation 14 the high replicates of the P line (PH) were heavier but had similar carcase composition to the low replicates (PL) mice. Carcase composition was also analysed in generation 20 (Hastings and Hill, 1989). The PH mice again showed greater (approximately 50% of the control line mean) 10 week body weight and lean body mass than PL mice but similar body composition between lines.

The F lines (F=fat) have been selected using the ratio of GFP weight to body weight as the selection criterion. Selection was carried out in 10 week males in the same way as in the P lines. To prevent confusion the high and low lines will be referred to as 'Fat' or 'Lean' lines. Bishop and Hill (1985) showed that in generation 14 the fat line was heavier but had similar lean body mass to

the lean line. Analyses in generation 20 (Hastings and Hill, 1989) showed that the fat line mice were slightly heavier (approximately 9% of the control mean) than lean line mice but were very much fatter than lean lines (150% of the control line mean in terms of GFP/body weight and 90% of the control line mean in terms of % fat).

Inbred lines of mice were used in one experiment were obtained from Bantam and Kingman Limited.

Mice were obtained at weaning and maintained until needed at IAPGR, Roslin. Mice were allowed free access to standard mouse ration and water at all times.

2.2 MUSCLES

In the chicken, all muscle analyses were carried out on two muscles: the *m. pectoralis superf.* (pectoralis muscle: PM) and the *m. gastrocnemius* (gastrocnemius muscle: GM).

The PM is the major breast muscle of the chicken. It originates on the sternum, clavicle and sternal ribs and inserts on the humerus acting to pull the wing down (Nickel *et al.*, 1977). This muscle forms part of the most desirable portion of the chicken and is therefore important to the poultry industry. Its size and shape may have been under selection in the form of selection for conformation. The GM is a muscle of the lower leg. It has three heads: lateral, tibial and medial, originating on the lateral epicondyle of the femur, the tibia and the medial epicondyle of the femur respectively. It inserts on the tarsometatarsus and acts to flex the toe and knee joints (Nickel *et al.*, 1977). This muscle was chosen for ease of dissection and because it contrasts the PM in many respects. It contains a different proportion of fibre types to the PM (Barnard *et al.*, 1982) and may not

have been under selection to the same extent as the pectoralis muscle.

In the mouse, analyses were carried out on homologous muscles: the pectoralis and the gastrocnemius. These muscles were chosen for several reasons. Both these muscles are relatively easy to dissect out, they are large in comparison to many of the muscles of the mouse and they allow direct comparison of homologous muscles between species.

In both species and for all experiments the protocol for dissection was similar. Animals were killed (by cervical dislocation) and the pectoral muscles followed by the gastrocnemius muscles were dissected out as quickly as possible. Whole muscles were weighed and whole muscles or samples of muscle tissue were placed on ice until it was convenient for them to be placed in a -80°C freezer for storage or for further treatment.

2.3 MEASUREMENT OF DNA, RNA AND PROTEIN IN MUSCLE

The quantitative separation and determination of major constituents of biological tissues was exhaustively investigated and reviewed in the 1960's (Hutchison and Munro, 1961; Munro and Fleck, 1965 and Munro and Fleck, 1966). In these three reviews the authors survey and test all published methods of separating and quantifying protein, DNA, RNA and lipids in animal tissues. After considering all these methods they suggest optimal procedures for estimating the amounts of DNA, RNA, protein and lipids. Munro and Fleck (1966) suggest a modification of the Schmidt-Thannhauser method for quantification DNA and RNA in animal tissues. In this method RNA is separated from DNA by alkaline digestion following acid-precipitation of the homogenate. DNA is solubilised by further alkaline treatment or by treatment with hot acid. RNA levels can be estimated by reading the

absorption at 260nm after correction for contaminating peptides. DNA levels can be estimated using Giles and Myers (1965) modification of the Burton diphenylamine method.

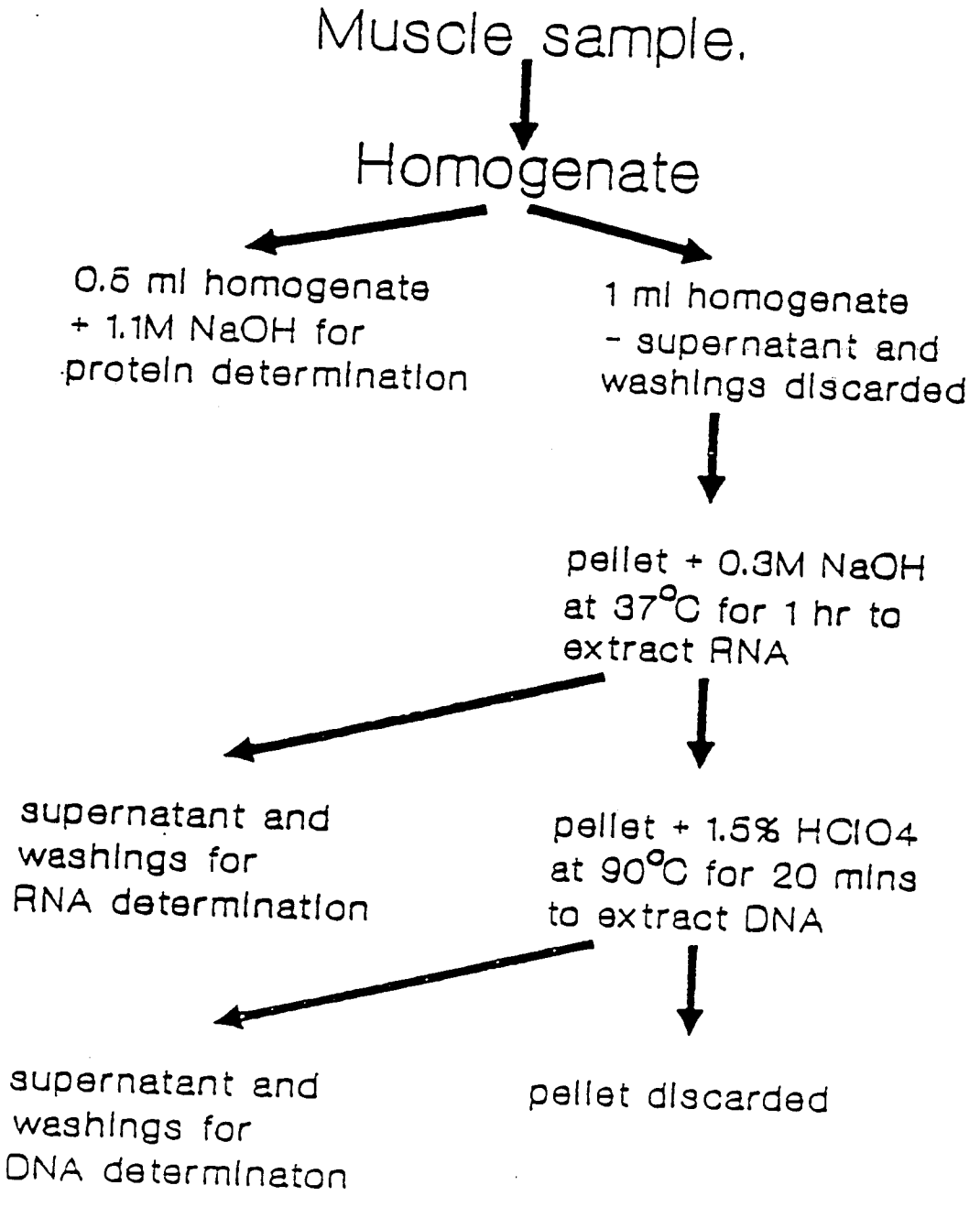
Following these recommendations, Shibko *et al.* (1967) developed a method for the sequential separation of protein, RNA, DNA, lipid and glycogen from a tissue homogenate. This involves the separation of glycogen by acid-precipitation of the homogenate. RNA and DNA are separated by alkaline digestion and hot acid digestion as recommended by Munro and Fleck (1966). The lipids are then extracted using solvents and the protein can then be determined gravimetrically. This is basically the method employed in this thesis to determine DNA, RNA and protein levels in muscle. However, there are several differences between these methods and the method of Shibko *et al.* (1967). Firstly, the method is scaled down to allow it to cope with small muscle samples. Munro and Fleck (1966) show that this is possible. Secondly, the muscle samples are homogenised in an acid medium as opposed to adding concentrated acid after homogenisation (Munro and Fleck, 1965). Thirdly, instead of determining protein gravimetrically, protein was determined in a sample of the homogenate after solubilisation. None of these alterations affect the rationale of the separation procedure.

Between 0.2 and 0.3 grams of muscle was weighed out to the nearest 0.1mg and the muscle sample was finely minced with dissecting scissors. During the entire separation procedure the sample was kept on ice. Ten volumes of cold 20% perchloric acid ($HClO_4$) was added and the sample was homogenised with a Polytron homogeniser with a PTA-7 head. Two 30 sec bursts at 80% of maximum speed with cooling on ice in between bursts was found to be sufficient to homogenise the sample. A 0.5ml sample of

the homogenate was solubilised in an equal volume of 1.1M sodium hydroxide (NaOH) for protein determination by Lowry (Waterborg and Matthews, 1984) or by the BCA method (Pierce Chemical Company, 1986). A 1ml sample of the homogenate was used for the extraction of RNA and DNA. The sample was centrifuged (2900g for 5 minutes) after which the supernatant was discarded. The pellet was washed with 0.5ml of 5% $HClO_4$, centrifuged (2900 g for 5 mins) and the washings discarded. Throughout the extraction procedure pellets were resuspended using several bursts of a sonicator. The pellet was resuspended in 0.9ml, 0.3M NaOH and incubated at 30°C for 1 hour (water bath) after which 0.1ml 70% $HClO_4$ was added and the sample was left on ice for 10 minutes to allow the precipitate to flocculate. The sample was centrifuged (11600g for 5 mins) and the supernatant removed. The pellet was resuspended in 0.5ml 5% $HClO_4$, centrifuged (11600g for 5 mins) and the supernatant removed and combined with the previous supernatant for subsequent RNA determination. The pellet was suspended in 0.4ml 1.5% $HClO_4$ was added and the sample left on ice for 10 mins to allow the precipitate to flocculate. The sample was centrifuged (11600g for 5 mins) and the supernatant removed. The pellet was resuspended in 0.5ml 1.5% $HClO_4$, centrifuged (11600 g for 5 mins) and the supernatant removed and combined with the previous supernatant for subsequent DNA determination. This method is shown diagramatically in Figure 2.1

Protein levels were determined using one of two techniques. The Lowry method (Waterborg and Matthews, 1984) was initially used since it is an accepted method in the literature. However, the BCA method (Pierce Chemical Company, 1986) was then adopted since this only involves the addition of one reagent and was found to be as accurate as the Lowry method.

Figure 2.1. Stratagey for extraction of DNA, RNA and protein.



The Lowry method was carried out as follows. To 0.2ml of sample (or standard) 1 ml of freshly mixed complex-forming reagent was added and the mixture was immediately vortexed. The complex-forming reagent was prepared by mixing 2% sodium carbonate solution, 1% copper sulphate solution and 2% sodium potassium tartarate in the proportion 100:1:1. The sample plus reagent mixture was allowed to stand at room temperature for 10 mins after which 0.1ml of Folin reagent was added and the mixture was immediately vortexed. Folin reagent was obtained from BDA chemicals Ltd. The mixture was allowed to stand at room temperature for 20 mins whereupon absorbance at 550nm was measured against a reagent blank. Standards containing 1mg/ml, 5mg/ml and 10mg/ml of bovine serum albumen run in parallel were used to calculate the linear regression of protein concentration on absorbance and the resulting equation was used to calculate the unknown protein concentrations. The samples for this assay were prepared by addition of 0.9ml distilled water to 0.1ml of the homogenate plus 1.1M NaOH solution in order to bring the protein concentration within the operating range of the assay. The standards contained homogenising medium and 1.1M NaOH in the correct proportions and were treated identically.

The BCA protein assay was carried out as follows. To 0.5ml of sample (or standard) 1ml of BCA working reagent was added and the mixture was immediately vortexed. The BCA working reagent was made up by mixing a reagent containing sodium carbonate, sodium bicarbonate, BCA detection reagent, sodium tartrate and sodium hydroxide with a reagent containing 4% copper sulphate in the ratio 50:1. Both these reagents were obtained from the Pierce Chemical Company Ltd. The sample plus reagent mixture was incubated at 37°C for 30 mins, cooled and the absorbance at 562nm was measured against a water plus reagent blank. The same standards were used in the same way as in the

Lowry assay to calculate unknown protein concentrations. Also in common with the Lowry assay, samples and standards were diluted 1 in 10 with distilled water to bring them within the range of the assay.

The estimation of RNA concentration was carried out by the method of Munro and Fleck (1965). The absorbance at 260nm of 1 in 10 dilutions of the sample were read against a water blank. Correction for contaminating peptides was made by determining the concentration of protein in the sample by the Lowry or BCA methods. Correction was carried out by subtracting 0.001 optical density units from the absorbance at 260nm for every 1mg of peptide per ml of solution. The concentration of RNA can then be calculated since a reading 1.00 optical density unit is equivalent to 32µg of RNA per ml of solution.

DNA concentration was estimated using Giles and Myers (1965) modification of Burton's diphenylamine method. To 0.4ml of sample (or standard) 0.4ml of 4% diphenylamine in glacial acetic acid was added and the mixture was immediately vortexed. To this mixture 0.020ml of aqueous 1.6mg/ml acetaldehyde solution was added and the resulting mixture was immediately vortexed. The sample plus reagents mixture was incubated at 30°C overnight (air oven) and the absorbance at 595nm and 700nm measured against a reagent blank. Standards containing 10µg/ml, 50µg/ml, 100µg/ml and 150µg/ml of salmon sperm DNA in 10% $HClO_4$ were run in parallel to the samples in this assay. These standards showed that the difference between absorbance at 595nm and absorbance at 700nm ($A_{595}-A_{700}$) is a curvilinear function of DNA concentration in this assay. This curvilinear function was transformed to a linear function by taking the exponential of the difference in absorbance at 595nm and 700nm (i.e. $e^{A595-A700}$). The linear regression of DNA concentration on this

transformed value was used to calculate the DNA concentration of unknown samples.

In all of the assays described above, duplicates of samples and standards were run. All reagents were of analytical grade. Samples were run through the separation and determination in groups of twelve. The separation and extraction of DNA, RNA and protein could be completed in a single day. The samples from this extraction process were kept at -80°C for analyses on subsequent days. Samples were analysed on a random basis.

2.4 ESTIMATION OF FIBRE TYPE, SIZE AND NUMBER

Sectioning and histochemistry of the muscle sections was carried out in the histochemistry section of the Metabolic Pathology Department of the Institute for Grassland and Animal Production, Poultry Department, Roslin. Materials and methods for these procedures are described in Appendix 1. Nine sections from each muscle sample were taken. Two of these sections were stained and a photograph of a representative field of view taken. If staining of a section was unsatisfactory, further sections were stained and photographed. At the end of each batch of photographs, a photograph of stage micrometer was taken to allow calibration. Sections and photographs were given numbers which represented the muscle sample from which they were derived. The muscle samples associated with specific numbers were not revealed until analysis of all sections was complete.

In the chicken sections, fibre types were recognized from the description of Ashmore and Doerr (1971a and b) and Barnard *et al.* (1982). Although it was possible to detect three fibre types (type I, IIA and IIB) in preliminary experiments and by using serial sections, it was not possible to consistently detect these three fibre types on a single section throughout the subsequent

experiments. Since the pectoralis muscle of the chicken is predominantly of one fibre type (>98% type IIB) it was decided to carry out analysis with only distinction between type I and type II fibre types which could be consistently distinguished. In the mouse sections all three fibre types could be consistently recognized throughout the experiment. It was therefore possible to carry out analysis for all three fibre types.

Fibre size was estimated using a Reichert Jung, Mop-2 digital image analyser connected to a Prime P550 computer. Photographs of muscle sections were placed on the digitising tablet of the digital analyser and using a magnetic stylus with an attached pen, the boundaries of the muscle fibres were traced. When the boundary of each muscle fibre was completed the digital analyser calculated the area of each fibre in square microns and sent the information to the computer. At the beginning of each run of analyses the digital analyser was calibrated using the photographs of the stage micrometer. The cross sectional area of each fibre was therefore calculated in square microns. After a sufficient number of fibres were measured (between 100 and 300) the diameter of the individual fibres in μ^2 was calculated. This calculation was made using the formula

$$\text{diameter} = 2 \times \sqrt{\pi/\text{area}}$$

This assumes that each fibre has a circular cross sectional area, a justified assumption since each fibre is polygonal when held within the structure of the muscle but will become circular in cross section isolated (Swatland, 1984). Throughout the experiment, it was attempted to measure all the fibres within a fibre bundle. Fibre types were analysed separately within a muscle section.

Percentage fibre type was calculated by taking relative numbers of the different fibres within a section and expressing this as a percentage of the total number of fibre within a section. This assumes that each section is representative of the muscle as a whole. This appeared to be justified for the chicken samples where distribution of fibre types appeared homogeneous throughout the muscle. In the mouse this calculation was not made since a non-homogeneous distribution of fibre types was obvious in single sections.

Fibre number was calculated using the relationship -

$$\text{Fibre number} = \frac{\text{muscle weight}}{(\text{mean fibre diameter})^3}$$

Mean fibre diameter was calculated using all the fibre diameters estimated on a section irrespective of fibre type. Mean fibre diameter is raised to the power of 3 in the above relationship since it is a linear dimension of muscle size, whereas fibre number and muscle weight are proportional to muscle volume. This relationship gives an index of fibre number, not an absolute estimate and is equivalent to the apparent fibre number discussed by Swatland (1984). This relationship assumes that fibre diameter and fibre length maintain a constant ratio during growth. This is justified since Moss (1968a) shows this to be the case for the chicken up to 266 days of age. It was felt that this calculation was justified in the case of chicken muscle but not in mouse muscle for the same reasons as are given for percentage fibre type.

Using the above methods, the following fibre dimensions were calculated. Chicken: mean fibre diameter of type I and type II fibres, percentage of type I and type II fibres, mean fibre diameter, apparent fibre number. Mouse: mean fibre diameter of type I, type IIA and type IIB fibres.

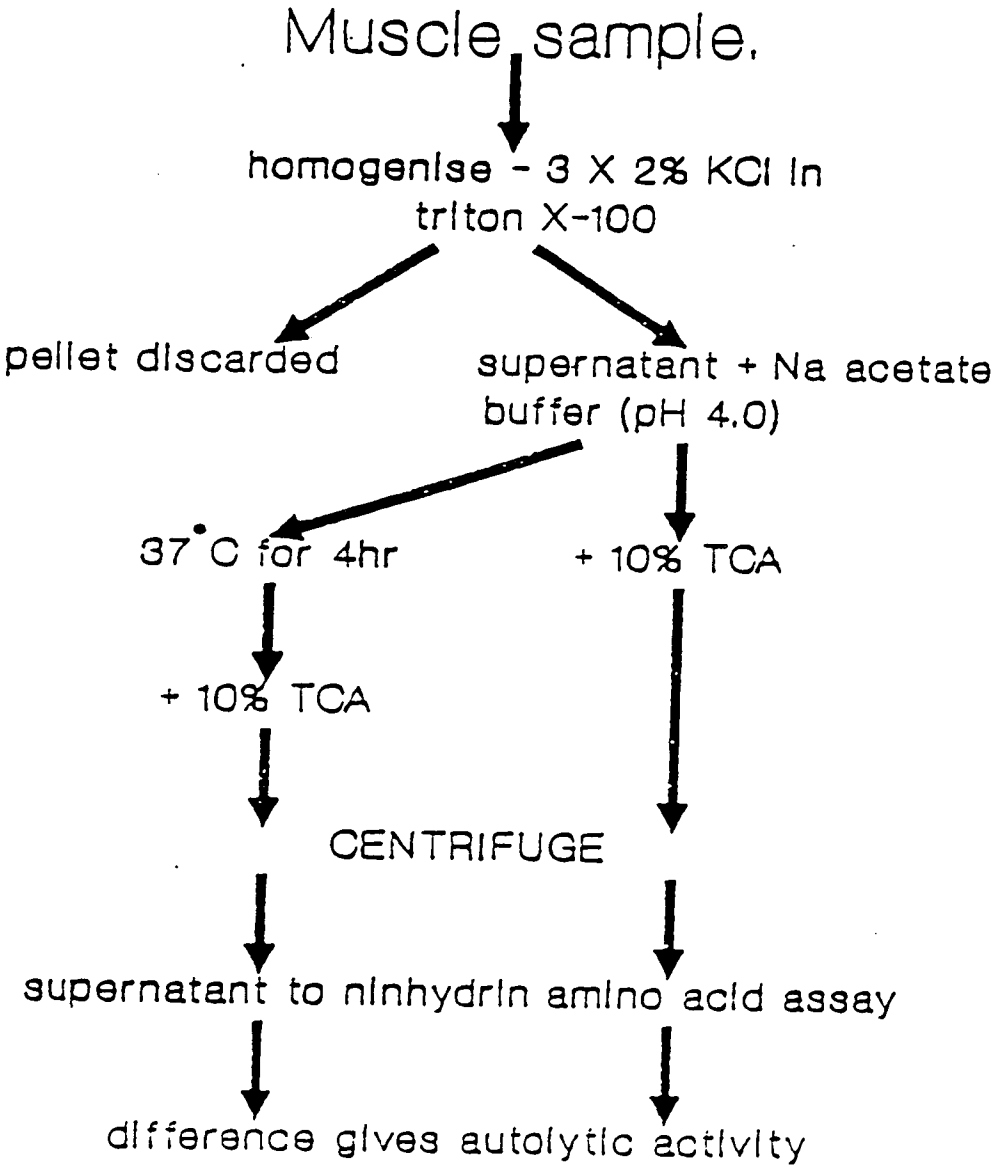
2.5 ESTIMATION OF RATE OF AUTOLYSIS

This assay was developed from the assay described by Iodice *et al.* (1966) and Iodice *et al.* (1972). The technique was scaled down to allow analysis of muscle samples from mice and very young chickens.

The PM was dissected as described previously, weighed (to the nearest 0.1mg) and the whole muscle (or a 0.2 to 0.3g sample of muscle) minced with dissecting scissors. Three volumes of 2% potassium chloride in 0.267% tritonX-100 was added. This mixture was homogenised using a Polytron homogeniser with a PTA-7 head (two 30-second bursts at 80% of maximum speed with chilling on ice between bursts). The homogenate was then placed at -80°C until analysis could be carried out. Throughout this and subsequent treatments the sample was kept on ice.

The homogenate was thawed and vortexed. It was centrifuged at 15600g for 30 mins at 4°C after which the supernatant was transferred to ice and the pellet was discarded. Two 0.08ml aliquots of the supernatant were placed in two 'eppendorf' tubes (1.5ml) and 0.02ml of sodium acetate buffer (pH 4.4) was added to give a final concentration of 0.5M. One tube was incubated at 37°C for 4 hours (water bath) after which 0.1ml of 10% trichloroacetic acid (TCA) was added to precipitate the proteins and stop autolysis. 0.1ml of 10% TCA was added to the other tube at time 0. After addition of 10% TCA the samples were heated to 55°C for 10 mins (heating block). Precipitated protein was removed by centrifugation for 5 mins at 11600 g. The supernatant was diluted 1 in 40 with distilled water in preparation for the ninhydrin amino acid assay. The above describes the procedure for mouse muscle samples. The procedure for chicken samples was identical except that volumes were doubled. The extraction is shown diagramatically in Figure 2.2.

Figure 2.2. Determination of autolytic activity.



The ninhydrin amino acid assay was carried out as described by Blackburn (1968). To a 0.2ml aliquot of sample or standard, 0.2ml of ninhydrin solution (2% ninhydrin, 0.3% hydrindantin in 75% methyl cellusolve in 1 M sodium acetate buffer at pH 5.51) was added and the solutions were mixed by shaking. This mixture was incubated at 100°C for 15 mins (heating block) after which 1ml of 50% ethanol was added. The tubes were allowed to cool after which they were shaken for 30 seconds. The absorbance at 570nm was recorded against 50% ethanol blank. Standard solutions containing 50, 150 and 250µmol tyrosine were run in parallel with this assay. Linear regression of amino acid concentration on absorbance at 570nm was used to calculate unknown amino acid concentrations. The difference in amino acid concentration (µmol/g wet weight) between incubated and unincubated samples was defined as the autolytic activity.

Throughout this assay, duplicates of samples and standards were run. The ninhydrin assay was run twice for each sample and the mean difference between samples was used to calculate autolytic activity.

2.6 INJECTION OF PROTEASE INHIBITORS INTO GROWING MICE

These experiments was designed in an attempt to repeat observations made by Chelmicka-Shorr *et al.* (1978) and Hollenberg Sher *et al.* (1981). Two attempts were made to repeat the results. The first attempt followed the published methods as closely as possible. The second attempt was a modification of the injection regime in the first.

The first run was carried out as follows. Each mouse was injected intra-peritoneally 5 days out of 7, from 3 weeks to 8 weeks of age with a 0.2ml control solution or treatment solution. The treatment solution contained

0.1mg/ml pepstatin and 0.75mg/ml leupepin in 10% ethanol in saline. The control solution contained 10% ethanol in saline. Ethanol was included since pepstatin will only dissolve in water if initially dissolved in ethanol. Mice were weighed at the beginning of each week. At 8 weeks mice were killed by cervical dislocation and both PM's and GM's were dissected out and weighed. Protein concentration in these muscles was estimated by the BCA protein assay as described in Section 2.3. Three strains of mice were used: C57BL/10-dy/dy (from G. Bulfield, IAPGR, Roslin), C57BL/6 and C57BL/10 (Bantan and Kingman Ltd). 10 males and 10 females of each strain were divided equally into treatment or control groups.

The second run was different from the first run in several ways. During the first run several mice from control and treatment groups had died. It was assumed that this was due to the relatively large injection volume and associated ethanol. It was therefore decided to reduce the number of injections by increasing concentrations of leupeptin and pepstatin and scale the size of the injection to the size of the mouse. The concentrations of leupeptin and pepstatin were increased to give similar weekly doses of the inhibitors to those given in Experiment 1. Male mice from each replicate of the P line, were allocated to control or treatment groups on a random basis. 5 or 6 mice were allocated to each group. Injections were given 3 days out of 7 from 4 weeks of age to 8 weeks of age. On each day of injection, mice were weighed to the nearest 0.1g and were given an intra-peritoneal injection of 0.01ml per gram body weight containing 1.2mg/ml leupeptin and 0.2mg/ml pepstatin in 10% ethanol in saline treatment) or 0.01ml per gram body weight of 10% ethanol in saline (control). Body weights were recorded at the beginning of each week. At 8 weeks of age mice were weighed, killed by cervical dislocation and the left and right Pm's and left and right Gm's

muscles dissected out and weighed. These muscles were then placed at -80°C before being assayed for the activity of cathepsins B and H. These assays were carried out by I.R. Paton of the Gene Expression Group at the Institute of Animal Physiology and Genetics Research, Roslin. as described by Barrett (1980).

CHAPTER 3. CHANGES IN MUSCLE DNA, RNA AND PROTEIN LEVELS DURING THE GROWTH OF CHICKENS DIFFERING IN GROWTH RATE.

3.1 INTRODUCTION

Modern breeds of broiler chicken grow much faster than their predecessors as a result of selection for increased weight at a fixed age. The muscles of broilers therefore grow at a faster rate, but there is little information on how increased muscle growth is reflected in the levels of DNA, RNA and protein. Such data are important because they help us understand how the cellular units (number, size and activity of the DNA units) are changed to increase the size and growth rate of muscle and may suggest methods by which selection for muscle growth rate may be improved.

This experiment examines the DNA units in growing muscles of a fast growing strain of broilers and a slow growing strain of layers. In this way the effect of selection on muscle nuclei and associated cytoplasm will be determined.

3.2 EXPERIMENTAL DETAILS

Seventy as-hatched broilers (Ross 101, Ross Breeders Ltd.) were grown to 70 days of age under the system of management described in Chapter 2. At the ages of 1 day, 7 days, 14 days, 28 days, 42 days and 70 days, ten birds of each strain were taken and the following measurements made: body weight, left and right pectoralis muscle (PM) weight and left and right gastrocnemius muscle (GM) weight. At each of these ages body weight of the remaining birds was recorded. The left PM and left GM were sampled and stored for biochemical analysis as described in chapter 2. The right PM and right GM were sampled and frozen in isopentane cooled with solid CO_2 for analysis of fibre type, size and number. This

technique proved unsuccessful, however, and estimates of fibre type, size and number could not be made.

3.3 RESULTS

3.3.1 Body and muscle weights

As expected, broilers are heavier than layers at each age apart from 1 day, when layers are heavier (Table 3.1). The difference in weight at 1 day may reflect differences in egg size between strains and is not, therefore, of consequence to this study.

The liveweight performance of the broiler strain is below that expected under commercial conditions (Ross Breeders Ltd.- personal communication). This is probably due to the non-ideal conditions under which these birds were grown.

In line with the strain differences in body weight, broilers have heavier PM's than layers at all ages except 1 day when layers have heavier PM's. GM's are similar in weight between strains until 14 days after which broilers have heavier GM's. As expected, broilers and layers differ in muscle size and rate of muscle growth.

3.3.2 Concentrations of DNA, RNA and protein

DNA concentration (mg/g wet weight) is an estimate of the number of nuclei per unit of muscle. DNA concentration falls with age in both the PM and GM (Table 3.2, Figure 3.1). DNA concentration is higher in the PM at younger ages but after 28 days DNA concentration is similar in both muscles.

DNA concentration is higher in broiler PM at 1 day, but falls at a faster rate than in layer PM; layers have a larger DNA concentration at 14 days and at subsequent ages (Table 3.2, Figure 3.1). A similar trend is seen for

Table 3.1. Mean (± sem) body weight, pectoralis muscle (PM) weight and gastrocnemius muscle (GM) weight, in a strain of broilers and a strain of layers from 1 to 70 days of age. The ratio of broiler to layer for each of the weights at each age is also given.

Strain	Age(d)	Body Wgt.(g)	PM Wgt.(g)	GM Wgt.(g)
Broiler	1	36.5± 0.9	0.20±0.01	0.17±0.01
	7	83.0± 3.5	1.16±0.11	0.21±0.01
	14	181.4± 6.7	4.92±0.27	0.54±0.02
	28	637.8±26.7	21.25±1.18	2.40±0.15
	42	1414.7±56.2	57.97±2.72	5.55±0.22
	70	2363.8±89.6	125.05±5.58	12.18±0.60
Layer	1	40.7± 0.9	0.28±0.01	0.17±0.01
	7	60.0± 1.6	0.79±0.04	0.20±0.01
	14	103.4± 3.3	2.29±0.10	0.30±0.01
	28	252.5± 6.6	6.90±0.27	0.96±0.02
	42	480.6±12.3	14.80±0.47	2.08±0.06
	70	976.9±23.5	32.00±0.85	4.63±0.10
Ratio B/L	1	0.90 **	0.71 **	1.00
	7	1.38 **	1.53 **	1.05
	14	1.75 **	2.15 **	1.80 **
	28	2.52 **	3.07 **	2.50 **
	42	2.94 **	3.92 **	2.67 **
	70	2.42 **	3.91 **	2.63 **

** strains differ with $P<0.01$

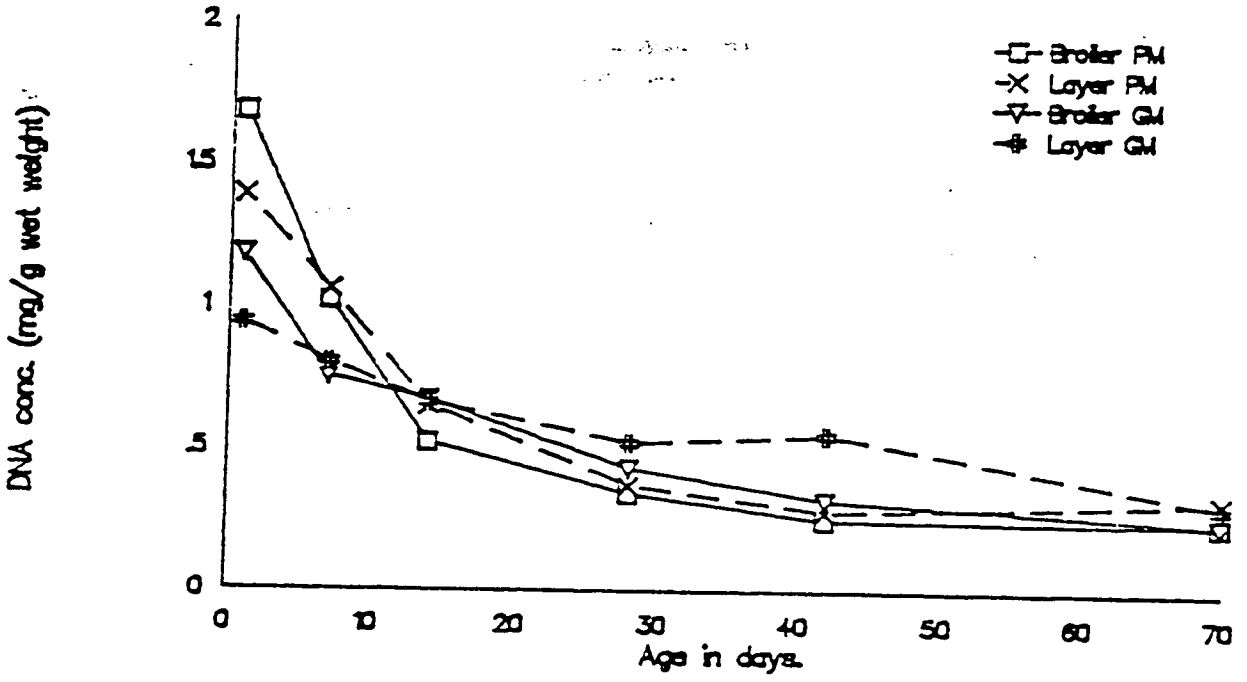
Table 3.2. Mean (± sem) DNA, RNA and protein concentration in the pectoralis and gastrocnemius muscle of a strain of broilers and a strain of layers from 1 to 70 days of age. Ratio of broiler to layer for each age is also given.

