EFFECTS OF LOW BIRTHWEIGHT ON POSTNATAL DEVELOPMENT OF SKELETAL MUSCLE IN THE PIG

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DECLARATION

I declare that this thesis contains the presentation and discussion of results from my own research. It was prepared and written by myself.

Susan E Handel
I particularly wish to express my gratitude to Dr NC Stickland, my supervisor, for his invaluable help and friendship throughout the course of this work.

Dalrymple Partners of Oxenfoord Mains, Midlothian, provided the pedigree Large White pigs. Mr D MacFarlane kindly recorded pig birthweights and marked appropriate littermates; I am extremely grateful to him for so cheerfully carrying out the extra work that this entailed.

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Technical staff of the Anatomy Department, Royal Veterinary College, London, were involved in the presentation of the photographs for this thesis. Miss J Wood's skills are evident in the photographic reproductions which were kindly mounted by Mrs S Evans; I am very grateful to them both.

My most sincere thanks also go to Mr P Kettle who exhibited great care and patience in the typing of this thesis.

The work for this thesis was conducted in the Anatomy Department of the R(D)SVS, the facilities of which were so kindly made available by Professor KM Dyce.
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SUMMARY

The aim of this work was to investigate the effects of low birthweight within litters of pigs on the development of skeletal muscle, as observed at the level of the light and electron microscope, and to relate these effects to general body growth and potential for meat production. An increase in body weight after birth imposes ever-increasing demands on muscle metabolism and by observing its inherent adaptation in animals of different birthweights some of the phenomena affecting muscle development were revealed.

This study was based on 49 purebred Large White pigs of various ages between birth and 128 days, ranging in liveweight from 650g to 49500g; 35 of these animals were sucklers. The pigs came from a total of 17 litters. Littermates were slaughtered at the same age and were chosen by their weight at birth. The largest male (average birthweight of 1544g) and the smallest male (average birthweight of 1144g) littermates were selected together with any 'runt' pigs of 950g or less (average birthweight of 776g).

The work performed in the study of these pigs can be considered under four sections according to the techniques employed.

(i) The liveweights and a length measurement, representative of the crown-rump length, were recorded for all pigs on the day of slaughter. Weights of various body organs together with long bone and backfat thickness measurements were also made to enable a complete investigation of the relative growth of
littermates and their constituent tissues.

Runt pigs maintained liveweights significantly below ($P < 0.001$) those of their large littermates throughout the period of study despite a few discrete incidences of catch-up growth. The relative growth rate of body organs was essentially equal between littermates. However the percentage composition of littermates with respect to organ weight remained different through to 128 days of age since the various degrees of growth retardation seen between littermates at birth were not compensated for postnatally.

Runt littermates were significantly lighter for their crown-rump length relative to both their small ($P < 0.05$) and large ($P < 0.025$) littermates and had backfat measurements comparatively lower ($P < 0.01$) than those of their large littermates. These were signs of the permanent nature of the prenatal growth restriction evident in runt pigs.

(ii) Fresh frozen, whole midbelly, sections from the semitendinosus muscle of each animal were stained for the demonstration of both acid and alkaline preincubated ATPase and were used for the determination of total fibre number, primary fibre number and secondary: primary fibre number ratio. Samples of m. trapezius were similarly prepared to give estimates of the secondary: primary fibre number ratio.

Low birthweight was associated with a reduced total muscle fibre number in the order of 19% ($P < 0.001$) between large and
runt littermates. A comparatively lowered muscle fibre number was not always associated with low birthweight but when this was the case it was generated through a reduced secondary: primary fibre number ratio ($P < 0.01$). Primary fibre number was not significantly affected in low birthweight pigs except in two extreme cases of very severe runting.

An apparent association between muscle fibre number and the animal's relative growth potential is discussed.

(iii) Further fresh frozen, serial sections of m. semitendinosus and m. trapezius were stained for the histochemical demonstration of SDHase and GPase. These sections, together with those stained for acid and alkaline preincubated ATPase, enabled profiles of the muscle fibre types of the littermates to be compiled.

In both muscles the number of slow oxidative (SO) fibres, that were arranged in groups within 'metabolic bundles', increased with growth between birth and 128 days from, approximately, 1.0 to 10.6, and from 2.4 to 10.9, in m. semitendinosus and m. trapezius, respectively. The TSA of m. semitendinosus increased in proportion to $2/3$ ($P > 0.50$) of liveweight whereas the area occupied by SO fibres increased at a rate significantly greater than $2/3$ ($P < 0.001$) and 1.0 ($P < 0.01$). Regression analysis revealed that the area of this muscle occupied by SO fibres was significantly greater in runt and small littermates ($P < 0.001$) relative to their large littermates when they were compared at an equal liveweight.
lower birthweight littermates were older but not at the same age; this appeared to reveal an age, as well as a weight-related parameter in the development of the muscle.

The greater TSA of m. semitendinosus classified as SO in the lower birthweight pigs relative to the heavy birthweight pigs, when compared at equal liveweights, was instigated through a combination of a higher percentage of SO fibres (P < 0.05) and significantly greater mean SO fibre TSAs (P < 0.001). The mean TSAs of all myofibre types were similar between littermates of the same age but most types were of greater TSA in the lower birthweight littermates when compared (by regression analysis) at the same liveweight; this suggested an age related aspect in muscle growth independent of that associated with liveweight.

The higher percentage of SO fibres in the low birthweight pigs, when compared at an equivalent liveweight to their large littermates, appeared to be related to their affected secondary: primary fibre number ratio. This phenomenon, plus the data on the number of slow fibres per metabolic bundle, indicated that it was the number of slow fibres per metabolic bundle that was regulated with liveweight gain rather than the resultant percentage of slow fibres within the muscle.

(iv) An ultrastructural investigation into oxidative and non-oxidative fibres from the 'deep' and 'superficial' portions of m. semitendinosus, respectively, was undertaken to evaluate the mitochondrial, lipid droplet and myofibrillar content of
these myofibres between littermates. The ultrastructural percentage composition of myofibres was not found to be impaired by reduced birthweight except when, as in two extreme cases, birthweight was severely reduced. In these instances the myofibrillar percentage volume of the non-oxidative fibres was greatly affected.

The oxidative and non-oxidative fibres, as well as possessing different compliments of mitochondria and lipid droplets showed differential rates of myofibrillar accumulation. The relatively small postnatal change in the percentage volume of myofibrils of oxidative fibres, as opposed to the high change within the non-oxidative fibres, presented a cytological basis by which to explain the differential effects of growth retardation on the comparative TSAs of these fibre types.

The quantitative ultrastructural development of the myofibre types are discussed in relation to their histochemical differentiation.

During the course of this study muscle fibre anomalies were evident in the form of 'giant' fibres. Data collected on their occurrence, histochemical and ultrastructural characteristics are reported and discussed.

A study of the prenatal skeletal muscle development of mice bred for disparate liveweights at 6 weeks of age was also performed. The results of this preliminary study suggested that, in a genetically small strain of mice, the relatively lower muscle fibre
number evolves through a reduced primary fibre population only, secondary fibre number being unaffected.
Porcine muscle development is not only of particular interest to those working in the field of general developmental biology but also to the meat industry where the interest is commercial. Over the past few years there has been a definite trend towards increased pig production and the perpetuating theme of research is to improve the efficiency of this production.

In adding to our general knowledge of the growth process, studies of porcine muscle development have applications in human, as well as animal biology. The neonatal pig is, in many ways, similar to its human infant counterpart, being at a comparable stage of development at birth unlike many other experimental animals, and its suitability has been exploited for studies of a number of postnatal problems associated with birthweight (Cooper 1975).

The pig is a multiparous species. The production of a large litter, especially from an animal with a relatively long gestation period, results in the birth of siblings of a considerable weight range (Widdowson 1971b). Thus one litter of pigs produces heavy littermates, that are representative of uninhibited prenatal growth, against which to gauge the postnatal development and meat producing potential of littermates disadvantaged by a comparatively low birthweight.

The piglet of lowest birthweight in a litter is often called the "runt". Definitions of a runt in the literature are based on absolute birthweight (Pomeroy 1960; Powell and Aberle 1980, 1981).
on birthweight relative to its littermates (McLaren 1965; Perry and Rowell 1969), or on both (Hegarty and Allen 1978). In the mouse (McLaren 1965) and the pig (Waldorf et al. 1957) foetal weight has been found to decrease from the ovarian end of the uterine horn towards the middle. The apparent effect of position from the middle of the horn to the cervical end in the pig is the production of a slight increase in foetal weight (Waldorf et al. 1957). The consistency of this 'position effect' in producing a low weight conceptus at a specific site in the uterine horn excludes many factors, in particular genetics, as the underlying cause of runting; birthweight within a litter is not, in any case, considered a heritable trait in the pig (Baker et al. 1943; Craig et al. 1956).

Evidence implicating the blood flow to the developing foetus, unfortunately positioned in the middle of the uterine horn, suggests that the cause of prenatal growth retardation is a limitation on nutrient supply. Garris (1984) noted that there were regional variations in blood flow during the latter part of gestation in the guinea-pig. Both the ovarian and cervical uterine regions were found to be preferentially perfused with respect to the middle uterine zone which, in uteri possessing three pregnancy sites in one horn, supported the smallest foeto-placental unit (Garris 1983).

Natural growth retardation in the pig appears to produce foetuses equivalent to the 'small-for-dates' situation reported in man (Flecknell et al. 1981). Prenatally growth retarded pig littermates appear to be characterised by lower weight and shorter length
(Wigmore 1982) in a similar way as small-for-dates babies (Davies et al. 1979; Davies 1981; Ounsted et al. 1982). Small-for-dates babies also appear to have absolutely and relatively smaller placentas (Woods et al. 1982) as do small pig foetuses (Wootton et al. 1977; Wigmore 1982), a phenomenon which is correlated with the low placental blood flow (Wootton et al. 1977). The general consensus of opinion of workers investigating human intrauterine growth retardation is that the probable cause is undernutrition (Davies et al. 1979; Dawkins 1964; Naeye 1965) which suggests that the growth retarded neonatal pig likewise results from inadequate prenatal nutrition.

The overall aim of the work presented here was to determine the effects of low birthweight, caused by the restriction of growth in utero, on different aspects of the postnatal development of pigs.

It is known that pigs with a relatively high content of lean meat have a greater number of fibres in their muscles than fat-type pigs with a low muscle bulk (Stickland and Goldspink 1975). Muscle fibre number is reduced by prenatal growth retardation (Wigmore and Stickland 1983) and remains fixed thereafter (Staun 1963). Even very severe levels of undernutrition after birth fail to affect the total number of muscle fibres (Stickland et al. 1975). These results indicate that inhibited prenatal growth, which appears to limit the postnatal growth potential of the pig (Widdowson 1971a; Hegarty and Allen 1978; Powell and Aberle 1980), has serious implications on meat production.
A study of the relationships between birthweight, muscle cellularity and general body growth was therefore undertaken to define the cellular parameters limiting the postnatal development of the low birthweight pig. This study also comprises a full and comprehensive investigation into the subsequent effects on muscle characteristics, as defined by histochemical fibre types and their relative sizes, effects only briefly alluded to in the literature. The ultrastructural development of muscle of siblings of different birthweights was also monitored quantitatively to augment these observations and fill an apparent void in this area of comparative developmental muscle research.
1. **GROWTH STUDY OF BODY AND ORGAN WEIGHTS**

1.1. **INTRODUCTION**

Literature concerning the influence of piglet birthweight on subsequent growth performance appears controversial when first regarded and has consequently resulted in misinterpretation. Powell and Aberle (1980) found reports of other workers concerning the effects of birthweight on postnatal growth conflicting while England (1974) claimed that the available data was supportive of the contention that healthy pigs undersized at birth are not inferior to their large littermates in production capacities. Most of this discrepancy is due to an inadequacy in the expression of results and the lack of work including consistent weighings of various birthweight animals through to slaughter weight. England (1974) concentrated on the growth rate of piglets to 5 days while Hemsworth et al. (1976) extended their study up to 3 weeks of age. Hegarty and Allen (1978), and Powell and Aberle (1980), considered slaughter weight animals but only the latter workers weighed the animals during the interim period, the age at which the swine reached 26 kg being recorded.

Taken overall, however, the data presented does in fact support the hypothesis that low birthweight animals grow at a slower rate than their heavier counterparts (England 1974; Hegarty and Allen 1978; Powell and Aberle 1980; Campbell and Dunkin 1982). Few workers have considered their results in view of the probability that comparative daily gain (ie liveweight per se.) will be influenced by the vantage position of the heavier birthweight
animal, as alluded to by Campbell and Dunkin (1982). Although their premise does not appear to have been validated statistically, other workers agree that comparisons of growth rate made on a weight-, rather than an age-related, basis eradicate the apparently inferior growth rate of the low birthweight pig (England and Keeler 1964). Indeed, it would be natural to expect some variation in weight between animals of different birthweights but of the same age (Lodge and McDonald 1959) unless catch-up growth, characteristic of rehabilitated, postnatally undernourished animals (Widdowson and McCance 1963; McCance and Widdowson 1974), had occurred. In such instances absolute growth rate would no longer be maintained in proportion to birthweight, the incremental growth rate being greater in the lower birthweight animal than its heavier littermate.

It was therefore considered valuable to conduct a comprehensive study of the growth of a large number of different birthweight pigs over a wide age range, reinforced by statistical data, with an aim to elucidating the possible occurrence of catch-up growth.

Most investigators exploring the consequences of the differential growth rates resulting from disparate prenatal growth retardation between animals on body composition, with respect to relative organ weights, have concentrated on neonates (Guinea-pigs: Widdowson 1974; Human: Naeye 1965; Monkey: Myers et al. 1971; Pig: Widdowson 1970 1971a 1974; Flecknell et al. 1981). Few workers have followed the resultant course of comparative changes in body component weights with postnatal growth although Widdowson (1971a)
allowed a runt pig and a large littermate to grow to maturity (3 years) and various organs were weighed and analyzed for protein and DNA. Since animals are chosen by weight at slaughter it seemed appropriate to consider animals on a similar weight basis in this study and to extend the work initiated by Widdowson, and therefore test statistically the hypothesis that runts become well proportioned adults (Widdowson and McCance 1974).

The growth rate observations on liveweight were therefore combined with various measurements on the gross dimensions of the body and weights of certain soft tissues to evaluate the consequences imposed by the evident retardation exhibited at birth on the animal's subsequent growth performance.

1.2. MATERIALS AND METHODS

1.2.1. Selection Of Animals

All the animals used in this study were offspring of a commercial, pedigree Large White herd owned by Dalrymple Partners of Oxenfoord Mains, Midlothian. Pregnant animals were maintained from conception through to lactation on 'Sowmix Meal' (BOCM SILCOCK of Basingstoke, Hampshire; sole suppliers to this establishment) mixed with barley to give a final protein concentration of approximately 17%. This was fed at a basic level of 3.2 kg per day, then increased to 6.8 kg per day postpartum to supply just over 1.0 kg of protein per day to each sow. While suckling the sow's milk was supplemented with a 21% protein creep feed of '402 Suckler Care Pellets'. Male piglets were castrated at 3 - 4 weeks of age. Wean-
ing was at 4 - 5 weeks when pigs were transferred to a weaning house and maintained on an 18% protein diet, a mixture of 'Growacon Gold' and barley. On reaching light pork weight (approximately 40 kg) they were transferred to a fattening house where a 17.5% protein pellet with 10% added maize ('Elite Red Pellets') was fed until bacon weight, at approximately 90 kg. The management aimed to maintain all animals, growing, fattening and breeding, in a very high condition score at all times.

Selection of animals for this study was by weight at birth. Where possible three animals were chosen from a litter; the largest male, smallest (not less than 1000 g) male and the runt (950 g or less). Small littermates were chosen to have birthweights just above that considered 'critical' (about 1000g) by Hegarty and Allen (1978), and Powell and Aberle (1980), while the runt littermates were born below this 'critical' weight. In total 17 litters were used of different ages ranging from 0 to 128 days. This total incorporated 17 large, 13 small and 19 runt pigs.

1.2.2. Choice Of Muscles

Two muscles were chosen for investigation; m. semitendinosus, a caudal muscle of the thigh, and m. trapezius (Pars thoracis), a muscle of the shoulder girdle.

M. semitendinosus was chosen for the following reasons:

i It is of commercial value, being a major muscle of the ham. Therefore results from this muscle may be of value in meat production.
The existing literature already covers certain aspects of this muscle's pre- and postnatal structural and histochemical development in some depth (Ashmore et al. 1973; Beermann et al. 1978; Hegarty and Allen 1978; Wigmore and Stickland 1983).

This muscle is adapted for a dual function of posture and propulsion (Sivachelvan and Davies 1981) by possessing distinct fibre type regions (Beecher et al. 1965), hence enabling the examination of physiologically different muscle within one anatomical muscle.

The main considerations in the choice of m. trapezius were:

i Relative maturity at birth with respect to muscles of the hind limb (Davies 1974; Suzuki and Cassens 1980).

ii Relatively lower postnatal impetus with respect to m. semitendinosus (Butterfield and Berg 1966)

iii Predominant function of postural support as opposed to the dual function of m. semitendinosus.

1.2.3. Preparation Of Material

At ages 0, 1, 2, 4, 6, 9, 12, 15, 19, 27, 33, 46, 64, 84, 100, and 128 days representative animals from chosen litters were weighed then slaughtered at the Royal (Dick) School of Veterinary Studies Field Station, Bush, by exsanguination after electrical stunning. Ice was packed around the carcasses to decrease the rate of cell lysis on returning to the R(D)SVS for dissection.
Prior to dissection of the carcass a measurement was made from the nuchal crest to the base of the tail, with the spine straightened, to represent the crown-rump length. A ratio of live-weight to the cube of this length, designated the Ponderal Index (Lubchenco et al. 1966; Davies 1981), was used to evaluate the nutritional status of the animal at slaughter.

Dissection commenced with the uncovering of m. semitendinosus of the left hind limb and the marking of the exposed superficial surface with a fine strip of Latex Injection Mass Blue (Philip Harris Biological Ltd). This facilitated the orientation of the deep and superficial portions of the muscle for both the histochemical sections and the electron microscopy (EM) samples. This muscle and m. trapezius were then removed, cleaned of superficial fat and connective tissue, and weighed. Likewise the brain (including medulla oblongata and pons), liver, kidneys and adrenals were dissected, cleaned and weighed; the cerebellum was weighed as a separate entity. Measurements of long bone length on the left humerus and femur were made; from the most proximal to the most distal aspects in each case. A measurement of backfat thickness was taken to include fat and skin depth at the surface exposed when the carcass was cut at the level of the last rib.

1.2.4. Method Of Studying Relative Growth

The relative growth of the body, and its components, between littermates must be quantified for a comprehensive analysis. For a valid comparison a reference parameter must be defined. Where growth of the body, or component of it (Y) bears a constant
'allometric' relationship with the chosen reference parameter (X) the relationship can be described by the equation,

\[ Y = a \cdot X \]  

(Huxley 1932)

where 'a' is a constant and 'b' is the growth or regression coefficient, a statistical term describing the relationship of the dependent variable, Y, on the independent variable, X, (Snedecor and Cochran 1967).

A linear relationship is established graphically for an allometric situation by a double logarithmic plot (ie log Y = log a + b.log X). The regression coefficients, thus computed, were used to investigate growth between body components of littermates, and to categorise their respective growth impetuses as high, low, or average (regression coefficients significantly higher, lower, or not significantly different from 1.0). For non-allometric relationships, which are non-linear, logarithmic transformation of the X and/or Y variate must be performed to obtain a linear relationship between the variates. This concept enables the evaluation of relationships between dissimilar parameters.