Strain	Age (d)	DNA conc. (mg/g)	RNA conc. (mg/g)	Protein conc. (mg/g)
Pectoralis muscle				
Broiler	1	1.68±0.08	2.07±0.06	92.1±3.9
	7	1.02±0.07	4.08±0.13	122.5±4.2
	14	0.52±0.04	2.59±0.08	152.5±5.6
	28	0.34±0.02	1.79±0.08	171.6±3.8
	42	0.25±0.03	1.40±0.05	166.1±5.0
	70	0.24±0.02	1.00±0.05	175.6±5.6
Layer	1	1.39±0.05	1.82±0.08	80.7±3.4
	7	1.06±0.04	3.75±0.12	141.4±3.3
	14	0.65±0.04	2.32±0.07	154.9±3.8
	28	0.37±0.03	1.46±0.08	151.6±4.4
	42	0.28±0.03	1.23±0.04	173.6±5.6
	70	0.32±0.05	1.10±0.04	164.6±9.8
Ratio B/L	1	1.21 *	1.13 *	1.14 *
	7	0.96	1.09	0.87 **
	14	0.80 *	1.11 *	0.98
	28	0.92	1.23 *	1.13 *
	42	0.89	1.13 *	0.96
	70	0.75	0.91	1.07
Gastrocnemius muscle				
Broiler	1	1.18±0.07	2.10±0.06	115.3±6.2
	7	0.75±0.07	2.09±0.13	126.7±6.9
	14	0.67±0.06	2.08±0.16	129.6±5.9
	28	0.43±0.04	1.61±0.09	147.0±3.6
	42	0.32±0.03	1.27±0.02	149.7±4.6
	70	0.24±0.05	0.96±0.05	156.0±4.3
Layer	1	0.94±0.11	1.94±0.10	106.1±4.6
	7	0.80±0.09	1.57±0.07	121.6±6.3
	14	0.66±0.05	1.71±0.07	131.8±4.4
	28	0.52±0.05	1.50±0.08	135.7±2.8
	42	0.55±0.04	1.37±0.06	143.8±4.9
	70	0.30±0.04	1.08±0.06	158.3±7.2
Ratio B/L	1	1.11	1.08	1.09
	7	0.93	1.33 *	1.04
	14	1.01	1.21	0.98
	28	0.82	1.07	1.08
	42	0.58 *	0.92	1.04
	70	0.80	0.88	0.98

* strains differ with $P<0.05$
** strains differ with $P<0.01$

Figure 3.1.

DNA conc. vs age in the PM and GM of a strain of broilers and layers.



the GM. Broilers have more nuclei per gram muscle than layers at hatch, but this falls faster during growth resulting in less nuclei per gram muscle in broilers at ages above 7 days (Table 3.2, Figure 3.1).

RNA concentration is an estimate of the amount of protein synthetic machinery per gram muscle (Waterlow *et al.*, 1978) and as such measures the capacity of the muscle for protein synthesis. RNA concentration is similar in the PM and GM at hatch (Table 3.2, Figure 3.2). GM RNA concentration falls slowly from hatch to 70 days contrasting the picture in the PM where there is a peak at 7 days after which RNA concentration falls to reach similar levels as the GM at 70 days.

Young broilers have higher RNA concentrations than layers in both muscles suggesting that their muscles have a greater capacity for protein synthesis during growth than layers (Table 3.2, Figure 3.2).

Protein concentration increases with age in both muscles but is lower in the PM at 1 day and shows a greater rate of increase resulting in a higher ultimate protein concentration (Table 3.2, Figure 3.3). Significant differences are seen between strains for both muscles, but these differences are not in a consistent direction (Table 3.2). The relevance of this inconsistent variation between strains to the differences in muscle growth is not known. Selection for growth has not systematically altered the amount of protein per gram muscle.

3.3.3 Number, size and activity of DNA units

Total DNA (mg) is an estimate of the number of nuclei in an individual muscle. Total DNA increases with age in both muscles although in the GM no increase is seen in the first week (Table 3.3, Figures 3.4a and b). Total DNA increases at a greater rate in broiler muscle than layer

Figure 3.2.

RNA conc. vs age in the PM and GM of a strain of broilers and layers.

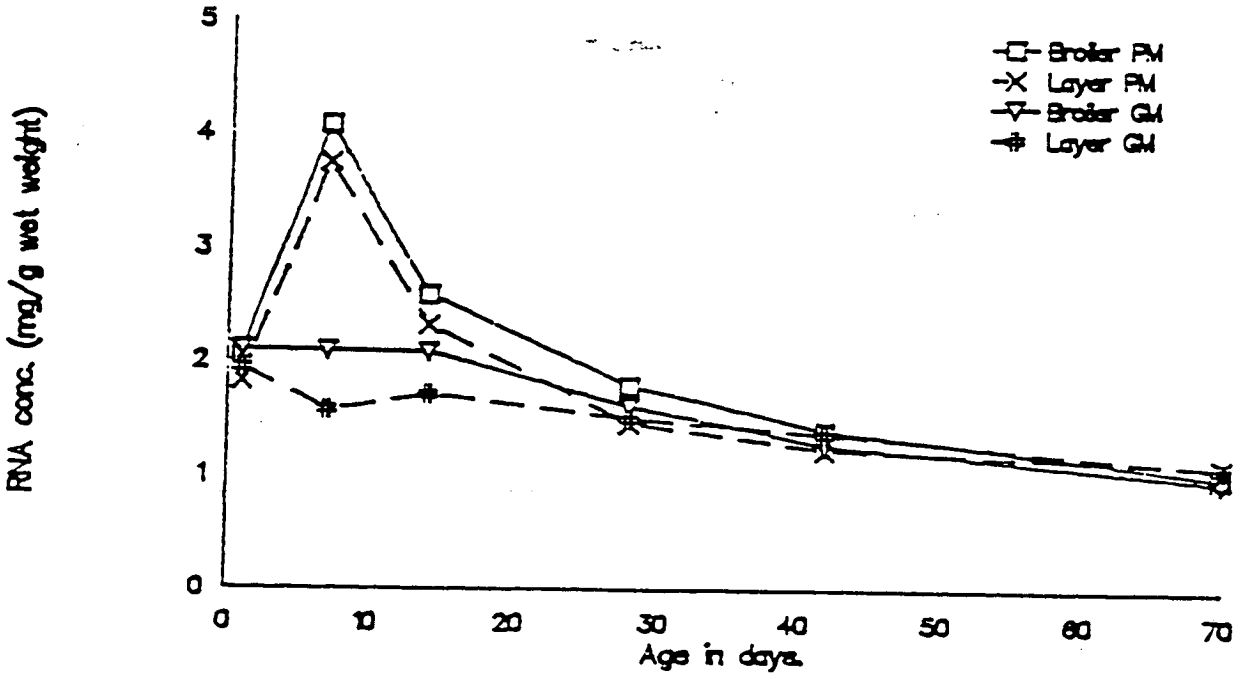


Figure 3.3.

Protein conc. vs age in the PM and GM of broilers and layers.

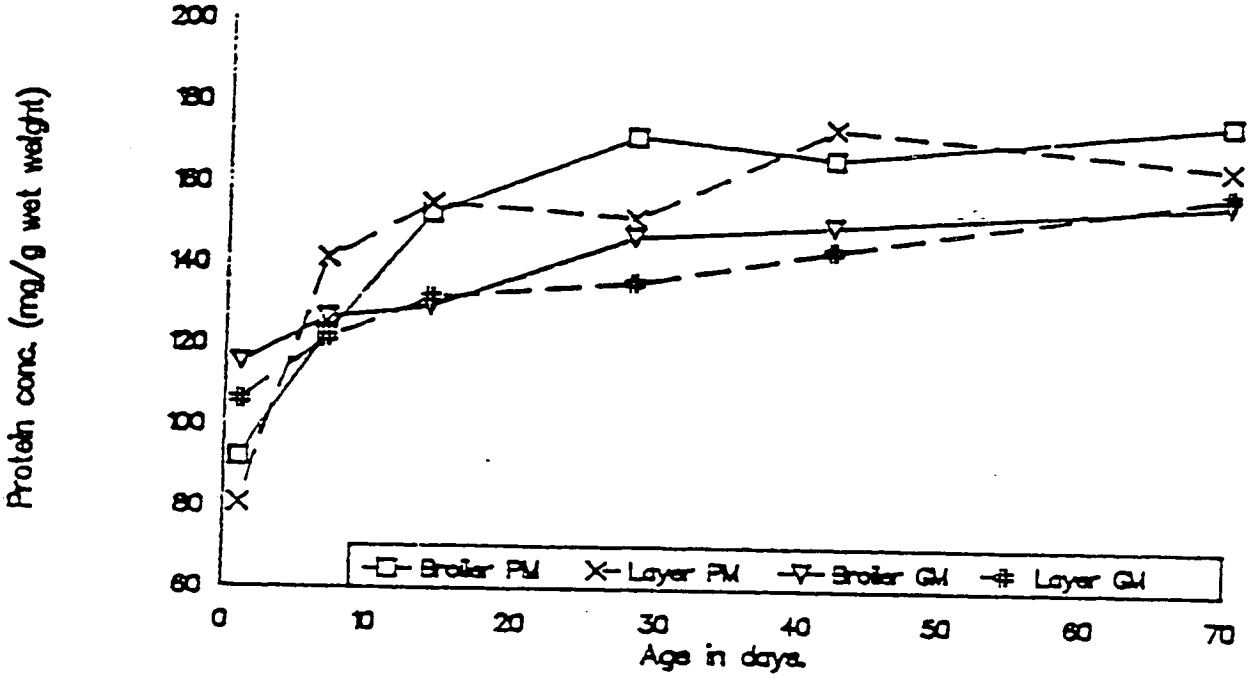


Table 3.3. Mean (± sem) total DNA, protein:DNA ratio and RNA:DNA ratio in the pectoralis muscle and gastrocnemius muscle of a strain of broilers and a strain of layers from 1 to 70 days of age. The ratio of broiler to layer at each age is also given.

Strain	Age (d)	Total DNA (mg)	Protein:DNA ratio	RNA:DNA ratio
Pectoralis muscle				
Broiler	1	0.34±0.02	56.3± 4.0	1.27±0.09
	7	1.15±0.12	128.3± 13.7	4.30±0.53
	14	2.52±0.19	317.9± 36.3	5.33±0.53
	28	7.28±0.44	504.2± 19.1	5.21±0.12
	42	14.86±1.94	796.0±138.0	6.54±1.03
	70	30.11±2.46	780.1± 83.1	4.28±0.24
Layer	1	0.39±0.02	58.7± 3.1	1.32±0.06
	7	0.83±0.05	135.1± 5.4	3.58±0.16
	14	1.46±0.07	250.0± 22.5	3.69±0.22
	28	2.58±0.23	444.8± 54.8	4.23±0.57
	42	4.26±0.49	699.0±102.0	4.90±0.75
	70	10.34±1.71	609.8± 88.9	3.92±0.48
Ratio B/L	1	0.87	0.96	0.96
	7	1.38 *	0.95	1.20
	14	1.72 **	1.27	1.44 *
	28	2.82 **	1.13	1.23
	42	3.49 **	1.14	1.33
	70	2.91 **	1.28	1.09
Gastrocnemius muscle				
Broiler	1	0.20±0.02	103.6± 13.1	1.84±0.11
	7	0.16±0.02	182.8± 18.2	3.09±0.40
	14	0.36±0.03	227.2± 45.2	3.20±0.14
	28	1.05±0.13	359.1± 25.4	3.86±0.24
	42	1.81±0.19	523.6± 84.3	4.46±0.71
	70	3.01±0.70	1128.0±404.0	6.68±2.36
Layer	1	0.16±0.02	138.1± 27.5	2.48±0.47
	7	0.16±0.02	176.4± 30.6	2.31±0.39
	14	0.20±0.02	211.4± 17.8	2.68±0.12
	28	0.50±0.05	288.5± 34.0	3.07±0.23
	42	1.16±0.12	277.5± 26.9	2.61±0.24
	70	1.40±0.23	641.9± 97.5	4.38±0.71
Ratio B/L	1	1.25	0.75	0.74
	7	1.00	1.04	1.34
	14	1.80 **	1.07	1.19 *
	28	2.10 **	1.24	1.26 *
	42	1.56 **	1.89 **	1.71 *
	70	2.15 **	1.76	1.52

* strains differ with $P<0.05$
** strains differ with $P<0.01$

Figure 3.4a.

Total PM DNA versus age in a strain of broilers and layers.

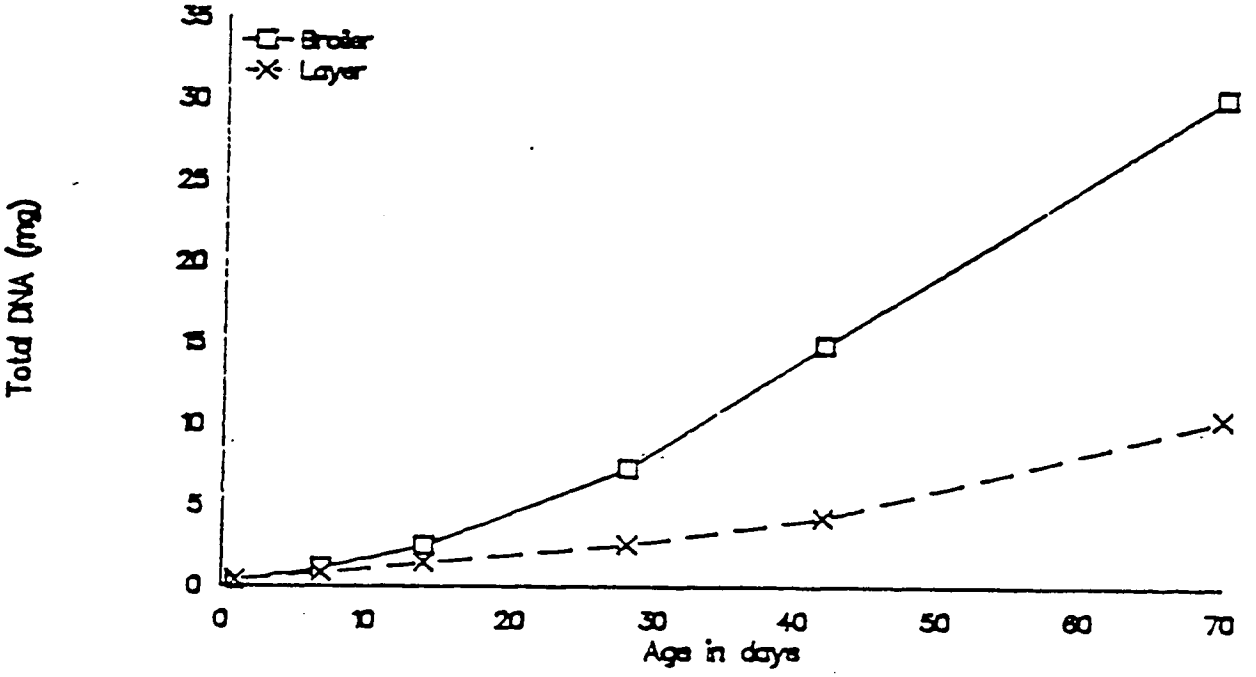
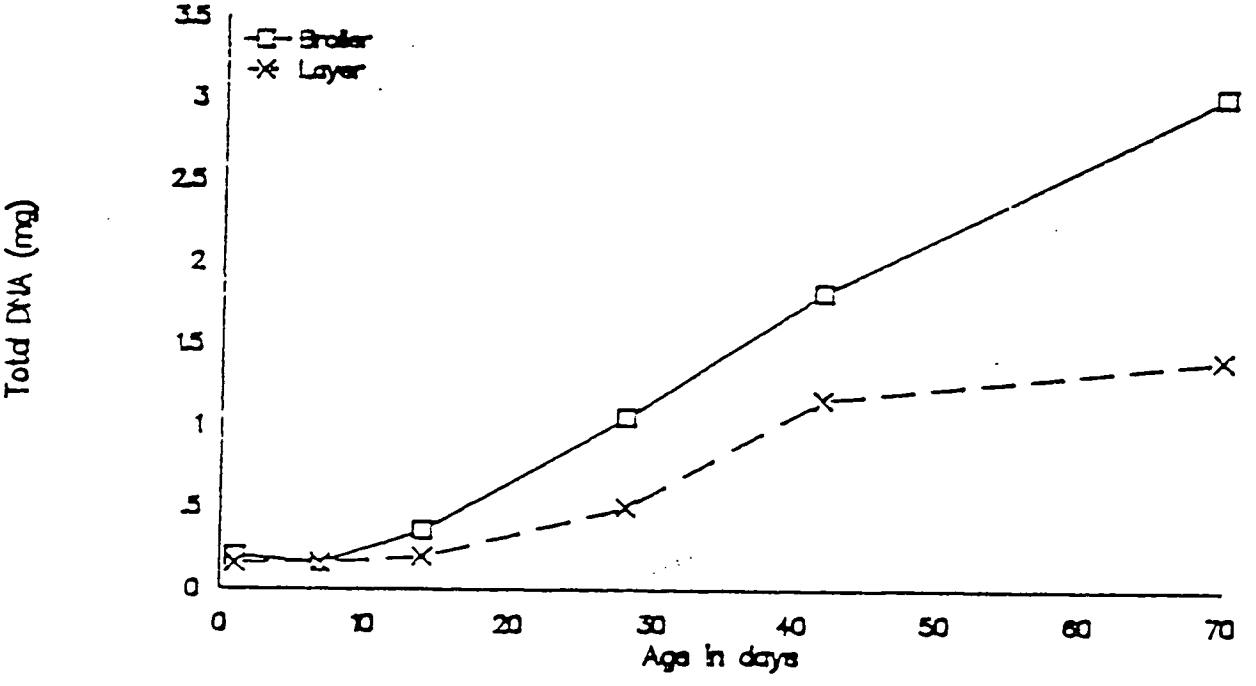


Figure 3.4b

Total GM DNA versus age in a strain of broilers and layers.



muscle showing that selection for increased growth has increased the number and rate of accumulation of muscle nuclei (Table 3.3, Figures 3.4a and b).

In order to determine whether strain differences in number of nuclei are due to difference in muscle weight at an age, total DNA is plotted against muscle weight (Figures 3.5a and b). Linear regressions of total DNA on muscle weight are shown on these graphs. The gradient of these regression lines is greater for layer muscle showing that selection for growth has slightly decreased the number of nuclei in broiler muscle compared with layer muscle of the same weight. An attempt was made to compare regression lines using the method described by Snedecor and Cochran (1967, chapter 14) but because of inhomogeneous residual variation this test was not meaningful.

The ratio of protein:DNA (DNA unit size) in muscle has been accepted as an estimate of the size of the nucleus plus cytoplasm unit (Cheek, 1985). Both muscles exhibit an increase in DNA unit size with age (Table 3.3, Figure 3.6) showing that muscle growth occurs by both an increase in the number and size of nuclear units. The PM has a smaller DNA unit size than the GM at hatch but increases at a faster rate resulting in a larger DNA unit size in the PM at 42 days (Table 3.3).

Broilers have a greater DNA unit size than layers after 7 days of age in the PM and after 14 days in the GM (Table 3.3, Figure 3.6). Selection for growth has increased muscle size at a given age by increasing the size of the nucleus plus cytoplasm unit.

When DNA unit size is plotted against muscle weight, broilers are seen to have smaller PM DNA unit sizes than layers at a given weight of muscle (Figure 3.7a). No consistent trend is seen for the GM (Figure 3.7b).

Figure 3.5a.

Total PM DNA vs muscle weight in a strain of broilers and layers.

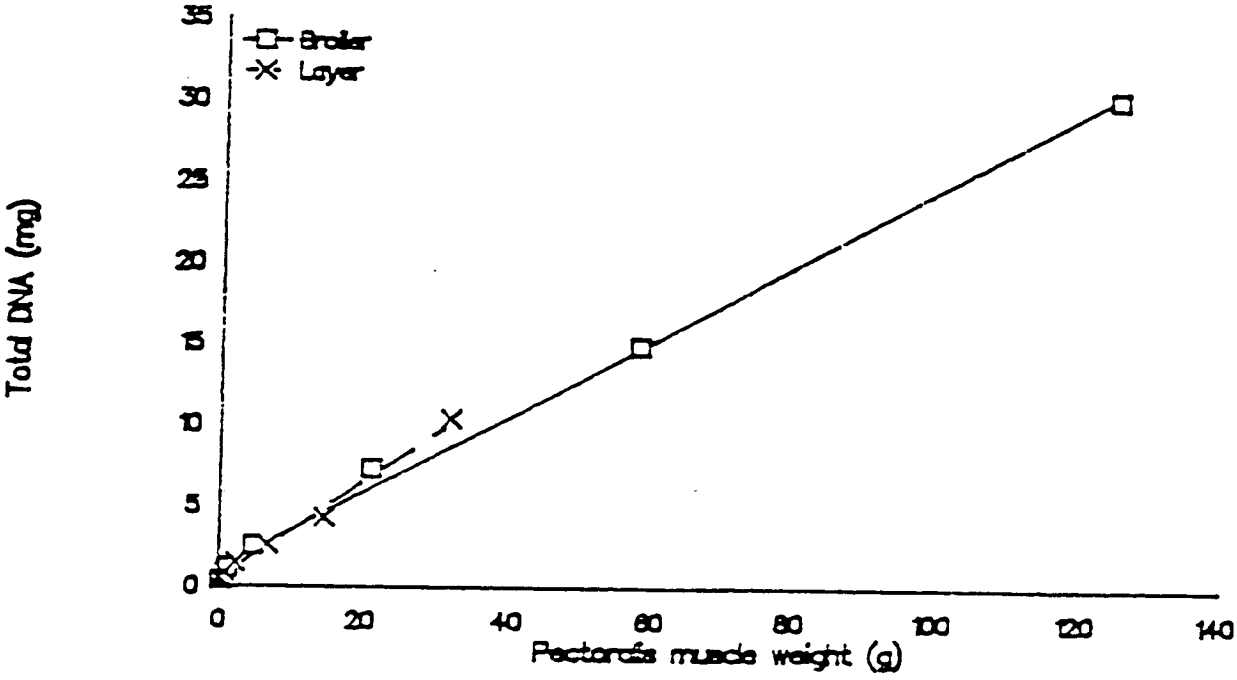


Figure 3.5b.

Total GM DNA vs muscle weight in a strain of broilers and layers.

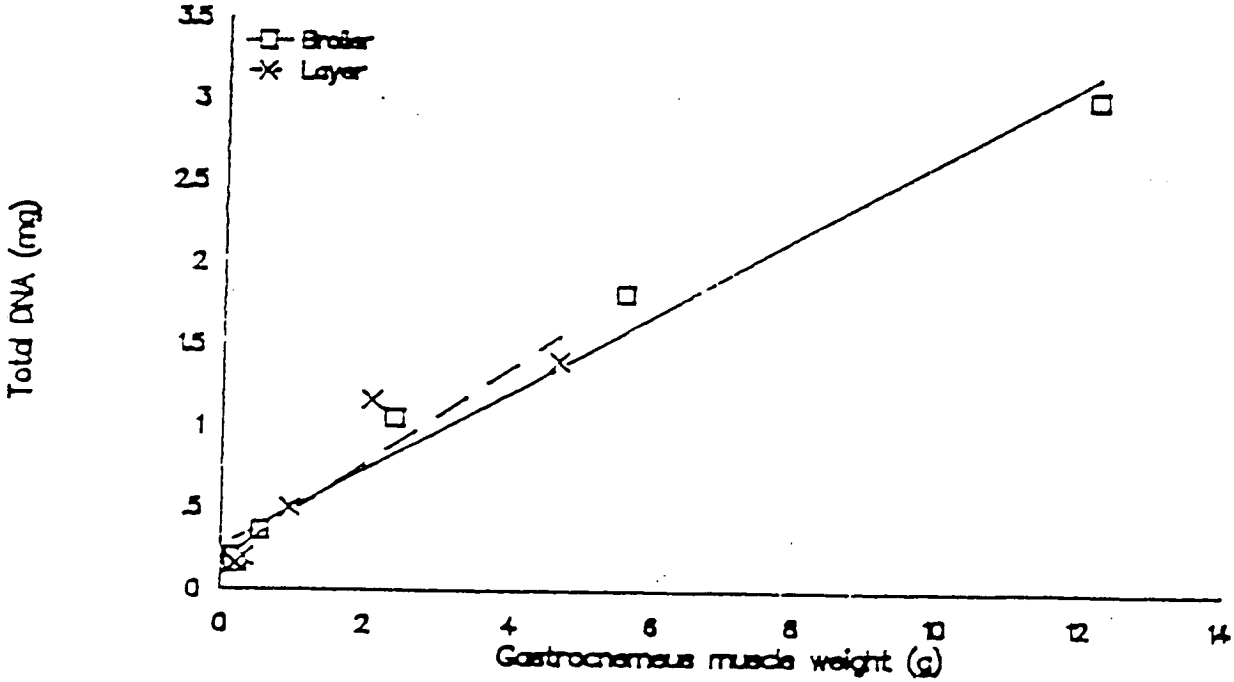


Figure 3.6.

Protein : DNA vs age in the PM and GM of broilers and layers.

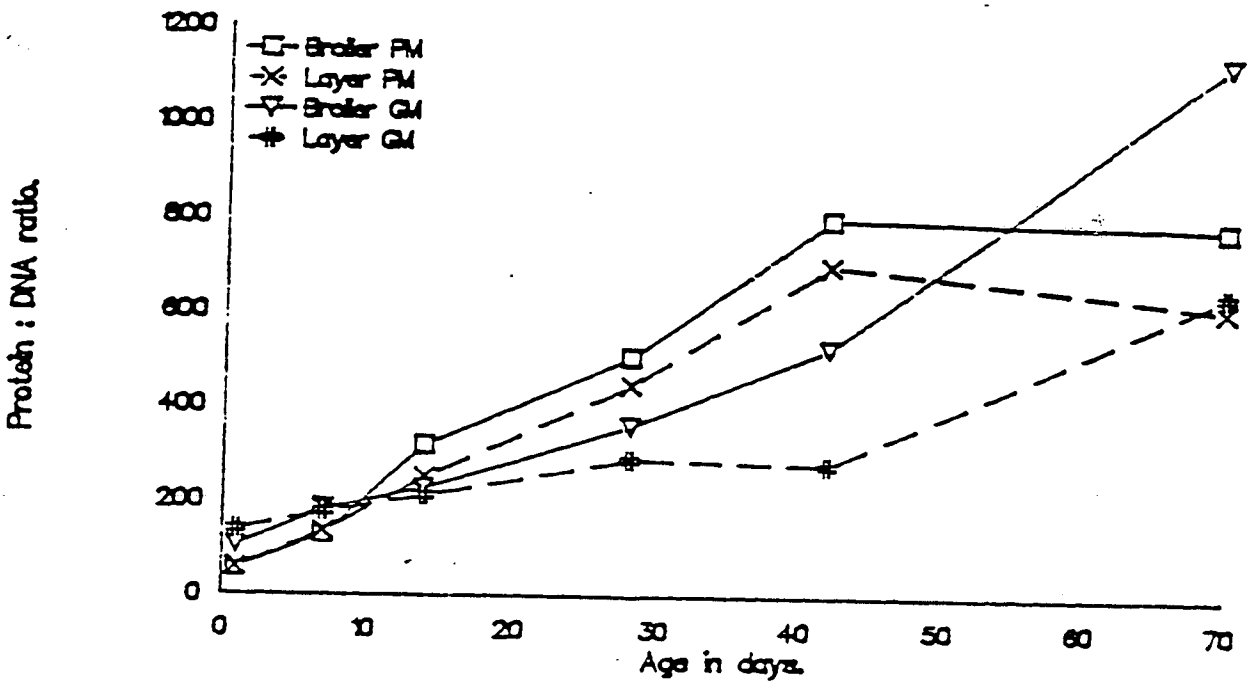


Figure 3.7a.

PM protein : DNA ratio vs muscle weight in broilers and layers.

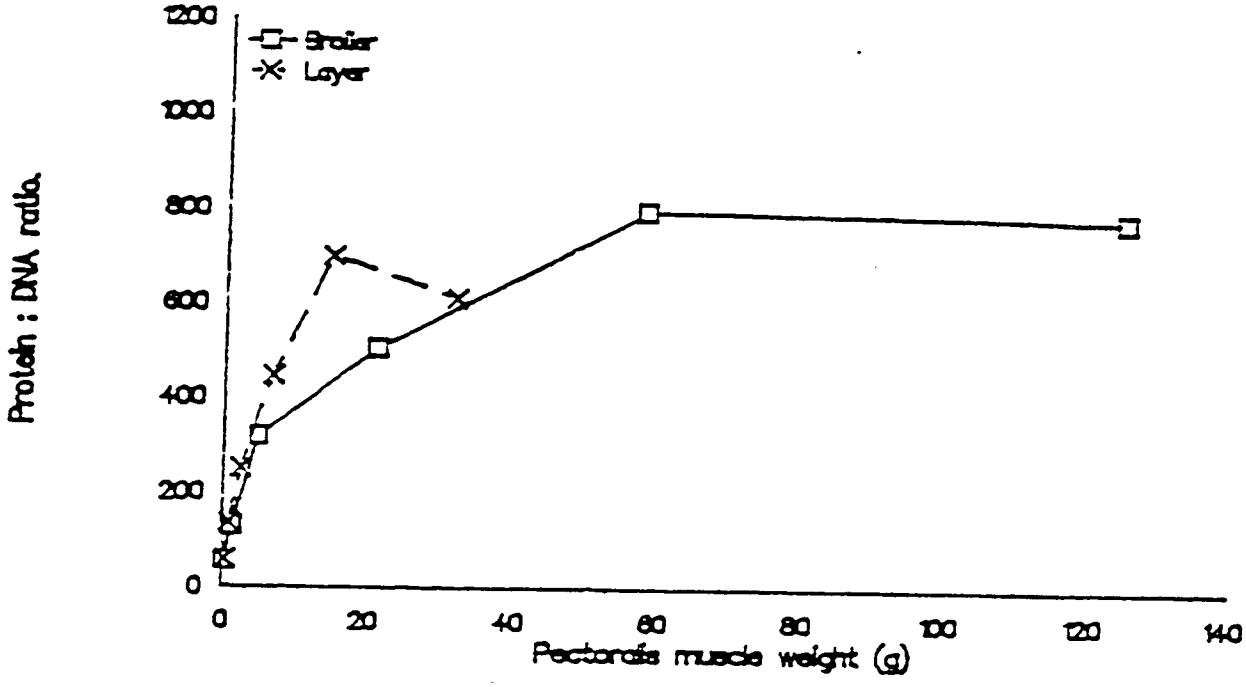
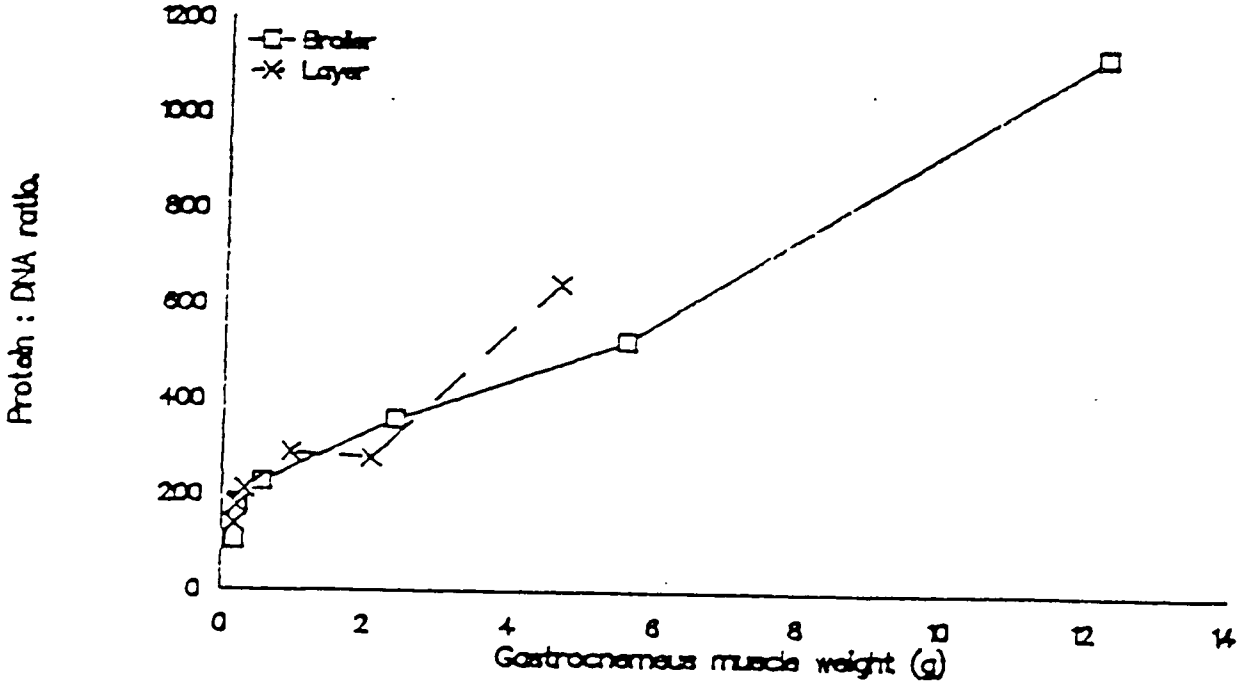


Figure 3.7b.

GM protein : DNA ratio vs muscle weight in broilers and layers.



Selection for improved growth in the chicken has produced PM muscles which reach mature DNA unit size at a smallIer weight.

The ratio RNA:DNA is an estimate of the capacity of a nuclear unit to synthesize protein. In the PM RNA:DNA increases dramatically over the first week after which it plateaus, falling slightly between 42 and 70 days (Table 3.3, Figure 3.8). In the GM, RNA:DNA increases steadily with age. Broilers have a greater RNA:DNA ratio from 7 days of age in both muscles (Table 3.3, Figure 3.8) suggesting that selection for growth has increased the capacity of nuclear units to synthesize protein.

When strains are compared at the same muscle weights, broilers have more RNA per nucleus than layer muscles (Figure 3.9a and 3.9b). Selection has increased the capacity of broiler muscle nuclei to synthesize protein compared with layer muscles of the same weight.

3.4 DISCUSSION

3.4.1 How do muscles grow?

The two muscles examined in this experiment exhibit different patterns of growth, particularly over the first two weeks. The PM and GM are of similar weight at hatch, but the former grows to be 8 to 10 times larger at 10 weeks of age. Possible reasons for differences in growth pattern are discussed by Ricklefs (1983) and Swatland (1984). The GM is a leg muscle and must be able to function at hatch to enable the chick to find food and water. The provides power for flight and need not be functional until the bird is ready to fly. Evolution may have favoured a stratagem whereby the leg muscles develop to functional maturity by hatch, but flight muscle development is delayed until after hatch.

Figure 3.8.

RNA : DNA vs age in the PM and GM of broilers and layers.

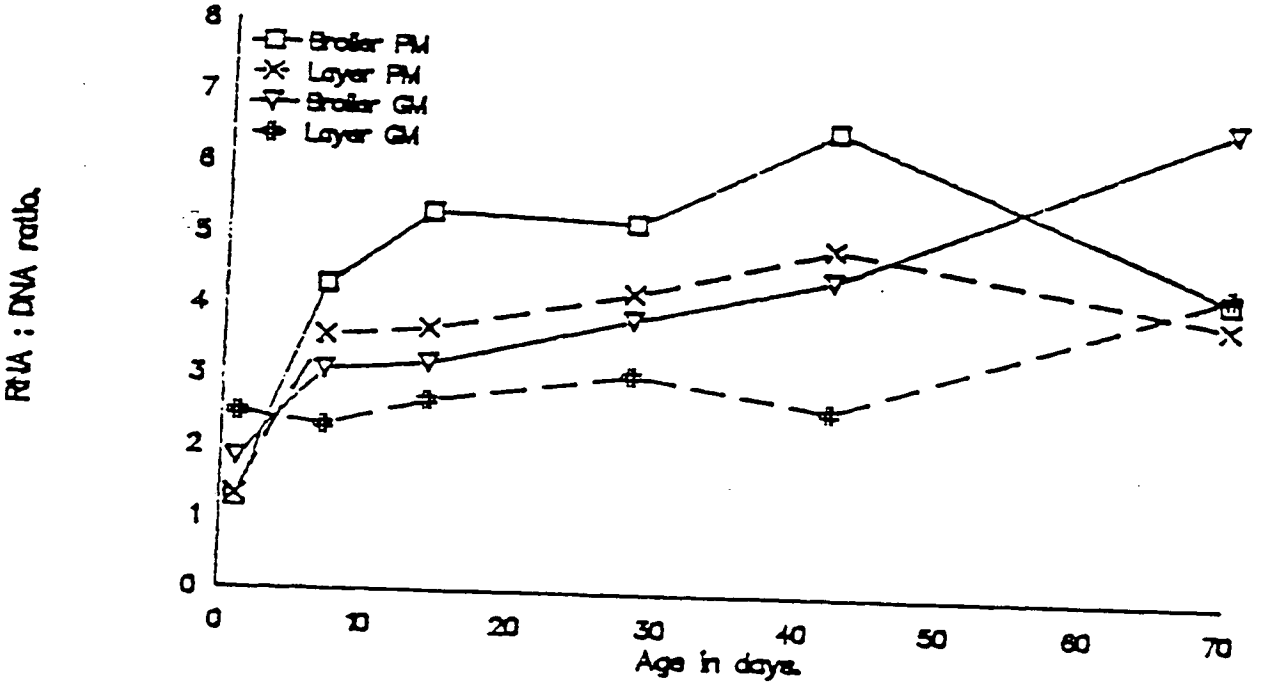


Figure 3.9a.

PM RNA : DNA ratio vs muscle weight in broilers and layers.

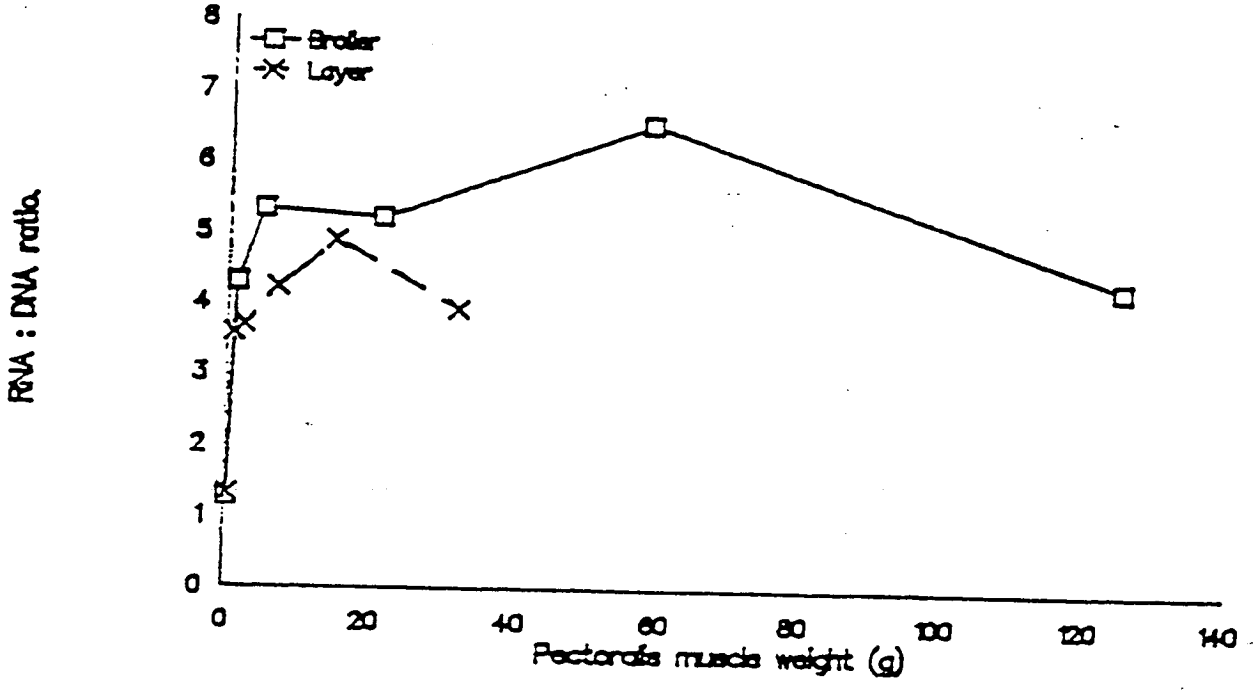
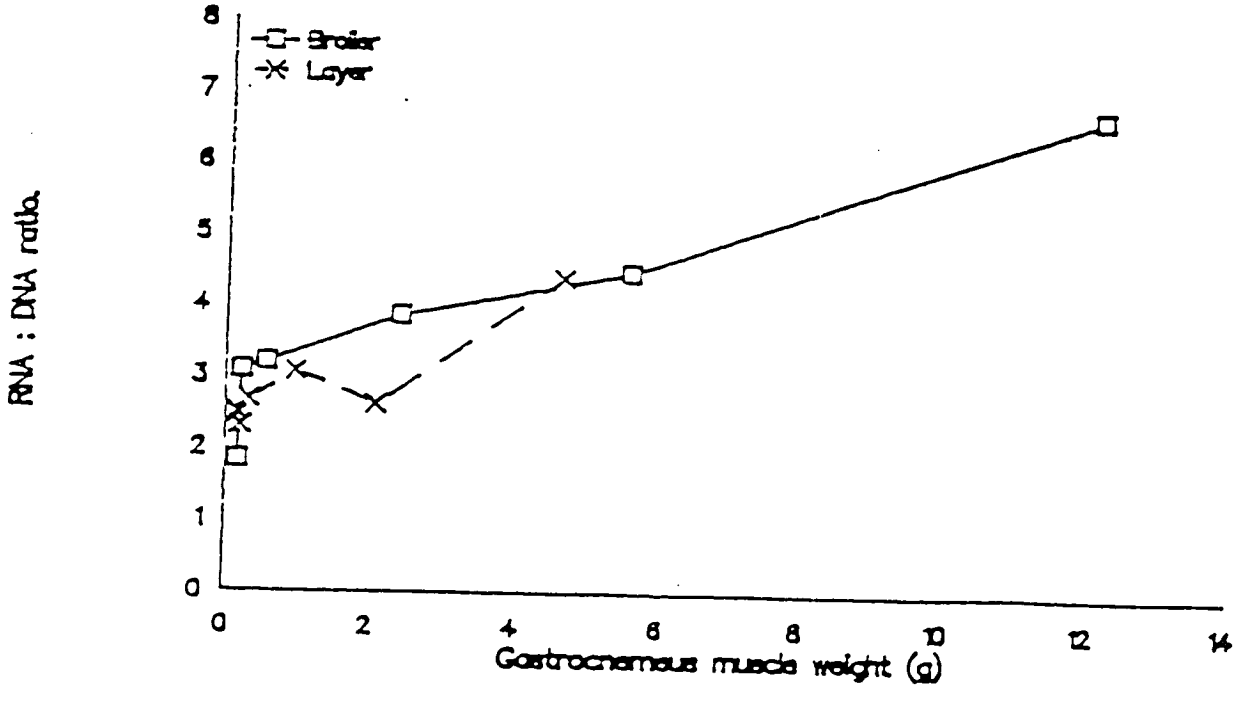


Figure 3.9b.

GM RNA : DNA ratio vs muscle weight in broilers and layers.



As described in chapter 1, post hatch increase in number of nuclei involves the transfer of nuclei into the muscle fibres from satellite cells. Increase in nuclear unit size involves protein accretion. Since both number and size of nuclear units increase during muscle growth, the rate of protein accretion and the rate of accumulation of nuclei from satellite cells are important in determining the rate of muscle growth.

3.4.2 How has selection increased muscle growth?

Broilers and layers have similar numbers of nuclei in their PM and GM at hatch, but by 10 weeks of age broiler muscle has a larger number of bigger nuclear units than layer muscle. Selection for improved growth must therefore have favoured birds with additional or more active satellite cells. In addition, selection has raised the size of the DNA unit at a given age by increasing the amount of protein associated with an individual nucleus.

Although it is not possible to determine whether number or rate of division of satellite cells is increased by selection, a clue may come from an examination of the size of DNA units during growth. Since satellite cells have a low cytoplasm to nucleus ratio (Swatland, 1984), a muscle which contains a larger number of these would have a smaller DNA unit size. A larger proportion of satellite cells may be the cause of the smaller DNA unit size seen in broiler PM when compared to the GM on the basis of muscle weight (figure 3.7a) and may also explain why the broiler PM is smaller and contains more nuclei per unit weight than layer PM at 1 day (Tables 3.1 and 3.2).

Other authors have recently published results which can be compared with these (Henteges *et al.*, 1983; Kang *et al.* 1985a; Jones *et al.*, 1985a; Klasing *et al.*, 1987). Slight differences in the absolute size of some of the variables are seen between this study and these published studies

(Kang *et al.* 1985a, Jones *et al.* 1985a). This may be due to slight differences in technique and in the genotypes compared. The muscles used by Henteges *et al.* (1983) and Klasing *et al.* (1987) are different to those examined here so comparison may not be relevant. The results from this study therefore confirm published results.

As mentioned above, in order to grow faster, broiler muscle must accumulate protein at a greater rate than layer muscle. This has been observed on many occasions and, using a variety of methods, increased protein accretion has been shown to be due to decreased rates of protein degradation relative to synthesis (see Waterlow *et al.*, 1978). The fact that the decrease is relative is emphasised by the results from this experiment which show that broiler muscle has a larger amount of RNA per gram muscle and per nucleus suggesting that broiler muscle has an increased capacity for protein synthesis.

The repeated demonstration that increased growth of muscle is brought about by decreased protein degradation has led to the acceptance of this process as the major factor causing increased muscle growth. The results described here emphasise that other factors are also important. The number of nuclei in a muscle is closely associated with muscle size and, because of the central role of nuclei in biological processes it can be suggested that the most likely site for the control of protein accretion is the muscle nucleus. Transfer of a nucleus into a muscle fibre would then be a prerequisite for an increase in fibre, and therefore, muscle weight.

The relative importance of increases in number and size of DNA units in increasing muscle weight can be assessed using a technique developed by Falconer *et al.* (1978) to compare the number and size of cells in organs of mice selected for high and low body weight. These authors show that the regression of logarithm of cell number on the

logarithm of organ weight at a particular age is an estimate of the association betwen cell number and size at that age. Using this method Falconer *et al.* (1978) demonstrate that variation in cell number accounts for about 70% of the strain difference in lung and spleen weight and about 50% of the difference in liver and kidney weight.

Table 3.4 shows regression coefficients of log number of nuclei and log DNA unit size on log muscle weight for the PM and GM of both strains at 42 days. At this age 92% of the variation in PM weight is associated with differences in the number of nuclei and 4% with DNA unit size. Equivalent figures for the GM are 49% and 55% respectively. Selection has affected these two muscles differently, increasing the PM weight at 42 days by raising the number of nuclear units and increasing the GM weight by raising both number and size of nuclear units. These results again stress the importance of satellite cells in increasing the growth of muscle. Selection must have either increased the number or rate of division of these cells in broiler muscle.

It is important to consider why different muscles are affected in different ways by selection for growth. A relevant point may be that the DNA unit in the PM is larger than in the GM. There may be an upper limit to DNA unit size, in which case the nuclei of the PM may already be near this. Increase in the size of the PM could only then occur by increase in number of nuclei but the GM, because of its smaller DNA unit size, may be able to respond to selection by increasing both nuclear number and size.

An alternative method for examining the effects of selection is to compare muscles from different strains at the same muscle weight. This demonstrates whether selection has altered the cellular composition of the

Table 3.4. Regression coefficients for regression of log (total DNA) on log (muscle weight) and log (protein:DNA) on log (muscle weight) for the PM and GM in broilers and layers at 42 days of age.

Muscle	Regression Coefficient	
	tot.DNA on musc.wgt.	protein:DNA on musc.wgt
Pectoralis	0.925±0.141**	0.043±0.147
Gastrocnemius	0.489±0.146**	0.554±0.164**

** $P<0.01$ for $b \neq 0$

muscle, as opposed to merely changing the rate of growth. Comparing number, size and activity of nuclear units at a given weight shows that the cellular composition of the muscle is indeed altered. At a weight of around 20g the broiler PM has 10% less, 30% smaller and more active DNA units than the layer PM. At a similar point on the growth curve, the broiler GM has 10% fewer, similarly sized, more active units. Broilers with muscles of these weights are almost half the age of layers. Layer muscles are reaching a mature state in terms of number, size and activity of nuclear units at a much smaller weight. The major difference between muscles of similar weights from different strains is the stage of development.

3.5 CONCLUSIONS

1. Selection for improved growth in chickens has increased the rate of muscle growth by increasing the number and rate of accumulation of nuclei.

2. Selection has also acted on the size of the DNA unit although the extent of this change depends on the muscle.

3. The amount of RNA per gram muscle and per unit DNA is slightly increased in growing broilers suggesting that the capacity for protein synthesis is increased by selection for growth.

4. Comparing strains at a given muscle weight shows that layer muscle matures in terms of number and size of DNA units at a smaller weight than broilers.

CHAPTER 4. A COMPARISON OF MUSCLE DNA, RNA AND PROTEIN LEVELS IN STRAINS OF CHICKEN WHICH DIFFER IN BODY WEIGHT.

4.1 INTRODUCTION

The experiment described in chapter 3 examined muscle growth with respect to the muscle nucleus in two strains of chicken with large differences in body weight at market age and showed that selection had improved muscle growth by increasing the rate of accumulation of muscle nuclei and the amount of cytoplasm per nucleus at a given age. The extent of these changes depended on the muscle; the pectoralis muscle (PM) showing an increase predominantly in the number of nuclei and the gastrocnemius muscle (GM) having equal increases in number and size of nuclear units. The protein synthetic capacity of the muscles, as measured by the concentration of RNA and RNA:DNA ratio was also slightly enhanced by selection. The experiment described in this chapter examines these components in several different strains at one age to see if the above conclusions hold in general, or are specific to the strains used in the earlier experiment

4.2 EXPERIMENTAL DETAILS

Dissections and biochemical assays were carried out as described in Chapter 2.

The strains used in this experiment represent strains available at the Institute of Animal Physiology and Genetics Research, Roslin over a three month period in Winter 1986-1987. Most of these strains were part of other experiments and therefore management and husbandry varies between strains. Also included in this experiment is the broiler strain used in Chapter 3 raised under ideal conditions (deep litter, ad lib feeding and 23.5h L: 0.5h D) and under normal IAPGR conditions (wire runs,

ad lib feed and 14h L:10h D). The extent to which these non-ideal conditions affect body and muscle growth can thus be measured. 10 male birds of each strain at 7 weeks of age were used.

One-way analysis of variance is used to detect the effect of strain and treatment on each of the variables.

4.3 RESULTS

An exact description of the strains used in this experiment is given in Table 4.1. The strains represent a large range of growth rates; a seven-fold difference in 7 week body weight being evident between the largest and smallest strains. The effect of ideal versus non-ideal rearing conditions on broiler growth can be seen by comparing the performance of the 1972-relaxed broiler line (MK) and the contemporary broiler grandparent (101) under both sets of conditions. Birds raised under ideal conditions show a 20% and 48% higher 7 week body weight respectively.

Strain differences in muscle weight grossly reflect differences in body weight (Table 4.2).

DNA concentration is an estimate of the number of nuclei per unit muscle. Strains of chicken with heavier bodies, and therefore muscles, tend to have lower DNA concentrations in both the PM and GM (Table 4.3). Selection for growth has reduced the number of nuclei per unit muscle (PM $P<0.05$, GM $P<0.01$).

RNA concentration is a measure of the protein synthetic capacity of a unit weight of muscle. As with DNA concentration, RNA concentration in both muscles falls with increasing body weight of the strains (Table 4.3) showing that selection for growth has reduced the amount of RNA per unit muscle ($P<0.01$). Two strains have PM RNA concentrations which stand out from the other strains.

Table 4.1. A description of strains and their rearing conditions. Mean (± s.d.) 7 week body weight is also given and strains are arranged in order of this weight.

Strain	Description	Body weight(g)
Bantam	Random-bred bantam line. maintained at IAPGR.	376.0± 43.1
71	Layer grandparent line. White Leghorn, brown egg layer. IAPGR, Roslin.	522.0±105.8
MK non-ideal	Control broiler grandparent line. Random-bred since 1972. IAPGR, Roslin	1366.3±161.6
MK ideal	As MK but obtained from Ross Breeders Ltd and reared under ideal conditions	1635.2±163.4
101 non-ideal	Broiler grandparent line, obtained from Ross Breeders Ltd. and reared at IAPGR, Roslin.	1891.3±221.8
VLDL lean	Broiler line selected for low plasma VLDL (Whitehead et al., 1986). IGAP, Roslin.	2206.4±239.1
M4	Broiler line, obtained from D.B. Marshall Ltd. IAPGR, Roslin.	2217.3±122.2
VLDL fat	Broiler line selected for high plasma VLDL. IGAP, Roslin.	2494.4±161.0
101 ideal	As 101 but reared under ideal conditions.	2793.9±164.1

Table 4.2. Mean (± se) pectoralis muscle weight, gastrocnemius muscle weight and proportional size of these muscles relative to the 71 layer strain. The strains are given in order of ascending 7 week body weight.

Strain	Pectoralis		Gastrocnemius	
	wgt(g)	:71	wgt(g)	:71
Bantam	12.84±0.64	0.90	1.44±0.06	0.67
71	14.26±0.75	1.00	2.14±0.09	1.00
MK non-ideal	45.27±1.18	3.17	5.69±0.23	2.66
MK ideal	59.67±2.75	4.18	7.04±0.17	3.29
101 non-ideal	81.87±6.33	5.14	7.31±0.48	3.41
VLDL lean	92.86±4.59	6.51	10.10±0.53	4.72
M4	95.59±3.98	6.70	8.59±0.24	4.01
VLDL fat	92.72±2.71	6.50	10.82±0.42	5.06
101 ideal	147.51±6.03	10.34	12.20±0.57	5.70

Table 4.3. Mean (± se) DNA, RNA and protein concentration in the PM and GM of strains of chicken. The proportional size of these concentrations relative to layer strain 71 are also given for each strain. Strains are arranged in order of ascending body weight.

Strain	DNA conc. (mg/g)	:71	RNA conc. (mg/g)	:71	Protein conc. (mg/g)	:71
Pectoralis muscle						
Bantam	0.246±0.011	0.85	1.290±0.056	1.00	196.3±3.7	1.06
71	0.288±0.017	1.00	1.295±0.023	1.00	185.1±4.7	1.00
MK non-ideal	0.268±0.022	0.93	1.309±0.044	1.01	193.9±5.8	1.05
MK ideal	0.224±0.012	0.78	1.290±0.054	1.01	207.8±5.5	1.12
101 non-ideal	0.204±0.012	0.71	1.374±0.046	1.06	201.4±4.8	1.10
VLDL lean	0.233±0.009	0.81	1.187±0.033	0.92	204.1±4.6	1.10
M4	0.226±0.018	0.78	1.256±0.038	0.97	202.1±3.8	1.09
VLDL fat	0.200±0.008	0.69	1.079±0.036	0.83	200.0±3.1	1.08
101 ideal	0.196±0.017	0.68	1.210±0.016	0.93	202.9±2.8	1.10
Gastrocnemius muscle						
Bantam	0.529±0.050	1.23	1.434±0.053	1.07	170.5±4.2	0.97
71	0.429±0.025	1.00	1.345±0.042	1.00	175.9±4.2	1.00
MK non-ideal	0.421±0.036	0.98	1.302±0.031	0.97	177.7±3.1	1.01
MK ideal	0.357±0.036	0.83	1.216±0.050	0.90	171.5±5.9	0.97
101 non-ideal	0.308±0.021	0.71	1.310±0.061	0.97	178.0±4.2	1.01
VLDL lean	0.345±0.026	0.80	1.122±0.033	0.83	178.2±4.4	1.01
M4	0.340±0.018	0.79	1.257±0.048	0.93	180.4±2.7	1.02
VLDL fat	0.231±0.021	0.54	1.011±0.056	0.75	172.0±4.1	0.97
101 ideal	0.284±0.033	0.66	1.034±0.058	0.77	169.2±3.8	0.96

The 101 grown under non-ideal conditions and the VLDL fat birds have exceptionally high and low RNA concentration in their PM's respectively.

Protein concentration in the PM and GM behave differently as 7 week weight of the strains increases (Table 4.3). The PM shows a slight increase in protein concentration with increasing muscle weight ($P<0.01$). The GM, on the other hand, shows no trend: strains which differ in 7 week body weight have similar protein concentrations in their GM's. One way analysis of variance shows no significant variation between strains in GM protein concentration.

Total DNA is an estimate of the number of nuclei in a muscle and plots of total DNA versus muscle weight for each muscle are shown in Figure 4.1 (data in table 4.4). Linear regressions of total DNA on muscle weight are shown in these plots to indicate outlying strains. The strains show a steady increase in number of nuclei with muscle weight. Seven week muscle weight is very closely related to number of nuclei in the muscle as judged by the small spread of means about the regression line. The only exception to this close relationship is the GM of the VLDL fat strain which has a lower number of nuclei than would be expected for a muscle of that weight.

The ratio of protein:DNA (DNA unit size) is an estimate of the amount of cytoplasm associated with an individual muscle nucleus and plots of protein:DNA ratio against muscle weight are given in Figure 4.2 (data in table 4.4). In both muscles there is a significant positive relationship between DNA unit size and muscle weight. Again the GM of the VLDL fat strain is exceptional to the trend having a larger DNA unit size than would be expected for a GM of that weight.

Total DNA is changed to a greater extent than protein:DNA ratio as demonstrated by the greater relative change in

Figure 4.1a.
PM total DNA vs PM weight in strains of chicken.

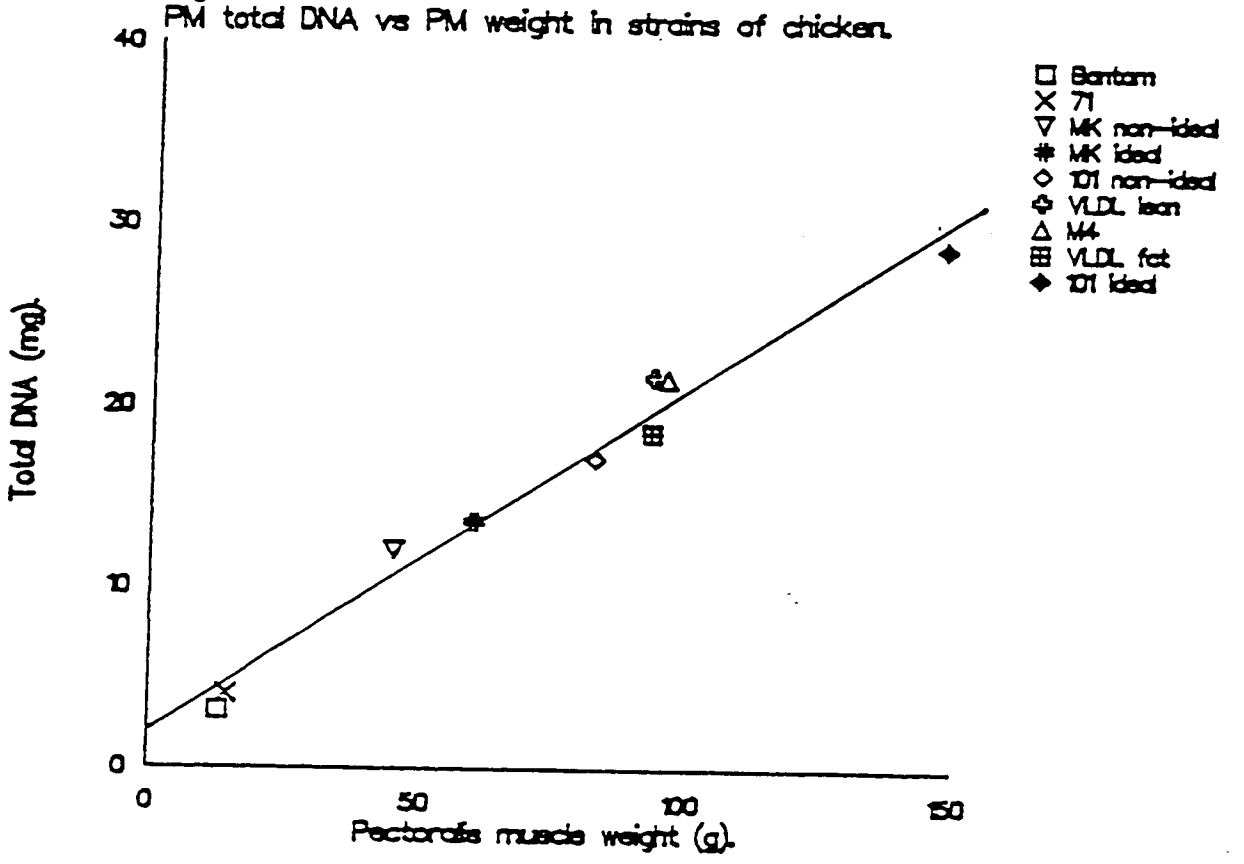


Figure 4.1b.
GM total DNA vs GM weight in strains of chicken.

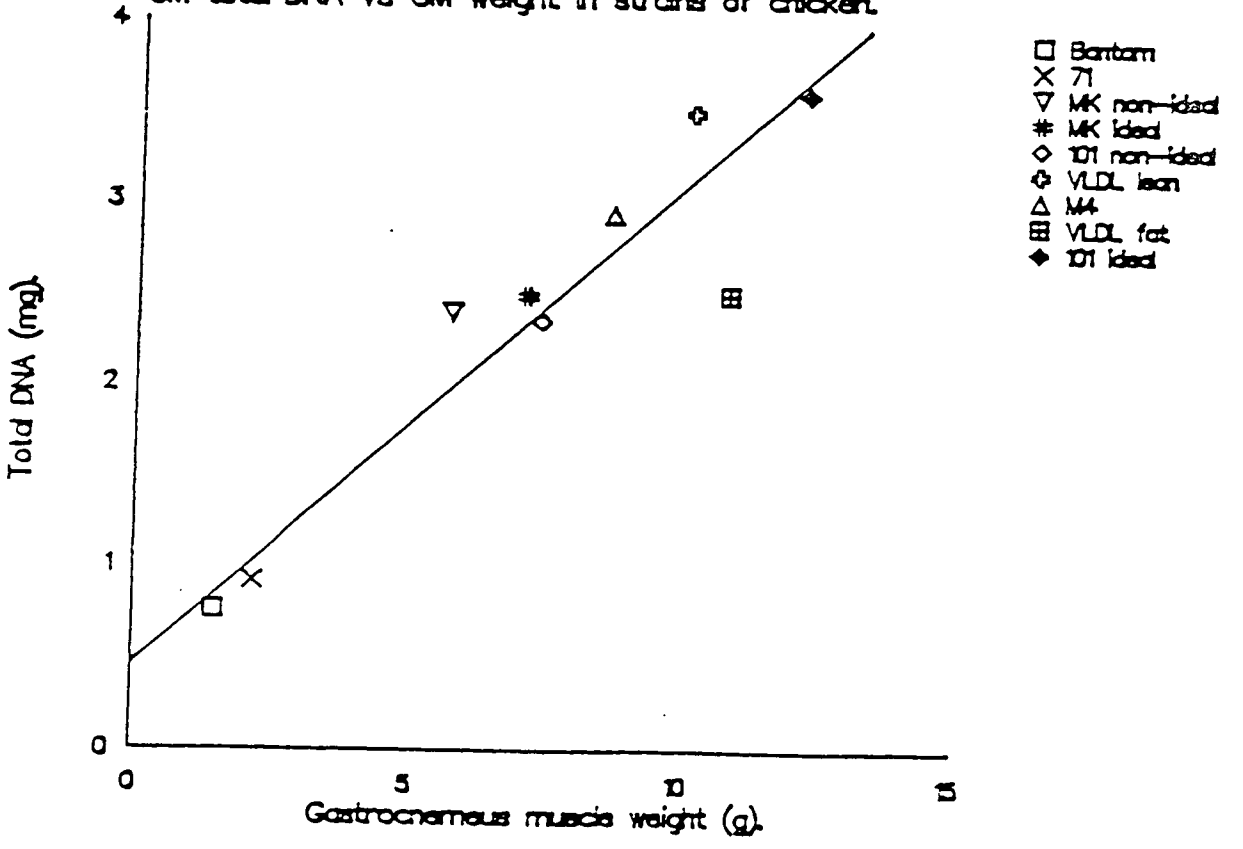


Figure 4.2a.
PM protein:DNA ratio vs PM weight in strains of chicken.