When no statistically significant difference exists between the calculated regression coefficients (ie the rate of change in the dependant variables Y₁ and Y₂, with X is essentially the same) at a given value of X the corresponding Y values may not be equal. Elucidation of this contention is by statistical comparison of the intercepts (a) of the regression lines (Quenouille, 1969). A significant diversity between intercepts (when regression coefficients
are not dissimilar) denotes significantly different values of \( Y \) at a given value of \( X \).

One of the main aims in this chapter was to define the consequences of the influence of birthweight on liveweight gain. Therefore the difference in change in liveweight \( (Y) \) with age \( (X) \) between littermates was the first consideration. The age curve of growth is generally determined by two opposing forces; a growth-accelerating, and a growth-decelerating, force (Brody 1945). No equation adequately represents the whole growth cycle so the age curve has to be broken up into its constituent segments, each described by an individual equation, with the logarithm of liveweight plotted against age, as discussed by Brody (1945).

The rate of growth of body organs is most appropriately compared between littermates on a weight-related basis since it is well established that organ weights bear a strong relationship with the attainment of body weight (Brody 1945; Davies 1974; Doornenbal and Tong 1981) irrespective of the animal's age. Dissimilar ranges in liveweight between littermates at any age made comparison of such parameters as these, on an age-related basis, not feasible. Liveweight is the primary consideration in selection of slaughter animals in meat production and the obvious choice for a reference parameter in this study.

In all transformations the natural logarithm was employed. Regression data was computed on a Research Machines 380Z using an MBASIC CP/M 2.2 statistics package.
1.3. RESULTS

1.3.1. Growth Rates Of Littermates

The mean birthweights (± standard deviation) of the large, small, and runt littermates were 1544 g ± 189 (n = 17), 1144 g ± 190 (n=13), and 776 g ± 107 (n = 19), respectively, the difference between each group being highly statistically significant (P < 0.001). The runt was, on average, about half the weight of the large at birth, and the small, as well as above the aforementioned 'critical' birthweight, represented, approximately, the median birthweight between that of the large and runt. 59% of runts were gilts.

Liveweight, prior to slaughter, was used to compute age curves of growth for the three categories of animals (Figure 1) as an exponential plot. Animals that failed to exhibit a normal liveweight gain were excluded from the computations. These included the large littermate at 15 days, which showed growth retardation due to a general infection, and two animals from the runt category. The latter two pigs were born with an exceptionally low birthweight of more than 2.5 SD below their mean litterweight and 50% the weight of their respective large littermates. Attention was drawn to their extreme birthweight condition by the fact that they failed to show any substantial weight gain to their slaughter ages of 9 and 15 days. Figure 1 illustrates two distinct phases of growth; from birth to about 12 days and from 15 to 128 days. The regression coefficients of the liveweight: age growth curves for both growth periods were not significantly different (P > 0.1) between the
The natural logarithm of liveweight, at slaughter, against age of littermates categorised by birthweight. The regression lines define two phases of growth (i) and (ii).

Growth phase (i) 0 - 12 days
Regression equations: Large, \( \log_e Y = 0.10X + 7.28 \)
Small, \( \log_e Y = 0.12X + 6.94 \)
Runt, \( \log_e Y = 0.10X + 6.59 \)

Growth phase (ii) 15 - 128 days
Regression equations: Large, \( \log_e Y = 0.02X + 8.18 \)
Small, \( \log_e Y = 0.02X + 7.76 \)
Runt, \( \log_e Y = 0.03X + 7.45 \)
littermates which indicates that their respective incremental growth rates were essentially equal. The combined regression coefficients, for the large, small and runt categories, for liveweight against age were 0.101 ± 0.020 (mean ± SE) for the first, and 0.024 ± 0.002 for the second growth phase. When equal growth rates are coincident with intercepts that are not significantly different the absolute growth rates are similar; such is the case from 0 - 12 days between large: small and small: runt littermate groups, and from 15 - 128 days between the small and runt littermates (Table 1). This shows, surprisingly, that the growth rate of small littermates fell behind that of the large during the second growth phase.

Table 1 - Differences Between Intercepts Of Liveweight: Age Curves Of The Large, Small And Runt Littermates During The Two Phases Of Growth.

<table>
<thead>
<tr>
<th>Compared littermates</th>
<th>Growth Phases</th>
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<tr>
<td></td>
<td>Birth - 12 days</td>
</tr>
<tr>
<td>Large: Runt</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>Large: Small</td>
<td>NS</td>
</tr>
<tr>
<td>Small: Runt</td>
<td>NS</td>
</tr>
</tbody>
</table>

The runt and large littermates are the only category combination to show a significant difference (P < 0.001) in absolute growth rate from birth to 128 days, so, on average, maintaining their relative
weight differences evident at birth. Despite the apparent trend for catch-up growth in the runts, indicated by the growth curve (Figure 1), and indeed real catch-up growth in specific cases, this was evidently not statistically significant.

1.3.2. **Growth Rates Of Muscles And Organs**

The absolute organ weights were always less in the lower birthweight littermates, when their liveweights were accordingly less, the most frequent exceptions being brain, cerebellum and adrenals. When relative liveweight among the littermates was not maintained in order of birthweight the organs that apparently failed to reflect this 'catch-up' growth were, usually, also brain, cerebellum and adrenals.

The difference between the three groups of littermates at each age with regards to percentage body composition, and other measured parameters, (as specified in 'Preparation Of Materials'), are shown in Table 2. At any age all three littermates were equal only in their percentage occupied by m. semitendinosus, liver and heart. In contrast, the percentage of m. trapezius was found to be significantly less (P < 0.05) in the runts when compared to the small, but not the large, littermates. The percentage liveweight occupied by kidneys was greater in the runts than in the large littermates (P < 0.001). However, the adrenals were not only proportionally heavier in the runts than in their large littermates (P < 0.001) but also compared to their small littermates (P < 0.05). The percentages of total brain weight were significantly different between all littermates, and were inversely related to birthweight.
Table 2 - Differences Between Littermates Of The Same Age With Respect To The Measured Parameters. Probabilities Calculated By A Paired T-Test.

<table>
<thead>
<tr>
<th>Compared Parameter</th>
<th>Large - Runt</th>
<th>Large - Small</th>
<th>Small - Runt</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. semitendinosus Z</td>
<td>6.4</td>
<td>4.9</td>
<td>4.4</td>
</tr>
<tr>
<td>M. trapezius Z</td>
<td>5.4</td>
<td>0.9</td>
<td>7.1 *</td>
</tr>
<tr>
<td>Total brain Z</td>
<td>-79.8 ***</td>
<td>-30.5 **</td>
<td>-57.6 *</td>
</tr>
<tr>
<td>Cerebellum Z ($)</td>
<td>-77.6 ***</td>
<td>-24.4 ***</td>
<td>-64.3 ***</td>
</tr>
<tr>
<td>Liver Z</td>
<td>-0.8</td>
<td>-3.7</td>
<td>-5.1</td>
</tr>
<tr>
<td>Kidneys Z</td>
<td>-13.9 ***</td>
<td>-8.0</td>
<td>-10.2</td>
</tr>
<tr>
<td>Adrenals Z</td>
<td>-61.1 ***</td>
<td>-17.2</td>
<td>-50.7 *</td>
</tr>
<tr>
<td>Heart Z</td>
<td>-4.0</td>
<td>1.8</td>
<td>-7.5</td>
</tr>
<tr>
<td>Crown-rump length (cm)</td>
<td>13.4 ***</td>
<td>7.6 **</td>
<td>8.2 *</td>
</tr>
<tr>
<td>Back-fat thickness (mm)</td>
<td>24.8 **</td>
<td>10.4</td>
<td>17.0</td>
</tr>
<tr>
<td>Humerus length (cm)</td>
<td>15.0 ***</td>
<td>12.1 ***</td>
<td>5.7</td>
</tr>
<tr>
<td>Femur length (cm)</td>
<td>19.3 ***</td>
<td>10.9 **</td>
<td>6.1</td>
</tr>
</tbody>
</table>

All Z values are an expression of organ weight as a percentage of liveweight.

* = significant difference at P<0.05
** = significant difference at P<0.01
*** = significant difference at P<0.001

($) - n values for cerebellum are 17 for Large-Runt, and 11 for both Large-Small and Small-Runt group comparisons.
Table 3 - Regression Coefficients And Growth Impetuses Of Muscles And Organs Relative To Liveweight Of Large, Small And Runt Littermates From Birth To 128 Days Of Age.

<table>
<thead>
<tr>
<th>Muscle/organ</th>
<th>Large</th>
<th>Small</th>
<th>Runt</th>
<th><strong>Total regression coefficient impetus</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>M. semitendinosus</td>
<td>1.22 (0.03)</td>
<td>1.18 (0.03)</td>
<td>1.20 (0.06)</td>
<td>1.20 (0.03) High</td>
</tr>
<tr>
<td>M. trapezius</td>
<td>0.96 (0.02)</td>
<td>0.93 (0.02)</td>
<td>1.00 (0.02)</td>
<td>0.98 (0.01) Av.</td>
</tr>
<tr>
<td>Total brain</td>
<td>0.25 (0.02)</td>
<td>0.26 (0.02)</td>
<td>0.19 (0.03)</td>
<td>0.23 (0.01) Low</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>0.33 (0.03)</td>
<td>0.33 (0.04)</td>
<td>0.20 (0.05)</td>
<td>0.28 (0.03) Low</td>
</tr>
<tr>
<td>Total brain</td>
<td>0.25 (0.02)</td>
<td>0.25 (0.02)</td>
<td>0.18 (0.03)</td>
<td>0.22 (0.02) Low</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>0.85 (0.03)</td>
<td>0.84 (0.04)</td>
<td>0.93 (0.04)</td>
<td>0.89 (0.02) Low</td>
</tr>
<tr>
<td>Liver</td>
<td>0.75 (0.02)</td>
<td>0.81 (0.04)</td>
<td>0.77 (0.02)</td>
<td>0.77 (0.01) Low</td>
</tr>
<tr>
<td>Kidneys</td>
<td>0.69 (0.07)</td>
<td>0.67 (0.04)</td>
<td>0.57 (0.03)</td>
<td>0.62 (0.03) Low</td>
</tr>
<tr>
<td>Adrenals</td>
<td>0.79 (0.05)</td>
<td>0.87 (0.04)</td>
<td>0.83 (0.02)</td>
<td>0.84 (0.02) Low</td>
</tr>
</tbody>
</table>

* No significant difference existed between growth rates of muscles or organs, relative to liveweight, of any littermate.

The regression coefficients of all organ weights against liveweight for the three littermates are displayed in Table 3. No significant difference (P > 0.1) existed between the three growth coefficients for any of the organs examined. A collated regression coefficient (b) was calculated to represent the growth of these organs in this strain of Large White pig which, thereby, enabled evaluation of the respective growth impetuses. All organs, except the muscles, grew at a slower rate than the whole body; in order of decreasing rate they were liver, heart, kidneys, adrenals, cerebellum, and brain. M. trapezius gained weight at the same rate as the body while the growth of m. semitendinosus was significantly greater (P < 0.001) than that of the whole body. Significant differences existed between littermates in their percentage...
liveweight composition with respect to certain of the weighed organs (Table 4). At an equal liveweight the runts possessed (i) a significantly higher ($P < 0.001$) proportion of m. semitendinosus, brain and cerebellum, and adrenals, (ii) a significantly lower ($P < 0.001$) proportion of m. trapezius and kidneys, and (iii) essentially equal proportions of heart and liver, with respect to their large littermates. Comparing the large and small littermates the percentage composition, with respect to liveweight, was only different regarding m. semitendinosus, adrenals, kidneys and heart; the former two tissues comprising a significantly higher ($P < 0.05$), and the latter two organs a significantly lower ($P < 0.001$) proportion of the small littermate. Runts were only similar in composition to their small littermates regarding m. trapezius; brain and cerebellum, kidneys, adrenals and heart all being relatively significantly greater ($P < 0.001$) while m. semitendinosus and liver were significantly lower ($P < 0.001$).

Humerus and femur lengths were significantly different between two of the three littermate groups, being shorter in the lower birthweight groups, the exception being the small to runt group comparisons. Crown-rump lengths were significantly longer ($P < 0.025$) in littermates of a heavier birthweight at any age postnatally. Crown-rump length was always longer in the large than the runt littermates except in one case (84 days) when the runt was heavier than its large littermate. A possible relationship between crown-rump length and liveweight was confirmed by the calculation of the Ponderal Index of each animal. This parameter remained approximately constant at a value of $0.094 ± 0.023$ (mean ± SD) for
all littermates through to 128 days. Ponderal Index was significantly lower in runts than in both the large (P < 0.025) and the small (P < 0.05) littermates.

Table 4 - Intercepts For Regression Coefficients Comparing The Relative Liveweight Composition Between Large, Small And Runt Littermates From Birth To 128 Days Of Age.

<table>
<thead>
<tr>
<th>Muscle/organ</th>
<th>Large</th>
<th>Small</th>
<th>Runt</th>
<th>Significance of difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Large: Runt</td>
</tr>
<tr>
<td>M. semitendinosus</td>
<td>-7.75</td>
<td>-7.41</td>
<td>-7.48</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>M. trapezius</td>
<td>-6.43</td>
<td>-6.20</td>
<td>-6.81</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>Total brain</td>
<td>1.67</td>
<td>1.65</td>
<td>2.19</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>-0.84</td>
<td>-0.83</td>
<td>0.15</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>Total brain-cerebellum</td>
<td>1.55</td>
<td>1.58</td>
<td>2.10</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>Liver</td>
<td>-2.35</td>
<td>-2.30</td>
<td>-3.05</td>
<td>NS</td>
</tr>
<tr>
<td>Kidneys</td>
<td>-3.06</td>
<td>-3.66</td>
<td>-3.23</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>Adrenals</td>
<td>-6.43</td>
<td>-6.18</td>
<td>-5.28</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>Heart</td>
<td>-3.35</td>
<td>-4.09</td>
<td>-3.79</td>
<td>NS</td>
</tr>
</tbody>
</table>

* Natural logarithms of intercepts.

Backfat thickness was only found to be significantly different between runts and large littermates, being less in the runts (P < 0.01), increasing from an average of 2.0mm in the neonatal littermates to an average of 8.0mm in the littermates at 128 days.

All the raw data, on which these results were based, is detailed in Appendix 1.
1.4. DISCUSSION

1.4.1. Relative Growth Of Littermates

The term 'low birthweight' was found to be synonymous with 'small size at birth' as runt neonates had shorter crown-rump and long bone lengths than their heavier littermates. The results presented here, through demonstrating similar regression coefficients for liveweight: age curves for the three littermate groups, confirm that absolute growth is proportional to liveweight and so will be less in a smaller animal as inferred by Campbell and Dunkin (1982). This demonstrates that the smaller animal is not disproportionately subordinate to its larger littermate in its incremental growth rate, which supports the findings of England and Keeler (1964), Widdowson (1971b) and England (1974). The dependence of growth rate on liveweight is indicative of a correlation with birthweight, since this is simply a certain liveweight at a specific age. Such a correlation has been found by Hemsworth et al. (1976) in suckling pigs, although this may not be the most important factor influencing weight gain, as discussed later. However, in this study the inevitable limits imposed by size at birth were shown statistically to persist throughout, and at any given liveweight runts were chronologically older than their large littermates as found by Powell and Aberle (1980). In this study at 26 kg liveweight the average age of the large littermate was estimated to be 90.5 days (calculated from the regression data provided with Figure 1), the runts requiring a further 9.5 days, 2.5 days longer than Hegarty and Allen's (1978) runts, to achieve this weight. The
number of animals in the present study appears to be insufficient to deduce indisputable trends in liveweight gain between littermate groups after 64 days; consideration of specific litters reveals that runts can 'catch-up' with their small littermates and achieve weights approaching those of their large littermates, although statistically this is not indicated. The age after which animals were seen to present this situation was within the range of 56 - 112 days, determined by Blunn et al. (1953) to be the period when expression of the animal's genetic potential is most prevalent.

No cases of catch-up growth of prenatally retarded pigs have previously been reported at any age, indeed it is thought to be a phenomenon solely of the postnatally undernourished animal (Widdowson and McCance 1974), and Widdowson (1974) has concluded that catch-up growth will not occur in animals small in size at birth. This prompts the query as to the nature of the mechanism by which small size at birth could impose such a limit on the expression of the animal's genetic potential for growth.

Small size at birth imposes a physical disability on the animal which may have an adverse effect on the animal's normal development in various ways. The rate of weight gain in young pigs is primarily influenced by milk consumption (Lodge and McDonald 1959; Lucas 1968) and low birthweight piglets compete less successfully for food, especially during suckling, than their large littermates (Hartsock and Graves 1970; England 1974; Hemsworth et al. 1976). Campbell and Dunkin (1982) have also suggested that light birthweight pigs may in fact be particularly vulnerable to
nutritional stress, more so than their larger littermates.

During the course of this work it has been revealed that the runt’s ability to compete with its heavier littermates could not possibly be confounded by any specific defects in its motor coordination incurred by restricted cerebellar growth (Bedi et al. 1982). Although the offspring of undernourished rats show a significant reduction in cerebellar weight, above the reduction in the weight of the whole brain, when compared to control littermates (Howells et al. 1977; Bedi et al. 1982), this situation did not appear to be reflected in the prenatally undernourished pig. There was, in fact, evidence of cerebellar sparing, with respect to the rest of the brain, in pigs of low birthweight. The results of Bedi et al. (1982) showed that at the same age their prenatally undernourished rats had a cerebellar and a forebrain weight below that of the controls, by 25% (P < 0.01) and 12% (P < 0.01), respectively. However, in the present study, at the same age, the weight of cerebellum in the runt was not significantly below that of the large littermates although the brain minus cerebellum (the definition of forebrain by Bedi et al. 1982) was significantly less (P < 0.001) by 13%; a similar value to the deficiency seen in the experimental rats of Bedi et al. (1982) but 4% greater than seen in those of Howells et al. (1977). However these latter workers found an average reduction of 14% in cerebellar weight in their prenatally undernourished animals, while in the present study, of naturally occurring prenatal undernutrition, no such effect was evident. The discrepancy in the results these workers presented and those presented here, in the pig, may be due to the severity, and the
duration, of the prenatal food restriction imposed in the rats, and hence the tremendously high degree of growth retardation suffered by them. At 27 days the experimental rats af Bedi et al. (1982) showed a deficit in body weight, comparative to the control animals, 17% below that seen between the runt and large littermates in this study at birth. Dickerson and Dobbing (1967) have shown that the cerebellum of the pig has a higher rate and shorter period of growth, as indicated by weight and chemical composition, relative to the rest of the brain. Although these authors considered that the cerebellum would, therefore, be more prone to nutritional stress than the rest of the brain it may be that, in utero, the nutritional stress normally evident in siblings of large litters is not severe enough to bare its effects on the cerebellum. Indeed, this part of the brain might be actively spared under such natural conditions due to its greater growth rate and, therefore presumably, higher metabolic rate (Hammond 1944); a concept which will be discussed later.

Powell and Aberle (1980) reduced the different levels of behavioural competition during pre-weaning growth by cross-fostering piglets to create small groups of similar sized, suckling animals. Despite these expediences, runt pigs failed to grow as fast, and utilise feed for weight gain as efficiently, as their large littermates. The success of artificial rearing schemes is dubious and they are certainly beyond the scope of the average pig farm (Lecce 1975). The lack of advantage gained by fostering, and with artificial rearing studies, suggests a physiological rather than a behavioural reason for the failure of low birthweight pigs to
catch-up. Widdowson (1971b; reviewed by Widdowson and McCance 1975) proposed a theory on the hypothalamic regulatory mechanism of voluntary food intake which postulates that an animal's size, at a critical time in development, determines its appetite from then on, and hence its dimensions at maturity. This would exclude any possibility of catch-up growth in prenatally growth retarded pigs, as Widdowson (1974) foresaw, and which is supported statistically in this study, although discrete instances of catch-up growth occurred. This poses the question, "What innate difference exists within the biological make-up of the low birthweight animal that fails to show, and that which does show, catch-up growth?". By this study of the composition of the body the key to this phenomenon, which is obviously associated with birthweight, has begun to be elucidated. The rest of this, and the succeeding chapter help to shed some light on the enigma of relative growth.

1.4.2. Birthweight And Growth Of Body Organs

Prenatal growth retardation affects body composition, with respect to the percentage of liveweight occupied by organs, an effect which is evident at birth and maintained postnatally. The weighing of body organs of the three littermate groups has shown that there was no difference in the growth rates of these organs between animals (Table 3). This infers that the differential effect on organ growth prenatally, that is manifest in relative organ weights at birth, will not be compensated for postnatally, and results in unequal body composition in pigs of either the same age, or liveweight, but disparate birthweight.
It is generally accepted that during development of the foetus the organ tissues with the highest metabolic rates, at that time, receive priority in obtaining the nutrient supply they require (Hammond 1944), and that these organs develop early in foetal life so are most mature at birth (McMeekan 1940). When nutrient supply is limited, as in the growth retarded pig foetus, it is the later developing organs that suffer the most reduced weight gain. The prenatally growth retarded neonate is thus more reminiscent of a foetus of the same weight than its large littermate with respect to the absolute weight of maturing soft tissues such as liver, kidneys, heart and small intestines (Widdowson 1971a). However the brain weight is similar to that of the large littermate since the nervous tissue develops prior to other tissues, while the muscle weight is suggestive of that of a foetus of lighter weight, as muscle is one of the tissues most greatly affected by prenatal undernutrition. Flecknell et al. (1981) found that not only brain, but also pituitary, adrenal and thyroid weights showed no significant change amongst piglets of decreasing body weight at birth, implying preferential protection of these organs from the effects of intra-uterine growth restriction, unlike other body organs. This work was substantiated by the present finding that the sparing effect on brain and adrenal was so great that no matter whether the large and runt littermates were compared at the same age (Table 2), or the same liveweight (Table 4), the runt always had a greater percentage of total brain (P < 0.001) and adrenals (P < 0.001) than its large littermate. However, although in agreement with Flecknell et al. (1981) this is in discrepancy with the results of Hegarty and Allen
with respect to adrenal weight. These workers showed that in the adult control of 106 kg the adrenal weight was significantly heavier than in runt pigs of the same weight. In the light of the present work such a situation would not be expected as the sparing effect on adrenal weight, evident in the neonate, appeared persistent.

Large and runt littermates were found to be similar in their percentage of body weight occupied by both liver and heart at the same age and at equal liveweights. This is in accordance with the fact that the weights of these organs are highly related to body weight as stated by Flecknell et al. (1981) and deduced from the results of Widdowson (1970, 1971a, 1974). The kidneys were, however, proportionally heavier in the runt than in the large littermate at the same age (P < 0.001) but compared on a similar liveweight basis the percentage weight of the kidneys was deficient in the runt (P < 0.001).

1.4.3. Birthweight And Muscle Growth

The work of Campbell and Dunkin (1982), and Hegarty and Allen (1978), showed that on an equal liveweight basis, of 6.5 kg and 106 kg respectively, the weight of m. semitendinosus was not significantly affected by birthweight; the results presented here show that at a similar liveweight m. semitendinosus was significantly heavier in the runt than the large littermate (P < 0.001). The disparity between these findings and those of Campbell and Dunkin (1982) could well be a consequence of the larger number of animals included in the present study; these workers only considered six pigs, although they were all Large Whites. The breed of swine may
be of tremendous significance. Hegarty and Allen (1978) looked at a selection of both Yorkshire (an alternative name for the Large White pig) and Yorkshire crossbred swine at variance with the use of solely purebred Large Whites in the present, and Campbell and Dunkin's (1982), investigation. The relative growth of muscles is dependent on the breed (Davies 1974) and thus differential muscle growth within a breed determines the ultimate carcass composition with respect to the muscles under consideration. That is to say, in breeds where two muscles have significantly different growth impetuses disparity in liveweight gain between littermates can result in different proportions of the muscles at equal liveweights. The slower growing animal will be significantly older at a given liveweight and will possess a greater, or smaller, proportion of a muscle of a higher, or lower, growth impetus respectively, as discussed later. This premise cannot be verified specifically for the breeds used by Hegarty and Allen (1978) as no data on their relative muscle growth impetuses is available.

M. trapezius was, in contrast to m. semitendinosus, significantly lighter in the runts than their large littermates \( P < 0.001 \) when the animals were compared at equal liveweights; the results for this muscle being similar to those of Hegarty and Allen _dorsi_ (1978), and Powell and Aberle (1980) for m. longissimus\(^d\) As opposed to the relatively different weights of m. semitendinosus and m. trapezius among littermates considered at equal liveweights, the percentage weight of the two muscles studied here were similar among animals of the same age.
The different growth impetuses of m. semitendinosus and m. trapezius postnatally (Table 3) is indicative of dissimilar states of maturity at birth; this fact being verified subsequently with regard to their respective histochemical development (see Chapter 3). M. semitendinosus is less mature than m. trapezius at birth and so, having spent less time developing in utero, will probably have been subjected to a lower degree of retardation prenatally. The fact that low birthweight pigs were deficient, on an equal liveweight basis, with respect to m. trapezius but not to m. semitendinosus belies the discovery of different prenatal growth rates of m. semitendinosus among pig foetuses within a litter, producing a proportionally lighter muscle ($P < 0.05$) in the smaller neonate (Wigmore and Stickland 1983). However, when large and runt littermates were compared (by regression analysis) at equal liveweights postnatally, m. semitendinosus was 31% heavier in the runt. This phenomenon is associated with the different growth impetuses of the two muscles. M. semitendinosus grows at a significantly greater ($P < 0.001$) rate, while m. trapezius grows at the same rate as the whole body. Thus the carcass of this breed of pig will comprise an increasing percentage of m. semitendinosus with age; this, combined with the fact that runts are chronologically older than their large littermates at an equal liveweight explains why runts have a higher proportion of this muscle when comparisons are made on a weight-constant basis.
1.4.4. **Backfat Thickness And Relative Skeletal Dimensions Of Littermates**

The relatively lower (P < 0.01) backfat thickness of the runts in comparison with their large littermates, at the same age, corresponds to the fact that runts have fewer adipocytes for lipid filling initially (Powell and Aberle 1981), suggesting that adipocyte differentiation is limited during prenatal growth inhibition. However, these workers and Hegarty and Allen (1978) found that by slaughter weight (96 kg) runt pigs generally had the same proportion of fat deposits as their large littermates. Through the recruitment of preadipocytes for lipid filling runts accumulated fat stores proportionally equal to those of their large littermates (Hegarty and Allen 1978; Powell and Aberle 1981). This suggests that the runts were as well nourished postnatally as their large littermates, as those in this study were believed to be. Despite the absolutely thinner backfat of the runts, and an average Ponderal Index significantly lower than that of their small (P < 0.05) and their large (P < 0.025) littermates, consideration of specific litters older than 64 days (and heavier than 16 kg) suggests that compensation for the consequences of prenatal undernutrition were beginning to occur. It is, therefore, quite likely that, by conventional slaughter weight, the runt pigs of this study would have had equal fat accumulations to those of their large littermates and so agree with the findings of Hegarty and Allen (1978) and Powell and Aberle (1981).

The long bones grow in proportion with the body's dimensions
so are comparatively shorter in the smaller pig foetus (Adams 1971) and are of similar length in runt and large littermates at the same slaughter weight (Hegarty and Allen 1978). In the present study the humerus and femur were significantly shorter in groups of animals of comparatively lower growth rates, and therefore smaller size, between 15 and 128 days. The muscles associated with these bones will likewise be significantly shorter in the runt than the large littermate.

1.4.5. Birthweight And Postnatal Growth

As previously mentioned, the effect of prenatal growth retardation irreparably alters the percentage organ weight composition of the body among littermates, so no animal is ever identical in body composition. If the percentage body composition of the large littermate represents the ideal there appears to be no evidence that the ideal is pertinent for adequate body functioning (Widdowson 1974). However it would conceivably benefit the runts bodily functioning not to outgrow its larger birthweight littermates because, despite specific incidences of body catch-up growth, the absolute weights of the brain minus cerebellum, and cerebellum, of the runts concerned were consistently less than those of the ‘small’ littermates they had outgrown, with respect to liveweight, by, on average, 16% and 4% respectively. Maybe it is within the concept that limited prenatal development restricts postnatal development, that the reason for the low incidence of catch-up growth lies. As shown by Widdowson (1971a), certain body organs such as the liver, heart and kidneys have, in addition to lower
weights, lower total DNA content in the runts, as opposed to the large littermates and hence a lower number of cells. The growth of these vital organs is therefore restricted postnatally by the hypertrophy of their constituent cells (Winick and Noble 1966), with the exception of the liver. A restriction on organ growth and, therefore, on cell number prenatally, may be a factor limiting the amount of postnatal growth. The implications of prenatal growth retardation at the cellular level are discussed in Chapter 2 with specific reference to muscle.
2. HISTOLOGICAL INVESTIGATION OF MUSCLE FIBRE NUMBER

2.1. INTRODUCTION

All features that contribute to the determination of the ultimate size of a muscle are reviewed in this introduction to emphasise the importance of one of these features, to which this chapter is devoted, namely, the number of myofibres within a muscle.

The muscle mass of an animal is determined by the size and the number of its constituent myofibres. The dimensions of a myofibre change by both an increase in length, through the addition of sarcomeres at the musculo-tendinous junction (Speidel 1934; Goldspink 1968), and in transverse sectional area (TSA). The mean fibre TSA increase is directly responsible for the TSA increase of the whole muscle (Davies 1972). Myofibre diameter increase commences at a rapid rate in the young animal, and gradually decreases as mature body dimensions are attained (Chrystall et al. 1969; Stickland and Goldspink 1973).

Moss (1968), from work on the growth of chicken skeletal muscle, has shown that during normal muscle growth a direct relationship exists between the mean TSA of its component fibres and the total number of nuclei within the fibres. Hogberg and Zimmerman (1979) also found a consistent relationship between muscle fibre TSA and nuclear number in young pigs, as did Powell and Aberle (1975) in a study of two breeds of swine selected for their differing muscle bulk. Increasing the dimensions of muscle fibres thus
requires a proportional increase in the total number of nuclei.

The source of DNA contributing to this increase in the number of myofibre nuclei is thought to exist outside the fibre after the myotube is formed (Allen et al. 1979). It is characteristic of muscle fibre precursor cells, that, once they have fused to form myotubes, and hence myofibres, they permanently withdraw from the cell cycle and are no longer capable of proliferation (Bintliff and Walker 1960; Stockdale and Holtzer 1961). The increase in muscle DNA cannot, therefore, originate from mitotic division of muscle fibre nuclei. Much evidence has now accumulated that satellite cells (as described by Mauro 1961 and Muir et al. 1965) are the progenitors of myofibre nuclei postnatally (Moss and Leblond 1971; Swatland 1977; Allen et al. 1979). It is clear that, since there appears to be a limitation on the volume of cell cytoplasm over which a nucleus has jurisdiction (Widdowson 1970; Cheek et al. 1971), muscle nuclear proliferation during postnatal growth is one of the determinates of the animal's potential for muscle development. Nevertheless, there appears to be no information indicating that prenatal growth restriction might limit the number of satellite cells developing in utero, or that this condition hinders the proliferation of satellite cell nuclei after birth for incorporation into the myofibre as true muscle fibre nuclei. The existing literature does, however, report histological studies, the results of which imply that prenatal nutritional stress does not, in fact, limit the cross-sectional growth of the myofibres of the affected littermates postnatally (Hegarty and Allen 1978; Powell and Aberle 1981).
The proliferation of nuclei is not the only factor limiting cell size, there are also physiological restrictions on the dimensions a myofibre can achieve, above which metabolic function is impaired. Among these physiological restrictions is that the time taken for oxygen to diffuse into the centre of a fibre is proportional to its TSA (Hill 1956); it is therefore energetically inefficient to exceed such physiological limitations of cell size.

Although the existence of phenomena restricting the degree of myofibre hypertrophy warrants consideration, the role of total muscle fibre number in determining muscle mass in the mature animal is far more important. Hooper (1982) found that fibre number was responsible for two thirds of the increase in muscle bulk following selection for altered body weight while Luff and Goldspink (1967) attributed three quarters of the variation in muscle weights between mice to differences in total muscle fibre numbers. Miller et al. (1975) found that m. longissimus mass was more related to estimated total fibre numbers than myofibre diameters in pigs with differential postnatal growth rates and therefore differential accumulations of muscle bulk. The number of fibres in the muscle therefore appears to be the most prominent factor in limiting the ultimate size of the muscle.

There may be an increase in muscle fibre number after birth depending on the state of maturity of the muscle at birth. The occurrence of hyperplasia after birth can be regarded as an extension of the embryonic differentiation of the muscle in neonatal animals of relative immaturity such as the rat (Enesco and Leblond
1962; Chiakulas and Pauly 1965). However, some animals fail to show any increase in fibre number postnatally (for example, the pig: Staun 1963, Stickland and Goldspink 1973; and the mouse: Timson 1982). In fact hyperplasia has been shown to cease during the gestational period in the pig (Swatland 1973) and in the lamb (Everitt 1968). In animals, such as these, limited prenatal hyperplastic growth is not compensated for during postnatal development (Widdowson and McCance 1960; Winick and Noble 1966; Robinson 1969). Therefore the genetic legacy for fibre number (Luff and Goldspink 1967 1970; Hooper 1982) cannot be expressed unless manifest at birth.

The prenatal period obviously holds the key to an animal’s potential for meat production. It has been deduced by many workers that low birthweight animals present a reduced total muscle fibre number. Hegarty and Allen (1978) and Powell and Aberle (1981) formed this premise from the finding that runts usually had larger fibre diameters (depending on the muscle) than their large littermates of the same liveweight, despite their similar muscle weights (Hegarty and Allen 1978), or as Powell and Aberle (1981) found, their lower total carcass muscle at equal liveweights. More recent papers have made actual estimates of the total myofibre numbers of divergent birthweight animals (Bedi et al. 1982 in rats; Wigmore and Stickland 1983 in pigs) so verifying these deductions.

Muscle fibre number is achieved prenatally through the development of two populations of myofibres. These two types of muscle fibres can be demonstrated prenatally in meat producing
animals using the histochemical test for adenosine triphosphatase (ATPase) as shown in the porcine foetus between 75 (Ashmore et al. 1973) and 90 (Beermann et al. 1978) days of gestation. These myo-fibre types are known to originate from two morphologically distinct fibre populations that develop during myogenesis from precursor, myoblast, cells. This phenomenon is known as the 'Biphasic Theory' of myofibre development and has been observed in the foetal muscle of many mammalian species including the ovine (Ashmore et al. 1972b), the bovine (Omner 1971) and the porcine (Ashmore et al. 1973; Swatland and Cassens 1973a; Beermann et al. 1978; Wigmores and Stickland 1983) foetus. From the work of these researchers it is apparent that all fibres formed during the initial, short duration, stages of fusion (the primary myotubes) serve as a structural framework around which the secondary myofibres (which rarely have a myotube conformation) form. Initially the secondary myofibres constitute the fibre population with the smallest diameters in foetal mammals, up until approximately half term, thereby distinguishing these two groups of myofibres. The primary fibres alone stain positively for acid ATPase (an indication of slow contractility manifest in the mature animal, as discussed in Chapter 3) during late gestation (Ashmore et al. 1972b, 1973; Beermann et al. 1978) and are thought to determine the location within the muscle of groups of slow fibres, so characteristic of postnatal porcine muscle (Davies 1972). The slow fibres, so grouped, together with their juxtapositioned fast fibres, are termed, in this work, 'metabolic bundles' due to their significance regarding the metabolism of the muscle (see Chapter 3).
Wigmore and Stickland (1983) investigated the mechanism by which the reduced fibre number, found in prenatally growth retarded animals, is executed. They concluded that, since the surface area of the primary fibre acts as a template for the development of the secondary fibre population, a restriction on its size would limit the number of fibres able to develop around it. The mean primary fibre diameter of m. semitendinosus was found to be positively correlated with the weight of the individual pig foetus and accordingly resulted in a lower ratio of secondary to primary myofibres, and hence a lower total fibre number in this muscle of the smaller foetus.

Muscle fibre number, apart from being highly correlated with the quantity of meat produced by an animal, as previously discussed, may influence its quality. Stickland and Goldspink (1975) related the fibre number, and fibre density, of m. flexor digiti v brevis from Large White and Landrace pigs to carcass assessment parameters and concluded that high fibre number was associated with a greater lean meat content. These workers also found that fibre number was inversely related to fibre size and, as fibre size is related to meat quality (Staun 1983) then, within a breed, high fibre number is not only associated with a greater lean meat content but also, probably, with better meat quality.

These findings have special relevance in a study of muscle development of meat producing animals with respect to the amount and quality of the end product. Elucidating the mechanism whereby growth restriction, prior to birth, affects muscle development
after birth, may hold the key to the manipulation of this consequential development.

The aim of the work discussed in this chapter was, therefore, to investigate the effects of prenatal growth retardation on the postnatal cellularity of the muscle, as demonstrated by histological techniques. The consequences of the differential prenatal growth retardation between littermates on the relative postnatal growth of the muscle with regards to the TSA of individual myofibres is discussed in the ensuing chapter.

2.2. MATERIALS AND METHODS

2.2.1. Preparation Of Muscle Sections

The muscles (m. semitendinosus and m. trapezius) for this histological investigation were dissected from all animals as described in Chapter 1. After the dissection and weighing of the two muscles a complete midbelly section was taken from m. semitendinosus, and for m. trapezius a strip, up to 2cm in length, was cut from midway along the posterior edge of the thoracic portion. In the older animals the midbelly slice of m. semitendinosus had to be halved to produce a size suitable for sectioning. The muscle slices, of 2 - 3mm thickness, were mounted with water on a piece of 5mm thick cork sheeting placed on a cryostat chuck and rapidly frozen in dichlorodifluoromethane (Artton 12, ICI Ltd) cooled to its melting point of -158°C with liquid nitrogen. The cork helped prevent splitting of the muscle samples during freezing. 10µm serial sections, cut on a Slee retracting rotary cryostat at -22°C,
were picked up on coverslips and allowed to thaw and dry out at room temperature. The quality of the sections was checked periodically during cutting by staining with methylene blue and examining under a microscope.

The muscle samples excised for this work were all prerigor; optimal histochemical staining is ensured by freezing as soon as possible after death. The large nature of the samples made freezing at resting length impractical.

2.2.2. Procedure Of Obtaining Histological Data

Sections from both muscles were used in a series of four histochemical staining techniques as discussed in the following chapter. The ATPase staining was considered appropriate for the histological study as, apart from its undeniable advantage in distinguishing the original position of primary fibres (see 'Introduction'), its characteristically intense staining amongst positively reacting fibres made fibre outlines very clear. The alkaline ATPase stain was more beneficial than the acid ATPase stain in segregating fibres, however poor differential alkaline ATPase staining in the young animal made the latter stain more employable in such cases.

Calculated areas of the stained muscle sections were projected onto white card with a projection microscope. Five areas, containing an average of 500 - 1500 fibres, were randomly selected from each m. trapezius section while areas from m. semitendinosus, containing a similar number of fibres, were projected to amount to at least 10,000 counted fibres, approximately 2.5% of the estimated
total myofibre number for this muscle. Fibre counting commenced with the recording of the number of metabolic bundles within the known area, followed by the counting of all fibres within that area. The former figure enabled the assessment of the number of primary fibres per unit area which, together with the total number of fibres within that area, contributed to the evaluation of the secondary: primary fibre number ratio for both the muscles observed. M. semitendinosus provided total myofibre and primary fibre number estimates, given by the product of the appropriate values per unit area (mm$^2$) and the total TSA of the muscle (also in mm$^2$). The TSA of m. semitendinosus was calculated either by outlining the whole projected section, or by obtaining the impression of the frozen sectioned large muscle blocks on tracing paper; the area was measured on a Reichert-Jung videoplan.