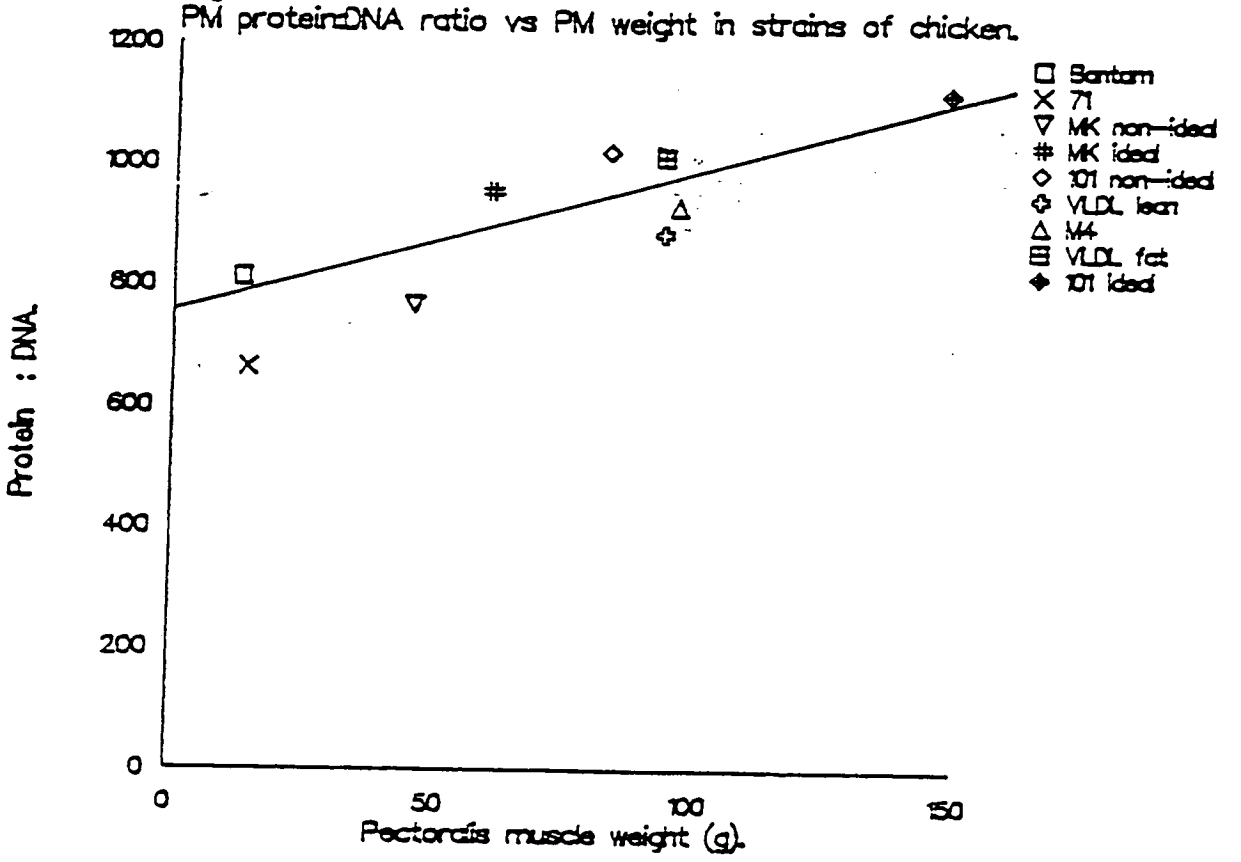


Figure 4.2b.
GM protein:DNA ratio vs GM weight in strains of chicken.

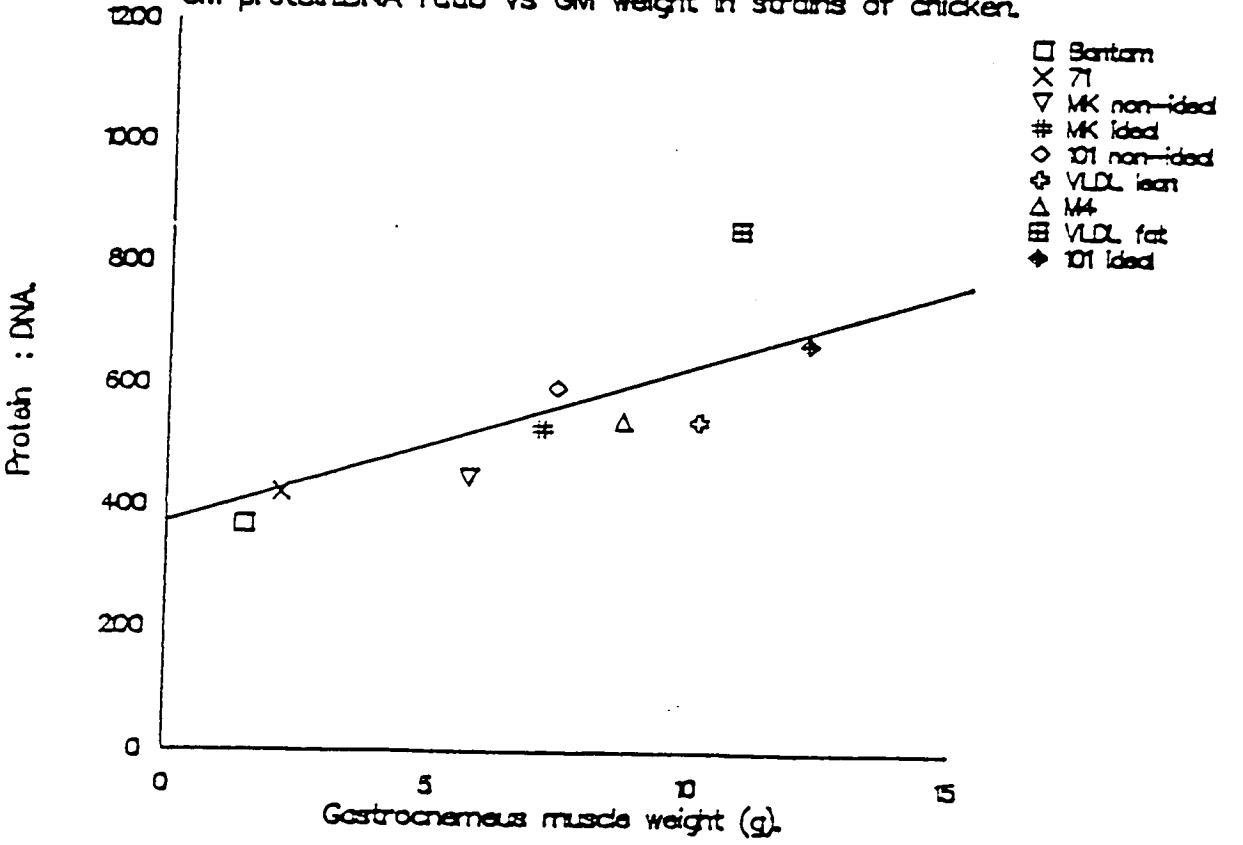


Table 4.4. Mean (± se) total DNA, protein:DNA ratio and RNA:DNA ratio in the pectoralis and gastrocnemius muscles of strains of chicken. The proportional size relative to layer strain 71 is also given. The strains are arranged in ascending 7 week body weight.

Strain	Total DNA (mg)	:71	Protein:DNA (mg:mg)	:71	RNA:DNA (mg:mg)	:71
Pectoralis muscle						
Bantam	3.17±0.23	0.77	812.5± 43.5	1.22	5.38±0.44	1.16
71	4.10±0.32	1.00	664.7± 43.9	1.00	4.63±0.28	1.00
MK non-ideal	12.07±0.94	2.94	767.6± 67.9	1.15	5.21±0.50	1.12
MK ideal	13.66±1.16	3.33	955.3± 57.4	1.45	5.92±0.40	1.28
101 non-ideal	17.15±1.80	4.18	1021.0± 85.3	1.54	6.95±0.53	1.50
VLDL lean	21.68±1.43	5.29	886.9± 41.5	1.33	5.15±0.24	1.11
M4	21.58±1.92	5.26	931.6± 57.3	1.40	5.77±0.34	1.24
VLDL fat	18.59±1.11	4.53	1013.6± 38.3	1.52	5.46±0.24	1.18
101 ideal	28.80±2.70	7.02	1118.0±111.0	1.68	6.61±0.60	1.43
Gastrocnemius muscle						
Bantam	0.76±0.08	0.83	369.1± 59.0	0.87	3.00±0.37	0.93
71	0.92±0.06	1.00	422.3± 26.3	1.00	3.23±0.21	1.00
MK non-ideal	2.39±0.20	2.60	447.3± 35.5	1.13	3.29±0.30	1.02
MK ideal	2.48±0.25	2.69	528.4± 48.2	1.25	3.63±0.24	1.12
101 non-ideal	2.34±0.34	2.54	594.8± 38.9	1.41	4.44±0.45	1.37
VLDL lean	3.49±0.31	3.79	540.8± 38.8	1.29	3.41±0.25	1.06
M4	2.93±0.18	3.18	541.7± 25.2	1.28	3.75±0.17	1.16
VLDL fat	2.49±0.24	2.71	856.0±138.0	2.03	5.08±0.90	1.57
101 ideal	3.59±0.57	3.90	668.6± 80.1	1.58	4.00±0.47	1.23

total DNA seen when the values for each strain are indexed to strain 71 (Table 4.4). This confirms the effect seen in the previous chapter.

The RNA:DNA ratio estimates capacity for protein synthesis of a nuclear unit and plots of this variable against muscle weight are shown in Figure 4.3 (data in table 4.4). Both muscles show a slight increase in RNA:DNA as 7 week body weight of the strains increases ($P<0.01$) showing that capacity for protein synthesis increases with increasing 7 week body weight. Two strains are behave differently to the others. The 101 strain grown under non-ideal conditions has a larger RNA:DNA ratio than would be expected in both muscles. The VLDL fat strain has a greater RNA:DNA ratio than expected for a GM of that weight.

4.4 DISCUSSION

The strains used in this experiment represent a wide variety of growth profiles created by selection for different characters including increased weight (all of the broiler strains), reduced weight (bantam), food efficiency (101), egg production (71), increased fatness (VLDL fat) and reduced fatness (VLDL lean). As a result of their different selection histories strains have a range of different muscle weights at 7 weeks of age and are therefore ideal material with which to investigate the effects of selection on the nuclear unit.

A close relationship was observed between the number of nuclei in a muscle and the weight of the muscle. Increased 7 week muscle weight was associated with greater number of nuclei in the PM and GM (Table 4.4 and Figure 4.1). DNA unit size also showed a positive relationship with muscle weight (Table 4.4 and Figure 4.2) but the relationship does not seem as close as that between number of nuclei and muscle weight. The range of

Figure 4.3a.
PM RNA:DNA ratio vs PM weight in strains of chicken.

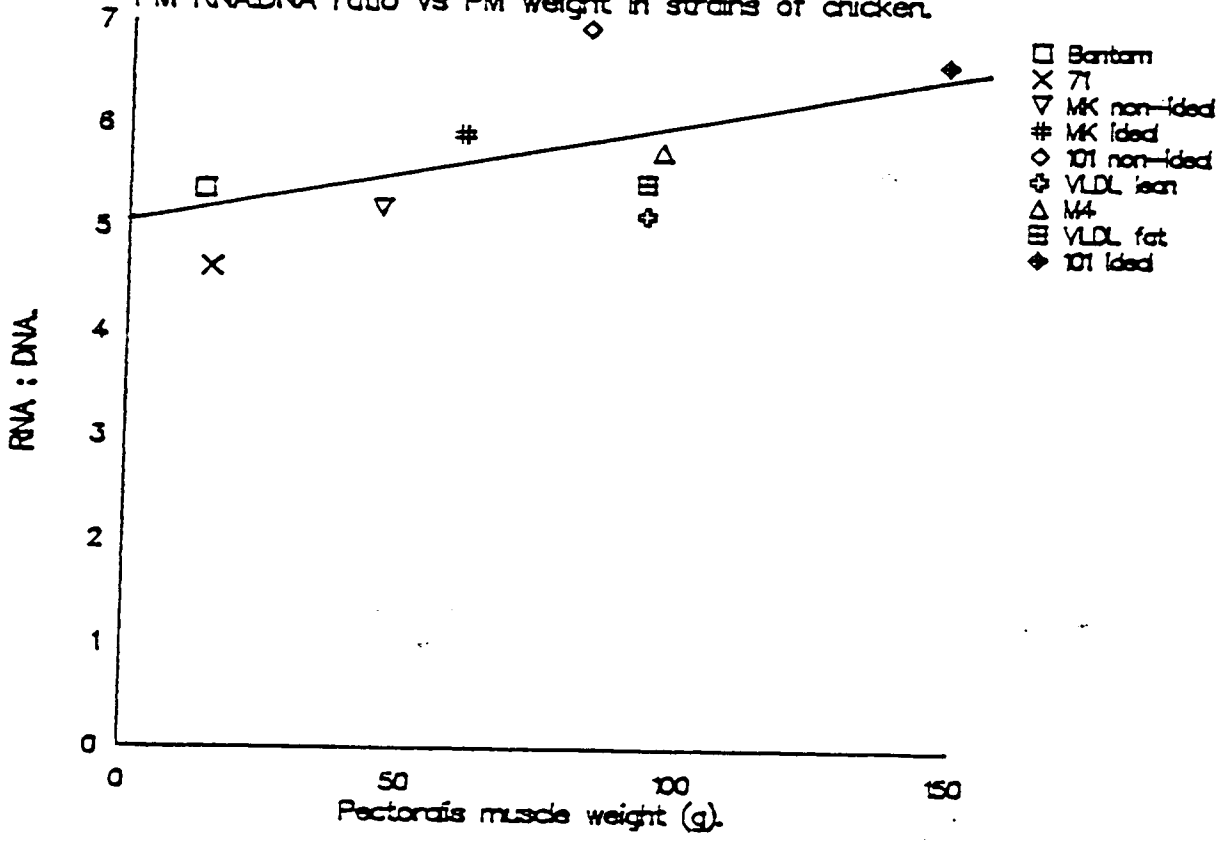
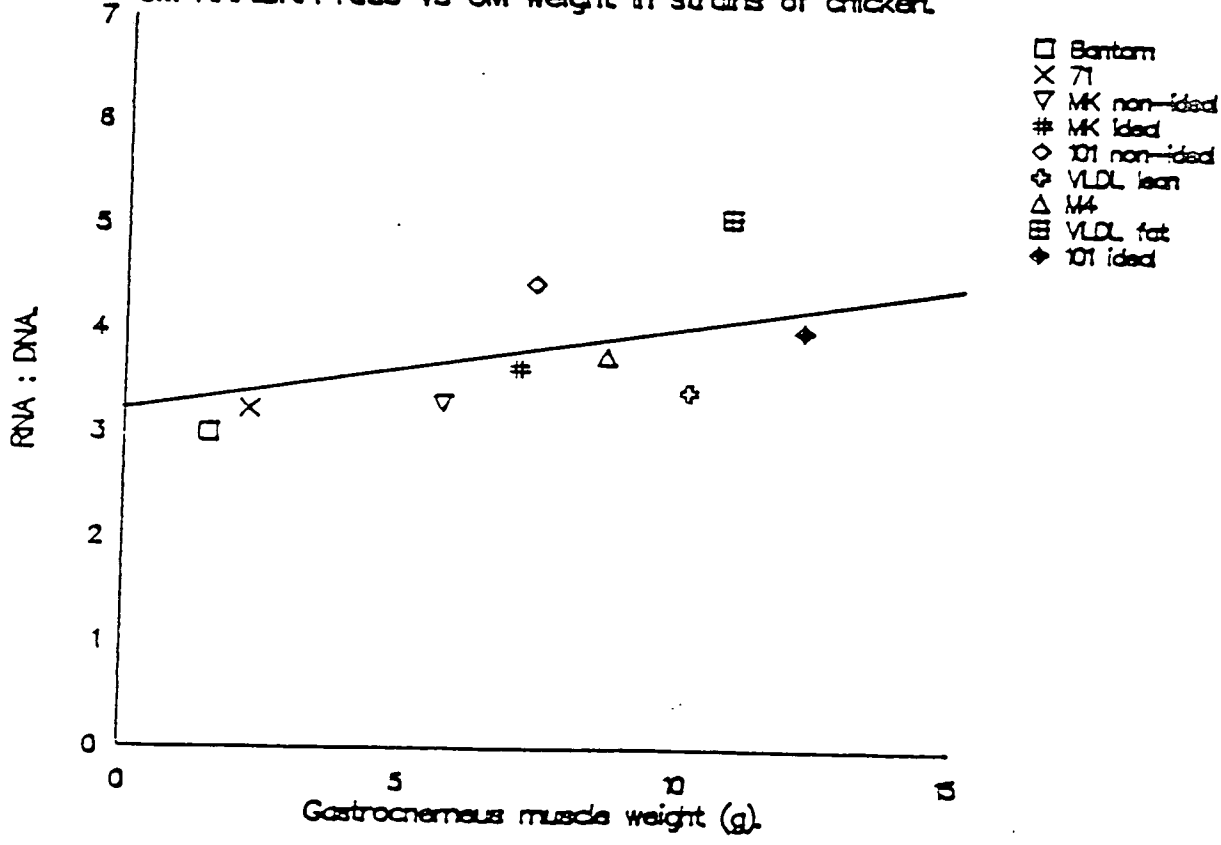


Figure 4.3b.
GM RNA:DNA ratio vs GM weight in strains of chicken.



variation between strains in number of nuclei is far greater than for DNA unit size and follows more closely the observed differences in muscle weight. This supports the results of the previous experiment (chapter 3) and also emphasises the important role that satellite cells play in muscle growth *per se* and in increased muscle growth caused by selection.

Coefficients of regression for log(total DNA) on log(muscle weight) and log(DNA unit size) on log(muscle weight) can be used to estimate the relative effects of increase in number and size of nuclear units (Table 4.5) (Falconer *et al.*, 1978). This analysis shows that 88% of the variation between strains in PM weight is associated with difference in number of nuclei and 15% associated with DNA unit size. Figures for the GM are 74% and 26% respectively. These results support the suggestion made above that the major cause of variation in muscle weight between strains is variation in number of nuclei.

The results for the PM are similar to those given in chapter 3 (Chapter 3: 93% - number of nuclei, 4% - DNA unit size. Chapter 4: 88% - number of nuclei, 15% - DNA unit size.). The GM, however, shows a larger effect of increase in number of nuclei and a smaller effect of increase in DNA unit size than was seen in chapter 3 (Chapter 3: 49% - number of nuclei, 55% - DNA unit size. Chapter 4: 74% - number of nuclei, 26% DNA unit size.). The latter result is probably more reliable since it is based on a greater number of birds (90 versus 20) and strains (7 versus 2).

The VLDL fat strain is exceptional in that has less nuclei but larger DNA units in the GM than would be expected for muscle weight. This strain has been selected for increased levels plasma very low density lipoprotein in order to change fat levels (Whitehead *et al.*, 1986). Different fibre types contain different amounts of lipid,

Table 4.5. Coefficients for regression of log (total DNA) on log (muscle weight) and log (protein:DNA) on log (muscle weight) for the pectoralis muscle and gastrocnemius muscle in chickens of various strains.

Muscle	Regression Coefficient	
	tot.DNA on musc.wgt.	protein:DNA on musc.wgt
Pectoralis	0.881±0.026**	0.148±0.028**
Gastrocnemius	0.742±0.044**	0.262±0.049**

** regression significant with $P<0.01$.

type IIA (red) fibres contain more lipid than type IIB (white) (Barnard *et al.*, 1982). Since the different fibre types also seem to have different DNA unit sizes (PM has larger DNA unit size than GM), selection for increased VLDL may have altered DNA unit size by changing fibre type.

Non-ideal rearing conditions reduce the liveweight of birds but do not have large effects on the relationships between number of nuclei, nuclear unit size and muscle weight. Both number of nuclei and DNA unit size are affected by non-ideal conditions. Examination of the graphs of these variables versus muscle weight shows that non-ideal conditions retard the growth of muscle by moving birds back along the curve seen for the other strains. This observation indicates that the strain differences observed in chapter 3 are due to selection and are not the result of non-ideal rearing conditions.

A small increase in activity of nuclear units, as measured by RNA:DNA ratio, is seen with increase in 7 week muscle weight of the strains. This is in keeping with the trends seen at 6 weeks in chapter 3 and emphasises that increased muscle growth is associated with a small increase in the capacity of a muscle nucleus for protein synthesis. The protein synthetic capacity of a gram of muscle (RNA concentration) decreases slightly as a result of selection for growth. Increased muscle growth requires a greater protein synthetic capacity from a larger DNA unit.

Two strains behave exceptionally as regards the relationship between RNA:DNA ratio and muscle weight: the 101 strain grown under non-ideal conditions has a larger than expected RNA:DNA ratio in the PM and GM and the VLDL fat strain has a larger ratio in the GM. It could be argued that the 101 strain in non-ideal conditions is trying to grow as fast as possible but is being held back

through an increased rate of protein degradation associated with the poor environment. This could be tested by examining rates of protein degradation in a strain in ideal and non-ideal conditions. The increased RNA:DNA activity seen in the GM of the VLDL fat strain is the result of the lower DNA concentration in the GM of the VLDL fat strain which may be caused by changes in fibre type in the GM of this strain (see above).

4.5 CONCLUSIONS

1. Strain variation in muscle weight is associated with variation in number and size of nuclear units.

2. Muscle weight is more closely related to number of nuclei than nuclear unit size, emphasising the role of the satellite cell in increasing muscle growth rate.

3. Increase in muscle weight is also associated with a slight increase in the capacity of the muscle nucleus to synthesize protein.

4. The conclusions made in chapter 3 are confirmed and extended.

CHAPTER 5. A COMPARISON OF MUSCLE FIBRE TYPE, SIZE AND NUMBER IN FAST AND SLOW GROWING STRAINS OF CHICKEN.

5.1 INTRODUCTION

As discussed in chapter 1, muscle is unusual in that it has two basic cellular units from which muscle growth may be analysed: the muscle nucleus and the muscle fibre. In the two previous chapters, experiments were discussed which describe how selection for increased growth has affected the muscle nucleus. This chapter investigates the effect of selection on the muscle fibre by examining the muscle fibres during the growth of a fast growing, broiler strain and a slow growing, layer strain. Three components are measured: muscle fibre type, size and number and by examination of these it should be possible to determine how selection has altered the structure of the muscle in order to increase muscle growth.

5.2 EXPERIMENTAL DETAILS

The experiment is similar to that described in Chapter 3. The same strains as used in Chapter 3: Ross 101 (a broiler grandparent line) and Ross 71 (a White Leghorn layer grandparent line) were grown in parallel and sampled at 1 day, 7 days, 14 days, 28 days, 49 days and 105 days. At each age, 3 males and 3 females of each strain were weighed and dissected. These sample sizes are small in comparison to the experiments described in previous chapters because of the time and labour required to measure fibre diameter and fibre type. Sacrifice of sample size at the expense of accuracy is the strategy of other workers in this field (H.J. Swatland - personal communication). The right pectoralis muscle (PM) and gastrocnemius muscle (GM) were weighed and sampled, frozen, sectioned and stained for determination of fibre type, size and number. Sectioning and staining was kindly carried out by I. Anderson and L. Dick of the Metabolic

Pathology Group of the Institute for Grassland and Animal Production, Roslin. Methods are described in Appendix 1. Details of methods by which fibre diameter and number were calculated from these sections are described in Chapter 2. The significance of effects of strain, sex and age was tested by analysis of variance.

5.3 RESULTS

5.3.1 Body and muscle weights

As expected broilers have significantly heavier bodies and muscles ($P<0.01$) at all ages apart from at 1 day of age (Table 5.1). Females are significantly heavier and have heavier PM's at 28 and 49 days ($P<0.05$). This observation is unexpected since it is well documented that male chickens are heavier than females (for example: Wilson, 1977). This difference could be an effect of sampling, however, this was excluded since the body weight of sampled birds was similar to those remaining at each age. Alternatively, this difference may be due to the sexes being differentially affected by non-ideal rearing conditions.

5.3.2 Muscle fibre type

It was not possible to consistently distinguish the sub-types of type II fibres (i.e. type IIA and type IIB). As time and resource constraints did not permit this trial to be repeated analysis was only carried out for type I and type II fibres. The percentage of type II fibres apparent in the PM and GM at each age for the strains and sexes is shown in Table 5.2. In both muscles, type I fibres are rare, making conclusions about the effects of age, sex and strain hard to draw. Only three general observations may be made. The PM has a higher proportion of type II fibres than the GM. The percentage of type II fibres increases towards 100 % with age in the GM

Table 5.1. Mean (± s.e.) body weight, pectoralis muscle weight and gastrocnemius muscle weight for both sexes of strains of broiler and layer from day old to 105 days of age. The ratio of weights in broiler relative to layer is also given for each variable at each age.

Strain	Age(d)	Male	B/L	Female	B/L
Body weight (g)					
Broiler	1	38.9± 1.2	0.93	43.3± 4.7	1.08
	7	114.0± 8.9	1.69	115.4± 7.8	1.76
	14	261.5± 12.5	2.53	297.5± 18.0	2.44
	28	765.6± 32.6	3.21	892.1± 34.7	3.45
	49	1966 ±146	3.25	2000.5± 65.2	3.56
	105	4860 ±240	3.15	4143 ±397	3.66
Layer	1	41.6± 0.5		40.0± 1.3	
	7	67.3± 2.0		65.4± 2.0	
	14	103.1± 1.7		121.7± 7.1	
	28	238.4± 13.6		258.8± 11.0	
	49	597.1± 32.6		562.5± 31.7	
	105	1542.3± 49.6		1131.0± 42.9	
Pectoralis weight (g)					
Broiler	1	0.22± 0.01	0.96	0.22± 0.05	0.81
	7	2.05± 0.18	1.95	2.39± 0.40	2.46
	14	6.88± 0.23	3.07	8.36± 0.85	2.83
	28	21.70± 1.82	3.46	30.66± 2.21	4.48
	49	73.25± 9.89	4.27	94.86± 1.30	5.47
	105	239.2 ±31.6	4.78	204.9 ±26.6	4.87
Layer	1	0.23± 0.01		0.27± 0.02	
	7	1,05± 0.04		0.97± 0.05	
	14	2.24± 0.22		2.95± 0.18	
	28	6.28± 0.65		6.84± 0.38	
	49	17.14± 1.50		17.35± 0.90	
	105	50.01± 2.28		42.04± 2.87	
Gastrocnemius weight (g)					
Broiler	1	0.12±0.01	1.00	0.11±0.01	0.92
	7	0.31±0.02	1.93	0.32±0.03	2.13
	14	0.78±0.02	3.12	0.83±0.11	2.67
	28	2.94±0.27	3.72	3.31±0.15	4.07
	49	7.40±0.68	3.07	7.48±0.28	3.34
	105	25.00±1.71	3.02	17.50±2.14	2.98
Layer	1	0.12±0.00		0.12±0.01	
	7	0.16±0.01		0.15±0.01	
	14	0.25±0.03		0.31±0.04	
	28	0.79±0.07		0.81±0.05	
	49	2.41±0.11		2.24±0.14	
	105	8.27±0.53		5.86±0.56	

Table 5.2. Mean (± s.e.) percentage of type II fibres in sections of PM and GM from both sexes of a strain of broilers and layers from day old to 105 days. The ratio of percentage fibre types in broiler relative to layer at each age is also given.

Strain	Age	% type II in PM		% type II in GM	
Broiler		Male	Female	Male	Female
	1	100 ±0	99.51±0.49	82.57±1.32	84.46±1.23
	7	97.84±1.66	99.29±0.71	82.21±4.63	91.30±4.26
	14	100 ±0	99.92±0.08	91.25±0.20	89.89±0.64
	28	95.82±0.18	100 ±0	95.29±2.90	97.17±2.83
	49	98.74±1.26	100 ±0	99.76±0.23	95.75±2.34
	105	100 ±0	100 ±0	91.87±2.71	97.82±1.96
Layer	1	97.75±2.25	99.66±0.34	91.98±3.27	87.45±3.11
	7	100 ±0	100 ±0	93.28±3.41	92.95±1.54
	14	97.88±1.51	100 ±0	92.83±4.39	92.35±0.12
	28	100 ±0	100 ±0	99.56±0.44	95.60±1.16
	49	98.76±1.23	99.77±0.23	97.94±2.06	100 ±0
	105	100 ±0	100 ±0	99.42±0.58	98.88±1.12
B/L	1	1.02	1.00	0.90	0.96
	7	0.98	0.99	0.88	0.98
	14	1.02	1.00	0.98	0.97
	28	0.96	1.00	0.96	1.02
	49	1.00	1.00	1.02	0.96
	105	1.00	1.00	0.92	0.99

($P<0.01$). Broilers have less type II fibres in their GM's than layers ($P<0.01$).

5.3.3 Muscle fibre diameter

Mean fibre diameter increases with age in all cases (Figure 5.1, Table 5.3). After 1 day of age broilers have larger fibre diameters than layers ($P<0.01$ after 7 days). Selection for increased growth has increased the rate of post-hatch growth in fibre diameter but reduced the diameter of fibres at hatch.

Females tend to have larger fibre diameters than males but the extent to which this is due to the unexpected differences seen between sexes in body weight and muscle weight is not known. Some association is likely since in the PM the ages at which sex differences in fibre diameter are most visible coincide with the ages at which PM weights show the biggest differences.

When mean fibre diameter is plotted against muscle weight, much of the variation seen between strains and sexes disappears (Figure 5.2). Male broilers, however, have smaller fibre diameters than female broilers when muscles of the same weight are compared. Another noticeable difference concerns the GM of female layers which has a larger fibre diameter than male layers and broilers of both sexes when muscles of the same weight are compared.

5.3.4 Muscle fibre number

An index of fibre diameter is calculated using mean fibre diameter and muscle weight in the following way. Let V, D and L be the volume, depth and length of a muscle fibre, then

$$V = \pi.(\tfrac{1}{2}.D)^2.L$$

Figure 5.1a.

PM mean fibre diameter vs age in broilers and layers.

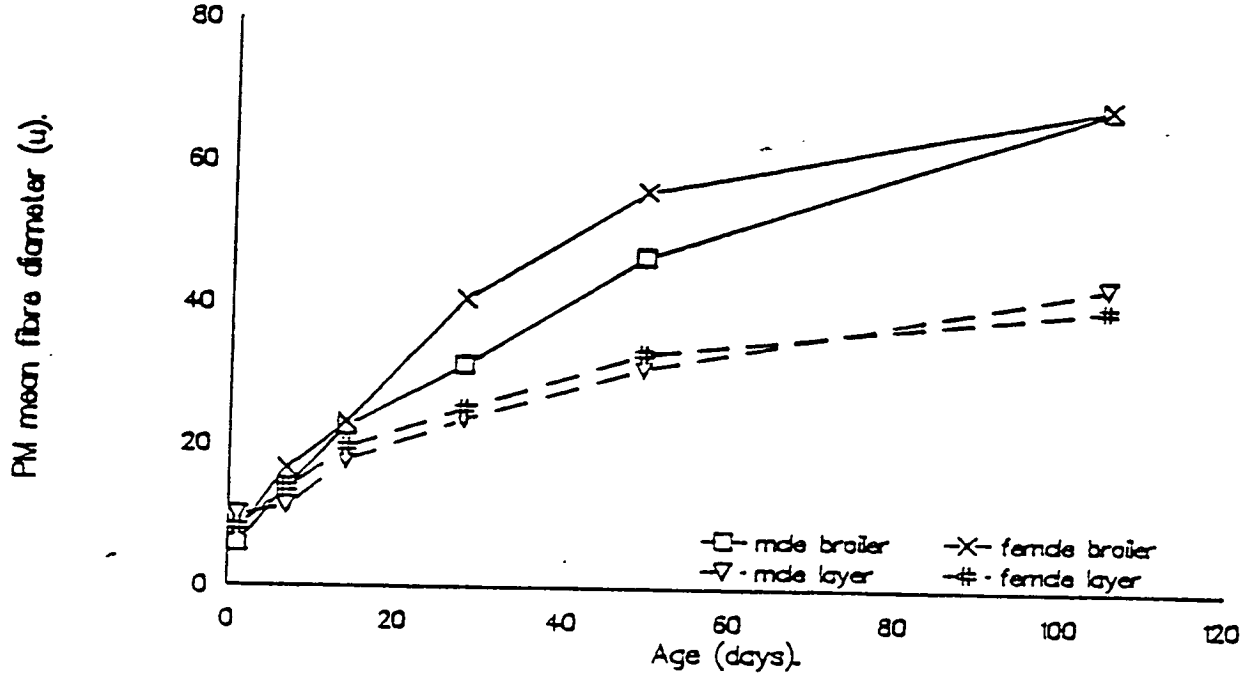


Figure 5.1b.

GM mean fibre diameter vs age in broilers and layers.

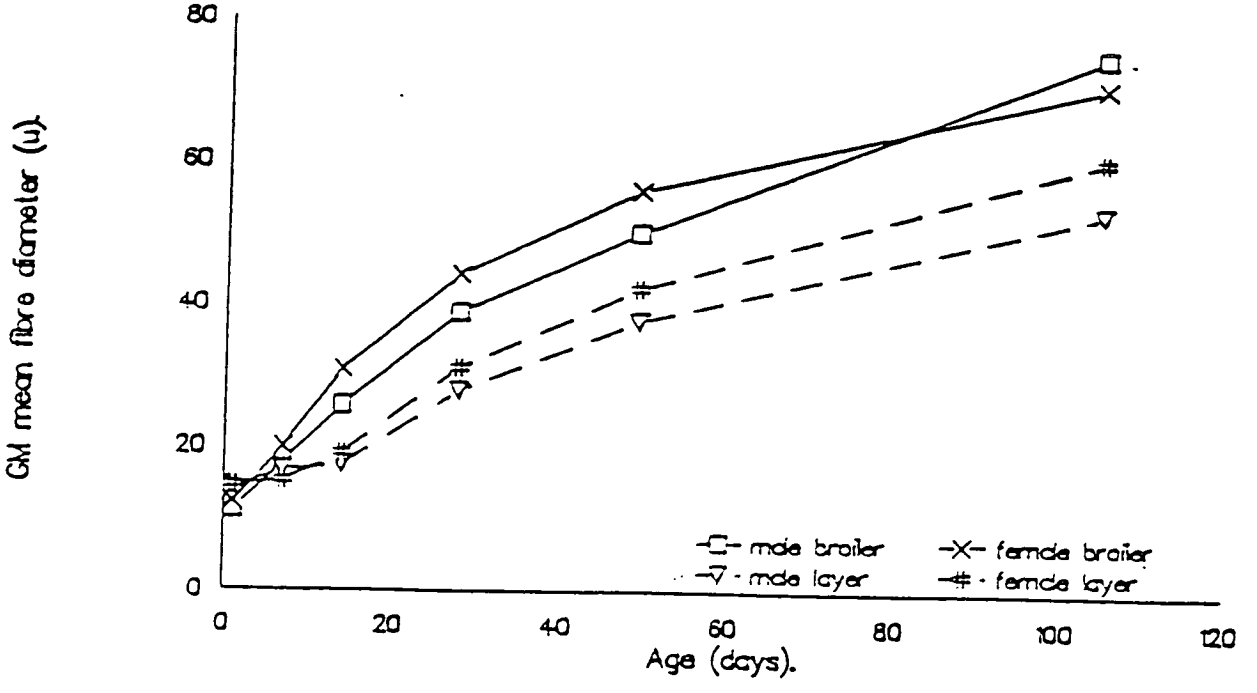


Table 5.3. Mean (± s.e.) fibre diameter in the pectoralis and gastrocnemius muscle for both sexes of strains of broiler and layer from day old to 105 days. The ratio of fibre diameters in broiler relative to layer at each age is also given.

Strain	Age	Pectoralis muscle		Gastrocnemius muscle	
		Male	Female	Male	Female
Broiler	1	6.01±0.96	7.85±1.89	11.24±0.50	12.28±0.39
	7	13.92±1.08	16.59±2.21	17.74±0.94	19.94±1.36
	14	22.61±1.58	23.19±0.55	25.74±1.03	30.76±1.03
	28	31.41±3.00	40.69±3.66	38.87±1.89	44.20±1.82
	49	46.76±3.83	55.95±7.12	50.00±2.21	55.92±1.91
	105	67.95±1.05	68.20±7.13	75.03±5.34	70.80±2.36
Layer	1	10.01±2.07	8.29±1.05	14.37±0.86	14.63±0.96
	7	11.29±0.89	13.81±0.84	16.76±1.43	15.27±0.63
	14	17.77±0.53	19.66±1.48	17.46±0.72	19.13±0.54
	28	23.71±0.38	25.23±0.93	27.82±1.14	31.04±0.73
	49	31.10±1.89	33.17±0.51	37.66±1.29	42.23±3.09
	105	42.92±1.33	39.87±1.22	53.03±3.53	60.48±1.56
B/L	1	0.60	0.94	0.78	0.84
	7	1.23	1.20	1.06	1.30
	14	1.27	1.18	1.47	1.61
	28	1.32	1.61	1.40	1.32
	49	1.50	1.69	1.32	1.32
	105	1.58	1.71	1.41	1.17

Figure 5.2a.

PM mean fibre diameter vs PM weight in broilers and layers.

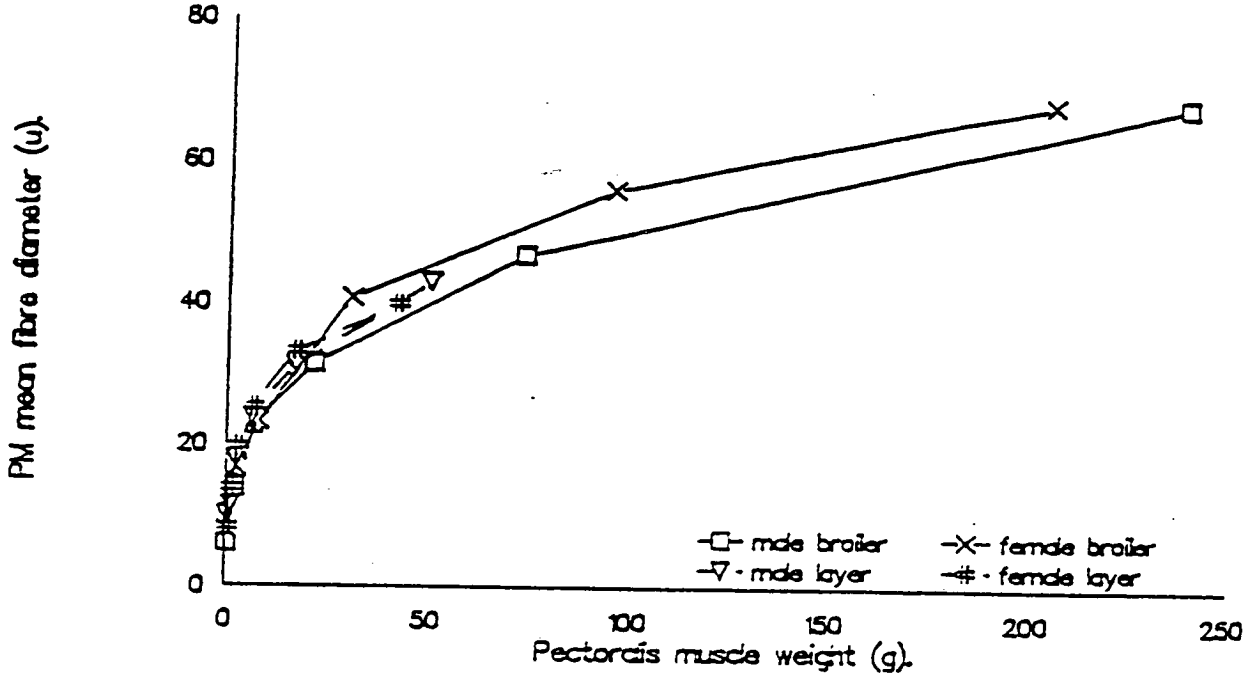
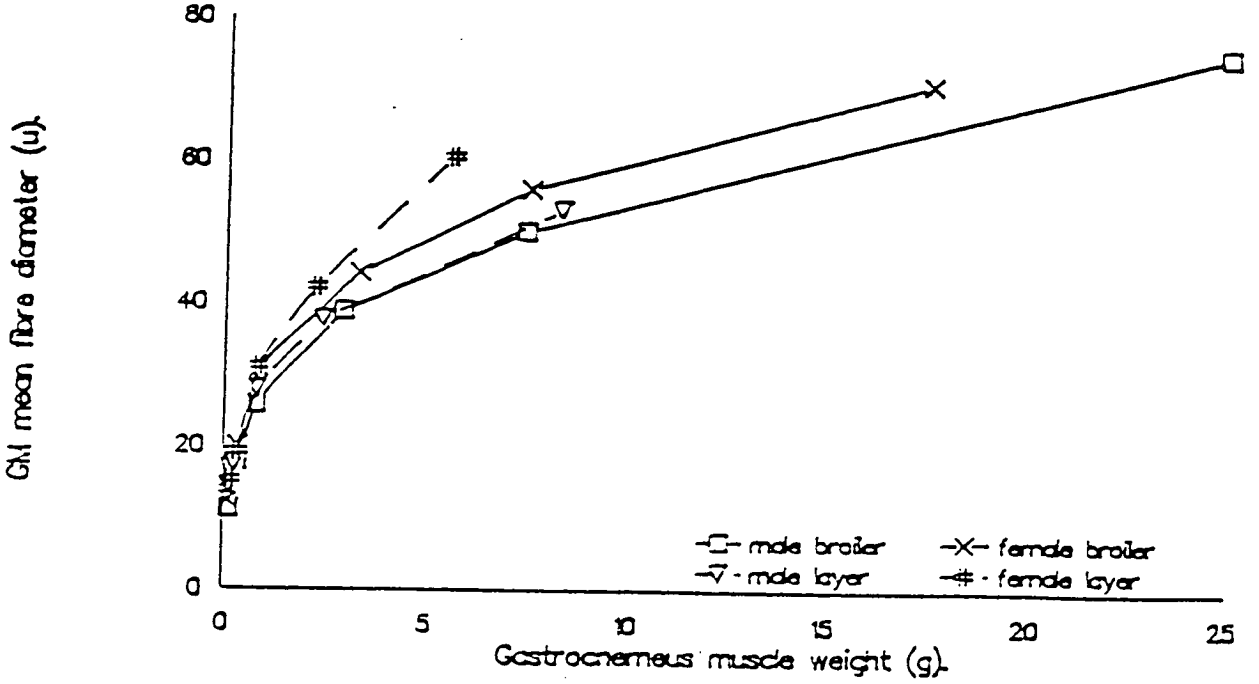


Figure 5.2b.

GM mean fibre diameter vs GM weight in broilers and layers.



It is assumed from evidence discussed later that during this period of growth, L is directly proportional to D. The weight of a fibre is proportional to its volume, so if W is the weight of a muscle and N is the number of fibres in it, then

$$W \propto N.D^3$$

and

$$N \propto W/D^3$$

The number of fibres in a muscle may therefore be estimated using the index W/D^3. This is not the actual number of fibres since the relationships between fibre volume and weight and between fibre length and diameter are not known.

When fibre number is plotted against age, no consistent trends are obvious (Figure 5.3, Table 5.4). Broilers tend to have more fibres in the PM than layers and males tend to have more fibres than females (35% in broilers and 15% in layers). Three way analysis of variance by strain, sex and age supports these observations showing significant effects of strain ($P<0.01$) and sex ($P<0.01$) but not age.

The GM behaves slightly differently. Estimated fibre number falls over the first two weeks but broilers still tend to have more fibres than layers and males still tend to have more fibres than females. Three way analysis of variance shows significant effects of strain ($P<0.01$), sex ($P<0.01$) and age ($P<0.05$).

Both the PM and GM grow without increase in fibre number. Selection for increased growth has increased the number of fibres in both muscles and males have more muscle fibres than females.

Figure 5.3a.

PM estimated fibre number vs age in broilers and layers.

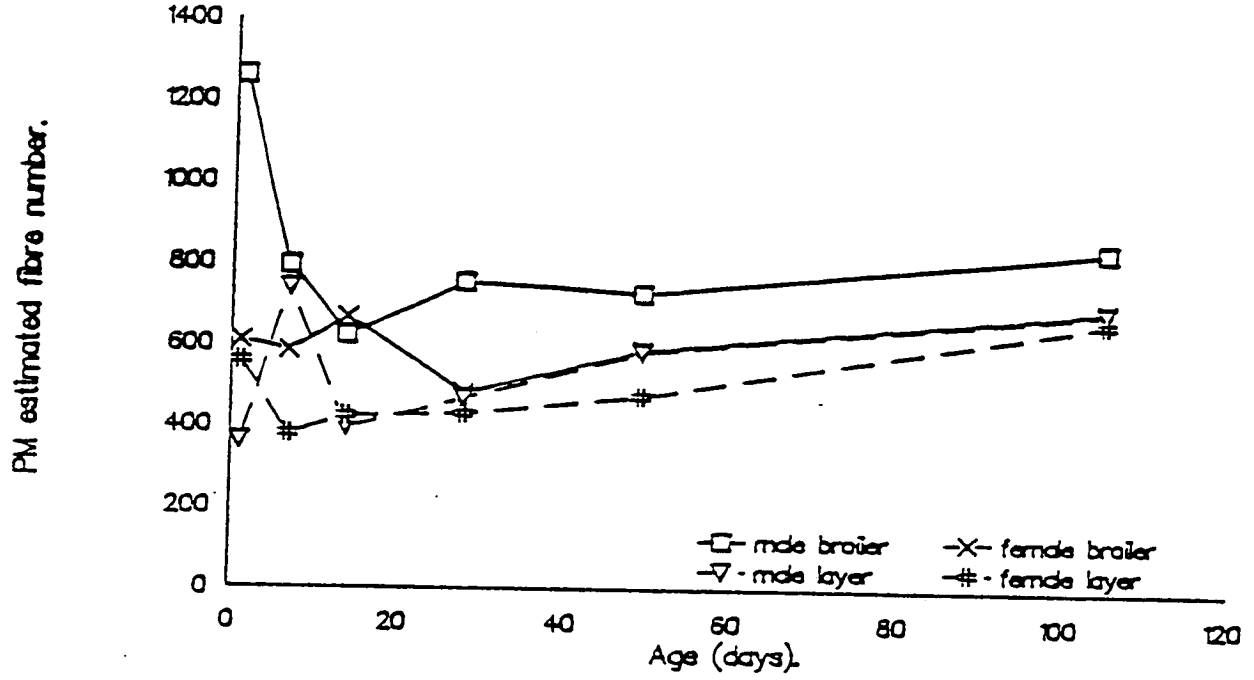


Figure 5.3b.

GM estimated fibre number vs age in broilers and layers.

Table 5.4. Mean (± s.e) estimated number of fibres in the pectoralis muscle and gastrocnemius muscle of both sexes of a strain of broilers and layers. The ratio of fibre number in broiler relative to layer at each age is also given.

Strain	Age	Pectoralis muscle		Gastrocnemius muscle	
		Male ($\times 10^{-6}$)	Female ($\times 10^{-6}$)	Male ($\times 10^{-6}$)	Female ($\times 10^{-6}$)
Broiler	1	1261 ±430	609 ±220	84.7 ±14.9	62.9 ±11.1
	7	794 ±149	584 ±170	51.19± 7.01	42.37± 7.85
	14	623 ±109	667.9± 51.0	46.42± 5.80	28.77± 4.67
	28	754 ±179	581.8± 87.2	50.03± 2.65	38.66± 3.24
	49	728.2± 90.5	589 ±217	61.4 ±11.8	48.10± 3.39
	105	838.4± 67.3	695 ±139	61.9 ±11.2	49.07± 4.07
	pooled	834.3± 64.4	601.5± 64.4	60.73± 3.39	44.33± 3.39
Layer	1	360 ±164	560 ±212	42.05± 6.83	39.43± 8.59
	7	739 ±202	379.3± 58.1	38.2 ±12.1	41.55± 3.48
	14	395.3± 11.1	424 ±107	47.37± 8.69	45.51± 8.27
	28	468.9± 38.5	430.8± 45.1	36.78± 1.50	27.16± 0.78
	49	583.8± 86.3	475.5± 20.7	45.37± 2.91	30.78± 4.26
	105	688.7± 75.3	663.0± 26.1	60.3 ±17.3	26.37± 1.35
	pooled	532.9± 64.4	488.8± 62.1	43.43± 3.39	35.13± 3.27
B/L	1	3.50	1.09	2.01	1.60
	7	1.07	1.54	1.34	1.02
	14	1.58	1.57	0.98	0.63
	28	1.61	1.35	1.36	1.42
	49	1.25	1.24	1.35	1.56
	105	1.21	1.04	1.03	1.79
	pooled	1.56	1.23	1.40	1.26

5.4 DISCUSSION

5.4.1 Sex and muscle growth

As expected, broilers grew faster than layers, but for most of the experiment females were actually heavier than males. This contradicts the established observation that male chickens are heavier than females (for example: Wilson, 1977) meaning that conclusions about the effect of sex on muscle growth cannot be made from these data. The unexpected sex differences do not, however, invalidate conclusions on the effect of selection on muscles or their constituent fibres.

5.4.2 Muscle fibre type

As discussed in chapter 1, muscle fibre type describes the biochemical characteristics of a muscle fibre and as such may be important to the poultry industry as an indicator of meat quality (Greaser *et al.*,1986). Broiler chickens have been shown to have a greater proportion of anaerobic fibres (type IIB) than layers (Aberle *et al.*, 1979) and a similar effect of selection has been observed in the pig (Swatland, 1984). Unfortunately, due to problems with the staining technique, type IIA and IIB fibres could not be reliably distinguished in the strains examined here preventing an analysis of the effects of selection on the proportions of type IIA and IIB fibres for this strain of broiler chicken.

Analysis was possible for type I and type II fibres. Type I fibres are thought to derive from primary myotubes and type II fibres from secondary myotubes (Swatland, 1984), therefore, differences in the proportions of these fibre types may show how selection has affected muscle growth in the embryo. Broilers tend to have less type II fibres in the GM than layers suggesting that selection has

increased the number of myotubes produced during the primary phase of myotube generation.

5.4.3 Muscle fibre diameter

Broiler muscles are larger than layer muscles partly because they have muscle fibres with larger diameters. This difference is generated after hatch, since day-old broiler chicks have smaller muscle fibre diameters than layer chicks (Table 5.3). Ricklefs (1985) has suggested that a constraining functional relationship may exist between developmental maturity and potential growth rate of avian muscle causing the muscle of selected lines to be less well developed than in unselected lines. Until now, the only evidence in support of this hypothesis was indirect. Chicks from selected lines are less able to control their body temperature by shivering in cold temperatures. The smaller fibre diameter seen in broilers at hatch is direct evidence that selection for increased growth proceeds by delaying the development of muscles. Selection for increased growth has favoured birds with more, less mature muscle fibres at hatch.

Strain differences seen in muscle fibre diameter are reduced when plotted against muscle weight (Figure 5.2). The increased muscle weight of broilers at market weight seems to be caused, at least in part, by the increased rate at which broilers move along the fibre diameter - muscle weight curve.

5.4.4 Muscle fibre number

Muscle fibre number is estimated by dividing muscle weight by the cube of mean fibre diameter. This assumes that fibre length increases in direct proportion to fibre diameter. Moss (1968a) showed that muscle fibres in the chicken PM maintain a constant ratio of fibre diameter to length throughout growth. In the GM, this ratio remained

constant up until two months of age (the age at which mature frame size was reached) after which fibre diameter continued to increase without change in fibre length. The assumption that fibre diameter is directly proportional to fibre length is therefore justified for the chicken.

Moss (1968a) shows the assumption that fibre length and diameter increase in direct proportion holds within strains, however, if this ratio were to differ between strains, then the estimation of fibre number would be inconsistent. Selection for increased growth might increase the length of fibres relative to their diameters causing fibre weight to be underestimated and differences in fibre number between strains to be overestimated. There is no evidence to show how fibre length is affected by selection (Swatland, 1984). The role of fibre length in increasing muscle weight by selection is not known at this time.

The observation that fibres in the GM maintain a constant ratio of length to diameter until mature frame size is reached (Moss, 1968a) may explain why female layers have larger fibre diameters in their GM than male layers or broilers (Figure 5.2b). Layer females may have reached mature frame size and further growth of the GM occurs only by increase in fibre diameter.

Although the PM grows without significant change in muscle fibre number, the PM of broiler males and the GM of both sexes of broilers exhibit a fall in estimated fibre number over the first week. The estimates of fibre number at 1 day may be less reliable than at other ages because of the very small size of the fibres at this age and because fibres are less densely packed than at older ages resulting in a different relationship between fibre weight and muscle weight. Assuming that the figures for day 1 are a result of these inaccuracies, chicken muscle is seen to grow without increase in fibre number. Pooling

figures over ages gives a more reliable estimate of the relative numbers of fibres in different strains. Selection has increased PM fibre number by between 23% (female) and 56% (male) and in the GM by 26% and 40%.

5.4.5 Selection and the muscle fibre

Broilers have larger muscles than layers of the same age because they have more fibres with larger fibre diameters. The extent of the difference in muscle fibre number is estimated above by pooling over ages. Pooling is only possible for fibre number since this does not change during post-hatch growth (Swatland, 1984). In order to estimate the extent of changes in characters (such as fibre diameter and muscle weight) which change relative to each other with age, the method described by Falconer *et al.* (1978) and used in chapters 3 and 4 can be used. The coefficient of the regression of log(size) or log(number) of units in an organ on log(weight) of the organ calculated using results for different strains at one age estimates the proportion of the difference in weight at that age which is associated with size or number of units. Regression coefficients for regressions of log(fibre diameter) on log(muscle weight) and log(fibre number) on log(muscle weight) at 49 days for the PM and GM are given in Table 5.5. Forty nine days is chosen because this is approximately the age at which market weight is reached. At this age 11% of the variation in PM weight and 32% of the variation in GM weight is associated with difference in fibre number. The figures of 30% and 23% for the proportion of the variation in PM and GM weight associated with difference in fibre diameter underestimate the real effect of change in fibre diameter for the following reason. Fibre diameter maintains a constant ratio with fibre length during growth so an increase in fibre diameter will increase muscle weight by a function of

Table 5.5. Regression coefficients for regressions of log fibre diameter and log fibre number on log muscle weight for the PM and GM at 49 days of age.

Muscle	Regression Coefficient	
	fibre diam. on musc.wgt.	fibre number on musc.wgt
Pectoralis	0.298±0.036**	0.106±0.108
Gastrocnemius	0.227±0.044**	0.319±0.132**

** regression significant with $P<0.01$

(fibre diameter)^3. The real figures for the proportion of the difference in PM and GM weight at 49 days associated with fibre diameter are 89% and 69% respectively. Selection has increased muscle weight at 49 days primarily by increasing fibre size.

The difference in magnitude of the effects of fibre diameter and (fibre diameter)^3 emphasises the importance of fibre size in determining muscle weight. Differences in fibre diameter between treatments and strains are documented in the literature but many of these papers underestimate the true effect of observed changes in fibre diameter on muscle size because the relationship between fibre diameter and fibre weight is ignored.

Similar work to this has been carried out previously in the chicken (Smith, 1963; Moss, 1968b; Mizuno and Hikami, 1971 and Knitzetova *et al.*, 1972). The broiler used in the experiment described here grows very much faster than the breeds used in these earlier studies. It has been generally concluded that fibre diameter is the major cause of strain differences in muscle weight (Smith, 1963, Moss, 1968b, Knitzetova *et al.* 1972) showing that the increased muscle growth of the modern broiler has occurs in a similar way as in the past. The fibre diameter measured for broilers in the experiment described here is larger than that measured by the above authors. Where comparison is possible the broilers in the current experiment have between 20% (Moss, 1968a) and 100% (Mizuno and Hikami, 1971) larger fibres in the PM. It is not known whether these differences reflect variation in sectioning techniques or are due to the continuing effect of selection for growth.

Fibre diameter and number have also been investigated in other species (Swatland 1984) and, in general, the predominant role of muscle fibre diameter in increasing

muscle size seen in the current experiment is also seen for other species.

5.5 CONCLUSIONS

1. Selection for increased growth in the broiler chicken has increased muscle weight by increasing the diameter and number of muscle fibres.

2. A greater proportion of the increase in muscle weight is associated with fibre diameter as opposed to fibre number.

CHAPTER 6. A COMPARISON OF THE NUMBER AND SIZE OF DNA UNITS AND TYPE, NUMBER AND SIZE OF MUSCLE FIBRES IN LINES OF MICE DIFFERING IN GROWTH CHARACTERISTICS.

6.1 INTRODUCTION

Lines of mice have been selected divergently for lean body mass (P line) and percentage fat (F line) by Prof. Hill's group at the University of Edinburgh for over 20 generations (Chapter 2; Sharp *et al.* 1984; Bishop and Hill; 1985, Hastings and Hill, 1989). Selection in the P line has resulted in differences in lean body mass but not composition whereas selection for percentage fat has caused the F lines to differ in body composition but not lean body mass. These lines of mice therefore represent ideal material for experiments designed to measure the effects of selection for growth on the structure of muscle.

The experiment described in this chapter examines the weight and composition of muscle from the P and F mouse strains in terms of the number and size of the nuclear units and the type, size and number of muscle fibres at 10 weeks of age to determine how selection has acted at the cellular level to change muscle growth.

6.2 EXPERIMENTAL DETAILS

The mice used were from generation 28 of the selection experiment discussed in Chapter 2. All six high and low replicates from the P line and the F line were used (i.e. PH1, PL1, PH2, PL2, PH3, PL3, FH1, FL1, FH2, FL2, FH3, FL3). Two lines, random bred from the same base population for the same number of generations, were used as controls (i.e. AC1 and AC2).

The analysis was based on 3 male mice at 10 weeks of age from each replicate. Animals were weighed and killed, the left pectoralis muscle (PM) and gastrocnemius muscle (GM)

were dissected out, weighed and pooled for determination of DNA, RNA and protein concentrations as described in Chapter 2. The contralateral muscles were dissected out, weighed and used for fibre type and diameter estimations as described in Appendix 1, Chapter 2 and Chapter 5.

Analysis of variance was used to measure the significance of effects of strain and replicate.

6.3 RESULTS

PH mice have heavier bodies and muscles than controls which have heavier bodies and muscles than PL mice (Table 6.1). No significant differences are seen between the PH, PL and control lines in %muscularity (sum of muscle weights/body weight X 100).

F-fat, F-lean and control mice do not differ significantly in 10 week body weight, PM weight, GM weight or total muscle weight (Table 6.1). The F fat line, however, has a significantly lower %muscularity than the control or F lean lines.

Previous work has shown that the P lines differ in body mass but not body composition and the F lines differ in body composition but not lean body mass (Bishop and Hill 1985; Hastings and Hill, 1989). The results in Table 6.1 reflect established differences.

Examination of DNA, RNA and protein concentrations (mg/g wet weight) in the PM and GM of the P and F lines (Table 6.2) shows that selection for lean body mass and percent fat has not significantly altered the composition of muscle.

Total DNA measures the number of nuclei in a muscle. Protein:DNA and RNA:DNA ratios measure the size of the nucleus plus cytoplasm unit and the protein synthetic capacity of this unit respectively. PH mice have more

Table 6.1. Mean (± se) body weight, right pectoralis weight, right gastrocnemius weight, total muscle weight (sum of right and left PM and GM) and % muscularity (total muscle weight/body weight X 100)for lines of mice selected divergently for lean body mass (P) and percentage fat (F).

Line	n	10 week body weight (g)	:Control
P.L.	9	27.28±1.08	0.86
P.H.	9	40.36±1.25	1.28
F. fat	9	34.10±0.97	1.08
F. lean	9	32.95±1.12	1.04
Control	6	31.58±1.01	1.00
Significance of P. line difference		P<0.01	
Significance of F. line difference		NS	

Line	n	Right PM weight (g)	:Control	Right GM weight (g)	:Control
P.L.	9	0.111±0.008	0.71	0.123±0.008	0.89
P.H.	9	0.204±0.007	1.30	0.186±0.008	1.33
F. fat	9	0.141±0.008	0.90	0.151±0.005	1.08
F. lean	9	0.163±0.007	1.04	0.157±0.010	1.12
Control	6	0.157±0.008	1.00	0.140±0.007	1.00
Significance of P. line difference		P<0.01		P<0.01	
Significance of F. line difference		NS		NS	

Line	n	Total muscle weight (g)	:Control	%muscularity (%)	:Control
P.L.	9	0.473±0.028	0.78	1.733±0.080	0.90
P.H.	9	0.781±0.023	1.29	1.945±0.064	1.01
F. fat	9	0.582±0.016	0.96	1.710±0.032	0.89
F. lean	9	0.634±0.025	1.05	1.927±0.048	1.00
Control	6	0.605±0.086	1.00	1.926±0.086	1.00
Significance of P. line difference		P<0.01		NS	
Significance of F. line difference		NS		P<0.01	

Table 6.2. Mean (± se) DNA concentration, RNA concentration and protein concentration in pooled left pectoralis and left gastrocnemius muscles for mice selected divergently for lean body mass and percentage fat. The proportional size relative to Control line value is also given for each line.

Line	DNA conc. (mg/g)	:Cont.	RNA conc. (mg/g)	:Cont.	Protein conc. (mg/g)	:Cont.
P.L.	0.609±0.020	1.00	0.973±0.032	1.03	180.8±2.8	0.97
P.H.	0.559±0.013	0.92	0.921±0.027	0.97	188.1±1.9	1.01
F. fat	0.583±0.020	0.96	0.935±0.034	0.99	182.0±3.5	0.98
F. lean	0.563±0.016	0.92	0.938±0.027	0.99	182.3±3.0	0.98
Control	0.610±0.033	1.00	0.945±0.021	1.00	185.8±3.9	1.00
Significance of P line difference	NS		NS		NS	
Significance of F line difference	NS		NS		NS	

nuclei in their muscles than control mice which have more nuclei than PL mice (Table 6.3). No significant differences are observed between the F fat, F lean and control mice in number of muscle nuclei. Selection for lean body mass which alters muscle size has altered the number of muscle nuclei, but selection for body composition does not affect the number of nuclei in a muscle.

The PH mice have significantly larger DNA units than controls or PL mice (Table 6.3) showing that selection for lean body mass has affected size as well as number of nuclear units in a muscle. No significant effect is seen on DNA unit size in the F line mice (Table 6.3) showing that selection for body composition does not affect DNA unit size.

RNA:DNA unit size is similar across lines (Table 6.3). Selection for lean body mass or body composition has not affected the protein synthetic capacity of the nuclear unit in muscle at 10 weeks of age.

When the mean fibre diameter in muscles of the P and F lines are grouped according to fibre type (Table 6.4), no significant differences are observed. Selection for lean body mass and body composition has not altered fibre diameter of the individual fibre types.

Percentage fibre type and fibre number were not measured since it was apparent that there was variation in distribution of fibre types within the PM and GM. This meant that the sections examined could not be assumed to be representative of the muscle as a whole in terms of fibre type. No conclusions about the effect of selection on percentage fibre type and fibre number can therefore be made from this experiment.

Table 6.3. Mean (± se) left PM weight, left GM weight, total DNA, protein:DNA ratio and RNA:DNA ratio for the muscles of mice selected divergently for lean body mass and percentage fat. The proportional size relative to Control line value is also given for each line.

Line	Left PM weight (g)	:Cont.	Left GM weight (g)	:Cont.
P.L.	0.114±0.009	0.74	0.124±0.008	0.81
P.H.	0.194±0.009	1.25	0.197±0.009	1.29
F. fat	0.136±0.005	0.88	0.154±0.005	1.01
F. lean	0.161±0.005	1.04	0.153±0.009	1.00
Control	0.155±0.009	1.00	0.153±0.010	1.00
Significance of P line difference	P<0.01		P<0.01	
Significance of F line difference	P<0.01		NS	

Line	Total DNA (mg)	:Cont.	Protein:DNA (mg:mg)	:Cont.	RNA:DNA (mg:mg)	:Cont.
P.L.	0.145±0.009	0.77	299.7±11.5	0.97	1.604±0.049	1.02
P.H.	0.218±0.007	1.16	338.1± 7.3	1.10	1.653±0.047	1.05
F. fat	0.169±0.007	0.90	316.3±15.9	1.03	1.611±0.052	1.03
F. lean	0.177±0.008	0.94	325.2± 9.2	1.06	1.668±0.040	1.06
Control	0.188±0.014	1.00	307.9±12.6	1.00	1.567±0.071	1.00
Significance of P line difference	P<0.01		P<0.05		NS	
Significance of F line difference	NS		NS		NS	

Table 6.4. Mean (± se) fibre diameter of the fibre types in pectoralis and gastrocnemius muscles of lines of mice selected divergently for lean body mass and percentage fat. The proportional size relative to Control line value is also given for each line.

Pectoralis muscle

Line	Type I (μ)	:Cont.	Type IIA (μ)	:Cont.	Type IIB (μ)	:Cont.
P.L.	33.81±2.13	1.00	36.33±1.59	0.95	59.12±3.01	0.90
P.H.	32.38±1.81	0.96	34.60±1.44	0.91	57.44±2.43	0.88
F. fat	32.47±3.99	0.96	35.23±1.46	0.92	58.98±3.02	0.90
F. lean	25.05±4.36	0.74	34.42±1.62	0.90	60.45±2.37	0.92
Control	33.63±2.62	1.00	38.05±1.91	1.00	65.41±2.30	1.00
Significance of P. line difference	NS		NS		NS	
Significance of F. line difference	NS		NS		NS	

Gastrocnemeus muscle

P.L.	37.27±2.43	1.06	35.46±1.26	0.99	54.80±2.36	0.96
P.H.	36.94±1.31	1.05	38.03±1.08	1.06	57.37±2.25	1.01
F. fat	35.01±2.11	0.99	37.73±1.45	1.05	56.82±2.59	1.00
F. lean	35.75±2.82	1.01	37.43±1.81	1.04	61.54±2.13	1.08
Control	35.24±2.01	1.00	35.93±1.23	1.00	56.93±2.19	1.00
Significance of P. line difference	NS		NS		NS	
Significance of F. line difference	NS		NS		NS	

6.4 DISCUSSION

6.4.1 Effects of selection on nuclear units in mice

The experiment described here demonstrates that selection in mice for lean body mass has altered muscle weight by changing number and size of the DNA units. Selection for body composition does not change muscle weight and does not affect the number and size of DNA units. The relative effects of changes in number and size of nuclear units can be assessed using the technique used in preceding chapters described by Falconer *et al.* (1978). The coefficient of a regression of log(total DNA) on log(muscle weight) and log(DNA unit size) on log(muscle weight) estimates the proportion of the variation in muscle weight associated with total DNA and DNA unit size respectively. The coefficients are 0.839 ± 0.083 and 0.257 ± 0.075 for the P and F line mice suggesting that 84% of the observed variation in muscle weight between lines is associated with variation in number of nuclei and 26% associated with difference in nuclear unit size.