Regression analysis of myofibre and primary fibre number on liveweight and on age was conducted to establish any postnatal changes. Paired observations were analysed to test the significance of the difference between mean values of the histological data for littermate groups.

2.3. RESULTS

2.3.1. Fibre Number

The regression coefficients of the number of fibres in the semitendinosus muscle with liveweight and with age (Figure 2) were not found to be significantly different from zero indicating that this muscle maintained a constant number of myofibres after birth.
**Figure 2**

M. semitendinosus fibre number against age of littermates categorised by birthweight.

Regression equation: $Y = -327X + 425610$
M. semitendinosus contained an average of 414760 ± 90750 (Mean ± SD) fibres for all animals. The maximum variation in fibre number found between litters (129310 to 657560) exceeded the highest intra-litter fibre number difference (175310 to 477600) by about 75%.

The total number of fibres estimated for m. semitendinosus was significantly lower (P < 0.001) in the runts than their large littermates by an average of 19%. The fibre number differences for this muscle between large: small and small: runt littermate groups were not significant.

The low birthweights exhibited by subjects of the runt category were not consistently associated with a reduced fibre number relative to their respective large littermates. When low birthweight was associated with a reduced fibre number, ie when the runt fibre number was less than 100% that of the large littermate, the relative importance of the primary and the secondary fibre populations, in determining the total fibre number effect, was investigated. When the fibre number difference between large and runt littermate groups was considered after exclusion of pairs of siblings that failed to exhibit such a disparity in muscle fibre number the difference was increased from 19% to 25%. This necessitated the consideration of the primary fibre numbers and the secondary: primary fibre number ratios of 15 pairs of littermates as discussed in the two ensuing sections.

The exclusion of two animals from the runt category was deemed necessary for some calculations (as explained in the Discussion) as
they appeared to constitute a subpopulation suffering from severe prenatal growth retardation, as alluded to in the Results of Chapter 1. These pigs had a birthweight more than 2.5 SD below their mean litterweight and 50% that of their respective large littermates. These two animals, which possessed total fibre number counts, for this muscle, 38% and 49% of their respective large sibling's fibre number, did not affect the significance of the difference in fibre number between these two littermate groups (Table 5).

Table 5 - Significance Of Difference Between Paired Histological Observations For Large And Runt Littermate Groups.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean % difference</th>
<th>Large: Runt minus extreme runts</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. semitendinosus Total Fibre Number</td>
<td>25 ***</td>
<td>21 ***</td>
</tr>
<tr>
<td>M. semitendinosus Primary Fibre Number</td>
<td>15 *</td>
<td>NS</td>
</tr>
<tr>
<td>M. semitendinosus Secondary: Primary Fibre Number Ratio</td>
<td>13 **</td>
<td>11 **</td>
</tr>
<tr>
<td>M. trapezius Secondary: Primary Fibre Number Ratio</td>
<td>10 **</td>
<td>6 *</td>
</tr>
</tbody>
</table>

* = significant difference at P<0.05  
** = significant difference at P<0.01  
*** = significant difference at P<0.001
An indication of possible catch-up growth between certain littermates (although not statistically significant) was expressed in the previous chapter, in animals older than 64 days. Table 6 shows the liveweight and fibre number, estimated for m. semitendinosus, of runt animals after this age, expressed as a percentage of their respective large littermates. The possible relevance of muscle fibre number to the attainment of liveweight in the mature animal is discussed later.

Table 6 - Relative Liveweights and M. Semitendinosus fibre numbers of large and runt littermates after 64 days of age.

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>Liveweight of Runt as % of Large</th>
<th>Fibre No. of Runt as % of Large</th>
</tr>
</thead>
<tbody>
<tr>
<td>84</td>
<td>106</td>
<td>100</td>
</tr>
<tr>
<td>100</td>
<td>87</td>
<td>68</td>
</tr>
<tr>
<td>128</td>
<td>81</td>
<td>82</td>
</tr>
</tbody>
</table>

2.3.2. Primary Fibre Number

Regression analysis of primary fibre number against liveweight and age demonstrated that the number of primaries in m. semitendinosus was independent of the age and liveweight of the animal; the regression line was not significantly different from the horizontal (i.e. zero). The average number of primary fibres estimated for m. semitendinosus, from all animals, was 17460 ± 3870 (Mean ± SD). The most extreme difference in primary fibre number between litters
was approximately two times that within litters (20110 to 31740).

When a disparity existed between the total fibre number of large and runt littermate groups the primary fibres of m. semitendinosus showed a significantly lower number in runts (P < 0.05) when compared to their large birthweight littermates (Table 5); the average reduction being about 15%. However, by eliminating the two severely runted piglets from the analysis the effect of birthweight on primary fibre number was no longer significant. The extremely low primary numbers of these two piglets, averaging at 59% the value estimated for their large littermates, were obviously responsible for the significance of the difference in primary fibre number seen between these two littermate groups.

2.3.3. Secondary: Primary Fibre Number Ratio

The fact that the myofibre and primary fibre number of m. semitendinosus did not change with liveweight or age demonstrated that the secondary: primary fibre number ratio likewise remained constant after birth in this muscle. As estimates of myofibre number and primary fibre number were not available for m. trapezius a comparison of the regression of secondary: primary fibre number ratios for this muscle was conducted and found not to be significantly different from zero (P > 0.50). It was therefore established that no significant change occurred in the secondary: primary fibre number ratio of m. trapezius after birth, as was deduced for m. semitendinosus. The secondary: primary fibre number ratios of m. semitendinosus and m. trapezius were, on average, 23.9 ± 3.3 (mean
± SD) and 22.0 ± 3.2, respectively, when all birthweight categories were considered. As with total myofibre and primary fibre numbers the maximum difference in secondary: primary fibre number ratio existing between litters far exceeded any variation found within individual litters; the former variation being about 250% that of the latter in m. semitendinosus and 300% of the latter in m. trapezius.

Runting, in the instances where it was associated with a reduced total fibre number in m. semitendinosus, reduced the average secondary: primary fibre number ratio (P < 0.01) by about 13% (Table 5), from 25.3 to 22.1. Since the fibre number of one muscle is indicative of other muscles in the body (Stickland and Goldspink 1973), runting was assumed to affect the number of myofibres in m. trapezius only in the cases where it affected m. semitendinosus fibre number. On investigation, the average secondary: primary fibre number ratio for m. trapezius of these runts showed a 10% reduction (P < 0.001) relative to that of the large litters (Table 5) from 22.9 to 20.7 (illustrated in Figure 3). Exclusion of the two extreme runts, which appeared to constitute a subpopulation, from the analysis of this data failed to render the difference in secondary: primary fibre number ratio between the large and runt categories insignificant, although the percentage difference was reduced slightly to 11% (P < 0.01) and 6% (P < 0.05), for m. semitendinosus and m. trapezius, respectively.

All the raw data on the different parameters of muscle fibre cellularity for m. semitendinosus and m. trapezius obtained for
Figure 3

M. trapezius sections, stained for the demonstration of acid ATPase activity, from littermates of 84 days of age, illustrating the relatively lower secondary: primary fibre number ratios in the muscles of runt littermates.

A Large littermate - secondary: primary fibre number ratio = 29.1

B Runt littermate - secondary: primary fibre number ratio = 23.5

The similar number of acid ATPase positive fibres (10-12) per bundle between littermates emphasises the reduced number of unstained fibres per metabolic bundle in the runt muscle which is indicative of a lower total number of secondary fibres.
this chapter are displayed in Appendix 2.

2.4. DISCUSSION

2.4.1. Muscle Fibre Number After Birth

The lack of change in the total number of myofibres in m. semitendinosus from birth to 128 days supports the general concensus that muscle fibre number is fixed at birth in the pig (Stickland and Goldspink 1973). However, although this is true of the real fibre number within any muscle, it is sometimes possible to see a change in the apparent fibre number, as emphasised by Swatland and Cassens (1972a). Swatland's data on porcine sartorius muscle (1976a) supports the hypothesis that muscle fibre arrangement is dynamic, rather than static, during growth and it is seldom possible to include all the fibres in a single plane of section. This phenomenon is due to the existence of intrafascicular terminations within the muscle that are able to extend into the plane of sectioning (Swatland and Cassens 1972a), so constituting an increase in apparent fibre number and, therefore, an extra parameter of muscle growth postnatally.

Decreases in muscle fibre number, observed postnatally by some authors, however, are more difficult to explain. Stickland (1983) found that the soleus muscle of the rat and the pectineus muscle of the dog, showing apparent decreases of up to 41% (Layman et al. 1980) and 32% (Ihemelandu 1980) respectively, were evident due to structural changes within these muscles with age. The alterations involved the relative arrangement of the muscle fibres and their
affixed tendons that changed in such a way as to decrease the number of fibres present in the plane of sectioning (Stickland 1983). This illustrates the necessity of a fundamental knowledge of the architecture of muscles prior to histological studies to ensure that results are not confounded by apparent changes in fibre number as opposed to real changes.

The absence of a significant change in m. semitendinosus fibre number after birth does not, however, exclude the possibility that this muscle possesses intrafascicularly terminating myofibres. Mackay and Harrop (1969) showed that plotting the number of muscle fibres in serial transverse sections of m. anterior gracilis of the rat against the relative position along the muscle length produced a plateau-shaped graph with a central peak. These results indicated that in m. anterior gracilis a double relay of myofibres were present, interdigitating over the central portion of the muscle. An increase in the length of the intrafascicularly terminating fibres would not, therefore, transect the midbelly and be detected as an apparent rise in fibre number at this plane of sectioning.

It is conceivable that the internal fibre arrangement of m. semitendinosus may contain intrafascicular terminations since, in the adult especially, it is a very long muscle but, as in the rat m. anterior gracilis, the arrangement prohibits myofibre growth into the midbelly. Although Wigmore and Stickland (1983) failed to observe any intrafascicular myofibre terminations in m. semitendinosus of the foetal pig their method utilised myofibre teasing and scanning electron microscopic examinations, and not the concise
method employed by Mackay and Harrop (1969) of total fibre counts along the length of the muscle.

Apart from the large size of m. semitendinosus, which makes total fibre counts impractical (though estimates are considered, by Jimenez et al., 1975, to be reliable), its apparent constancy of fibre number after birth, together with the parallel myofibre arrangement, make this muscle ideal for a quantitative study.

2.4.2. Relative Muscle Fibre Number Amongst Litters And Littermates

The considerable variation in fibre number seen between litters (up to 509%) was approximately 240% greater than that exhibited between the most diverse cases within a litter. This value (509%) is mainly due to the genetic effect on fibre number seen within any particular breed. Stickland and Goldspink (1973) reported fibre number counts for m. flexor digiti v brevis of pure Large Whites, from different herds, that showed a 176% variation. The 272% maximum fibre number difference for m. semitendinosus seen between any two littermates in the present study is primarily due to the effects of growth restriction in utero, the genetic factor within a litter being less.

The 19% (P < 0.001) reduction in fibre number seen overall in the runts, in comparison with the large littermates, irrespective of the exceptions where fibre number was not affected, was comparable with that seen in the m. semitendinosus from growth retarded pig foetuses by Wigmore and Stickland (1983), who found an average
Everitt (1968) found that the foetuses of severely underfed ewes have up to 31% fewer fibres in their semitendinosus muscles than foetuses of ewes on a high plane of nutrition at 140 days' term (full term being at about 10 days after this gestational age). Bedi et al. (1982) produced a decrease in soleus muscle fibre number, 19% (P < 0.01) below the normal value of male rats, by undernourishing their dams throughout gestation and lactation. Muscle fibre number reductions within a litter, as exemplified in this study, strongly suggest that prenatal nutritional stress does have a considerable effect on muscle cellularity.

Although muscle fibre number differences between large: small and small: runt groups were insignificant the difference between the two group comparisons was essentially equal at 11% and 14%, respectively. This coincides with the fact that the small littermates represented the median birthweight between the large and runt littermates. This is in agreement with data presented by Wigmore (1982) that illustrated a positive correlation between foetal pig weight and primary fibre diameter, and between primary fibre diameter and the number of secondaries developing on the surface of each primary. Hence there is a direct association between the animal's weight and its ultimate fibre number, resulting in a graded effect on fibre number with the various birthweights within a large litter; the relative degree of growth restriction in utero dictating the degree of muscle fibre hyperplasia through the secondary: primary fibre number ratio only, primary fibre number being unaf-
fected. However two extremely 'runted' piglets proved to be an exception to this rule.

In the present study a significant difference (P < 0.05) was found to exist between the primary fibre number of the highest and lowest birthweight littermates, but the significance of the difference was lost by excluding the two exceptional runts. These two animals probably constituted a subpopulation (Royston et al. 1982) of such severe growth retardation as to affect primary fibre formation, which usually appears exempt from nutritional stress. These piglets had birthweights of 700g and 600g, considerably lower than the 'critical' weight, in the range of 900 - 1000g, suggested by Hegarty and Allen (1978) and Powell and Aberle (1980, 1981). However the extremely low birthweights exhibited by these two runts was not the sole indication of their abnormally diminished muscle cellularity which appeared to be executed through an affected compliment of primary fibres. In all, 50% of the pigs in the 'runt' category had a birthweight below 800g, while 21% of this category were born of a weight less than 700g, but these pigs failed to show such a severely reduced muscle fibre number or an affected primary fibre number.

The phenomenon attributable to the two extreme runts, and presumably their severely affected muscle fibre number, was their respective birthweights relative to their siblings. Both runts were not only 50% the weight of their respective large littermates at birth but were also more than 2.5 SD below the mean litterweight, a combination which together accounted for their unique condition, i.e
severely reduced muscle fibre number, affected primary myofibre number and failure to grow.

It is, therefore, proposed that these stipulations, which are more stringent than those previously put forward in the literature, constitute a 'critical' weight at birth.

Within the normal range of growth retardation manifest in a litter of pigs muscle fibre number changes are imposed by effects on secondary fibre formation only. The reduced muscle fibre number of m. semitendinosus was accomplished by an average reduction in the secondary: primary fibre number ratio of 13% and was reflected in m. trapezius by a reduction of 10% between large and runt littermates. The similar degree to which the ratios of these two muscles were affected suggests that their total fibre numbers were equivalently affected between littermates, although an actual estimation of m. trapezius fibre number was not available.

2.4.3. Implications Of Muscle Cellularity

The present work, and that of Stickland and Goldspink (1973) on an 'indicator' muscle, suggests that the significantly lower (P < 0.001) muscle fibre number revealed in m. semitendinosus of the runts, compared to their large littermates, is accordingly exhibited by all of their muscles, with possible consequences on meat quantity and quality. Myofibre number shows a high correlation with muscle mass (see Introduction) and a greater muscle mass has been associated with a high density of thin fibres (Miller et al. 1975; Stickland and Goldspink 1975) which enhances meat quality.
1963). However the runt littermates of this study possessed a significantly greater (P < 0.001) mass of m. semitendinosus at an equal liveweight to their large littermates despite having a significantly lower (P < 0.001) fibre number in this muscle (see Chapter 1) which would denote myofibres of larger diameter and hence lower meat quality. This situation may not be reflected in all muscles for the following reason. M. trapezius, being a lower growth impetus muscle than m. semitendinosus (see Chapter 1), constitutes a lower percentage of body weight in the runt than the large littermate of the same liveweight while also, presumably, possessing fewer fibres. Results of actual fibre diameter measurements are presented and discussed in Chapter 3. One would assume that there comes a point when the limit to myofibre TSA is reached in all littermates and the largest littermate's inherent potential for greater muscle bulk, by virtue of its higher fibre number predominates, with the associated production of a meatier carcass (Luff and Goldspink 1967; Hooper 1982).

2.4.5. Muscle Cellularity And Its Relationship With Birthweight And Growth

Myofibre number in m. semitendinosus although, on average, less in the runt than the large, was essentially equal (± 5%) between littermates of 1, 19, 46, and 84 days of age. The oldest runt littermate of this collection had attained a liveweight greater than that of its large littermate, by 6%. In fact, the relative fibre numbers and liveweights of the runt and large littermates appear highly related after 64 days (Table 6) which, as
mentioned in the introduction of Chapter 1, is during the age range 56 - 112 days considered by Blunn et al. (1953) to be the probable time when an animal's genetic potential for growth is expressed.

That muscle fibre number is an indicator of the animal's potential for growth appears extremely plausible. The authors Hegarty et al. (1973), Miller et al. (1975) and Hegarty and Allen (1978) have all reasoned that ultimate body size is influenced by muscle cell number. The extent of muscle hyperplastic restriction in utero, resulting from limited nutrition, is probably a more crucial impediment to the pig's growth potential than its size at birth. Size at birth may not necessarily be a result of reduced cell hyperplasia but may arise solely from reduced hypertrophy, as the muscle fibre number results show (see Appendix 2). The relative contributions of decreased hyperplasia and of decreased hypertrophy to low birthweight is only evident in the mature, well fed, animal when cell hypertrophy has ceased and all the attained dimensions between littermates compared, ie the pig's potential for meat production has been expressed. The runt littermates in this study of ages 1, 19, and 46 days (with an m. semitendinosus fibre number of at least 99% that of their large littermates) may therefore have also possessed the capacity for catch-up growth evident in the runt littermate of 84 days.
3. HISTOCHEMICAL FIBRE TYPE DIFFERENTIATION

3.1. INTRODUCTION

As an introduction to this chapter, which incorporates the results of the histochemical techniques as applied to the muscle tissue incorporated in this study, the significance of these techniques is discussed and literature covering their implications reviewed. This provides background information on the mechanical and metabolic properties of muscle and the maintenance of functional harmony between the muscle and the animal as a whole, thus aiding the interpretation of the proceeding work.

Skeletal muscle is not composed of a homogeneous population of myofibres. The constituent fibres of a muscle differ in TSA, ultrastructural, biochemical, physiological and histochemical properties. A myofibre's indigenous histochemical qualities are reflective of its physiological and metabolic capacities that contribute to the effectiveness and efficiency of the skeletal muscle as an apparatus of extreme functional significance with regards to the support and movement of the body.

The most comprehensive system of nomenclature for fibre 'typing' gives a full description of these physiological and metabolic capacities, based on the relative contraction speed of the fibre and its propensity for oxidative and glycolytic metabolism, as does the classification system of Peter et al. (1972). Accordingly, these workers described three basic myofibre types in adult guinea-pig and rabbit limb muscles that are common to most mature
mammalian muscles:

i slow-twitch, oxidative metabolism (SO)

ii fast-twitch, oxidative and glycolytic metabolism (FOG)

iii fast-twitch, glycolytic metabolism (FG).

Although classification systems of other authors are based on one or more, or all three, of the aforementioned criteria (as reviewed by Muir 1974), the system of Peter et al. (1972) is most descriptive of myofibre properties and is easier to utilise in conveying information concerning these properties.

The SO and FG myofibres represent the two extremes of muscle fibre types. In general, SO muscle fibres are adapted for slow contraction sustained for long periods of time and are fatigue-resistant (Kugelberg 1973), whereas FG (fatiguable) fibres are adapted for fast speed, intermittent bursts of contraction (Burke and Tsairis 1974). The mechanical properties of a myofibre are related to the fibre's metabolic properties. The predominantly oxidative capacity of the SO fibre is coupled with a high capillary supply (Romanul 1965) to support its aerobic activity necessary to cope with its prolonged contractions. Fast fibres are generally larger in diameter (Burke and Tsairis 1974) with a lower capillary ratio (Romanul 1965) and a higher capacity for anaerobic glycolysis (Dalrymple et al. 1973), properties which are inversely related to the fibre's oxidative metabolism (Dubowitz and Pearse et al. 1960). Oxidative capacity is directly related to myofibre size (Howells and Goldspink 1974; Swatland 1983), thus a gradation is
seen in oxidative metabolism between FOG to F6 fibres.