As with chickens, (Chapters 3 and 4) selection has a greater effect on the number of muscle nuclei than on the size of the nuclear unit. This again emphasises the importance of satellite cells in changing the size of muscles as a result of selection, but the effect in mice is not so clear cut for the following reason. The experiment in chapter 3 established that the number of nuclei in muscle was similar between strains of chicken at hatch meaning that the greater number of nuclei in 6 week old broilers can only derive from post-hatch division of satellite cells. In the experiment described in this chapter, measurements are made only at 10 weeks preventing the direct implication of satellite cells as the cause of post-natal changes in muscle weight since it is possible that strain differences in number of nuclei

and/or muscle weight may exist at birth. The origin of line differences in number of nuclei must be either myoblasts (pre-natal) or satellite cells (post-natal). In order to identify which cell population is responsible, observations need to be made on the P and F line mice at birth. Due to the small size of mice at birth, such experiments would be difficult.

Other authors have examined the effects of selection on the number and size of DNA units in mouse muscle (Robinson and Bradford, 1969; Ezekwe and Martin, 1975 and Aberle and Doolittle, 1976). In all cases, increased muscle mass is associated with an increase in the number of nuclei again emphasising the importance of satellite cells or myoblasts.

The effect of selection on nuclear unit size is less clear. Ezekwe and Martin (1975) and Aberle and Doolittle (1976) do not estimate nuclear unit size. Robinson and Bradford (1969) show a reduced nuclear unit size in their fast growing line compared to controls contrasting the results described here. The positive relationship seen in the P line is more in line with observations in chickens (Chapter 3 and Chapter 4) and other species (Chapter 1 and Swatland, 1984). The negative relationship between nuclear unit size and muscle weight observed by Robinson and Bradford (1969) may be peculiar to their line.

In Chapter 3 and Chapter 4, RNA concentration and RNA:DNA ratio are used to estimate protein synthesis per unit muscle and per nucleus respectively (Waterlow *et al.*, 1978). No significant differences are observed between selected lines of mice in these variables in this experiment, but measurements were made at 10 weeks when mice are approaching adult size and are consequently growing slowly, if at all (Hill and Bishop, 1986). This may not be the correct age to find differences in amount of protein synthesis, since high rates of protein

synthesis are associated with high rates of growth. Differences in growth rate (and therefore protein synthesis rate) are more likely to be observed at earlier ages, during the period of maximum growth. RNA and DNA concentrations in muscle from lines of mice with high growth rates and controls were examined by Aberle and Doolittle (1976) who showed elevated RNA concentration (and RNA:DNA ratio since no differences in DNA concentration were observed) over the first two weeks of age. The experiment described in this chapter would not detect differences occurring early in growth.

In order to fully understand the role of protein synthesis and protein degradation in changing the rate of muscle growth of the P and F lines, these parameters must be estimated during growth.

6.4.2 Fibre diameter, number and type in mouse muscles

Previous experiments using lines of mice selected for growth show that an increase in muscle weight is associated with increased fibre number (Luff and Goldspink, 1967; Byrne *et al.*, 1973; Ezekwe and Martin, 1975 and Aberle and Doolittle, 1976). In all these experiments except Aberle and Doolittle (1976), selected mice show increased fibre size, however fibre type is not measured. The results described here contradict previous results to a certain extent since, in this experiment, fibre diameter does not vary when fibres are grouped according to fibre type. Because previous results are unanimous in showing that selection increases fibre number, it must be accepted that fibre number is likely to have altered in the P lines. The different conclusions of this and previous results are hard to reconcile until the relationship between fibre type and diameter in mice is considered. The diameter of type IIB fibres (fast, glycolitic) is approximately $^{2}/_{3}$ greater than the other fibre types (mostly type IIA (fast, oxidative) since type

I fibres (slow, oxidative) are rare in mouse muscle). If there were a shift from type IIA to IIB then the weight of a muscle would increase without a change in fibre number. Such changes are known to occur during muscle growth (Swatland, 1984). If fibre type were omitted from an analysis of fibre diameter and number, then such a change would appear as an increase in fibre diameter. This may be what other authors have observed.

The magnitude of the effect on muscle weight of a change in fibre type depends on the initial proportions, the relative diameters and the percentage change in fibre types. Calculations show that in a muscle with two fibre types initially present in equal proportions and one fibre type with fibres $^{2}/_{3}$ greater in diameter than the other, a 10% change in fibre type will cause a 9.5% increase in muscle weight. This effect is too large to be ignored. The role of this process in changing muscle weight in mice and other species must be investigated further.

6.5 CONCLUSIONS

1. Selection in P line mice which has altered lean body mass but not body composition has changed muscle weight at 10 weeks by changing the number and size of the nuclear units. The number of nuclei have changed to a greater extent than the nuclear unit size, emphasising the role of satellite cells and/or myoblasts in changing muscle growth.

2. Selection in F line mice which has altered body composition, but not lean body mass has not changed muscle weight, the number of muscle nuclei or the size of the nuclear units.

3. Muscle fibre diameter of the individual fibre types has not been changed in any of the selected lines.

Changes in fibre number, fibre length or the proportion of fibre types must account for line differences in muscle weight, but the magnitude of each of these effects is not known.

CHAPTER 7.AUTOLYTIC ACTIVITY IN MUSCLE OF STRAINS OF MOUSE AND CHICKEN DIFFERING IN GROWTH RATE

7.1 INTRODUCTION

The importance of protein degradation in determining the rate of protein accretion and therefore growth of muscle is discussed in Chapter 1. One of the unresolved problems in investigating protein accretion and its components during muscle growth is the time and expense of accurately measuring protein synthesis and protein degradation in a large number of animals. Iodice *et al.* (1966 and 1972) describe a technique which may be a useful alternative. They determined the 'autolytic activity' of a muscle by measuring the increase in amino acid concentration during incubation of a homogenate by measuring the increase in amino acid concentration during incubation at acid pH. This is considered to estimate the *in vivo* rate of protein degradation via lysosomes. Such a technique would be a useful alternative to the current methods of measuring rates of muscle protein degradation if it could be shown that the two methods were estimating similar parameters. Another potential use of such a technique would be as a selection criterion. Such a technique could be used in model species (e.g. mice) to investigate the role of muscle protein degradation in determining the rate of muscle growth and in determining the efficiency of growth. It could also prove useful in a selection programme as a potential biochemical correlate of muscle growth or efficiency.

In order to test if this autolytic activity would be of use in measuring differences in rates of muscle protein degradation, two experiments were carried out. In the first experiment, lines of mice thought to differ in rates of muscle protein degradation were compared. In the second experiment, strains of chicken known to differ in protein degradation (Bryan, 1985) were compared. If

variation in autolytic activity is associated with variation in muscle protein degradation, then lines of mice and chickens which differ in growth characteristics should show systematic differences in autolytic activity.

7.2 EXPERIMENTAL DESIGN

7.2.1 Experiment 1. Autolytic activity in mice

The mice used in this experiment were from generation 21 of the mouse selection experiment described in Chapter 2. Mice from three lines were used: the F lines selected divergently for percentage fat, the P lines selected divergently for lean body mass and the C6 line generated by crossing the control replicates of the F and P lines at generation 20. Five males and five females from each replicate from the F, P and C6 replicates were taken at 10 weeks of age, weighed, killed and the right pectoralis muscle (PM) removed, weighed and stored at -80°C. The muscle was homogenised and the autolytic activity measured as described in Chapter 2.

The data for the F, P and C6 lines are analysed using a nested three-way analysis of variance to detect effects of direction of selection, replicate within direction and sex.

7.2.2 Experiment 2. Autolytic activity in chickens

Forty as-hatched broilers (M4, D.B. Marshall Limited) and 35 as-hatched inbred layers (LC maintained at IAPGR, Roslin) were grown to 28 days of age under the conditions described in Chapter 2. Body weight of all remaining birds was recorded at the ages of 1 day, 3 days, 6 days, 10 days, 14 days, 20 days and 28 days. At each of these ages, 5 broilers and 5 layers were taken, weighed, killed, and the right PM and right gastrocnemius muscle (GM) dissected out and weighed. The PM and GM were then

sampled and stored at -80°C until autolytic activity could be measured as described in Chapter 2.

Body weights, muscle weights and autolytic activities are analysed by analysis of variance to detect effects of age and strain.

7.3 RESULTS

7.3.1 Experiment 1

Variation in 10 week body weight, muscle weight and %muscularity is observed between the G lines of mice (Table 7.1) and follows expectation from previous examinations of body composition (Bishop and Hill 1985, Hastings and Hill, 1989). Variation observed in body weight, muscle weight and body composition is not seen for autolytic activity showing that autolytic activity would not be a useful predictor of muscle protein degradation.

Significant variation in autolytic activity is seen between the sexes perhaps accounting for the differences in muscle size between males and females.

7.3.2 Experiment 2

As expected, broilers have heavier bodies and muscles than layers at all ages (Table 7.2). Autolytic activity is significantly greater ($P<0.01$) in broiler PM at all ages (Figure 7.1a, Table 7.2). No consistent differences in GM autolytic activity are seen between broilers and layers (Figure 7.1b, Table 7.2). Peaks in autolytic activity are seen at 10 days in broiler PM, layer PM and broiler GM and at 6 days in layer GM. Since the rate of muscle protein degradation is known to be less in this strain of broilers in comparison to layers (Saunderson and Leslie 1983) and the only observable difference in autolytic activity is in the opposite direction, then

Table 7.1. Mean 10 week body weight, PM weight, PM weight/body weight and autolytic activity in F and P line mice according to line and sex (replicates pooled). The proportional size relative to the control strain (C6) is also given for each variable. The significance of effects of direction of selection, replicates within direction and sex calculated from analysis of variance are also tabulated.

LINE	FEMALE		MALE		s.e. (pooled)	Effect	Sig.
Body weight							
	(g)	:C6	(g)	:C6			
Fat	28.53	1.09	34.59	1.07			
Lean	28.83	1.10	31.75	0.98		direction	P<0.01
PL	23.03	0.88	27.95	0.86	0.38	replicate	P<0.05
PH	34.55	1.32	40.13	1.24		sex	P<0.01
C6	26.22	1.00	32.30	1.00			
PM weight							
	(mg)	:C6	(mg)	:C6			
Fat	114.6	0.96	158.1	0.98			
Lean	120.1	1.01	179.5	1.11		direction	P<0.01
PL	103.3	0.87	137.2	0.85	3.2	replicate	P<0.01
PH	153.6	1.30	209.5	1.30		sex	P<0.01
C6	119.0	1.00	161.5	1.00			
PM weight / body weight							
	(mg/g)	:C6	(mg/g)	:C6			
Fat	4.03	0.89	4.57	0.91			
Lean	4.64	1.02	5.64	1.13		direction	P<0.05
PL	4.55	1.00	4.90	0.98	0.07	replicate	P<0.01
PH	4.43	0.97	5.21	1.04		sex	P<0.01
C6	4.54	1.00	5.00	1.00			
Autolytic activity							
	(mg/g/4hr)	:C6	(mg/g/4hr)	:C6			
Fat	16.14	0.88	13.45	0.96			
Lean	14.84	0.81	12.28	0.87		direction	N.S
PL	15.92	0.87	13.03	0.93	0.70	replicate	P<0.01
PH	14.18	0.77	11.85	0.81		sex	P<0.01
C6	18.30	1.00	14.07	1.00			

Figure 7.1a.

PM autolytic activity vs age in broilers and layers.

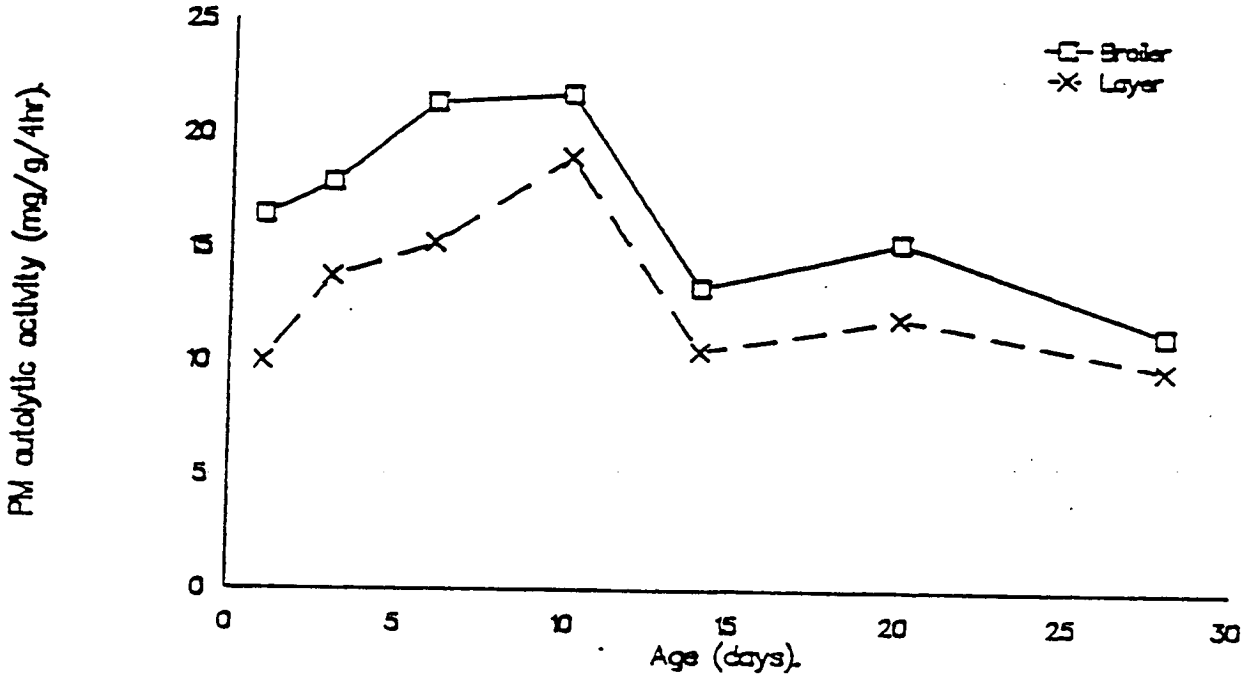


Figure 7.1b.

GM autolytic activity vs age in broilers and layers.

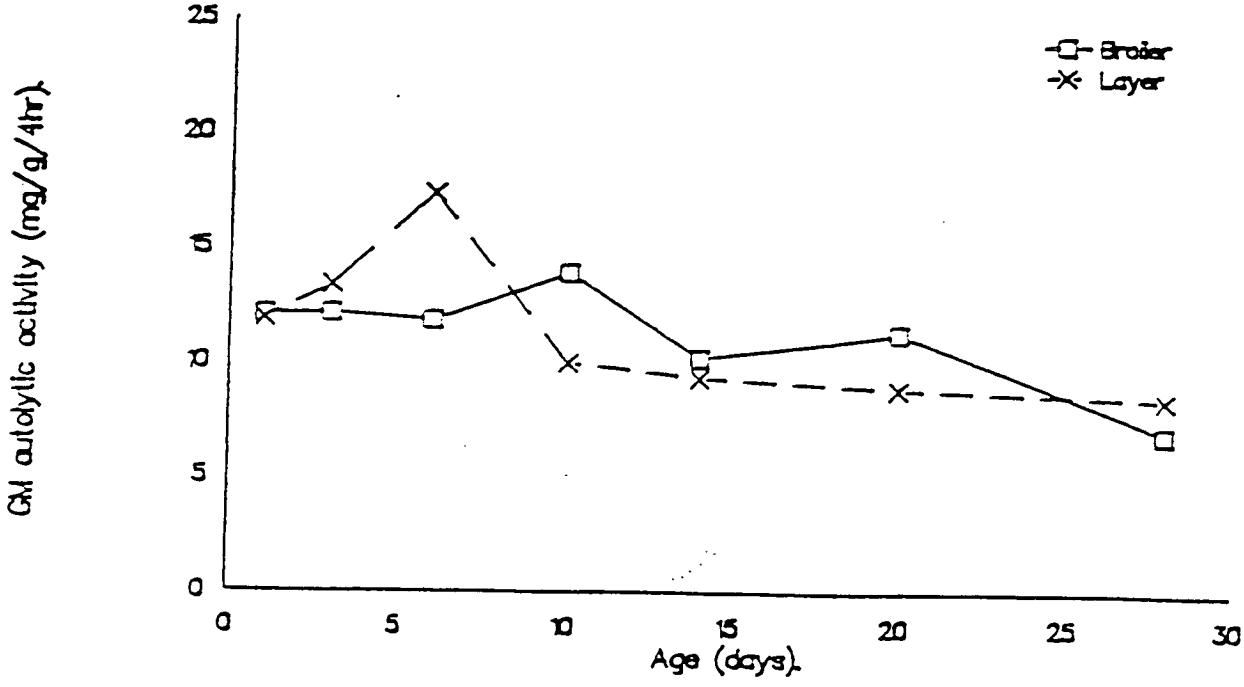


Table 7.2. Mean (± se) body weight, PM weight, GM weight, PM autolytic activity and GM autolytic activity in a strain of broilers and a strain of layers from 1 to 28 days. The proportional size of the variable in broiler relative to layer is also given in each case.

STRAIN	AGE (d)	Body Weight (g)	PM Weight (g)	GM Weight (g)
Broiler	1	43.78± 1.99	0.474±0.028	0.281±0.022
	3	56.78± 0.59	0.607±0.062	0.342±0.016
	6	78.56± 2.48	1.822±0.068	0.452±0.046
	10	126.64± 3.46	5.180±0.232	0.615±0.022
	14	182.96± 7.44	8.000±0.538	0.914±0.058
	20	348.0 ±22.9	19.58 ±2.09	2.100±0.140
	28	737.8 ± 4.99	48.98 ±4.80	5.616±0.065
Layer	1	34.57± 2.15	0.414±0.077	0.265±0.036
	3	37.07± 1.69	0.411±0.031	0.294±0.048
	6	48.32± 1.90	0.780±0.060	0.363±0.043
	10	64.15± 0.79	1.710±0.100	0.326±0.004
	14	88.22± 1.64	3.185±0.091	0.484±0.025
	20	142.17± 5.68	6.900±0.544	0.827±0.033
	28	251.3 ±12.4	13.04 ±0.66	1.765±0.135
B/L	1	1.26	1.14	1.06
	3	1.53	1.48	1.16
	6	1.62	2.34	1.24
	10	1.97	3.03	1.89
	14	2.07	2.51	1.89
	20	2.45	2.84	2.54
	28	2.94	3.76	3.18

		PM Autolytic Activity (mg/g/4 hr)	GM Autolytic Activity (mg/g/4 hr)
Broiler	1	16.44±2.05	12.05±2.20
	3	17.88±2.76	12.11±1.14
	6	21.37±1.56	11.81±0.88
	10	21.75±1.80	13.87±1.21
	14	13.29±2.73	10.16±0.63
	20	15.28±1.05	11.27±1.61
	28	11.30±3.49	6.92±1.28
Layer	1	10.00±1.66	11.86±2.13
	3	13.74±0.70	13.35±1.00
	6	15.72±1.81	17.34±2.24
	10	18.99±2.23	9.96±2.97
	14	10.48±1.55	9.30±1.31
	20	11.96±0.26	8.85±0.75
	28	9.75±1.23	8.42±0.93
B/L	1	1.64	1.02
	3	1.30	0.91
	6	1.36	0.68
	10	1.14	1.39
	14	1.27	1.09
	20	1.28	1.27
	28	1.16	0.82

autolytic activity is not an accurate predictor of the rate of muscle protein degradation in the chicken.

7.4 DISCUSSION

7.4.1 Autolytic activity

The results of the experiments described here show that autolytic activity is not a good method for measuring muscle protein degradation. The G line mice vary in growth characteristics and should therefore show systematic differences in muscle protein degradation. The chickens used were shown by Saunderson and Leslie (1983) to differ in muscle protein degradation; the broiler strain excretes approximately 25% less 3-methyl-histidine per g body weight than the layer strain. In neither the G line mice or strains of chicken was autolytic activity shown to vary in the manner expected of an estimator of muscle protein degradation.

This is not to say that autolytic activity does not measure a biological process. Autolytic activity differed significantly between the sexes of the mouse strains and peaks of autolytic activity were seen coinciding with periods of rapid growth in chicken muscle. In addition, autolytic activity has been shown to be closely related to the activity of the catheptic enzymes which are active in the lysosome (Iodice *et al.*, 1966 and 1972). It may be that muscles of male and female mice differ in size because of differences in the activity of their lysosomes, or alternatively, this difference may be due to the differential degradation of a muscle component between the sexes. Further experiment would be necessary to determine which is the case. Although autolytic activity is not directly related to the rate of muscle protein degradation, such variation suggests that it does reflect the rate of an important biological activity associated with lysosome function.

The function of the peak and consequences of the strain differences in autolytic activity seen in chicken muscle is unknown. The peak may have some role in muscle growth, since muscle growth rate is high at this stage (Ricklefs , 1983). Alternatively, it may be a consequence of high activity in the muscle at this stage since the experiment in chapter 3 revealed high levels of RNA at a similar stage. Again, further experiment would be necessary to determine the actual role and effects of this peak and these strain differences in autolytic activity.

Work carried out subsequently to this revealed that cathepsin D activity (one of the main lysosomal enzymes) in the PM is similar between the lines used in this study of chicken (Saunderson and Leslie 1987). Taking these two pieces of evidence into account, it can be concluded that the decreased rate of muscle protein degradation seen in broiler chickens is not caused by changes in the activity of the muscle lysosomes.

Work in other species is also relevant in this regard. Lowell *et al.* (1986) use rat muscle perfused with inhibitors of lysosomal enzymes to show that myofibrillar protein degradation during normal growth does not occur via the lysosome. Although decreased lysosomal activity (cathepsin D) has been shown to increase muscle protein accretion during induced hypertrophy of chicken muscle (Waterlow *et al.* 1978), muscle protein degradation via the lysosome does not appear to be directly associated with normal muscle growth.

7.4.2 Protein degradation and muscle growth

Since the lysosome does not appear to be the site of 'normal' muscle protein degradation, this raises the issue of the location and point of control of muscle protein degradation in growing muscle. Two possibilities can be suggested:

1. The control of lysosomal protein degradation may be altered in broiler muscle.

   The rate of protein degradation may depend on the redox state of the cell which is controlled by NADH, NADPH and glutathione couples (Ballard, 1977, Lindsay, 1983 and Tischler *et al.*, 1985). Changes in redox state may cause conformational changes in proteins, changing the rate of uptake of protein molecules by the lysosome, thus changing the rate of protein degradation. Also, glutathione is known to bind to proteins by breaking down disulphide bridges and may act as a marker for proteins about to be degraded via the lysosome. Changes in the redox state of the cell, perhaps associated with changes in the levels of free glutathione, may therefore alter rates of protein degradation.

2. General protein degradation may occur via another pathway.

   A system which may involve a similar marking process to glutathione, but not using the lysosome, covalently binds the peptide ubiquitin to a protein, whereupon the conjugate is rapidly degraded (Hershko and Ciechanover, 1982). Until very recently, the initial binding of ubiquitin was regarded as a marking step and the proteinases involved had not been identified (Ferber and Ciechanover, 1987). Ubiquitin has now been shown to have intrinsic proteolytic activity and it has been suggested that linking ubiquitin to a protein might form a self-degrading conjugate (Freid *et al.*, 1987). The fact that ubiquitin has been found in all cell types investigated (Fried *et al.*, 1987) would suggest that its proteolytic role is important to living cells. The role of this system in general protein

degradation during muscle growth is, as yet, unknown.

Another proteolytic system thought to be active in muscle involves a calcium-activated-neutral-protease (CANP) which selectively degrades a specific Z-line protein: 3α-actinin (Waterlow *et al.*, 1978; Bird, 1980). Although CANP does not degrade muscle specific proteins to constituent amino acids, it may be important in disrupting the structure of the myofibril to allow protein degradation to commence.

Another proteolytic enzyme of possible importance is multicatalytic proteinase which is located within the muscle fibre of rats and is suggested to have at least two active sites as shown by studies with inhibitors and synthetic substrates (Dahlman *et al.*, 1985a and b).

As discussed above, degradation of protein within muscles is complex, having several possible pathways each under independent control. Simple methods of estimating the rate of protein degradation within muscle may not be developed until the important pathways of protein degradation in muscle are identified.

7.5 CONCLUSIONS

1. Autolytic activity in growing muscle does not vary between strains of mice and chickens in the way expected of a predictor of muscle protein degradation. Autolytic activity is not a useful technique for measuring muscle protein degradation in an experimental situation or as a selection criterion.

2. Other degradative systems are known to operate in muscle. Investigations of these may identify a more useful method than autolytic activity in providing

an alternative technique for estimating the rate of muscle protein degradation.

CHAPTER 8. CAN PROTEASE INHIBITORS BE USED TO INCREASE MUSCLE GROWTH IN MICE ?

8.1 INTRODUCTION

As discussed in Chapter 1 protein accretion in muscle is the difference between protein synthesis and protein degradation. Reduction in the rate of protein degradation has commonly been cited as the route taken by animals when muscle growth is increased (Chapter 1, Waterlow *et al.*, 1978). If the rate of protein degradation could be decreased by administration of an inhibitory agent, this could provide a useful method of increasing muscle growth in meat producing species.

Actinomycetes produce several peptide protease inhibitors to counteract possible attack by proteolytic enzymes (McGowan *et al.*, 1976). Amongst these protease inhibitors are leupeptin (LEU) and pepstatin (PEP) which have been used with some success to alleviate the symptoms of muscular dystrophy in mice (Enomoto and Bradley, 1977; Chelmicka-Shorr *et al.*, 1978 and Hollenberg-Sher *et al.*, 1981) and in chickens (Stracher *et al.*, 1978). In addition, work with cell culture showed that LEU, PEP and antipain (another microbial protease inhibitor) will delay degeneration of dystrophic and normal muscle fibres (McGowan *et al.*, 1976). These effects are assumed to be due to inhibition of protease enzymes in affected muscle.

In addition to their success in relieving symptoms of MD in mice, Chelmicka-Shorr *et al.*, (1978) noted that non-dystrophic mice have an increased rate of growth over a period of injection with PEP compared with saline injected controls. This effect is proposed to be the result of reduction in the rate of protein degradation in the muscles of injected animals. If this effect is repeatable, and could be shown to work in farm animals,

then such an approach may be of use in increasing the efficiency of meat production.

The two experiments described in this chapter use similar techniques to those used by Chelmicka-Shorr *et al.*, (1978) to determine whether normal muscle growth is affected by injection of LEU and PEP.

8.2 EXPERIMENTAL DETAILS

8.2.1 Experiment 1

This experiment was an attempt to repeat the results of Chelmicka-Shorr *et al.* (1978) and Hollenberg-Sher *et al.* (1981) by using a similar injection regime and similar mouse strains. Three strains were used: C57BL/6, C57BL/10 and C57BL/10-dy/dy. The latter strain is the dystrophic strain used by Chelmicka-Shorr *et al.*, (1978). Exact methods are described in Chapter 2.

8.2.2 Experiment 2

The second experiment was a further attempt to repeat the observed effect using a modified injection regime on strains of mice thought to differ in rates of muscle protein degradation - the P line mice. These mice were considered to be appropriate for this type of experiment because of the proposed differences between lines in muscle protein degradation. If protease inhibitors affect protein degradation, then these lines should magnify any effect. The slower growing lines should be affected more by these inhibitors since they are expected to have a higher rate of protein degradation. Exact methods are described in Chapter 2. Muscles from mice used in experiment 2 were also used to determine effects of protease inhibitor injection on lysosomal enzyme activity.

Analysis of variance is used to estimate the significance of relevant effects in each experiment.

8.3 RESULTS

8.3.1 Experiment 1

Heavy mortality was seen in experiment 1, particularly in the dystrophic strain (C57BL/10-dy/dy). This strain was therefore omitted from further analysis.

Protease inhibitor injected animals have consistently greater gain (8 week body weight - 3 week body weight) and %gain (gain/3 week body weight) than control injected animals (Table 8.1) showing that protease inhibitors have a small, positive effect on body growth. C57BL/6 mice are significantly heavier than C57BL/10 mice (Table 8.1) and males have significantly greater body weights, gain and % gain than females (Table 8.1).

No significant effect of protease inhibitor injection is seen on muscle weights but, in all cases apart from the C57BL/6 females, protease inhibitor injection decreases %muscularity (PM+GM/body weight X 100) (Table 8.2). Injection of protease inhibitors does not increase muscle growth in these mice. Significant differences are seen between the sexes and strains in muscle weights and %muscularity (Table 8.2)

Only one significant difference is seen for muscle protein concentration: male mice have greater levels of protein in their PM's (Table 8.3). Protease inhibitor injection does not affect the amount of protein per gram of muscle.

The injection of the protease inhibitors LEU and PEP does not significantly improve body growth and muscle growth in these two strains of mice.

Table 8.1. Mean 8 week body weight, gain (8 week weight - 3 week weight) and percentage gain (gain/3week body weight X 100) for both sexes of mice given injections of protease inhibitors or saline. The proportional size in protease inhibitor injected mice relative to control mice and the significance of effects of strain, sex and treatment calculated from analysis of variance are also given.

Strain	Sex	Treatment	n	Body wgt (g)	Gain (g)	%Gain
		saline	5	18.30	9.23	102
	female	inhibitor	5	19.08	10.11	114
		inhib./sal.		1.04	1.10	1.12
C57BL/6						
		saline	5	23.35	11.73	102
	male	inhibitor	5	23.61	12.94	126
		inhib./sal.		1.10	1.01	1.24
		saline	5	21.05	9.87	90
	female	inhibitor	2	21.26	10.10	91
		inhib./sal.		1.01	1.02	1.01
C57BL/10						
		saline	5	24.68	13.06	112
	male	inhibitor	4	24.77	13.70	124
		inhib./sal.		1.00	1.05	1.11
pooled s.e.				0.29	0.27	4
Effect of strain				P<0.01	NS	NS
Effect of sex				P<0.01	P<0.01	P<0.01
Effect of treatment				NS	NS	NS

Table 8.2 Mean PM weight, GM weight (right + left) and % muscularity (sum of PM amd GM muscles/body weight X 100) according to strain, sex and treatment for both sexes of mice given injections of protease inhibitors or saline. The proportional size in protease inhibitor injected mice relative to control mice and the significance of effects of strain, sex and treatment calculated from analysis of variance are also given.

Strain	Sex	Treatment	PM weight (mg)	GM weight (mg)	%musc. (%)
		saline	162	206	2.01
	female	inhibitor	178	206	2.01
		inhib./sal.	1.10	1.00	1.00
C57BL/6					
		saline	306	304	2.61
	male	inhibitor	268	302	2.42
		inhib./sal.	0.87	0.99	0.93
		saline	190	218	1.94
	female	inhibitor	170	215	1.80
		inhib./sal.	0.89	0.99	0.93
C57BL/10					
		saline	250	278	2.13
	male	inhibitor	210	265	1.91
		inhib./sal.	0.84	0.95	0.90
pooled s.e.			15	9	0.02
Effect of strain			P<0.05	NS	P<0.01
Effect of sex			P<0.01	P<0.01	P<0.01
Effect of treatment			NS	NS	NS
strain X sex			P<0.01	NS	P<0.01
strain X treatment			NS	NS	NS
sex X treatment			P<0.01	NS	NS

Table 8.3. Mean protein concentration (mg protein/g wet weight) in the PM and GM according to strain, sex and treatment. The proportional size in protease inhibitor injected mice relative to control mice and the significance of effects of strain, sex and treatment calculated from analysis of variance are also given.

Strain	Sex	Treatment	PM protein concentration (mg/g wet wt)	GM protein concentration (mg/g/wet wt)
		saline	163.0	170.4
	female	inhibitor	160.3	178.0
		inhib./sal.	0.98	1.05
C57BL/6				
		saline	173.4	174.7
	male	inhibitor	166.6	174.9
		inhib./sal.	0.96	1.00
		saline	166.1	174.9
	female	inhibitor	154.9	167.8
		inhib./sal.	0.93	0.96
C57BL/10				
		saline	181.8	172.8
	male	inhibitor	169.0	171.2
		inhib./sal.	0.92	0.99
pooled s.e.			2.4	1.2
Effect of strain			NS	NS
Effect of sex			P<0.05	NS
Effect of treatment			NS	NS

8.3.2 Experiment 2

The modified injection regime used in this experiment prevented the mortality seen in experiment 1 - no mice died during the course of injections.

As in experiment 1, protease inhibitor injected mice have higher gain and %gain than their control injected littermates, but these differences are insignificant (Table 8.4). As expected, the PH mice grow to heavier weights than the PL mice (table 8.4).

No effect of protease inhibitor injection is seen on muscle weight or %muscularity in the P line mice, but PH mice have heavier muscles and are slightly less muscular than PL mice (Table 8.5).