The functional properties characteristic of a myofibre can be demonstrated by a battery of histochemical tests.

The histochemical test for succinate dehydrogenase (SDHase), which follows the diformazan deposition caused by the activity of this enzyme (Nachlas et al. 1957; Pearse 1960), is indicative of aerobic metabolism. An enzyme of the Tricarboxylic Acid Cycle, SDHase is tightly bound to the inner mitochondrial membrane. The reduced form of this enzyme is an electron donor, oxygen being the ultimate electron acceptor of the electron transport chain, culminating in the generation of energy in the form of ATP. Glycogen phosphorylase (GPase) causes the phosphorolysis of glycogen and thus its entry into the Glycolytic Cycle, generating energy in anaerobic conditions. This enzyme is, therefore, indicative of glycolytic metabolism, its reversibility being used as a test for its activity (Takeuchi and Kuriaki 1955) by the staining of the glycogen produced.

Bárány (1967) first suggested a role for the adenosine triphosphatase (ATPase) activity of myosin in determining the speed of muscle contraction from his findings that the actin- and calcium-activated ATPase activities of the myosins of 14 different muscles (from mammals, other vertebrates and invertebrates) were proportional to their respective times to peak contraction. The conclusion was that a greater intrinsic speed of shortening relates to a higher ATPase activity. The histochemical reaction used to locate the cellular activity of ATPase (Padykula and Herman 1955; Guth and
Samaha 1970) relies on the release of phosphate from adenosine triphosphate by this enzyme, the intensity of staining for the phosphate being indicative of the ATPase activity at the pH employed. The correlation between ATPase activity and the preincubation pH (see Materials and Methods of the present chapter) is due to the existence of the different myosins of fast and slow muscles (Sreter et al. 1966) as two electrophoretically distinct proteins (Samaha et al. 1970). The ATPase activity of slow myosin in the adult animal has been shown to be relatively acid-stable and alkali-labile, while that of fast myosin is characterised by alkaline stability and acid lability (Burke et al. 1971; Burke and Tsairis 1974; Kugelberg 1976) with the apparent exception of neonatal muscle.

Skeletal muscle of the neonatal cat (Buller et al 1960) and rat (Close 1964) have been demonstrated to be physiologically slow initially while possessing histochemical profiles, with respect to ATPase, characteristic of mature fast and slow muscles (Karpati and Engel 1967). Also, the ATPase activity of actomyosin isolated from young (5 and 10 day) rabbit muscles was shown by Guth and Samaha (1972) to be biochemically the same as that of adult slow muscle while the majority of myofibres stained histochemically as intensely as adult fast fibres. These authors attributed this phenomenon partly to the pH stability of the immature myosin ATPase, indicative of the fact that structural differences exist between foetal and adult forms of myosin (Trayer et al 1968). However Rubenstein and Kelly (1978) felt that some characteristic, other than the type of myosin or its ATPase activity, was
responsible for determining the contraction speed of young muscles and hence the disparity seen between the physiologically and histochemically determined muscle properties of the neonate. After investigating the existence of "slow" foetal muscles Kelly and Rubenstein (1980) proposed that early slow muscle movements were an outcome of the fact that primary myotubes, which contained significant proportions of slow myosin and constituted the fundamental motor units of the developing neuromuscular system, continued to dominate muscle function for a short time after birth.

Innervation has been shown to be essential for the initiation of secondary muscle fibre development in the foetal rat (Harris 1981) and has also been implicated in the fibre type differentiation of foetal (pig: Beermann et al. 1978) and of neonatal (rat: Brown 1973) muscle.

The unique and vital role of neural control, persisting throughout life, maintaining the composition and inherent properties of muscle fibres, appears to exercise its influence through the contractile activity induced in the myofibre by the characteristic impulse pattern of the innervating nerve. By denervating the extensor digitorum longus muscles of newborn rats, Brown (1973) showed that at 6 weeks this muscle, which was potentially fast, failed to develop the time to peak contraction normally occurring at this age. However, this author showed that by artificially stimulating this muscle in similarly treated experimental rabbits its contractile properties altered to resemble those of a normal muscle. Therefore it appears likely that the inability of dener-
vated fast muscle to differentiate may partly be a result of the absence of impulse activity following denervation. Studies of denervated cross-reinnervated, or artificially stimulated, mature muscle have not only produced physiological alterations (Close 1965; Salmons and Sréter 1976) but also metabolic and anatomical changes in the fibre types (referred to later). The denervated muscle usually adopts the characteristics of the muscle type that is normally innervated by the 'foreign' nerve with which it has been reinnervated. In the case of artificial stimulation a denervated fast muscle receiving a sustained, low frequency pattern of electrical activity, in general, assumes the metabolic and ultrastructural properties of a slow fibre while, similarly, slow fibres can be induced to adopt the characteristics of fast fibres with the application of intermittent bursts of more intense electrical activity to the muscle.

Metabolic alterations are reflected through enzyme activities, determined either biochemically (Dubowitz and Newman 1967; Prewitt and Salafsky 1967; Guth et al. 1968) or histochemically (Karparti and Engel 1967; Yellin 1967; Guth et al. 1968; Beermann et al. 1977), and by myoglobin concentrations (McPherson and Tokunaga 1967). Anatomical changes are manifest at the subcellular level with regards to the extent of the T-tubules, terminal cisternae and sarcoplasmic reticulum (Eisenberg and Salmons 1981), as well as Z-band width and mitochondrial volume (Salmons et al. 1978; Eisenberg and Salmons 1981). Alterations in the electrophoretic properties of cross-reinnervated fast and slow, cat and rat muscles, found by Guth et al. (1968), indicated structural changes in the
muscle proteins. As the synthesis of proteins is specified qualitatively by gene action this change suggests that the nerve influences the muscle at the level of gene expression (Samaha et al. 1970) although the mechanism by which this might be accomplished has yet to be elucidated.

It must be noted that the conversion of fast to slow, and slow to fast muscle, by cross-reinnervation is not always complete (as found by Dubowitz and Newman 1967; Guth et al. 1968) which implicates factors other than those specific to neural influence in the conversion process.

Experimental studies involving nutritional stress (Raju 1975; Howells and Jordan 1978; Howells et al. 1978) and exercise (Kowalski et al. 1969; Howells and Goldspink 1974; Herbison et al. 1980; Salmons and Henriksson 1981; Henckel 1983) have accentuated the versatility of skeletal muscle in these situations, which require modifications in the relevant metabolic and mechanical processes to maintain harmony of function within the animal.

The functional role of skeletal muscle is maintained in the growing pig without an equivalent increase in muscle bulk (Stant et al. 1968) or muscle fibre TSA (Elson et al. 1963). An increase in body weight imposes greater mechanical and metabolic demands on the functioning of the muscle which must be satisfied by consequential changes within the myofibres to maintain metabolic efficiency and support, both of which are imperative to the animal's survival. Possibly a sequence of events, culminating in the adaptation of muscle to the increased work load, triggers alterations in the
nervous system which have an ultimate, long-term effect on the skeletal muscle.

This chapter describes changes in the histochemical profiles of the deep and superficial portions of m. semitendinosus and of m. trapezius, and their relative myofibre type TSAs, and provides information on the adaptive transformations occurring in pig skeletal muscle with growth.

Studying dissimilar birthweight littermates whose relative weight differences were maintained throughout (see Chapter 1) produced the advantage of enabling the disassociation of the cumulative effects of age and liveweight on the muscle development in the interpretation of the results.

A comprehensive study of piglets of disparate birthweight also provided evidence of the effects of differential prenatal growth rates on the histochemical development and adaptive performance of skeletal muscle postnatally.

3.2. MATERIALS AND METHODS

3.2.1. Histochemical Techniques

Fresh frozen sections were prepared from m. semitendinosus and of all 49 animals studied m. trapezius and mounted on coverslips as described in Preparation Of Muscle Sections (Chapter 2). A minimum of four sections from every dissected muscle were selected for the application of each of the following histochemical techniques. The muscle sections were allowed to thaw and dry out at room temperature for about 1.5 hours.
prior to commencement of the techniques.

2.2.1.1. Acid Stable Myosin Adenosine Triphosphatase (Acid ATPase)

Muscle sections were exposed to the following procedure of Guth and Samaha (1970). The preincubation and incubation times and pHs stated here were those found to produce the most well defined differential staining in the muscle sections of this study after the results of a comprehensive range of these particulars had been considered. Consequently the times and pHs of the preincubations and incubations may not be optimal for muscle from other species.

1 Preincubation at pH 4.35 in a solution of 50mM potassium acetate and 18mM calcium for 25 minutes.

2 Rinsing (two changes, one minute each) in Rinse solution [18mM calcium chloride in 100mM tris (hydroxymethyl) aminomethane (Tris) pH 7.8].

3 Incubation in a solution containing 2.7mM adenosine triphosphate, 50mM potassium chloride and 18mM calcium chloride in 100mM Tris buffer, pH 9.4 at 37°C for 50 minutes.

4 Washing (three 30 second changes) in a 1% (w/v) calcium chloride solution.

5 Immersion in a 2% (w/v) cobalt chloride solution for 3 minutes.

6 Washing (four 30 second changes) in an alkaline washing solution of 100mM Tris buffer, pH 9.4.
Immersion in a 1\% \text{(v/v)} ammonium sulphide solution for 3 minutes.

Washing in at least two changes of distilled water.

Mounting of wet sections onto slides with Glycerine jelly.

Between each solution change excess solution was drained on tissue paper.

\subsection{3.2.1.2. Alkaline Stable Myosin Adenosine Triphosphatase (Alkaline ATPase)}

Muscle sections were exposed to the following procedure as outlined by Guth and Samaha (1970) after optimum preincubation and incubation times and pHs had been established.

1. Fixation for 5 minutes in 5\% formalin buffered at pH 7.6.

2. Rinsing (two changes, one minute each) in Rinse solution (see Acid ATPase), with agitation.

3. Preincubation at pH 10.4 in a solution of 18mM calcium chloride in 100mM Tris buffer for 15 minutes.

4. Rinsing (two changes, one minute each) in Rinse solution (see Acid ATPase).

5. Incubation for 40 minutes at 37\textdegree\ C in a solution of identical composition to that used for the Acid ATPase incubation.

The succeeding steps of this technique were the same as those for the Acid ATPase following incubation (steps 4 to 9 inclusive).
3.2.1.2. **Succinate Dehydrogenase (SDHase)**

Muscle sections underwent the following technique:

1. Incubation at 37°C in a solution containing one volume of 0.2M phosphate buffer (1.56g sodium dihydrogen orthophosphate and 32.22g disodium hydrogen orthophosphate to 500 ml of distilled water) pH 7.6, one volume 0.2M sodium succinate and two volumes of nitro BT (1mg/ml) for 30 minutes (Nachlas et al. 1957).

2. Washing in two changes of distilled water.

3. Drying in oven to eliminate air bubbles if necessary.

4. Fixation in 10% formal for 10 minutes.

5. Washing in distilled water.

6. Mounting wet sections in Glycerine jelly.

3.2.1.4. **Glycogen Phosphorylase (GPase)**

Muscle sections were subjected to the following procedure as described by Takeuchi (1956); a modification of the method outlined by Takeuchi and Kuraki (1955).

1. Incubation in the substrate which consisted of 75mg glucose-1-phosphoric acid, 15mg adenosine-5-monophosphoric acid, 10mg insulin, 3mg glycogen, 22.5ml distilled water, 15ml 0.1M acetate buffer (0.1M sodium acetate and 0.1M acetic acid) pH 5.9 and 7.5ml ethanol for 3 hours at 37°C.
2 Washing in distilled water.

3 Drying in oven at 37°C.

4 Immersion in absolute ethanol for 2 minutes.

5 Drying at room temperature.

6 Immersion in Lugol’s Iodine (1g iodine and 2g potassium iodide to 100ml distilled water) for 3 minutes.

7 Mounting in Glycerine jelly directly from iodine solution.

3.2.2. Procedure Of Myofibre Typing

3.2.2.1. Classification Of Myofibre Types

Classification of myofibres was achieved with the four histochemical techniques as follows:

**Alkaline ATPase**

A range in staining intensity was apparent in the muscle sections of young animals stained for this enzyme. A staining intensity ‘intermediate’ to that of the distinct positive and negative staining was classified. Fibres were, therefore, marked according to whether they showed a positive, negative, or intermediate reaction for alkaline ATPase.

**Acid ATPase**

As for alkaline ATPase, fibres were recorded as staining either positively, negatively, or intermediately.
SDHase

Fibres were classified as SDHase-positive (designated '0') or SDHase-negative. Although a continuous spectrum of SDHase activity, from weak to strong, is apparent in skeletal muscle (Nemeth et al. 1979; Swatland 1983) fibres possessing weak SDHase activity were recorded as non-oxidative.

GPase

Fibres were recorded as either GPase-positive (designated 'G') or GPase-negative if staining was negligible.

A total of 10 myofibre types were recorded and categorised under 5 major titles, as displayed in Table 7.

3.2.2.2. Slow Myofibres Per Metabolic Bundle

The average number of slow (alkaline ATPase labile, acid ATPase stable) fibres per metabolic bundle was determined from the mixed myofibre areas, i.e. the deep portion of m. semitendinosus and all of m. trapezius. This was accomplished by using a projection microscope to form an image of the muscle sections, stained with alkaline ATPase, onto a piece of white card at a low magnification. The number of negatively stained alkaline ATPase fibres associated with a known number of (at least 150) metabolic bundles were counted to obtain the average value for each animal.
Table 7 - Classification Of Myofibre Types And A Corresponding Classification System.

<table>
<thead>
<tr>
<th>Histochemical Staining Pattern</th>
<th>Myofibre Type Classification</th>
<th>Classification Of Suzuki And Cassens (1980)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid ATPase</td>
<td>Alkaline ATPase</td>
<td>SDHase</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>+</td>
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<tr>
<td>+</td>
<td>*</td>
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<td>-</td>
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</tr>
</tbody>
</table>

+ Positive staining reaction  
* Intermediate staining reaction  
- Negative staining reaction

3.2.2.3. Percentages Of Myofibre Types

Fascicles, from two randomly selected sites within the three relevant sections of muscle, ie from (i) deep and (ii) superficial portions of m. semitendinosus and from (iii) m. trapezius, were employed to estimate the percentage of myofibre types of these three muscle sections.

The fascicle was considered a representative sample unit as it incorporates complete metabolic bundles, whereas a selected area partially transects bundles which is uncharacteristic of the whole muscle. The two samples from each muscle section contained fibres
amounting to a total of 611 ± 242 (mean ± SD). The adequacy of this sample size was established by comparing fibre type percentages from fascicles with compilations performed from areas used to estimate fibre number (see Chapter 2), containing up to 30 times as many fibres.

The GPase stain was found to lose its contrast dramatically after mounting, resulting in complete loss of staining within a few days. Areas of the muscle sections were therefore photographed (Leitz Ortholux photomicroscope, black and white FP6 film) immediately after staining at a magnification as low as feasibly possible to aid identification with similar muscle sections of an alternative histochemical stain.

Muscle fascicles, from sections stained with alkaline ATPase, from areas corresponding to those photographed for GPase, were projected onto white card, approximately 30 x 60cm. Each myofibre within the chosen fascicle was outlined and the staining intensity recorded (as previously described). The alkaline ATPase stain was employed to outline fibres for the same reasons as it was employed for fibre counts; it distinguished between fibres to a higher degree than the other histochemical stains. The appropriate fascicle, stained with the other histochemical techniques, in subsequent serial sections, was then projected onto the outlined myofibres and their corresponding staining intensities marked accordingly. The myofibre types of the two fascicles from each muscle section were summated and percentages of each type determined.
3.2.3. Determination Of Different Myofibre Type Mean TSAs

From each muscle section the mean area of those mature fibre types classified as either SO, FOG, or FG, were determined. Random areas were selected from the two fascicles of outlined fibres of each muscle section, and from these areas 50 fibres of each type were measured on a Reichert-Jung Videoplan. The mean fibre areas, as opposed to diameters, were determined to equate with the whole muscle TSA in m. semitendinosus and enable the evaluation of the TSA of slow muscle within this muscle.

3.2.4. Determination Of Area Of Slow Muscle Within The Midbelly TSA Of M. Semitendinosus

The determination of the TSA of slow muscle within m. semitendinosus incorporated the use of the mean TSA of slow fibres from the deep and superficial portions and the percentages of this fibre type within these respective portions of the muscle. The boundary of the deep portion of m. semitendinosus was marked when the whole projected section was outlined (as described in Chapter 2) thus enabling the evaluation of the respective deep and superficial portion areas. The number of fibres within the deep and superficial portions of the muscle were then estimated from the total number of fibres in the section and the percentage of the total TSA occupied by these portions. These values, together with the percentage of slow fibres determined for the deep and superficial portions, enabled the number of slow fibres within the two portions to be ascertained; these figures were multiplied by the mean TSA of slow fibres, determined for the respective muscle portions, to give the
total area of the slow muscle within the midbelly TSA of m. semitendinosus.

3.1. RESULTS

3.2.1. Adequacy Of Myofibre Typing Procedure

The mean (± SE) percentage difference between the percentage of slow fibres determined for the deep portion of m. semitendinosus using two fascicles, amounting to a total of 551 ± 211 (mean ± SD) fibres, and an area containing 6395 ± 2000 fibres was 0.66 ± 2.42. For m. trapezius the difference was 1.19 ± 1.97 when the percentage of slow fibres was determined by the typing of two fascicles containing 689 ± 245 fibres and an area containing 5418 ± 1758 fibres. These results verified that the fibre typing employing two fascicles, with a total of approximately 600 fibres, adequately represented the percentage of fibre types within the area of muscle section from which they originated.

3.2.2. Changes In The Proportions Of Myofibre Types With Growth

3.2.2.1. Alkaline And Acid ATPase

The histochemical fibre type staining pattern of the superficial portion of m. semitendinosus characterises it as a predominantly "fast" muscle, while that of the deep portion of this muscle (Figures 4 and 5) and that of m. trapezius makes them representative of "mixed" muscles.

The neonatal litter presented poor alkaline ATPase differential staining in its muscles. However, 1.0 slow fibres per
Whole fresh frozen section of m. semitendinosus (TSA of approximately 680mm²) from the 64 day old runt littermate (Liveweight of 8410g) stained for the demonstration of SDHase.

The 'deep' portion (D) of this muscle occupies about 40% of the whole TSA and possesses a greater oxidative capacity than the 'superficial' portion (S).

A section of fresh frozen m. semitendinosus, stained for the demonstration of acid ATPase activity, from the 2 day old runt littermate (liveweight of 600g).

This figure compliments Figure 4 by demonstrating the disparity between the deep (D) and superficial (S) portions with respect to their compliment of slow (acid ATPase positive) fibres in the young pig.
'metabolic bundle' (illustrated in Figure 11) was apparent in the deep portion of m. semitendinosus of each littermate, while m. trapezius possessed an average of 2.4 slow fibres per bundle. With acid ATPase, groups of positively stained fibres were clearly visible, the primary fibre often existing as a myotube in the neonate (and up to 9 day old piglets), or having a slightly greater TSA than the other fibres in the bundle. On average, 3.7 and 4.5 fibres per bundle were stained positively for acid ATPase at birth in the deep portion of m. semitendinosus and in m. trapezius, respectively. The disparity in the number of acid ATPase-positive and alkaline ATPase-negative fibres in the young pig will be reconciled in a subsequent section.