Neither of the lysosomal enzymes (cathepsin B and cathepsin H) measured in this experiment shows a significant decrease in activity, but cathepsin B is consistently less active in the muscles of treated animals (Table 8.6).

Injection of the protease inhibitors LEU and PEP does not alter body growth, muscle growth or activity of cathepsin B and cathepsin H in the P lines of mice.

8.4 DISCUSSION

8.4.1 Protease inhibitors and growth

The work which stimulated the experiments described in this chapter reported a 31% increment in body weight in PEP injected, non-dystrophic mice compared with 16% in saline injected, non-dystrophic controls, (i.e. gain is almost doubled) (Chelmicka-Shorr *et al.*, 1978). In experiment 1, the respective figures are 126% for LEU and PEP injected and 102% for control injected mice, but, this difference is not statistically significant (Table 8.2) in spite of the fact that mice injected with LEU and

Table 8.4. Mean 8 week body weight, gain and percentage gain according to direction and treatment for P line mice. The proportional size in protease inhibitor injected mice relative to control mice and the significance of effects of direction, replicate within direction and treatment calculated from analysis of variance are also given for each variable.

Direction	Treatment	n	8 week body wgt. (g)	Gain (g)	% gain (%)
	saline	16	23.54	9.75	73.8
P L	inhibitor	17	24.78	10.60	81.7
	inhib./sal.		1.02	1.09	1.11
	saline	17	35.82	20.60	148.6
P H	inhibitor	16	34.78	21.24	166.3
	inhib./sal.		0.97	1.03	1.12
pooled s.e.			0.56	1.04	12.1
Effect of direction			P<0.01	P<0.01	P<0.05
Effect of replicate within direction			NS	P<0.01	P<0.01
Effect of treatment			NS	NS	NS

Table 8.5. Mean PM weight, GM weight and %muscularity at 8 weeks in P line mice according to direction and treatment. The proportional size in protease inhibitor injected mice relative to control mice and the significance of effects of direction, replicate and treatment calculated from analysis of variance are also given for each variable.

Direction	Treatment	PM weight (mg)	GM weight (mg)	%musc. (%)
	saline	247	243	2.09
P L	inhibitor	233	244	1.98
	inhib./sal.	0.94	1.00	0.95
	saline	366	326	1.94
P H	inhibitor	352	310	1.90
	inhib./sal.	0.96	0.95	0.97
pooled s.e.		8	5	0.02
Effect of direction		P<0.01	P<0.01	P<0.05
Effect of replicate within direction		NS	NS	NS
Effect of treatment		NS	NS	NS

Table 8.6. Cathepsin B and cathepsin H activity (nmol per min per g protein) in the PM and GM of P line mice according to direction and treatment.The proportional size in protease inhibitor injected mice relative to control mice and the significance of effects of direction and treatment calculated from analysis of variance are also given for each variable.

Direction	Treatment	Pectoralis muscle		Gastrocnemeus muscle	
		Cathepsin B	Cathepsin H	Cathepsin B	Cathepsin H
	saline	15.26	29.00	10.76	17.74
P L	inhibitor	18.07	28.47	12.06	17.73
	inh./sal.	1.18	0.98	1.12	1.00
	saline	16.14	26.22	12.53	21.50
P H	inhibitor	17.43	26.77	12.95	20.84
	inh./sal.	1.08	1.02	1.03	0.97
pooled s.e.		0.52	0.92	0.60	0.94
Effect of direction		NS	NS	NS	NS
Effect of treat.		NS	NS	NS	NS

PEP consistently have greater gain and %gain. The differences in %gain between experiment 1 and Chelmicka-Shorr *et al.* (1978) are striking and may be due to the control strain used by Chelmicka-Shorr *et al.* (1978) which was C57BL/6J-dy/+. The differences in gain seen in experiment 1 and by Chelmicka-Shorr *et al.* (1978) may be an effect of the dy allele on growth.

The P line mice used in experiment 2 also show consistently increased gain and %gain as a result of injection of LEU and PEP (Table 8.5), but, again the effect is not significant.

The improved gain reported previously (Chelmicka-Shorr *et al.*, 1978) as a result of injection of protease inhibitors can be repeated, but the effect is small.

8.4.2 Protease inhibitors and muscle growth

All of the authors who show a beneficial effect of protease inhibitor injection on the symptoms of MD assume that this is a consequence of the inhibitory action of protease inhibitors on muscle protein degradation (McGowan *et al.*, 1976; Enomoto and Bradley, 1977; Chelmicka-Shorr *et al.*, 1978; Stracher *et al.*, 1978; Hollenberg-Sher *et al.*, 1981). In spite of this assumption no effect of injection of LEU and PEP on muscle growth is seen in the experiments described in this chapter. Further, no differences between treatments in protein concentration were observed in experiment 1 (Table 8.4). The lack of effect on protein concentration combined with the lack of effect on muscle weight suggests that total protein would also be unaffected. Muscle protein accretion is not increased by injection of protease inhibitors.

The activities of the lysosomal enzymes cathepsin B and cathepsin H are known to be elevated in MD and the

beneficial effects of injecting protease inhibitors are suggested to be the result of action on these enzymes (Chelmicka-Shorr *et al.*, 1978, Hollenberg-Sher *et al.*, 1981) . LEU is a potent inhibitor of cathepsin B and will inhibit cathepsin H (Bond and Butler, 1987) and PEP inhibits another lysosomal enzyme, cathepsin D (Ballard, 1977). No significant differences in activity of cathepsin B and cathepsin H observed between injected mice and controls (Table 8.7) showing that injection of LEU and PEP does not cause decrease activity of these enzymes in muscle.

8.4.4 Why do protease inhibitors affect growth?

The experiments described in this chapter show that the increased growth which occurs as a result of injecting protease inhibitors into growing mice is not a result of the action of these inhibitors on muscle. Indeed, as has been noted by Enomoto and Bradley (1977), there is no recognized mechanism whereby protease inhibitors may pass from the bloodstream into muscle fibres to inhibit protease enzymes. Such a pathway is needed to support the assumption that the action of protease inhibitors is via intracellular proteinases and, until such a mechanism is discovered, this assumption cannot be justified.

An alternative method by which protease inhibitors might have physiological effects is reviewed by Hubbard and Kahmi (1985). These authors describe evidence suggesting that protease inhibitors alter the properties of steroid receptors (particularly glucocorticoid receptors) *in vitro*. If protease inhibitors can also alter the behaviour of steroid receptors *in vivo*, then physiological effects of injection of protease inhibitors might be seen. These effects could occur without direct effects on protein degradation. The effect of protease inhibitors on steroid receptors might have positive or negative effects on growth depending on whether the

receptor is activated or blocked by the inhibitor. Until the primary action of protease inhibitors *in vivo* is elucidated, their effects on growth cannot be assumed to be via intracellular proteases.

8.5 CONCLUSIONS

1. The original observation that injection of protease inhibitors into mice will increase gain (Chelmicka-Shorr *et al.*, 1978) can be repeated. The effect is small, and in experiments of this size is insignificant. The effect does not occur at the level of muscle growth and seems independent of the rate of muscle protein degradation.

2. Protease inhibitors are known to affect the action of hormone receptors so any physiological effect that protease inhibitors may have *in vivo* may be via these receptors rather than via effects on intracellular proteinases. Until the action of protease inhibitors is elucidated, their physiological effects cannot be interpreted.

3. Considering the expense of these protease inhibitors, the large amount needed for treatment and their relatively small effects, the use of these inhibitors as growth promoters is not justified.

CHAPTER 9. GENERAL DISCUSSION.

9.1 AIMS

With the advent of transgenic technology and the increasing sophistication of the poultry industry there is a need to understand the mechanisms which cause increased muscle growth. Strains of animals selected for growth are the ideal material in which to examine how the physiology of muscle is altered to increase growth, but as discussed in chapter 1, the data for the chicken is largely out of date. The experiments described in this thesis use modern breeds of chicken and strains of mice selected as a model of commercial breeding programmes to investigate the effects of selection on the cellular components of muscle. This work was carried out as part of a concerted research programme into the physiological and genetic determinants of growth pursued at Poultry Research Centre, Roslin (now IAPGR).

The first four experiments (chapters 3 to 6) were designed to investigate the effects of selection for liveweight in the chicken and lean body mass and body composition in the mouse (G lines) on muscle growth in terms of cellular components. Two basic units of muscle were examined, the DNA unit and the muscle fibre. These experiments were carried out because knowledge of how the cellular components of muscle react to selection might suggest novel methods of improving muscle growth by selection or other means. In addition, the effects of selection on components of muscle in modern breeds of chicken and the lines of mice used were not available when these experiments were begun.

Experiments designed to investigate the use of alternative techniques for measuring or altering the rate of muscle protein degradation are also described (chapters 7 and 8). These techniques were developed

during research into Muscular Dystrophy and have not been previously used to measure or change rates of protein degradation during normal muscle growth.

9.2 THE EFFECT OF SELECTION ON THE NUCLEAR UNIT.

The protein:DNA ratio (DNA unit size) in muscle estimates the amount of protein associated with an individual muscle nucleus and because of the multinucleate nature of muscle fibres has been accepted as a logical unit from which to analyse muscle growth (Cheek *et al.*, 1971; Waterlow *et al.*, 1978; Cheek, 1985). The total amount of DNA in muscle estimates the number of nuclei in that muscle. Both the DNA unit size and/or the number of nuclei may be increased as a result of selection for increased growth. The experiments described in chapters 3, 4 and 6 show that both these variables are altered by selection for growth and that the extent of these changes depends on the species and muscle examined. In most cases, number of nuclei is affected to a greater extent than the DNA unit size (Table 9.1).

While this study was being carried out results were published which showed similar effects on the DNA unit of selection for increased growth in the broiler chicken (Henteges *et al.*, 1983; Kang *et al.*, 1985a; Jones *et al.*, 1985a; Klasing *et al.*, 1987). These results therefore confirm effects observed by other workers.

A possible reason why the number of nuclei is affected to a greater extent than the size of the DNA unit may concern the role of the nucleus in 'controlling' a finite volume of cytoplasm. It is not unreasonable to suppose that there is an optimal volume of cytoplasm which a nucleus can efficiently control and selection acting to increase this volume may therefore reduce fitness and be opposed by natural selection. This hypothesis is supported by the observation that the increase in number

Table 9.1. Coefficients of regression of log(total DNA) on log(muscle weight) and log(protein:DNA) on log(muscle weight) calculated for chicken and mice.

Species	Age (d)	Muscle	Regression Coefficients		Chapter
			total DNA on muscle wgt.	protein:DNA on muscle wgt.	
chicken	42	Pectoralis	0.925	0.043	3
		Gastrocnemius	0.489	0.554	
chicken	49	Pectoralis	0.881	0.148	4
		Gastrocnemius	0.742	0.262	
mouse	70	mixed	0.839	0.257	6

of nuclei is seen to a greater extent in the chicken PM where DNA unit size is already larger than in other muscles. If the DNA unit size in chicken PM muscle is near maximum, further increase may be more detrimental to fitness than equivalent increases in other muscles with smaller DNA unit size. The only way in which chicken PM muscle could respond to selection in such a situation would be to increase the number of DNA units.

The only source of nuclei for the growing muscle fibre is the satellite cell (Swatland, 1984). In the chicken, this group of cells must have increased in number or rate of division as a result of selection since the number of nuclei is similar between strains at hatch. In the lines of mice studied here, it is not possible to be quite so conclusive because mice were examined only at 10 weeks of age. The number of nuclei could be altered at hatch in mouse lines implicating the rate of pre-natal division as the cause of altered muscle nucleus number. This could only be tested by experiment and since the muscles of neo-natal mice are very small this would difficult. In any case, it is debatable whether myoblasts (pre-hatch) and satellite cells (post-hatch) are different cell populations (Swatland, 1984).

Increase in rate of accumulation of nuclei could be brought about by increase in the number and/or rate of division of satellite cells (or myoblasts). It is not possible to determine which of these possibilities is the case using the data in this thesis. The cause of increased accumulation of muscle can only be found by carrying out experiments which measure number and rate of division of satellite cells during growth.

The number of satellite cells in a muscle has been estimated by examination of electronmicrographs of muscle sections and calculating the proportion of nuclei within the basal laminae of muscle fibres that are satellite

cells. In this way, estimates of satellite cell number during normal growth or experimental manipulation in various species have been made although the technique is technically difficult and gives values with large standard errors (Campion *et al.*, 1982a; Campion, 1984). Quail selected for high 4-week growth have more satellite nuclei per muscle and a larger proportion of muscle nuclei are satellite nuclei at the ages of 4 days and 4 weeks (Campion *et al.*, 1982a). The greater rate of DNA accumulation seen in these selected quail is concluded to be due to an increase in the mitotic activity of satellite cells, although the differences are not statistically significant. Further work needs to be carried out before the contribution of satellite cell number to change in rate of DNA accumulation can be properly assessed.

A possible method of counting satellite cells would make use of proteins specific to satellite cells or to cells undergoing mitosis (normal muscle nuclei do not mitose). Monoclonal antibodies could be raised against satellite cells or proteins such as topoisomerase II (a specific marker for cell division - Heck and Earnshaw, 1986) and used in immunoflorescence microscopy of muscle sections allowing the number of satellite nuclei per unit muscle to be measured. Experiments could then be designed to see how this number varies between strains and changes with age. Such experiments are planned at IAPGR, Roslin but have not yet been carried out.

There are no experiments published in which attempts have been made to estimate the rate of satellite cell division *in vivo* since problems in counting satellite cells prevent measurement of the rate of division. Satellite cells can be grown *in vitro* (Bischoff, 1974) and attempts are being made at IAPGR, Roslin to quantify differences between strains in rates of *in vitro* satellite cell

division. Satellite cells from broilers proliferate 10 to 15 percent faster than cells from layers when grown under the same condition (C. Goddard - personal communication) but such differences may be due to variation between strains in response of cells to growth factors in the culture medium. Nonetheless, such an approach when combined with accurate methods of counting satellite cells will reveal how selection acts at the level of the satellite cell to increase the rate of muscle growth.

In order to improve meat producing animals using transgenic technology, genes which affect the control of muscle growth need to be identified. The efficiency of selection for muscle growth rate could also be improved if a trait highly correlated with muscle growth rate and measurable on the live animal were to be identified. Since satellite cells are the source of muscle nuclei and are implicated in the alteration of muscle growth rate it is likely that genes which affect satellite cell number or division rate will have equivalent effects on muscle growth rate. Techniques for *in vivo* measurement of satellite cell number and division rate may therefore be of use in identifying genes which control muscle growth and in the selection of animals for improved muscle growth. Since satellite cells have a fundamental role in muscle growth, control of their function, either by selection, biochemical or transgenic means, must be one of the targets for attempts to alter muscle growth rate in farm animals.

9.3 THE EFFECT OF SELECTION ON THE MUSCLE FIBRE

One of the aims of the experiments described in chapters 5 and 6 was to measure the effect of selection for growth on muscle fibre type. Neither of these experiments gives conclusive results since in the chicken there were technical problems which could not be solved due to lack of time and in the mouse there was an unexpected

difference in distribution of fibre types within muscles. The latter experiment does, however, suggest that changes in fibre type can have large effects on muscle weight when the diameter of different fibre types varies. The fibre types in mice vary in diameter (chapter 6) and this may also be the case for the chicken (Barnard *et al.*, 1982) and other commercially important species (Swatland, 1984). The size of any effect on muscle weight of changes in muscle fibre type depends on the initial proportion of fibre types, the size of the change in fibre type and the relative size (weight) of each fibre type, all of which need to be measured before the role of this factor in changing muscle weight can be quantified.

A further consequence of the relationship between fibre type and fibre diameter is that selection for growth has been concluded to increase fibre diameter in the mouse (Luff and Goldspink, 1967; Byrne *et al.*, 1973; Ezekwe and Martin, 1975 and Aberle and Doolittle, 1976) and other species (Swatland, 1984). This conclusion is quite justified since muscle fibre diameter does indeed increase, but the actual cause of increase in fibre diameter may be a shift in fibre type. In order to determine the actual cause of a difference in muscle weight it is necessary to measure fibre type since without this information, the effects of change in fibre type or fibre diameter cannot be separated.

In chapter 6 selection for growth in the chicken is shown to increase fibre diameter and number at a given age. Because there is a volumetric relationship between fibre diameter and fibre weight, the increase in fibre diameter has a greater effect than does fibre number as observed previously in the chicken and other species, including the mouse (Swatland, 1984). Whether this increase in fibre diameter is driven by a change in fibre type, as discussed above, is not known and would have to be tested

by experiment. Differences in fibre type between strains of chicken differing in growth rate have been observed previously (Aberle *et al.*, 1979).

Muscle fibres form as a result of fusion of myoblasts during embryonic development and the number of muscle fibres is fixed at birth or hatch (see chapter 1). Fibre number therefore depends on the number of myoblasts which are available to form the muscle and the number of myoblasts which fuse to form an individual fibre, so any change in fibre number must involve changes in either or both of these variables. The chicken and to a lesser extent the mouse have certain constraints on prenatal muscle development for the time taken for development is fixed, and muscle must be functional at hatch or birth. If generation of additional myoblasts takes more time, and a decrease in the number of myoblasts fusing to form a fibre reduces the ability to function at hatch, then such changes may be opposed by natural selection. This may explain why fibre number has a lesser role in increasing muscle weight than fibre diameter. Reductions in functional maturity as a result of selection have been observed in the chicken (Ricklefs, 1983).

The above discussion on how selection affects the muscle fibre reveals the importance of considering the muscle fibre as a whole. Change in muscle fibre type, size and number are not independent and until the relationships between these variables are fully elucidated, the way in which selection (or other factors) acts to alter muscle size can not be fully understood. Further, in the above discussion (and the literature in general) fibre diameter is used to measure fibre size but the muscle fibre is a three dimensional structure having length as well as diameter. Methods of measuring fibre length need to be developed before the effects of selection on muscle When the lines of chicken compared in chapter 6 are compared

at similar muscle weight, much of the variation in muscle fibre diameter disappears. As with the DNA unit, selection has altered the developmental rate of the muscle fibre more than the relationship between fibre diameter and muscle weight. In order to manipulate the growth of muscle by novel methods the site and mechanisms which control the rate of muscle fibre growth must be determined.

9.4 MEASUREMENT AND MANIPULATION OF PROTEIN DEGRADATION

The experiments described in chapters 7 and 8 are preliminary investigations into the usefulness of published methods of measuring and altering muscle protein degradation rate. A quick, cheap and accurate method of estimating the rate of protein degradation would be useful in experiments designed to determine the changes in muscle protein degradation brought about by drugs, exercise, etc. and such a technique could be used as a selection criterion in experimental or commercial programmes designed to improve muscle growth. Improvements in muscle growth brought about using drugs is one way of improving the efficiency of meat production and one set of compounds, the ß-agonists, have already shown their potential for improving meat yields in several species (Lamming and Peters, 1987) through reduction in the rate of muscle protein degradation (Lobley, 1988).

The autolytic activity of muscle varied in a systematic way during muscle growth, but was not related to the rate of muscle growth in the way expected of an estimate of protein degradation rate. Subsequent work by others has shown that reduction in the activity of lysosomal enzymes (autolytic activity is a gross estimate of the activity of these enzymes) is not the cause of the reduced rates of protein degradation seen in the muscles of animals selected for improved growth (Saunderson and Leslie,

1987). The use of autolytic activity as a correlate of muscle protein degradation was therefore not pursued any further.

The injection of protease inhibitors resulted in a slight increase in gain as reported by Chelmicka-Shorr *et al.* (1978) but this was not reflected in increased muscle growth. Protease inhibitors may effect growth by changing hormone receptor function (Hubbard and Kahmi, 1985) and not, as previously thought, by inhibiting protease enzymes in muscle. The use of protease inhibitors as muscle growth promoters cannot therefore be justified and was not explored any further.

9.5 APPLICATION

The attention of breeding companies is moving from fast growth to include other traits such as food conversion efficiency, meat yield and meat quality. As this change in emphasis occurs, the possible use in selection programmes of biochemical indicators of these novel characters may become necessary. Identification of reliable biochemical indicators of production traits can only come from an understanding of how an animal can and does respond to selection for such traits at the biochemical level. In addition, as transgenic technology advances, there is a need to identify physiological processes which have large effects on production traits in order that genes which control these traits can be isolated and inserted into animals. The need to understand how muscle responds to selection for increased growth is therefore fundamental to the above approaches for the genetic improvement of commercial species and stimulated the work described in this thesis.

The experiments described in chapters 3, 4, 5 and 6 show that a major determinant of the rate of muscle growth in chickens and mice is the rate of supply of muscle nuclei

deriving from the satellite cell. If a method could be found of measuring the number and rate of division of these cells *in vivo*, this would be a valuable indicator of the muscle growth potential of an animal and would be useful in a commercial selection programme. If a gene which has a large effect on the number and or rate of division of these cells could be identified it would be a candidate for insertion into commercial species to improve muscle growth using back-crossing or transgenics. A prerequisite to both these approaches is the measurement of the number and rate of division of the satellite cell so further experimental work with this aim in mind should be initiated. Such work may, in time, lead to improved methods for the selection of meat animals and may be the basis for the commercial application of transgenic technology to meat production.

9.6 CONCLUSIONS

1. Selection for growth in mice and chickens changes muscle size by increasing the number and size of DNA units at a given age. The number of nuclei are affected more than DNA unit size although muscles vary in the extent of these changes. When muscles of the same weight are compared, strains with different growth rates have similar numbers of DNA units showing that selection has increased the rate of growth of muscle rather than the relationship between components. The satellite cell is concluded to have a major role in altering muscle growth rate.

2. Selection for growth in chickens increases muscle weight by increasing the number and diameter of muscle fibres at a given age. Increase in fibre diameter has a greater effect than fibre number because of the volumetric relationship between fibre diameter and muscle weight. Change in fibre diameter may be driven by a change in fibre type. Comparing

chickens at the same muscle weight shows that selection has increased the rate of growth of muscle fibre diameter rather than changing the relationship between muscle fibre diameter and muscle weight. Selection for growth in the mouse has not altered fibre diameter when fibres of the same type are compared. Change in muscle weight in the G lines of mice is concluded to have occurred by a change in number of muscle fibres or by a change in fibre type.

3. The rate of autolysis is not a useful method of estimating the rate of muscle protein degradation during normal muscle growth.

4. Muscle growth is not improved by injecting the protease inhibitors pepstatin and leupeptin into growing mice.

REFERENCES

Aberle, E.D., Addis, P.B., Shoffner, R.N. (1979). "Fibre types in skeletal muscles of broiler and layer - type chickens". Poult. Sci. 58: 1210-1212.

Aberle, E.D., and Doolittle, P.D. (1976). "Skeletal muscle cellularity in mice selected for large body size and in controls". Growth. 40: 135-145.

Allen, R.E., Merkel, R.A., Young, R.B. (1979). "Cellular aspects of muscle growth: myogenic cell proliferation". J. Anim. Sci. 49: 115-127.

Allen, R.E. (1987). "Muscle cell culture as a tool in animal growth research". Fed. Proc. 46: 290-294.

Ashmore, C.R. and Doerr, L. (1971a)."Post-natal development of fibre types in normal and dystrophic skeletal muscle of the chick". Exp. Neurol. 30: 431-446.

Ashmore, C.R. and Doerr, L. (1971b). "Comparative aspects of muscle fibre types in different species". Exp. Neurol. 31: 408-418.

Ballard, F.J. (1977). "Intracellular protein degradation". In "Essays in biochemistry". Ed. Campbell, P.N. and Aldridge, W.N. Pub. Academic press.

Barnard, E.A., Lyles, J.M., Pizzey, J.A. (1982). "Fibre types in chicken skeletal muscle and their changes in Muscular Dystrophy". J. Physiol. 331: 333-354.

Barrett, A.J. (1980). "Fluorimetric assay for cathepsin B and cathepsin H with methylcoumarylamide substrates". Biochem. J. 87: 909-912.

Bird, J.W.C., Carter, J.H., Triemer, R.E., Brooks, R.M., Spanier, A.M. (1980). "Proteinases in cardiac and skeletal muscle". Fed. Proc. 39: 20-25.

Bischoff, R. (1974). "Enzymatic liberation of myogenic cells from adult rat muscles". Anat. Rec. 180: 645.

Bishop, S.C. and Hill, W.H. (1985). "Effects of selection on growth, body composition and food intake in mice. III Correlated responses: growth, body composition, food intake and efficiency". Gen. Res. Camb. 46: 57-74.

Blackburn, S. (1968). "Amino acid determination - methods and techniques". Pub. Arnold.

Bond, J.S. and Butler, P.E. (1987). "Intracellular proteases". Ann. Rev. Biochem. 56: 333-364.

Bryan, L. (1985). "Protein deposition in poultry". Ph.D. thesis. University of Nottingham.

Bryan, L., Buttery, P.J., Fisher, C. (1985a). "The relationship between fractional growth rate and fractional rate of protein synthesis within a population of growing broilers". Proc. Nutr. Soc. 44: 76A.

Bryan, L., Buttery, P.J., Fisher, C. (1985b). "Effect of protein deficiency on the breast muscle in the broiler". Proc Poult. Sci. Symp. 14: 211-214.

Byrne, I., Hooper, J.C., McCarthy, J.C. (1973). "Effects of selection for body size on the weight and cellular structure of seven mouse muscles". Anim. Prod. 17: 187-196.

Campion , D.R., Marks, H.L., Richardson, L.R. (1982a)."An analysis of satellite cell content in the semimembranosus muscle of Japanese Quail selected for rapid growth". Acta. Anat. 112: 9-13.

Campion, D.R., Marks, H.L., Reagan, J.O., Barrett, J.B. (1982b)."Composition and muscle cellularity of Japanese Quail after selection for high body weight under an optimal or sub-optimal nutritional environment". Poult. Sci. 61: 212-217.

Campion, D.R. (1984)."The muscle satellite cell: a review". Int. Rev. Cyt. 87: 225-251.

Cheek, D.B. (1985). "The control of cell mass and replication. The DNA unit - a personal 20 year study". Early Hum. Dev. 12: 211-239.

Cheek, D.B., Hutt, A.B., Hill, D.E., Talbert, J.L. (1971). "Skeletal muscle mass and growth: the concept of the DNA unit". Pediat. Res. 5: 312-328.

Chelmicka-Shorr, E.E., Arnason, B.G.W., Astrom, K.E. (1978). " Treatment of mouse Muscular Dystrophy with the protease inhibitor pepstatin". J. Neuropath. Exp. Neurol. 37: 263-268.

Dahlman, B., Kuehn, L., Rutschmann, M., Reinauer, H. (1985a). "Purification and characterisation of a multicatalytic high-molecular-mass proteinase from rat skeletal muscle". Biochem. J. 228: 161-170.

Dahlman, B., Rutschmann, M., Kuehn, L., Reinauer, H. (1985b). "Activation of the multicatalytic proteinase from rat skeletal muscle by fatty acids or S.D.S.". **Biochem J. 228**: 171-177.

Davies, S.R., Barry, T.N., Hughson, G.A (1981). "Protein synthesis in tissues of growing lambs". **Br. J. Nutr. 46**: 409-419.

Dubowitz, V. and Brooke, M.H. (1973). "Muscle biopsy: a modern approach". **Pub. Saunders Company Ltd.**

Durschlag, R.P. and Layman, D.K. (1983). "Skeletal muscle growth in lean and obese Zucker rats". **Growth. 47**: 282-291.

Dutson, T.R. and Carter, A. (1985). "Microstructure and biochemistry of avian muscle and its' relevance to meat processing industries". **Poult. Sci. 64**: 1577-1590.

Enomoto, A. and Bradley, W.G. (1977). "Theraputic trials in Muscular Dystrophy III: studies of microbial proteinase inhibitors in murine dystrophy". **Arch. Neurol. 34**: 771-773.

Ezekwe, M.O. and Martin, R.J. (1975). "Cellular characteristics of skeletal muscle in selected strains of pigs and mice and their unselected controls". **Growth. 34**: 95-106.

Falconer, D.S., Gauld, I.K., Roberts, R.C. (1978). "Cell numbers and cell sizes in organs of mice selected for large and small size". **Genet. Res. Camb. 31**: 287-301.

Ferber, S. and Ciechanover, A. (1987). "Role of arginine-tRNA in protein degradation by the ubiquitin pathway". **Nat. 326**: 808-811.

Field, R.A., Young, O.A., Asher, G.W., Foote, D.M. (1985). "Characteristics of male Fallow Deer muscle at a time of sex-related muscle growth". **Growth. 49**: 190-201.

Fowler, S.P., Campion, D.R., Marks, H.L., Reagan, J.O. (1980). "An analysis of skeletal muscle response to selection for rapid growth in Japanese Quail". **Growth. 44**: 235-252.

Fried, V.A., Smith, H.T., Hildebrandt, E., Weiner, K. (1987). "Ubiquitin has intrinsic proteolytic activity: implications for cellular regulation". **P.N.A.S. 84**: 3685-3689.

Giles, K.W. and Myers, A. (1965). "An improved diphenylamine method for the determination of DNA". Nat. 206: 93

Goldspink, D.F. (1977a) "The influence of immobilisation and stretch on protein turnover of rat skeletal muscle". J. Physiol. 264: 267-282.

Goldspink, D.F. (1977b) "The influence of activity on muscle size and protein turnover". J. Physiol. 264: 283-296.

Goldspink, D.F. (1981). "Physiological factors influencing protein turnover and muscle growth in mammals". In "The development and specialisation of skeletal muscle". Ed. Goldspink, D.F. Pub. Cambridge University Press.

Goldspink, G. (1977). "The growth of muscles". Proc. Poult. Sci. Symp. 12: 13-28.

Goldspink, G. (1980). "Growth of muscle". In "The development and specialisation of skeletal muscle". Ed. Goldspink, D.F. Pub. Cambridge University Press.

Goldspink, G. and Ward, P.S. (1979). "Changes in rodent muscle fibre types during post-natal growth, undernutrition and exercise". J. Physiol. 296: 453-469.

Greaser, M.L. (1986). "Conversion of muscle to meat". In "Muscle as food". Ed. Bechtel, P.J. Pub. Academic Press.

Guth, L. and Samantha, F.J. (1972). "Erroneous interpretations which may result from application of the myofibrillar ATP-ase histochemical procedure to growing muscle". Exp. Neurol. 34: 465-475.

Handel, S.E. and Stickland, N.C. (1988). "Catch-up growth in pigs: a relationship with muscle cellularity". Anim. Prod. 47: 291-295.

Hanrahan, J.P., Hooper, A.C., McCarthy, J.C. (1973). "Effects of divergent selection for body weight on fibre number and diameter in two mouse muscles". Anim. Prod. 16: 7-16.

Hanset, R. (1986). "Double muscling in cattle". In "Exploiting new technologies in animal breeding: genetic developments". Ed. Smith, C., King, J.W.B., McKay, J.C. Pub. Longman.

Harbison, S.A., Goll, D.E., Parrish, F.C., Wang, V., Kline, E.A. (1976). "Muscle growth in two genetically different lines of swine". **Growth. 40**: 253-283.

Harris, A.J. (1981). "Embryonic growth and innervation of rat skeletal muscles I: neural regulation of muscle fibre numbers". **Phil. Trans. Roy. Soc. (London) B. 293**: 257-277.

Harris, C.I. and Milne, G. (1980). "The urinary excretion of N^{τ} methyl histidine in sheep: an index of muscle protein breakdown". **Brit. J. Nutr. 44**: 129-140.

Harris, C.I. and Milne, G. (1981a) "The urinary excretion of N^{τ} methyl histidine by cattle: validation as an index of muscle protein breakdown". **Brit. J. Nutr. 45**: 411-422.

Harris, C.I. and Milne, G. (1981b). "The inadequacy of urinary N^{τ} methyl histidine excretion in the pig as a measure of muscle protein breakdown". **Brit. J. Nutr. 45**: 423-429.

Harris, C.I., Milne, G., Mcdairmid, R. (1987). "The retention and metabolism of N^{τ} methyl histidine by cockerels: implications for measurement of muscle protein breakdown determined by the excretion of N^{τ} methyl histidine in excreta". **Brit. J. Nutr. 57**: 467-478.

Hastings, I.M. and Hill, W.G. (1989). "A note on the effect of different selection criteria on carcase composition in mice". **Anim. Prod. 48**: 229-233.

Hausman, G.J. and Campion, D.R. (1986). "Skeletal muscle cellularity and histochemistry in young lean and obese pigs". **Growth. 50**: 287-295.

Hayashi, K., Tomita, Y., Maeda, Y., Shinagawa, Y., Hashizume, T. (1985). "The rate of degradation of myofibrillar proteins of skeletal muscle in broiler chickens estimated by N^{τ} methyl histidine in excreta". **Brit. J. Nutr. 54**: 157-153.

Heck, M.S. and Earnshaw, W.C. (1986). "Topoisomerase II: a specific marker for cell proliferation". **J. Cell. Biol. 103**: 2569-2581.

Henteges, E.J., Marple, D.N., Roland, D.A., Pritchett, F.J. (1983). "Growth and *in vitro* protein synthesis in two strains of chicks". **J. Anim. Sci. 57**: 320-327.