The superficial portion of m. semitendinosus failed to show any slow fibres until 15 days postnatally. In this portion of the muscle, in the neonatal pig, no primaries were discernible by size, the largest fibres existing around the periphery of fascicles. Once slow fibres were established in the superficial portion their proportion remained constant at an average value of 3.0% ± 2.6 (mean ± SD).

After birth the number of slow fibres per bundle, and therefore the percentage of slow fibres, in the deep portion of m. semitendinosus and in m. trapezius increased until a liveweight of approximately 8.5 kg was attained. Thereafter the percentage of slow fibres (and hence the number of slow fibres per bundle) was not shown to change significantly (P > 0.25). The mature percentages of slow fibres were, approximately, 38.5 ± 6.0 (mean ± SD),
and 40.4 ± 4.6, in m. semitendinosus (deep portion) and in m. trapezius, respectively; and the number of slow fibres per bundle being 9.2 ± 1.2, and 9.8 ± 1.8, respectively.

With the increase in the number of slow fibres per bundle an intermediate zone, absent in m. semitendinosus of the neonate, became apparent. This region consisted of a gradation in the number of slow fibres per bundle from the deep to the superficial portion.

The increase in the number of slow fibres in the deep portion of m. semitendinosus and in m. trapezius (until approximately 8.5 kg) and the area of muscle occupied by these fibres is considered in full in subsequent sections.

3.3.2.2. SDHase

In the neonatal pig all muscle fibres exhibited oxidative staining, with respect to SDHase, of an intense blue colouration (Figure 11). The deep portion of m. semitendinosus was, however, distinct from the superficial portion as its fibres produced a stronger reaction for this enzyme stain.

Differential staining began at 6 days with a reduction in the intensity of staining in the superficial portion of m. semitendinosus which was marked by a slightly purplish tinge to the blue stain. This portion of muscle lost its 100% staining, indicating the presence of fibres exhibiting non-oxidative metabolism, between 6 and 19 days; appearing specific to the attainment of a liveweight of between 2450 - 3100 kg (Figure 6). After a liveweight of 8.5 kg had been obtained this superficial portion of muscle presented no
Percentage of oxidative myofibres against liveweight.

After the mature percentage had been attained (indicated by regression lines) no significant change was evident with increasing liveweight.

Regression equations:

Superficial portion of m. semitendinosus, \( Y = -0.00028X + 28.6 \)

Deep portion of m. semitendinosus, \( Y = -0.00006X + 76.1 \)

M. trapezius, \( Y = -0.00014X + 64.4 \)
significant (P > 0.10) change with increasing liveweight in its percentage of oxidative fibres, maintaining a value of 27.8% ± 7.4 (mean ± SD). Furthermore, there was no significant difference (P > 0.10) between the mature fibre type percentages of littermate groups with respect to this enzyme after attainment of this liveweight.

Differentiation of non-oxidative fibres was possible between a liveweight of 8.0 - 15.5 kg (33 - 64 days) in the deep portion of m. semitendinosus and in m. trapezius (Figure 6). Once differentiation had occurred the proportion of oxidative fibres did not appear to change significantly (P > 0.50; after 8.5 kg attained). The mature percentages of oxidative fibres (mean ± SD) for this deep portion of m. semitendinosus and of m. trapezius were 76.2 ± 5.8 and 67.6 ± 9.4, respectively.

3.3.2.3. GPase

At birth the GPase activity in all myofibres was negligible. The attainment of GPase activity, and hence glycolytic metabolism, in fibres was detected histochemically by a liveweight of 2.5 kg (6 - 19 days) in all muscle sections (Figure 7). The activity of this enzyme in the myofibres of the superficial portion of m. semitendinosus was apparent simultaneous to the loss of 100% SDHase staining. Therefore, FOG and FG fibres were classified in the superficial portion of m. semitendinosus after 2.5 kg liveweight, while in the deep portion of this muscle and in m. trapezius, although FOG fibres appeared at this liveweight, FG fibres were not observed until between 8.0 and 15.5 kg.
Figure 7

Percentage of glycolytic myofibres against liveweight.

After the mature percentage had been attained (indicated by regression lines) no significant change was evident with increasing liveweight.

Regression equations:

Superficial portion of m. semitendinosus, \( Y = 0.00006X + 97.5 \)

Deep portion of m. semitendinosus, \( Y = -0.00001X + 58.5 \)

M. trapezius, \( Y = -0.00003X + 45.9 \)
After the attainment of approximately 8.5 kg the proportion of glycolytic fibres did not change significantly \((P > 0.10)\) with liveweight (Figure 7), and no significant difference \((P > 0.10)\) was found to exist between the mature glycolytic fibre percentages of the littermate groups.

From the results presented here it was evident that the muscles sampled exhibited constant myofibre type percentages after a liveweight of approximately 8.5 kg had been attained. The mature fibre type percentages are shown in Table 8.

**Table 8 – Myofibre Type Percentages In 'Mature' Muscle Of Pigs Over A Liveweight Of 8.5kg.**

<table>
<thead>
<tr>
<th>Fibre Type</th>
<th>M. semitendinosus 'Superficial'</th>
<th>M. trapezius 'Deep'</th>
</tr>
</thead>
<tbody>
<tr>
<td>SO</td>
<td>3.0 (2.6)</td>
<td>40.4 (4.6)</td>
</tr>
<tr>
<td>FO</td>
<td>1.0 (1.1)</td>
<td>2.7 (1.4)</td>
</tr>
<tr>
<td>FOG</td>
<td>26.9 (7.1)</td>
<td>35.7 (4.1)</td>
</tr>
<tr>
<td>FG</td>
<td>71.3 (7.8)</td>
<td>23.8 (5.8)</td>
</tr>
</tbody>
</table>

Myofibre type percentages of the deep and superficial portions of m. semitendinosus and of m. trapezius of all littermates at each age considered are expressed in Appendix 3, parts A and B. The number of alkaline ATPase-negative and acid ATPase-positive fibres per metabolic bundle for each littermate are displayed in Appendix 4.
3.2.3. **Growth Changes In The Number Of Slow Fibres**

It was found that the number of slow fibres per metabolic bundle and the percentage of slow fibres in the deep portion of m. semitendinosus, and in m. trapezius, increased to a liveweight of approximately 8.5 kg, after which no significant change (P > 0.25) was demonstrated in the percentage of slow fibres, and hence, in the number of slow fibres per bundle. These changes are portrayed in Figures 8 and 9 that show the changes, with liveweight, in the percentage of slow fibres in m. semitendinosus and m. trapezius, respectively.

From birth to this liveweight, of approximately 8.5 kg, the number of slow fibres per bundle increased from 1.0 ± 0.0 (mean ± SD) in the deep portion of m. semitendinosus, and from 2.4 ± 0.2 in m. trapezius to 9.2 ± 1.2 and 9.8 ± 1.8, respectively; the percentage of slow fibres increased from an average of 3.8 ± 0.1, and 11.8 ± 0.2, in the deep portion of m. semitendinosus and m. trapezius, respectively, to the mature percentages displayed in Table 8.

No significant difference (P > 0.10) was shown to exist between littermates of equal liveweight, or age, with respect to the number of slow fibres per bundle in either muscle. Likewise, the percentage of slow fibres was not found to be significantly different (P > 0.50) in littermates of equal age in either of the muscles studied. However, on an equal liveweight basis the percentage of slow fibres in m. trapezius was significantly greater (P < 0.05) in the runt than the small littermates, while in the deep portion of m. semitendinosus a difference existed between the
Figure 8

Percentages of fast, slow and intermediate fibres (as classified in Table 7) in the deep portion of m. semitendinosus against live-weight.
Figure 9

Percentages of fast, slow and intermediate fibres [as classified in Table 7] in m. trapezius against liveweight.
large: runt and large: small littermate groups. In this muscle the percentage of slow fibres in runts was on average, 3.7% higher ($P < 0.001$), while in the small littermates the average percentage of slow fibres was 6.4% higher ($P < 0.05$), than in the large littermates.

### 3.3.4. Changes In The TSA Of Muscle Occupied By Slow Myofibres

From birth to 128 days of age the TSA of m. semitendinosus and the area occupied by slow fibres was seen to increase and bear a numerical relationship with liveweight. The relationship established between these two parameters and both liveweight and age were essentially equal between littermates.

The regression coefficient of the relationship between m. semitendinosus TSA and liveweight (Figure 10) was not significantly different ($P > 0.50$) from $2/3$, while that of the area of slow muscle and liveweight (Figure 10) was significantly greater than $2/3$ ($P < 0.001$) and 1.0 ($P < 0.01$). The former result supports the hypothesis that the TSA of a muscle grows in proportion to liveweight, while the latter result suggests that within the muscle the area occupied by slow fibres increases at a proportionally greater rate than liveweight (the regression coefficient was not significantly different from $5/4$).

The increasing area of muscle occupied by slow fibres was similarly correlated with the liveweight ($r = 0.92$) and with age ($r = 0.90$) but while no significant difference existed in the area of slow muscle between littermates of the same age, consideration of
Changes in the whole TSA of m. semitendinosus and the area of the muscle occupied by slow fibres with liveweight.

Regression equations:

Whole muscle, \( \log_e Y = 0.74 \log_e X - 0.39 \)

Muscle occupied by slow fibres, \( \log_e Y = 1.23 \log_e X - 7.26 \)
MUSCLE OCCUPIED BY SLOW FIBRES

WHOLE MUSCLE

\( \log_e \text{ Liveweight (g)} \)

\( \log_e \text{ TSA (mm}^2) \)
this entity in littermates of an equal liveweight revealed differences. The runt and small littermates both possessed a significantly greater \( (P < 0.001) \) area of slow semitendinosus muscle than their respective large littermates on an equal liveweight basis. Values derived from regression slopes indicated that the runt littermates possessed, on average, 1.3 times the TSA of slow muscle, while the small littermates possessed an average of 1.4 times the TSA of slow muscle present in the m. semitendinosus of the large littermates.

3.3.5. Occurrence Of Transitional Myofibre Types

In mixed muscle from the deep portion of m. semitendinosus and from m. trapezius a gradation of myofibre histochemical types were shown to exist between the mature SO and FOG fibres. As evident from Table 7, six of these so called 'transitional' fibre types were identified and allocated to two classes, I0 and F0. All transitional fibres were oxidative and non-glycolytic, and possessed ATPase activities that ranged in staining intensity from alkaline ATPase-high and acid ATPase-low, to alkaline ATPase-intermediate and acid ATPase-high (Figures 11 and 12).

A group of these intermediate fibres exhibited both acid and alkaline ATPase-stability in young pig muscle (Figure 11). The ratio of acid ATPase-stable to alkaline ATPase-labile fibres was greater than 1.0 at birth (indicative of fibres exhibiting both acid and alkaline ATPase-stability), decreasing from \( 3.7 \pm 0.6 \) (mean ratio ± SD) in the deep portion of m. semitendinosus, and \( 1.9 \pm 0.2 \) in m. trapezius to \( 1.1 \pm 0.1 \) in both muscles after a
Immature muscle histochemical staining.

Fresh frozen sections from the deep portion of m. semitendinosus from the 9 day old small littermate (liveweight of 3100g) stained for the demonstration of:

A Alkaline ATPase
B Acid ATPase
C SDHase

Fibre types:

\[ X = SD \quad Y = 10a \quad Z = FO \]

(see Table 7 for classification of fibre types)
liveweight of 8.5 kg.

Those fibres classified as FO were considered transitional fibres only after GPase staining had been detected in muscle sections, prior to this they represented merely undifferentiated fibres and progenitors of the FOG fibre type.

Transitional fibres were juxtaposed to the groups of slow fibres (Figure 12). The decrease in the percentage of transitional fibres with increasing liveweight was inevitably related to the attainment of the mature fibre type ratios, especially those of the SO fibres, as demonstrated in Figures 8 and 9; the negligible quantity of transitional fibres demonstrated in muscle from pigs heavier than 8.5 kg coincided with the constancy of the fibre type percentages considered representative of mature muscle (Table 8).

3.3.6. Changes In The Mean TSAs of Myofibre Types With Growth, And Mean Value Differences Between Littermates

Correlation coefficients established between myofibre type mean TSAs and age or liveweight indicated that myofibre TSAs were similarly correlated with age ($r = 0.88$) and with liveweight ($r = 0.95$). The regression coefficients of these parameters were not significantly different ($P > 0.10$) between littermate groups, enabling a comparison of the intercepts of the slopes. Comparisons, made in this way, revealed that no significant differences ($P > 0.05$) existed between the intercepts of regression slopes of mean myofibre TSAs with age between littermates. However, the same was not true of the intercepts of regression slopes with liveweight.
Mature muscle histochemical staining.

Fresh frozen sections from the deep portion of m. semitendinosus from the 84 day old runt littermate (liveweight of 28250g) stained for the demonstration of:

A  Alkaline ATPase
B  Acid ATPase
C  SDHase
D  GPase

Fibre types:

V = SO  W = IOa  X = IOd
Y = F0G  Z = FG

(see Table 7 for a classification of fibre types)
Summated regression coefficients for mean fibre TSAs against liveweight for all littermate groups (Table 9) revealed that for the SO types of the three muscle sections and for the FOG (including FO) types of m. trapezius the coefficients were not significantly different (P > 0.05) from 2/3. This suggested that the TSAs of these fibre types, from their respective muscles, grew in proportion to the animal's liveweight. All the other types had regression coefficients significantly greater (P < 0.025) than 2/3 which indicated that they grew at a proportionally greater rate than liveweight. The FOG (including FO) and FG fibre TSAs of the superficial portion of m. semitendinosus in fact bore a direct relationship with liveweight. The regression coefficients of these two exceptional fibre types was not significantly different from 1.0 (P > 0.10). Of all regression coefficients those of the SO fibres, from both portions of m. semitendinosus and from m. trapezius had the lowest regression coefficients, while those of the fast fibres, especially those of the superficial portion of m. semitendinosus, had the highest regression coefficients.
Table 9 - The Rate Of Growth Of Myofibre Type Mean TSAs With Liveweight, And Comparison Of Myofibre Type Mean TSAs Between Littermates.

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Myofibre Type</th>
<th>Total Regression Coefficient b (SE)</th>
<th>Intercepts (log e) of regression slopes</th>
<th>Significance of difference between intercepts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Large</td>
<td>Small</td>
</tr>
<tr>
<td>M. semitendinosus F0+FOG</td>
<td>0.52 (0.04)</td>
<td>0.25  1.08  1.92</td>
<td>P&lt;0.001</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>'Deep' FG</td>
<td>0.89 (0.04)</td>
<td>-0.87 -0.34 -0.77</td>
<td>P&lt;0.05</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>M. semitendinosus F0+FOG</td>
<td>0.58 (0.05)</td>
<td>0.82  1.86  1.86</td>
<td>P&lt;0.001</td>
<td>NS</td>
</tr>
<tr>
<td>'Super.' FG</td>
<td>0.97 (0.05)</td>
<td>-2.30 -1.94 -1.87</td>
<td>NS</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td>M. trapezius F0+FOG</td>
<td>0.67 (0.03)</td>
<td>0.14  0.03  0.58</td>
<td>P&lt;0.01</td>
<td>NS</td>
</tr>
<tr>
<td>'Deep' FG</td>
<td>0.69 (0.03)</td>
<td>-0.37 -0.79 -0.10</td>
<td>NS</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>'Super.' FG</td>
<td>0.76 (0.04)</td>
<td>-0.85 -0.37 -0.35</td>
<td>P&lt;0.05</td>
<td>P&lt;0.001</td>
</tr>
</tbody>
</table>

In the deep portion of m. semitendinosus, the TSAs of the FOG and FG fibres did not increase with liveweight at a significantly different (P > 0.50) rate from each other while the increase in TSA of both these fibres was significantly greater than that of the SO fibres (P < 0.05). The same was true of the superficial portion of this muscle, except that the divergence in the growth rate of the TSAs was even more significant (P < 0.001) between the fast and slow fibre types. However, in m. trapezius all fibre type TSAs grew at essentially the same rate; even the regression coefficients of the SO and FG fibres were not significantly (P > 0.10) different.

Table 10 shows the relative size ratios of the fast to slow fibres between the neonatal littermates (0 and 1 days) and the most mature animals in this study (100 and 128 days). These ratios
emphasise that just after birth the fast fibres have a lower TSA than the slow fibres but grow to a relatively greater extent post-natally, to obtain a ratio of approximate unity with the slow fibres.

Table 10 - Ratios Of Average Fast: Slow Fibre TSAs In Neonatal And Adult Littermates.

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Combined ages (days)</th>
<th>Ratio of fast: slow fibre TSAs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Large</td>
</tr>
<tr>
<td>M. semitendinosus</td>
<td>0+1</td>
<td>0.65</td>
</tr>
<tr>
<td>'Deep' portion</td>
<td>100+128</td>
<td>1.11</td>
</tr>
<tr>
<td>M. trapezius</td>
<td>0+1</td>
<td>0.77</td>
</tr>
<tr>
<td></td>
<td>100+128</td>
<td>1.05</td>
</tr>
</tbody>
</table>

* Small littermates of 100 and 128 days of age exhibited relatively poor growth performance.

As previously mentioned, at equal liveweights the myofibre type mean TSAs of the littermate groups were not always equal. The results of the comparison of regression slope intercepts are displayed in Table 9, where all significant differences indicate that, at equal liveweights, the lower birthweight littermates have, on average, greater mean TSAs of the myofibre type concerned; the majority of fibre types do, in fact, have greater TSAs in the lower birthweight littermate groups. As evident from Table 9, the SO fibres of the deep portion of m. semitendinosus are most
significantly different (P < 0.001) between each littermate group, more so than any other fibre type.

Mean TSAs of the mature myofibre types SO, FOG (including FO) and FG are shown in Appendix 5.

3.4. DISCUSSION

3.4.1. Histochemical Differentiation Of Myofibre Types

The histochemical myofibre typing of m. semitendinosus showed that the deep (craniomedial) aspect of this muscle contained a higher percentage of slow fibres and occupied an oval shaped portion of approximately 40% of the total muscle TSA (Figure 4). The mixed fibre type percentage composition (50: 50, slow: fast) of the deep portion of m. semitendinosus, which resembled that of m. trapezius (Table 8), made it distinct from the superficial portion that contained a higher percentage (over 90%) of fast fibres. It is a well known phenomenon that relatively more slow fibres are found in the deeper aspects of many locomotory muscles of animals (Cat: Bodine et al. 1982; Dog: Armstrong et al. 1982; Pig: Beermann et al. 1978; Rat: Pullen 1977). These deeper muscle regions assist the solely postural muscles in supporting the animal in the standing position. Thus the fibre composition of the muscle closely reflects its functional role.

The initial histochemical differentiation of muscle fibre types with respect to SDHase and GPase was found to extend over a greater age range than has previously been determined in the pig. The initiation of GPase staining has been observed during the first
week (m. semitendinosus: Beermann et al. 1978) and up to 10 days (longissimus and diaphragm muscles: Davies 1972) postnatally, while in the present study differential staining commenced after 6 days and was not always evident in all three littermates until 19 days of age. Likewise, while Ashmore et al. (1972b) found SDHase-negative (non-oxidative) fibres at about 14 days in m. triceps brachii and m. cutaneous, and Davies (1972) demonstrated such fibres at about 12 days after birth, in the present study the initiation of differential SDHase staining was exhibited over a range of up to 20 days, the age of commencement of this differentiation being muscle type specific, beginning earlier in predominantly fast muscles than in mixed muscles.

The low birthweight piglets selected for this investigation were responsible for the evident delay in histochemical differentiation in that they produced, at any given age, a range in live-weights extending below the average litterweight. This brought to light the fact that myofibre type differentiation, with regard to SDHase and GPase, was weight specific and, therefore, probably more related to physiological than to chronological age.

The lack of significant change with growth in the mature percentages of oxidative and glycolytic fibres (after the attainment of a liveweight of approximately 8.5 kg in this study) was also demonstrated by the results of Davies (1972) on longissimus dorsi and diaphragm muscles of pigs up to a liveweight of 59.6 kg.

An increase in fibre size, above a certain TSA, has been found by some other workers to coincide with decreases in oxidative
capacity (Goldspink 1969; Howells and Goldspink 1974; Swatland 1983). Goldspink (1965) attributes this effect to the dilution of mitochondria by myofibrillar material, the oxygen supply to the larger fibre being insufficient to support a greater population of mitochondria due to the decrease in capillary density that coincides with the growth of the muscle (Sillau and Banchero 1977). As a corollary there will be an increase in the glycolytic capacity of the fibres in view of the fact that reduced oxidative capacity produces a concomitant rise in glycolytic capacity (Dubowitz and Pearse 1960). However the muscles observed in the present study and those of Davies's study (1972) either failed to show fibre TSA increases substantial enough to produce a reduction in oxidative capacity or, if oxidative activity was reduced, its modification was not of sufficient magnitude to warrant a negative classification. The second alternative may indeed be the case as the aforementioned studies, which have detected reductions in the oxidative capacity of growing muscles, employed cytochemical methods which are more sensitive to alterations in SDHase activity than the 'all-or-nothing' histochemical classification employed here.

3.4.2. Prenatal Growth Retardation And Myofibre Types. And TSAs. Postnatally

Histochemical methods have been successfully employed to show, by way of proportions of fibre types, alterations in muscle metabolism after a period of nutritional deprivation. Howells et al. (1978) demonstrated that the anterior tibialis muscle of rats, prenatally undernourished and then postnatally rehabilitated, exhi-
bited decreased oxidative capacity together with increased glycolytic capacity. These results are supported by the biochemical methods applied to the gastrocnemius muscles of similarly treated rats (Raju 1975), the resultant rise in glycolytic activity being related to the increased susceptibility to fatigue of the muscle that Raju (1975) demonstrated physiologically.

These authors claimed that prenatal undernutrition has marked and persistent effects on the oxidative and glycolytic metabolic capacities of mature muscle, despite adequate postnatal nourishment. The fact that the mature pig muscle (of animals over a live-weight of 8.5 kg) investigated in the present study exhibited similar proportions of oxidative and glycolytic myofibres amongst all categories of littermates is not consistent with this view that prenatal growth retardation does have such an irreversible effect on these aspects of muscle metabolism. As the birthweights of the experimental and control rats in these studies were not given the state of growth retardation of the undernourished group, relative to the low birthweight pigs of the present study, cannot be ascertained. Therefore it might be deduced that either the rats had suffered a higher degree of growth restriction or the number of pigs of mature fibre type percentages in the present study were not sufficient to show differences between birthweight categories.

Despite the evident lack of effect of prenatal growth retardation on the oxidative and glycolytic metabolism of the muscle of the pigs in the present study marked effects were observed on the relative TSAs of different myofibres, exhibiting these metabolic
properties to different degrees, especially in the newborn animals.

The relative TSAs of fast: slow fibres of the deep portion of m. semitendinosus (displayed in Table 10) of the neonatal runts was almost half that of the large littermates at this early age, while in m. trapezius (see Table 10 also) such a substantial difference was not evident. These results are consistent with observations that undernutrition (in this instance, undernutrition in utero) has a differential effect, not only on the myofibre types, but also on different muscles. Studies that have been conducted on protein deprived rats from 6 - 25 weeks after birth (Oldfors et al. 1983) have shown that type 1 (SO) and type 2A (FOG) myofibres of the extensor digitorum longus muscle failed to grow while type 2B (FG) fibres actually atrophied. Undernourished foetal and suckling rats (Bedi et al. 1982), even after rehabilitation on an ad lib. diet for 5 months, showed persistent and significant deficits in FG fibre areas of the extensor digitorum longus muscle; in this instance this growth effect was also reflected by the FOG fibres. These nutritional manipulations demonstrate the vulnerability of myofibres with a fast contraction speed to nutritional inadequacy, which means that muscles with different functions are differentially affected by virtue of their relative fast fibre type content. This is illustrated by the extensor digitorum longus (Bedi et al. 1982) and the anterior tibialis (Howells et al. 1978) muscles of rats, born to undernourished dams and fed ad lib. after birth, which both showed significant weight deficits relative to body weight (due to their relatively high fast fibre content) unlike the soleus, which in the adult consists primarily of slow
fibres (Kugelberg 1976).

The deep portion of m. semitendinosus exemplifies this apparent preferential sparing effect of slow fibres from growth restriction imposed by undernutrition. However there appears to be a somewhat different situation in the trapezius muscle which, in the neonatal pig, does not present such disparate fast and slow fibre type TSAs as does the deep portion of m. semitendinosus (Table 10). The apparent sparing of the size of all the myofibres of m. trapezius is either indicative of the fact that this muscle has a more important postural role than the deep portion of m. semitendinosus (despite their similar myofibre type compositions) and/or that it is relatively more mature than m. semitendinosus at birth. The average postnatal growth impetus of m. trapezius as opposed to the high growth impetus of m. semitendinosus in this study suggests that the predominant growth phase of the former muscle is before birth and that it is, therefore, less prone to nutritional inadequacy in utero (with respect to the concept of Hammond, 1944, which proposes that earlier developing foetal organs have priority over later maturing organs with regards to nutrient supplies, as discussed in Chapter 1).

That the relative effect of prenatal growth restriction on different muscles is not merely a factor of their fibre type composition is reflected within the semitendinosus muscle of very young pigs. It was observed that the deep portion of this muscle in severely undernourished pigs, as illustrated by the two extreme runts (referred to and defined in the Results section of Chapter
1), occupied approximately 60% of the whole TSA at the midbelly, which was 21%, and 3 SD, above the average for the other pig littermates studied; this meant that the area classified as 'superficial' was greatly diminished in these runts. Despite the fact that the deep portion contained slow fibres, whose TSAs have been shown to be exempt from nutritional effects, these fibres occupied less than 22% of the deep area in the two severe runts. The most pertinent phenomenon contributing to the differential effect of the deep and superficial portion relative TSAs was, therefore, a preferential sparing of the fast fibres of the deep portion. The TSAs of the fast fibres from the superficial portion of m. semitendinosus were considerably smaller, relative to those of the deep portion, in the two extreme runts than in their littermates; the ratio of fast fibre areas for the superficial: deep portion being 0.48: 1 for the runts and 0.85: 1 for their large and small littermates combined. This illustrates an interesting point that not only might nutritional effects be myofibre type specific but that this selective growth effect might embody the functional significance of the whole muscle so that, for example, fast fibres within a postural muscle are less prone to growth restriction than those within a primarily propulsive muscle in cases of undernutrition.

In the present study, however, the resultant effects of growth restriction in utero on the muscle fibres of m. semitendinosus, unlike in the previously described rat studies (Howells et al. 1978; Bedi et al. 1982), were not evident in the combined relative TSAs of the fast and slow myofibre types of mature, adequately nourished, pigs of 100 and 128 days' age (Table 10). The long-term
effects of prenatal undernutrition persisting in the adult rats, despite adequate nutrition after birth, suggests that the rats had indeed suffered a more severe degree of food deprivation in utero than the runt pigs of the present study, as previously mentioned.

The observation made, during the course of this work, that low birthweight pigs have significantly larger myofibre TSAs in their semitendinosus and trapezius muscles (the degree of significance depending on the myofibre type and muscle, see Table 9) than their heavier littermates, when considered on a weight-constant basis, has also been noted by other authors. Hegarty and Allen (1978) found that of the psoas major, semi-tendinosus, biceps brachii and dorsi longissimus muscles the former two muscles contained fibres of significantly greater \( P<0.05 \) TSAs in runt pigs (average birthweight 810g) than the large littermates (average birthweight 1570g) at 106 kg while the fibre areas of the latter two muscles were not significantly different between siblings. Likewise Powell and Aberle (1981) found that the fibre TSAs of m. semimembranosus of runts (birthweight under 1000g) were either equal to, or greater than, those in this muscle of their heavier birthweight (over 1500g) littermates at equal slaughter weights.

These authors failed to attribute this fibre size effect to the disparate ages of similar slaughter weight siblings of low and high birthweight. Since low birthweight pigs appear to maintain their lower weight to 128 days (Figure 1) they will be chronologically older at any specific weight which probably accounts for the difference in myofibre TSAs of similar liveweight pigs of disparate
birthweights. This highlights the very interesting and fundamentally important phenomenon that there must be an age-factor, unrelated to the growth-force of weight influencing the TSAs of myofibre types. The relative difference in myofibre TSAs seen between littermates on a weight-constant basis is of particular significance in another aspect of muscle development, namely that concerning the TSA of slow muscle within m. semitendinosus, which will be discussed later.

3.4.3. Growth Changes In Myofibre Type Proportions

Numerous observations have contributed to our concept of the muscle as a tissue of immense adaptability. As Salmons and Henriks-son (1981) drew attention to, in a review of the amazing propensity of muscle for compensatory change, exercise stimuli are accompanied by an enhancement of the capacity for oxidative metabolism. These exercise-stimulated modifications are exemplified by the training of young Standardbred trotters (Henckel 1983), the weight-lifting of hamsters (Howells and Goldspink 1974) and rats (Kowalski et al. 1969), and the muscle of free living wild rabbits as opposed to that of wild rabbits bred in captivity (Nimmo and Snow 1983); the contractile characteristics are, however, in most cases, fairly stable under these exercise conditions (Edgerton et al. 1969).

Changes in the proportions of fast and slow fibres of the soleus muscle of rats have been induced by the synergistic ablation of the gastrocnemius muscle (Tomanek 1975) and also by subjecting young rats to chronic rotation (Martin and Romond 1975), a process
that stimulates hypergravity. Both these procedures enhanced the postural function of the soleus through functional overload which resulted in an acceleration of the attainment of the mature complement of SO fibres. It therefore appears that the way in which the muscle maintains a supportive role is through an increase in the percentage of these fatigue-resistant fibres (Edström and Kugelberg 1968). Such an adaptation is seen during the normal growth of animals, a process associated with an increased functional load due to increased liveweight. This natural adaptive mechanism has been reported by Davies (1972) in the longissimus muscle of pigs from birth to a liveweight of 93 kg; by Swatland (1975) in the sartorius muscle of 2 - 10 day old pigs; by Kugelberg (1976) in the soleus muscle of 3 and 34 week old rats; by Suzuki and Cassens (1980) in the masseter, trapezius, longissimus, rectus femoris and vastus intermedius muscles of pigs between birth and 16 weeks of age; and by Suzuki and Cassens (1983) in m. serratus ventralis thoracis of sheep. The only case in the literature disagreeing with this general concensus is that of Cassens et al. (1968) which reported an actual decrease in the percentage of, and total muscle TSA occupied by, SO fibres between 13 and 200 days of age in the longissimus muscle of pigs. No apparent reason for this disparity is evident.

Muscles, or those parts of muscles, concerned with a propulsive function do not appear to change in composition, with respect to the percentage of slow fibres; this is exemplified by the superficial portion of m. semitendinosus in the present study, and that of Sivachelvan and Davies (1981) and the biopsies (and therefore, presumably, the superficial portion) of the semitendinosus muscle
of cattle in the work of Holmes and Ashmore (1972). This is in contrast with those muscles required to support the body which do, however, invariably show an increase in the proportion of, and area occupied by, slow myofibres as discussed above.

The precise way in which the postural function of a muscle actually adapts via changes in the proportions of slow (myosin ATPase low) fibres was considered by Davies (1972). Davies's results support the hypothesis that the TSA of a muscle increases at a rate equivalent to 2/3 the power of the body weight and that, within such a muscle, the TSA occupied by slow fibres maintains a direct relationship \( b = 1.0, P < 0.05 \) with liveweight. The results presented for m. semitendinosus of the Large Whites investigated in the present study (this muscle, like m. longissimus \( dorsi \), had a high growth impetus) showed that the regression coefficient of its TSA with liveweight was likewise not significantly different from 2/3 \( (P > 0.50) \) which suggests that the TSA of a muscle does indeed grow in proportion to the animal's liveweight. The TSA occupied by slow fibres adapted accordingly by increasing to a factor of 5/4 the power of liveweight (Figure 10) which was found to be significantly greater \( (P < 0.01) \) than 1.0. This rate of increase in the TSA of slow muscle within m. semitendinosus was achieved, up until the attainment of a liveweight of approximately 8.5kg, by an increase in the percentage of slow fibres within the muscle together with an increase in the TSA of the individual fibres. After this liveweight the only significant factor contributing to the increase in the area of slow muscle was the increasing TSA of the slow fibres.
The results presented for m. semitendinosus support the general hypothesis proposed by Davies (1972) that the TSA of a muscle occupied by slow fibres increases at a greater rate than the total TSA of the muscle. The differing regression coefficients determined for m. longissimus (Davies 1972) and for m. semitendinosus may be due to the individual methods for the estimation of the TSA occupied by slow muscle or, in fact, it may represent a real difference. The second alternative would suggest that the slow muscle TSA increase, relative to liveweight, is a reflection of a muscle's unique postural adaptation as a consequence of its comparative supportive role within the body. This is a highly plausible theory since the average alkaline ATPase-negative fibre type percentage dorsi was determined, by Davies (1972), to be 18.0% for m. longissimus of the mature Large White which indicates that this muscle contains less than half the percentage of 'slow' fibres than the deep portion of m. semitendinosus. Consequently the supportive role of m. longissimus is probably less pronounced than that of m. semitendinosus which is reflected by the significantly lower regression coefficient (P < 0.01) for the TSA of slow muscle against live-dorsi weight for m. longissimus than for m. semitendinosus.

When considered on an equal liveweight basis littermates did not show a significantly different number of slow fibres per bundle in either m. semitendinosus or m. trapezius. However, in m. semitendinosus the percentage of slow fibres, at a given liveweight, was significantly greater in the runts than in the large littermates (P < 0.001) and was also significantly greater than in the small littermates (P < 0.05). This indicates that it is possibly
the number of slow fibres within a metabolic bundle that is regulated to maintain a constant relationship with liveweight, the percentage of slow fibres merely being a consequence of this, and the number of fast fibres within the bundle; the latter event is attributable to the secondary: primary fibre number ratio which, being smaller in the lower birthweight littermate, causes these animals to possess a greater percentage of slow fibres (a factor shown significantly in m. semitendinosus).

Despite the essentially equivalent relationship, exhibited by all littermate groups, between the increasing TSA of slow muscle within m. semitendinosus with the increasing liveweight of the animal there was a significantly different amount of slow muscle between the large: small and large: runt littermate groups when they were compared on a weight-constant basis (by regression analysis), the lower birthweight littermates possessing the greater TSA of slow muscle. This would appear to be due to the significantly larger percentage of slow fibres evident in the lower birthweight littermates groups of these comparisons, together with the greater TSA of the slow fibres within the 'mixed' (deep) portion of this muscle of all lower birthweight, with respect to large birthweight, littermates at equal liveweights (as previously discussed).

Any implications on meat quality that the differential area of slow muscle between littermate groups of equivalent slaughter weight might induce are difficult to ascertain. Fibre type quality associations have been attributed, not to the respective contraction speeds, but to other metabolic properties (Ashmore 1974) of
muscle fibres. Oxidative capacity is highly correlated with intrafibre fat (see Chapter 4) and interfibre fat deposits (Ashmore 1974), imparting flavour to meat. Glycolytic capacity is associated with alterations in postmortem pH through the production of lactic acid from carbohydrates within the myofibres which affects meat quality (Wismer-Pedersen 1959). The greater percentage of slow muscle within the low birthweight, small and runt littermates, with respect to the large littermates of an equal liveweight was, however, not found to be associated with a lower proportion of glycolytic fibres. It is feasible that the SO fibres possessed a higher capacity for oxidative metabolism than the FOG fibres (Ashmore and Doerr 1971) and that the glycolytic capacity was not of the same intensity between littermate groups, as previously mentioned, but with the histochemical techniques employed here it was not possible to draw conclusions on the effects of low birthweight on meat quality.

3.4.4. Myofibre Type Transformation

The increasing proportion of slow fibres within the muscle of growing animals suggests, considering the well documented constancy of postnatal muscle fibre number, that muscle possesses the potential for fibre type transformation. This theory has been supported by the demonstration of fibres with staining intensities for acid and alkaline preincubated ATPase intermediate to the patterns exhibited by fast and slow myofibres (10a to 10d fibre types, see Table 7). These 'transitional' fibres are in abundance during the period of fibre type percentage change but occupy a negligible proportion
of the mature fibre type percentages after a limit of about 8.5 kg has been reached (Figures 8 and 9), at a similar age (8 weeks) to the attainment of stable fibre typing in various muscles of Chester White pigs (Suzuki and Cassens 1980).

The process of fibre type conversion from fast to slow contractility appears to proceed by way of those transitional fibre types described in Table 7, which are seen to parallel the intermediate types documented by Suzuki and Cassens in pig muscle (1980) and in sheep muscle (1983). The FO fibres appear to be the precursors of the transitional forms since, after birth, no decrease is seen in the percentage of glycolytic fibres which suggests that there is no contribution of FG or FOG fibres to the transitional fibre population. The population of FO fibres was apparently derived from fibres that had not yet adapted a contraction speed and metabolic properties characteristic of a 'mature' fibre type and, hence, did not appear to entail the interconversion (proposed by Suzuki and Cassens, 1980, 1983) of FG, FOG, and FO fibres.

Prior to the complete loss of alkaline ATPase-stable activity the myosin ATPase of the FO fibres appears to obtain acid stability (presented by certain subtypes within the I0 fibre type category, Table 7); a phenomenon documented by Kugelberg (1976) in rat soleus muscle, as well as by Suzuki and Cassens (1980) in pig muscles. These subtypes are already present in the neonatal muscle which exhibits ratios of acid-stable to alkaline-labile fibres of between 1.6 and 4.9, illustrating the occurrence of prenatal fibre type conversion (Beermann et al. 1978). After a certain degree of acid
stability has been achieved (demonstrated by types I0d and I0b, Table 7) the alkaline stability of myosin ATPase appears to decrease (seen in types I0c and I0a, Table 7) until the acid and alkaline stability is characteristic of that demonstrated by SO fibres (illustrated by myofibres in Figures 11 and 12). Thus a continuum of transitional fibres exhibiting myosin ATPase alkaline and acid stability intermediate to F0 and SO fibres is displayed on conversion to the latter fibre type.

The location of transitional fibres adjacent to the groups of slow fibres implies that, after transformation, they augment the number of slow fibres within a metabolic bundle. The very position of these converting fibre types suggests that, since the nature of the myofibre innervation governs its inherent properties (as discussed in the Introduction), its innervating neuron could, in some way, be influenced by the impulse pattern of the nervous tissue associated with adjacent slow fibres as a result of direct feedback to the increased functional load initiating adaptation to maintain support of the growing animal. This 'influence' of the adjacent slow fibres could be due to a local innervation of fast fibres with branching neurons from the foreign slow fibre's nerve followed by a gradual degeneration of the initially innervating 'fast' neuron. An extensive reorganisation of the innervation of rat lumbrical muscle in early postnatal development has been reported by Betz et al. (1979); a lumbrical motoneuron maintains a nearly constant number of synapses during this period while retracting old, and forming new, synapses with muscle fibres. Tuffery (1971) has also observed the growth and degeneration of motor end-plates in the normal adult
cat hindlimb muscles. However there is also evidence for the alternative hypothesis that the exposure to increased functional activity may trigger alterations within the innervating neuron itself; Gerchman (1968) found changes in the morphology and staining properties of motor neurons of rats subjected to prolonged exercise. The mechanism behind fibre type conversion therefore has yet to be defined but the apparently closer relationship between liveweight and the number of slow fibres per bundle in m. semitendinosus than the percentage of slow fibres favours the former hypothesis (due to the slow fibre organisation) as opposed to the latter one which might induce innervation changes, and hence fast to slow fibre conversion, at random.