Hershko, A. and Ciechanover, A. (1982). "Mechanisms of intracellular protein breakdown". **Ann. Rev. Biochem.** 51: 335-364.

Hill, W.G. and Bishop, S.C. (1986). "Genetic control of growth, carcass composition and food utilisation in laboratory animals". **Proc. III W. Cong. Genet. Appl. Livestock Prod.** 355-566.

Holcman, A. and Zagozen, F. (1980). "Heritability of muscle fibre number and diameter". **Vetinaria Yugoslavia. 29**: 70-77.

Hollenberg-Sher, J., Stracher, A., Shafiq, S.A., Hardy-Stashin, J. (1981). "Successful treatment of murine Muscular Dystrophy with the proteinase inhibitor leupeptin". **P.N.A.S.** 78: 7724-7744.

Hubbard, J.R. and Kalimi, M. (1985). "Influence of proteinase inhibitors on glucocorticoid receptor properties: recent progress and future perspectives". **Mol. Cell. Biochem.** 66: 101-109.

Hutchison, W.C. and Munro, H.N. (1961). "The determination of nucleic acids in biological materials, a review". **Analyst.** 86: 768-813.

Iodice, A.A., Chin, J., Perker, S., Weinstock, I.M. (1972). "Cathepsins A, B, C ,D and autolysis during development of breast muscle of normal and dystrophic chickens". **Arch. Biochem. Biophys.** 152: 166-174.

Iodice, A.A., Leong, V., Weinstock, I.M. (1966). "Proteolytic activity of skeletal muscle of normal and dystrophic chickens and rabbits". **Enzym. Biol. Clin.** 6: 269-278.

Jones, S.J., Judge, M.D., Aberle, E.D. (1986a). "Skeletal muscle turnover in broiler and layer chicks". **J. Anim. Sci.** 62: 1576-1582.

Jones, S.J., Aberle, E.D., Judge, M.D. (1986b). "Muscle protein turnover in sex-linked dwarf and normal broiler chickens". **Poult. Sci.** 65: 2082-2089.

Kang, C.W., Sunde, M.L., Swick, R.W. (1985a). "Growth and protein turnover in the skeletal muscles of broiler chicks". **Poult. Sci.** 64: 370-379.

Kang, C.W., Sunde, M.L., Swick, R.W. (1985b). "Characteristics of growth and protein turnover in skeletal muscle of turkey poults". **Poult. Sci.** 64: 380-387.

Klasing, K.C., Calvert, C.C., Jarrell, V.L. (1987). "Growth characteristics, protein synthesis and protein degradation in muscles from fast and slow growing chicks". Poult. Sci. 66: 1189-1196.

Knitzetova, H., Knitze, B., Kopecny, V., Fulka, J. (1972). "Concentration of nuclei in chicken muscle fibres in relation to the intensity of growth". Ann. Biol. Anim. Bioch. Biophys. 12: 321-328.

Lamming, G.E. and Peters, A.R. (1987). "Future developments in the manipulation of growth in farm animals". Vet. Rec. 120: 495-499.

Lauretio, G.J., Scanes, C.G., Olsen, R.F. (1983). "Comparison of muscle protein synthesis and degradation in domestic fowl with defined growth characteristics, thyroid status and varying protein content in diet". Poult. Sci. 62: 1455.

Lindsay, D.B. (1983). "Growth and fattening". In "Nutritional physiology of farm animals". Ed. Rook, J.A.F., Thomas, P.C. Pub. Longman.

Lobley, G.E. (1988). "Protein turnover and energy metabolism in animals: interactions in leanness and obesity". In "Leanness in domestic birds: genetic, metabolic and hormonal aspects". Ed. Leclerq, B. and Whitehead, CC. Pub. Butterworths.

Lowell, B.B., Ruderman, N.B., Goodman, M.N. (1986). "Evidence that lysosomes are not involved in the degradation of myofibrillar proteins in rat skeletal muscle". Biochem. J. 234: 237-240.

Luff, A.R. and Goldspink, G. (1967). "Large and small muscles". Life Sci. 6: 1821-1826.

Luff, A.R. and Goldspink, G. (1970). "Total number of fibres in muscles of several strains of mice". J. Anim. Sci. 30: 891-893.

McCarthy, F.D., Bergen, W.G., Hawkins, D.R. (1983). "Muscle protein turnover in cattle of differing genetic background as measured by urinary N^{τ} methyl histidine excretion". J. Nutr. 113: 2455-2463.

McCarthy, J.C. and Shiel, E. (1975). "A note on an attempt to select for muscle fibre diameter in mice". Anim. Prod. 20: 171-172.

MacDonald, M.L. and Swick, R.W. (1981). "The effect of protein depletion and repletion on muscle protein turnover in the chick". Biochem. J. 194: 811-819.

McGowan, E.B., Shafiq, S.A., Stracher, A. (1976). "Delayed degeneration of dystrophic and normal culturestreated with pepstatin, leupeptin and antipain". Exp. Neurol. 50: 649-657.

Maeda, Y., Hayashi, K., Toyohara, S., Hashiguchi, T. (1984). "Variation among chicken stocks in the fractional rates of muscle protein synthesis and degradation". Biochem. Genet. 22: 687-700.

Marks, H.L. (1983). "Direct and correleted responses to selection". Proc. XIIX Poult. Sci. Symp. 47-57.

Maruyama, K., Sunde, M.L., and Swick, R.W. (1978). "Growth and muscle protein turnover in the chick". Biochem. J. 176: 573-582.

Miller, J.B., Crow, M.T., Stockdale, F.E. (1985). "Slow and fast myosin heavy chain content defines three types of myotubes in early muscle cell culture". J. Cell. Biol. 101: 1643-1650.

Miller, J.B. and Stockdale, F.E. (1986a). "Developmental origins of skeletal muscle cultures: clonal analysis of myogenic cell lineages based on expression of fast and slow myosin heavy chains". P.N.A.S. 83: 3860-3864.

Miller, J.B. and Stockdale, F.E. (1986b). "Developmental regulation of the multiple myogenic cell lineages of the avian embryo". J.Cell. Biol. 103: 2197-2208.

Mizuno, T. and Hikami, Y. (1971). "Comparison of muscle growth between meat-type and egg-type chickens". Jap. J. Zootech. Sci. 42: 526-532.

Moss, F.P., Simmonds, R.A., McNary, H.W. (1964). "the growth and composition of skeletal muscle in the chicken 2: the relationship between muscle weight and the number of nuclei". Poult. Sci. 43: 1086-1091.

Moss, F.P. (1968a). "The relationships between the dimensions of the fibres and the number of nuclei during normal growth of skeletal muscle in the domestic fowl". Am. J. Anat. 122: 555-563.

Moss, F.P. (1968b). "The relationships between the dimensions of the fibres and the number of nuclei during restricted growth, degrowth and compensatory growth of skeletal muscle". Am. J. Anat. 122: 565-572.

Mulvany, D.R., Merkel, R.A., Bergen, W.G. (1985). "Skeletal muscle turnover in young male pigs". J. Nutr. 115: 1057-1064.

Munro, H.N. and Fleck, A. (1965). "Analysis of tissues and body fluids for nitrogeneous constituents". In "Mammalian protein metabolism III". Ed. Munro, H.N. Pub. Academic press.

Munro, H.N. and Fleck, A. (1966). "The determination of nucleic acids". **Meth. Biochem. Anal. 14**: 113-176.

Nickel, R., Schummer, A., Seiferle, E. (1977). "Anatomy of domestic birds". Pub. Verlag Paul Parey, Berlin.

Ontell, M. and Kozeka, K. (1984). "The organogenesis of murine striated muscle: a cytoarchitechtural study". Amer. J. Anat. 171: 133-148.

Orcutt, M.W. and Young, R.B. (1982). "Cellular differentiation, protein synthesis rate and protein accumulation in muscle cell cultures isolated from embryos of layer and broiler cultures". J. Anim. Sci. 54: 769-776.

Palmiter, R.D., Brinster, R.L., Hammer, R.E., Trumbaur, M.E., Rosenfield, M.G., Birnberg, N.C., Evans, R.M. (1982). "Dramatic growth of mice that develop from eggs microinjected with metallothionein - growth hormone genes". Nat. 300: 611-615.

Perry, S.V. (1985). "The biochemistry and physiology of the muscle cell". Proc. Nutr. Soc. 44: 235-243.

Pierce chemical company. (1986). "B.C.A. and B.C.A protein assay reagent". Instructions 23225.

Powell, S.E and Aberle, E.D. (1975). "Cellular growth of skeletal muscle in swine differing in muscularity". J. Anim. Sci. 40: 476-485.

Pursel, V.G., Rexroad, C.E., Bolt, D.J., Miller, K.F., Wall, R.J., Hammer, R.E., Pinkert, C.A., Palmiter, R.D., Brinster, R.L. (1987). "Progress on gene transfer in farm animals". Vet. Immunol. Immunopathol. 17: 303-312.

Quinn, L.S. (1985). "Generation of chick muscle cells in groups of 16 from stem cells". Nat. 313: 692-694.

Reeds, P.J. and Palmer, R.M. (1995) "Role of prostaglandins in the control of muscle protein turnover". In "Control and manipulation of animal growth". Ed. Buttery, P.J., Haynes, N.B., Lindsay, D.B. Pub. Butterworths.

Ricklefs, R.E. (1983). "Avian postnatal development". In "Avian biology VII". Ed. Farner, D.S., King, J.R., Parkes, K.C. Pub. Academic Press.

Ricklefs, R.E. (1985). "Modification of growth and development of muscles of poultry". Poult. Sci. 64: 1573-1576.

Ridpath, J.F., Huiatt, T.W., Trenkle, A.H., Robson, R.M., Bechtel, P.J. (1984). "Growth and differentiation of chicken embryo muscle cell cultures derived from fast and slow growing lines. Intrinsic differences in growth characteristics and insulin response". Diff. 26: 121-126.

Robinson, D.W. and Bradford, G.E. (1969). "Cellular response to selection for rapid growth in mice". Growth. 33: 221-229.

Salminen, A. (1985). "Lysosomal changes in skeletal muscles during the repair of exercise injuries in muscle fibres". Acta. Physiol. Scand. 124. Supp. 539.

Saunderson, C.L. (1984). "Muscle protein turnover in broilers". Proc. XXVI Brit. Poult. Breeders Roundtable.

Saunderson, C.L. and Leslie, S. (1983). "N^{τ} methyl histidine excretion by poultry: not all species exctete N^{τ} methyl histidine quantitatively". Brit. J. Nutr. 50: 691-700.

Saunderson, C.L. and Leslie, S. (1987). "Changes in cathepsin D activity in breast muscle of broiler chickens when dietary protein is decreased". Biochem. Soc. Trans. 15: 961.

Schafer, D.A., Miller, J.B., Stockdale, F.E. (1987). "Cell diversification within the myogenic lineage: *in vitro* generation of two types of myoblasts from a single myogenic progenitor cell". Cell. 48: 659-670.

Sharp, G.L., Hill, W.G., Robertson, A. (1984). "Effects of selection on growth, body composition and food intake in mice I: responses in selected traits". Genet. Res. Camb. 43: 75-92.

Shibko, S., Koivistoinen, P., Tratnyek, C.A., Newhall, A.R., Friedman, L. (1967). "A method for sequential quantitative separation and determination of protein, RNA, DNA, lipid and glycogen from a single rat liver homogenate or from a subcellular fraction". Anal. Biochem. 19: 514-528.

Smith, J.H. (1963). "Relation of body size to muscle cell size and number in the chicken". Poult. Sci. 42: 283-293.

Snedecor, G.W. and Cochran, W.G. (1967). "Statistical methods". Pub. Iowa State University Press.

Solomon, M.B., West, R.L., Henteges, J.B. (1986). "Growth and muscle development characteristics of purebred Angus and Brahman bulls". Growth. 50: 51-67.

Stickland, N.C. and Goldspink, G. (1973). "A possible indicator muscle for the fibre content and growth characteristics of porcine muscle". Anim. Prod. 16: 135-146.

Stickland, N.C. and Goldspink, G. (1975). "A note on porcine skeletal muscle parameters and their possible use in early progeny testing". Anim. Prod. 21: 93-96.

Stickland, N.C., Widdowson, E.M. Goldspink, G. (1975). "Effects of severe energy and protein deficiencies on the fibres and nuclei in skeletal muscle of pigs". Br. J. Nutr. 34: 421-428.

Stracher, A., McGowan, E.B., Shafiq, S.A. (1978). "Muscular Dystrophy: inhibition of degeneration *in vivo* with protease inhibitiors". Sci. 200: 50-51.

Stryer, L. (1981). "Biochemistry". 2nd Edition. Pub. Freeman.

Swatland, H.J. (1978). "Longitudinal growth and rate of new sarcomere formation in porcine muscle". J. Anim. Sci. 46: 118-124.

Swatland, H.J. (1984). "The structure and development of meat animals". Pub. Prentice Hall.

Timson, B.F. (1982). "The effect of varying postnatal growth rate on muscle fibre number in the mouse". Growth. 46: 36-45.

Tischler, M.E., Fagan, J.M., Allen, D. (1985). "Relationship of the redox state to muscle protein degradation". in "Intracellular protein catabolism". Ed. Khairallah, E. Pub. Liss Inc.

Trenkle, A., DeWitt, D.L., Topel, D.G. (1978). "Influence of age, nutrition and genotype on carcase traits and cellular development of the *m. Longissimus* of cattle". J. Anim. Sci. 46: 1597-1603.

Waterborg, J.H. and Matthews, H.R. (1984). "The Lowry method for protein quantitation". In "Methods in molecular biology I". Ed. Walker, J.M. Pub. Humana Press.

Waterlow, J.C. Garlick, P.J. Millward, D.J. (1978). "Protein turnover in mammalian tissues and the whole body". Pub. North Holland.

Weydert, A., Barton, P., Harris, A.J., Pinset, C., Buckingham, M. (1987). "Developmental pattern of mouse muscle heavy chain gene transcripts *in vivo* and *in vitro*". Cell. 49: 121-129.

Whitehead, C.C, Griffin, H.D., Bannister, D.W., Saunderson, C.L. (1986). "Selection against body fatness in meat type poultry on the basis of plasma lipoprotein concentration: consequences for performance, body composition and metabolism". Proc. VII Eur. Poult. Conf. 157-161.

Wilson, B.J. (1977). "Growth curves: their analysis and use". Proc. XII Poult. Sci. Symp. 89-116.

Zak, R., Martin, A.F., Blough, A.F. (1979). "Assessment of protein turnover by use of radioisotopic tracers". Physiol. Rev. 59: 407-447.

APPENDIX 1. STAINING OF MUSCLE FIBRES FOR MYOSIN ATP-ASE

A1.1 PEOPLE AND TECHNIQUES

The sectioning and histochemistry of muscle for fibre typing discussed in Chapters 5 and 6 were carried out by Laura Dick and Irene Anderson of the Department of Metabolic Pathology, Institute for Grassland and Animal Production, Poultry Department, Roslin, Midlothian. This appendix contains a summary of the techniques involved. A more thorough discussion, containing the basic methods and principles of histochemical techniques for muscle sections is given by Dubowitz and Brooke (1973).

A1.2 PREPARATION FOR SECTIONING

Muscles were dissected from the animal, visible tendon and adipose tissue removed and the muscle weighed. In mice, 1 and 7 day old chicks, whole muscles were prepared for sectioning. In older chickens approximately 1cm by 0.5cm strips of tissue from the belly of the muscle were cut in parallel to the muscle fibres. The muscles or muscle strips were placed on a dissection board, and cut transversely using a scalpel giving blocks of muscle approximately 0.5cm by 0.5cm by 0.5cm. Muscle blocks were placed (cut end uppermost) on 2 cm diameter by 0.3 cm deep round cork 'chucks' using OCT compound, (BDH Chemicals Limited) for support. The cork plus muscle was then frozen by immersion for several seconds in isopentane cooled in liquid nitrogen. The frozen muscle blocks were then transferred to a cryostat at -25°C for sectioning. Two muscle blocks were prepared for each muscle sample.

A1.3 SECTIONING

The muscle blocks were mounted on the chuck of a cryostat and 20 or 30 sections cut, before mounting on to slides to 'square' the end of the muscle block. 10µm sections

were cut and mounted on glass slides (2 sections per slide). Nine slides were taken for each muscle sample. Sections were dried by exposure to air at room temperature for approximately 30 minutes, and then transferred to a fridge at 4°C for storage. Sections were usually processed within two days, but sections could be stored at 4°C for several months without appreciable loss in staining quality.

A1.4 STAINING

A1.4.1 Solutions

Formal caccodylate buffer pH 6.8.

2.14g of sodium caccodylate in 6.3ml of 0.2M hydrochloric acid and 20ml of formaldehyde made up to 200ml with distilled water.

Walpole's acetate buffer pH 4.0.

50ml of 1M sodium acetate plus 39ml of 1M hydrochloric acid made up to 250ml with distilled water.

1.0 M tris buffer pH 10.0

24.2g of trishydroxymethylaminomethane in 50ml of distilled water and pH adjusted to 10.0 using 0.1M hydrochloric acid then made up to 200ml with distilled water.

Incubating solution.

4ml of 1.0M tris buffer plus 2ml 0.18M calcium chloride, 0.03g ATP disodium salt and 14ml distilled water.

A1.4.2 Method

Cryostat sections were incubated in formal caccodylate buffer for 2 min and then rinsed twice in distilled water. Sections to be studied at pH 4 (pH 4.6 in mice)

were preincubated in Walpole's acetate buffer for 5 min at room temperature. Sections to be studied at pH 10 (pH 10.6 in mice) were preincubated in incubating solution without ATP for 15 min at room temperature. Sections were then incubated at 37°C in incubating solution with ATP for 45 min. After incubation sections were rinsed twice in distilled water and placed in 2% cobalt chloride solution for 2 min, sections were rinsed twice in distilled water and then placed in 1% ammonium sulphate solution for 1 min. Sections were then rinsed twice in distilled water and then mounted in glycerine gel for examination by microscope.

All sections for the experiments described in Chapter 5 and Chapter 6 were incubated at pH 10 for chickens or pH 10.6 for mice. These pHs gave the most consistent staining patterns. This method of staining allows the three main fibre types present in the PM and GM of chicken and mouse to be distinguished. In the chicken, type I fibres stain lightly, type IIB stain darkly and type IIA stain intermediately (Ashmore and Doerr, 1971a; Barnard *et al.*, 1981). In the mouse, type I fibres stain lightly, type IIA stain darkly and type IIB fibres intermediately.

A1.5 PHOTOGRAPHY OF SECTIONS

Two representative fields of view from each muscle sample were photographed. Along with each batch of photomicrographs a photograph of a field micrometer with 10μm divisions was taken to allow calibration of the digitising equipment. The methods of estimating fibre size from these photographs are discussed in Chapter 2.

Figure A1.1. Sections from the PM (A1.1a) and the GM (A1.1b) of a 1 day old layer used in the experiment described in chapter 5.

Figure A1.2. Sections from the PM (A1.2a) and the GM (A1.2b) of a 49 day old broiler used in the experiment described in chapter 5.

Figure A1.1. Sections from the PM (A1.3a) and the GM (A1.3b) of a 10 week old mouse used in the experiment described in chapter 5.

All of the above sections are stained for myosin ATP-ase with preincubation at alkaline pH.

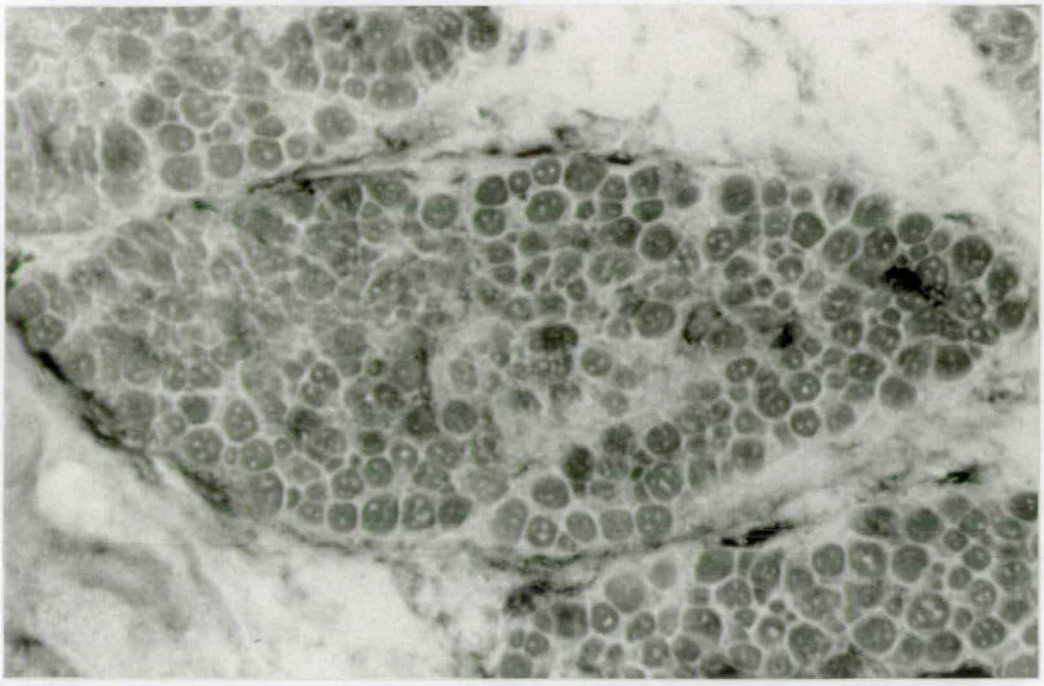


Fig. A1.1a

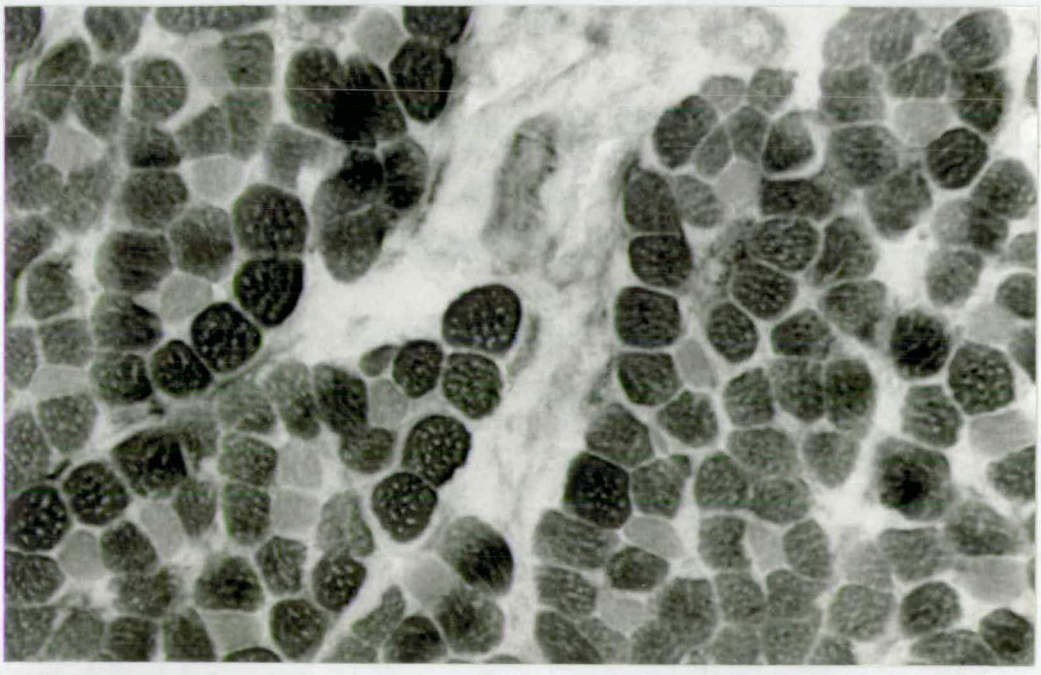


Fig. A1.1b

100μ

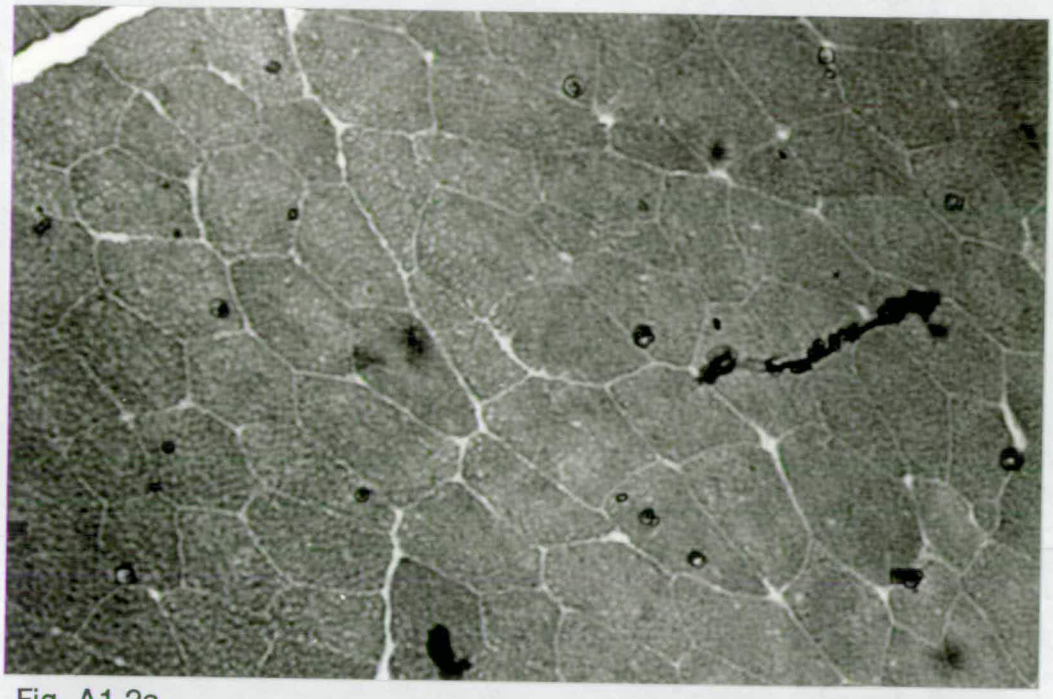


Fig. A1.2a

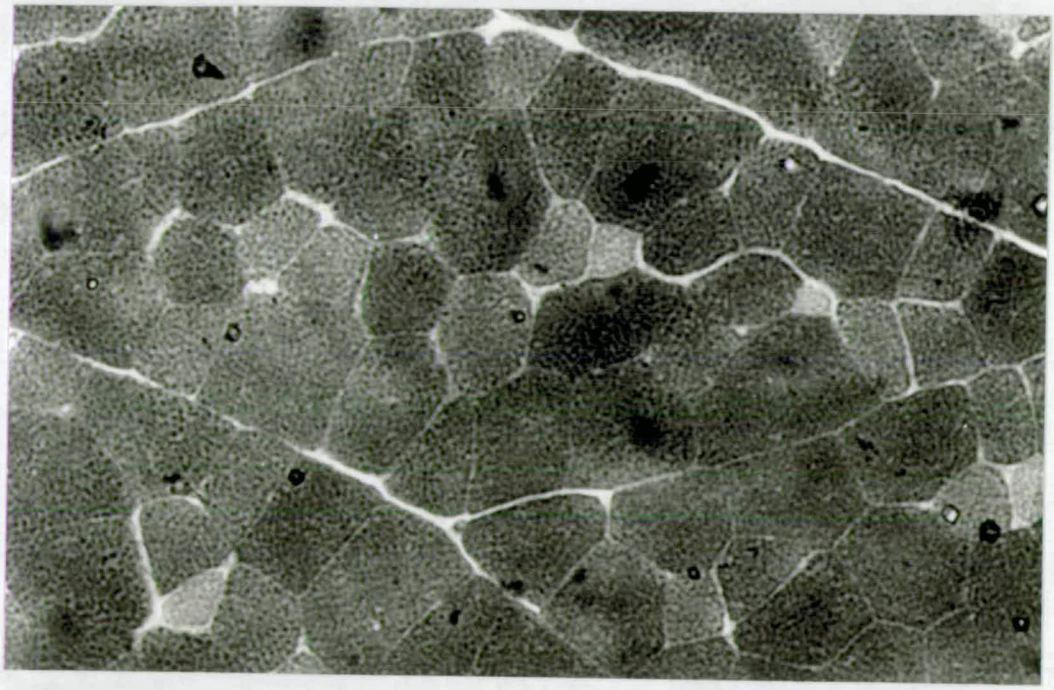


Fig. A1.2b

100μ

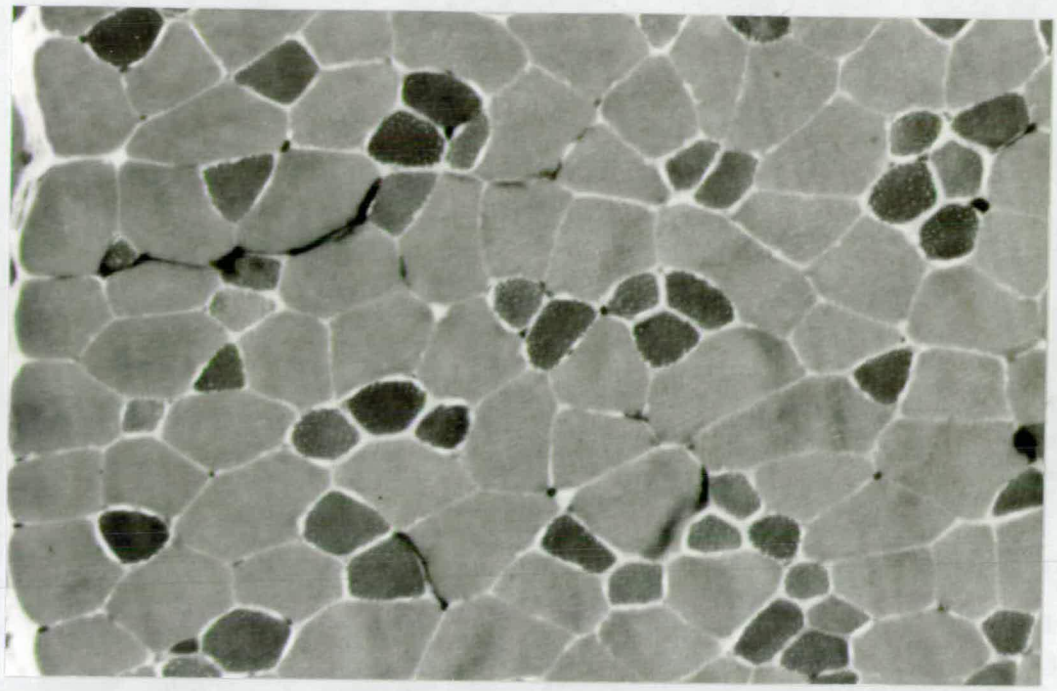


Fig. A1.3a

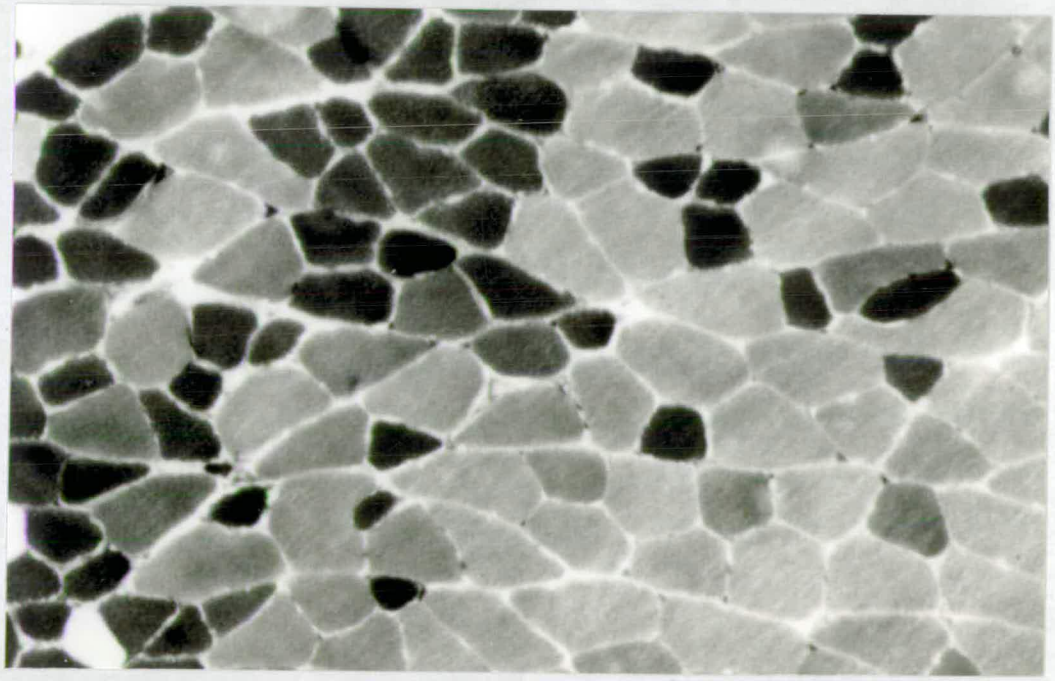


Fig. A1.3b

100μ