3.4.5. Relative Histochemical Development of The Muscles Studied

The comparative histochemical fibre type profiles of neonatal muscles are indicative of the relative extent of muscle development in utero and prompts speculations on the processes of many aspects of muscle growth. For example the concept that slow fibres from the rat soleus muscle are not the same as those of the extensor digitorum longus (Padykula and Gauthier 1967) is supported by observations on m. semitendinosus from neonates. The superficial portion of m. semitendinosus (primarily a fast muscle like the extensor digitorum longus) of the neonate lacks acid ATPase-stable fibres unlike the deep portion (which possesses a postural function like the soleus muscle) despite their similar morphological development in utero (Beermann et al. 1978). The mature superficial portion of m. semitendinosus does however contain histochemically demonstrable
slow fibres which obviously differ in development (as denoted by the delayed ATPase pH stability) from the slow fibres of a 'slower' muscle.

Obviously it is vital that a muscle with a supportive role should be prepared, at birth, to exert its postural function when the animal is expelled from an aquatic environment, free of gravitational forces, to one where the muscles must exert an equal force to oppose this and all those forces associated with functions essential to survival. From observations on the histochemical differentiation of neonatal m. semitendinosus in the present study it is apparent that the deep portion of this muscle possesses slow fibres at birth that are, presumably, able to exercise a postural role. The superficial portion, however, does not appear to be recruited for a 'slow' role until up to about 2 weeks after birth when slow fibres are evident through the application of the histochemical techniques. The functional adaptation of muscle fibres within a muscle to the external environment is therefore very different, and obviously closely related to their respective functional roles, a concept which extends to the different roles of the semitendinosus and trapezius muscles.

At birth m. trapezius contains a higher number of alkaline ATPase-labile (SO) fibres per bundle than m. semitendinosus which is, perhaps, indicative of its greater postural significance. Not only is development in anticipation of the functional role evident between muscles at birth but is also apparent between littermates; with respect to acid ATPase-stable fibres, large littermates pos-
sessed about 1.1 more of these fibres per bundle in both m. semitendinosus and m. trapezius. The effects of size differences within a litter in utero on muscle development may result from the mechanical forces associated with these size differences. The suggestion that quantitative aspects of contraction of skeletal muscle before birth (Swatland and Cassens 1973a; Hausman et al. 1982) are responsible for the initiation of the development of different fibre types in utero (Beermann et al. 1978) was, however, considered sceptical by Ashmore et al. (1972b). Sivachelvan and Davies (1981) proposed that the histochemical development of m. semitendinosus in foetal sheep was influenced genetically, the buoyant nature of the uterine environment protecting the foetus from the gravitational force to which the animal is subjected immediately after birth and which appears responsible for the histochemical adaptation of the muscle thereafter.

Genetic factors controlling prenatal fibre type formation could explain the different fibre type compositions between muscles (Komi et al. 1977) but it is unlikely to explain consistent differences between certain littermates, in the same way the right/left muscle fibre pattern asymmetry in man (Fugl-Meyer et al. 1982) cannot be explained genetically, a differential genetic factor having been completely eliminated in the latter case.

Hausman et al. (1982) considered that the prenatal conversion of acid ATPase-negative to positive fibres may be hormonally regulated. The growth retardation of the low birthweight pig is thought to be mediated through placental insufficiency (Waldorf et al.
1957; Wootton et al. 1977; Wigmore 1982) which will obviously coincide with a reduced supply of hormonal factors as well as nutrients. Therefore the apparent preparation of foetal muscle, demonstrated through ATPase activity, which appears related to weight at birth, may in fact be linked to the extent of anatomical development in utero, exemplified by the differential states of retardation that are manifest (by size) within large litters, be it through nutritional or hormonal deprivation.

The outcome of this histochemical study into the differentiation of myofibre types within skeletal muscle of pig siblings has been to accentuate the physiological adaptation of muscle to the postnatal environment and to help elucidate the modifications occurring in the muscle as a result of the extent of anatomical development in utero.
4. ULTRASTRUCTURAL DEVELOPMENT OF TWO MYOFIBRE TYPES

4.1. INTRODUCTION

Ultrastructural (electron microscopic) investigations are an ideal component of a comprehensive growth study, complementing and augmenting light microscopical observations. However, surprisingly few such studies have been undertaken on the course of muscle development.

There is a wealth of information in the literature concerning the ultrastructural characteristics of muscle fibre types in adult muscle, as classified for human (Sjöström et al. 1982), guinea-pig (Eisenberg et al. 1974; Eisenberg and Kuda 1976) and rat (Gottschall 1980) muscle. The criteria for myofibre typing by ultrastructural characteristics, from longitudinal sections of muscle in particular, are well documented and include Z-disc thickness (Gauthier 1968; Salmons et al. 1978), amount and distribution of sarcoplasmic reticulum (Padykula and Gauthier 1970; Ninomiya et al. 1981), quantity and internal structure of mitochondria (Gauthier 1968) and also myofibrillar array (Salmons et al. 1978; Ninomiya et al. 1981). The comparative fine structure of fast and slow myofibres is explicitly illustrated by the study of Eisenberg and Salmons (1981) which quantifies alterations in the aforementioned subcellular components of adult rabbit fast muscle on conversion to slow-type muscle induced by chronic, low-frequency electrical stimulation. Experimental procedures have also covered the extent of myofibrillar proliferation in muscle induced by stretch (Ashmore and Summers 1981). This type of work aids the elucidation of the
mechanisms involved in fibre structure modifications that normally occur during growth.

Work on the comparative ultrastructure of naturally developing diverse myofibre types described in the literature has been concerned mainly with the extent of sarcoplasmic reticulum (Walker et al. 1968; Schiaffino and Margreth 1969; Luff and Atwood 1971) and the T-tubular system (Schiaffino and Margreth 1969; Luff and Atwood 1971) within fast and slow fibres.

Campion et al. (1981) have described the development of fast fibres in porcine foetal muscle while Russell and Oteruelo (1981) have described the development of both fast and slow fibres in bovine foetal muscle; these two EM studies were, however, purely qualitative. Foetal, neonatal and adult pig muscle development has been further investigated by Swatland (1975, 1976b) in terms of myofibrillar proliferation (quantified in terms of the number of myofibrils per fibre) but there appear to be no comparative studies of the development of different myofibre types in the pig, from birth to maturity, that quantify the changes in the different subcellular organelles.

Comprehensive quantitative studies provide an invaluable reference in investigations of muscle pathology, aiding the early identification of myopathies and enabling the evaluation of the rate and extent of their manifestation in the affected tissue. The need for such studies to assist our understanding of pathological diseases of the musculature is well illustrated by 'Splayleg' of neonatal pigs; a condition which is manifest as a severe paresis of
the hind and forelimbs which 'splay' outwards (Deutsch and Done 1971). Light and electron microscopial observations of the muscle of affected piglets led Thurley et al. (1967) and Deutsch and Done (1971) to believe that the weakness of the limb muscles was related to an apparent deficiency of myofibrils. However it has been subsequently reported by Swatland (1975) and Bradley et al. (1980) that the relative lack of myofibrils (myofibrillar hypoplasia) cannot be considered a symptom of Splayleg as it is a natural occurrence of normal neonatal pig muscle and is not exclusive to the muscle of Splayleg pigs.

The EM was employed in the present study of pig muscle development to perform a stereological analysis of the ultrastructural changes occurring, with growth, in the two most diverse muscle fibre types (SO and FG) with respect to their myofibrillar, mitochondrial and lipid droplet content. These subcellular organelles were chosen in order to ascertain, as well as the general morphological changes in myofibres after birth, whether low birthweight had any permanent effect on muscle fibres with respect to myofibrillar protein content (a measure of muscle strength: Helander and Thulin 1962; Goldspink 1965) or capacity for oxidative metabolism (as the mitochondrial and lipid droplet values would reveal).

Stereology affords a quantification of the three dimensional structure of a tissue, or its cellular units, from two dimensional sections obtained from the tissue. Quantitative procedures are of immense practical value as, for example, they produce a graded
cytological indication of oxidative capacity (through investigation of mitochondrial content) unlike the exclusively positive or negative recordings of the histochemical (SDHase) technique applied in the present study to ascertain the percentage of oxidative fibres within muscle sections (see Chapter 3). Howells and Jordan (1978) showed cytophotometrically that undernutrition reduced the concentration of SDHase in the anterior tibialis and soleus muscles of rats. This effect on the oxidative metabolism of the rat muscle was permanent but was not reflected by the oxidative capacity of the muscle of low birthweight (apparently undernourished) pigs of the present study, as determined by the relative percentages of SDHase-positive staining fibres of low and high birthweight littermates (as discussed in Chapter 3). It was, therefore, considered important to apply a method specifically for detecting the effects of low birthweight on the ultrastructure of muscle fibres.

4.2. MATERIALS AND METHODS

4.2.1. Preparation Of Material For EM

Samples of prerigor m. semitendinosus, were excised for EM investigation from the midbelly of the left muscle after the removal of slices for the preparation of fresh frozen sections (Chapters 2 and 3). Longitudinal strips (approximately 1mm x 3mm) of muscle fibres were dissected from both the deep and superficial portions of the muscle, being orientated by the blue latex applied to the most superficial surface of the muscle before its excision from the animal (see Chapter 1). The existence of exceptionally small muscles in some animals necessitated the use of the right
muscle, the whole of the left muscle being used for the preparation of fresh frozen sections.

The muscle samples were fixed in 5% glutaraldehyde in 0.1M sodium cacodylate buffered at pH 7.2 for two hours. This was followed by two washes in buffer, post-fixation in 1% osmium tetroxide in buffer for one hour, two washes in double distilled water and, finally, dehydration through graded acetone. The samples were then embedded in Araldite. Sections were cut to a thickness of 60 - 80 nm with a Reichert OMU3 microtome and stained with uranyl acetate (20 minutes) and lead citrate (4 - 5 minutes). These ultrathin sections were examined with a Philips 400 electron microscope at an accelerating voltage of 100kV.

4.2.2. Stereological Analysis

The two myofibre type populations (oxidative and non-oxidative), chosen for stereological analysis of their ultrastructural composition, were selected from muscle samples by applying knowledge concerning the morphology of m. semitendinosus, with respect to fibre type location, gained from the histochemical demonstration of myofibre types as discussed in Chapter 3. Non-oxidative fibres (or, in the case of immature muscle exhibiting 100% SDHase-positive staining, the progenitors of non-oxidative fibres) were invariably located at the periphery of fascicles in the superficial portion of m. semitendinosus. Oxidative fibres were consistently demonstrated from birth to maturity at the centre of fascicles in the deep portion of this muscle. The choice of non-oxidative and oxidative fibres from these respective locations
meant that the non-oxidative fibres were from a population of histochemically determined fast fibres while the oxidative fibres were invariably defined as slow-contracting since the central fibres of fascicles in the deep portion of m. semitendinosus were alkaline ATPase-negative. Despite these further differences between the two chosen fibre populations their respective oxidative capacities produced the most prevalent ultrastructural disparities between them. Therefore, although these fibre type groups investigated are termed non-oxidative and oxidative it must be stressed that they are representative of the FG and SO fibre type populations, respectively, of m. semitendinosus.

Stereology relates two dimensional profiles of cell components seen in sections to their three dimensional structures in the original tissue. By the criteria of geometric probability the chance of finding, for example, mitochondrial profiles in a section will equal that of finding the total volume of mitochondria in the cell volume, a principle which permits the employment of point-counting techniques (Weibel et al. 1966). This method is described by Underwood (1970) and advocates the use of the following equation:

\[ V_v = P_o / P_m \]

where \( V_v \) = volume density of organelle in cell

\( P_o \) = points falling on organelle

\( P_m \) = points falling on cell (myofibre) profile

Muscle tissue, which is composed of fully orientated muscle fibres, is anisotropic (Elias and Hyde 1980) which means that it
displays a periodicity in the arrangement of some of its cytological structures. Muscle is composed of cells consisting of recurring units (sarcomeres) which exhibit a concentration of large mitochondria at the I band while small mitochondria are concentrated at the A band with a predominant number found in an annulus within 1μm of the sarcolemma (Eisenberg et al. 1974).

Transverse sections of muscle were cut obliquely and of an appropriate thickness to produce a silver interference colour which is less than mitochondrial diameter (Weibel et al. 1966), precautions which prevent an over-estimation of the volume fraction of mitochondria. Electron micrographs were taken of individual muscle fibres at a magnification of x6000 and were printed to give a final magnification of x12000 which was found adequate for the recognition of myofibre components. When myofibres failed to be completely represented by one electron micrograph, micrographs were taken across the centre of the fibre to create a montage on which a sector could be drawn (Underwood 1970). A sector is representative of the whole TSA of the fibre and allows for the localisation of mitochondria within circular zones. In neonatal pig myofibres myofibrillar hypoplasia often existed, in such instances the myofibrils were not evenly dispersed throughout the whole TSA (as is the case in myofibres of heavier pigs). When there was evidence of myofibrillar hypoplasia micrographs were taken to cover the whole TSA of the myofibre.

The number of each myofibre type sampled per animal was determined so as to produce volume estimates of a value within the lim-
its of 5 - 10% (Eisenberg et al. 1974) of the final accumulated mean for a sample of 15 myofibres (Underwood 1970). By this method the minimum sample size necessary to produce mitochondrial and lipid droplet percentage volume estimates within the acceptable limits was evaluated as 10 myofibres for this study; the myofibrilar percentage volume had a very much lower variation requiring a sample size of less than 5 myofibres to produce a representative value.

A 1cm squared test grid drawn on an acetate sheet (Eisenberg and Salmons 1981) was used for point-counting and hence the estimation of the volume density (expressed as a percentage) of myofibrils, mitochondria and lipid droplets for both the oxidative and non-oxidative fibre populations of m. semitendinosus from selected pig littermates. The two muscle fibre populations from 11 large, 9 small and 12 runt pigs were analysed stereologically.

Regression analysis of myofibre type organelle percentage volumes against liveweight and/or age was conducted to quantify postnatal changes. Paired observations were analysed to test the significance of the difference in the percentage volume of organelles between the two myofibre types and, also, to establish variations in these parameters between littermate groups.

4.3. RESULTS

The percentage volumes of myofibrils, mitochondria and lipid droplets of the oxidative and non-oxidative fibres of m. semitendinosus, of all littermates studied, are presented in Appendix 6, parts A and B, respectively.
4.3.1. Comparison Of Myofibre Type Ultrastructure

After birth until the piglets reached 4 days of age the mean myofibrillar percentage volume of non-oxidative fibres (21.2%) was significantly less (P < 0.001) than that of oxidative fibres (46.0%) by an average of 53.9% (as illustrated in figure 13A and B). In piglets between 4 and 6 days of age (corresponding to liveweights of about 1350 - 2200g) the increasing percentage myofibrillar volume of the non-oxidative fibres exceeded that of the oxidative fibres in all littermates except the two severely runted piglets noted (in Chapter 1) for their lack of liveweight gain and extremely low relative birthweight, and (in Chapter 2) for their abnormally low muscle fibre number; the myofibrillar hypoplasia of these littermates is discussed in the ensuing section.

The low myofibrillar percentage volume in the non-oxidative fibres from the superficial portion of m. semitendinosus of light piglets (liveweight of 2200g or less) was associated with a high content of granular material.

The mean myofibrillar percentage volume of non-oxidative fibres from piglets older than 6 days (and of a liveweight exceeding 2200g) was 62.9%, significantly greater (P < 0.001) than that of the oxidative fibres (51.9%), by an average of 21.2% (illustrated in Figure 13C and D). The change in the percentage volume of myofibrils between 46 and 84 days was only slight and suggested that the mature percentage values of myofibrillar volume for the two myofibre types had been attained by 46 days to give mature values of approximately 56.3% and 66.9% for oxidative and non-
Figure 13

Oxidative and non-oxidative myofibre type ultrastructure at birth and 84 days (x11000).

A Oxidative fibre from 'deep' portion of m. semitendinosus of runt littermate at birth (liveweight of 900g).

B Non-oxidative fibre from 'superficial' portion of m. semitendinosus of runt littermate at birth.

C Oxidative fibre from 'deep' portion of m. semitendinosus of runt littermate at 84 days (liveweight of 28250g).

D Non-oxidative fibre from 'superficial' portion of m.semitendinosus of runt littermate at 84 days.

My = Myofibrils
M = Mitochondria
L = Lipids
The increase in the percentage volume of myofibrils was significantly correlated with age (Figure 14) and with liveweight (Figure 15) in both the oxidative and non-oxidative fibres. The correlations of the percentage volume of myofibrils in the oxidative fibres with age \((r = 0.445, P < 0.01)\) and liveweight \((r = 0.450, P < 0.02)\) were not significantly different. The change in the percentage volume of myofibrils in the non-oxidative fibres was more highly correlated with both age \((r = 0.924, P < 0.001)\) and liveweight \((r = 0.727, P < 0.001)\) than in the oxidative fibres and was also found to show a significantly greater correlation with age than with liveweight \((P < 0.025)\) unlike the oxidative fibres.

The mitochondrial and lipid droplet percentage volume of the oxidative and non-oxidative myofibres were significantly different \((P < 0.001)\) at all ages from birth to 84 days; the oxidative fibres possessing a greater percentage volume of both these subcellular components (illustrated by the electron micrographs in Figure 13 and the graphs in Figure 16).

The percentage volume difference in mitochondria between the two fibre types was more or less consistent (at about 8%) throughout, even during the rise in the oxidative myofibres' mitochondrial content between 2 and 33 days of age. During this period the mitochondrial percentage volume of non-oxidative and oxidative fibres increased from a mean of 3.4% and 8.3%, respectively, before 2 days, and thence, until 33 days, had a mean value of 6.7% and 16.0%, respectively. In the oxidative myofibres changes in the
Figure 14

Myofibrillar percentage volume of oxidative and non-oxidative fibres for m. semitendinosus (from 'deep' and 'superficial' portions, respectively) of individual littersmates against the natural logarithm of their age.

Regression equations:

Oxidative fibres, \[ Y = 1.6 \log_e X + 45.7 \]
Non-oxidative fibres, \[ Y = 9.9 \log_e X + 28.0 \]
Figure 15

Myofibrillar percentage volume of oxidative and non-oxidative fibres for m. semitendinosus (from 'deep' and 'superficial' portions, respectively) of individual littermates against the natural logarithm of their liveweight.

Regression equations:

Oxidative fibres, \[ Y = 2.8 \log_e X + 27.1 \]
Non-oxidative fibres, \[ Y = 14.6 \log_e X - 68.7 \]
NON-OXIDATIVE FIBRES
OXIDATIVE FIBRES

Mylotribillary volume (%)

loge Liveweight (g)
percentage volume of mitochondria were mirrored by changes in the percentage volume of lipid droplets, but this phenomenon was not exhibited by the non-oxidative fibres. Consequently, the lipid droplet percentage volume differences between the oxidative and non-oxidative fibre types were greatest between 2 and 33 days. As demonstrated in Figure 16A, during this age-range the percentage lipid droplet volume of oxidative fibres more than trebled from a mean value of 3.8% (at 0 and 1 days) to a mean value of 13.3% (between 15 and 27 days). However, in the non-oxidative fibres (Figure 16B) there was little change between 2 and 33 days; the lipid droplet percentage volume at 6 days in Figure 16B which suggests an apparent rise was from one littermate only, and was probably not truly representative of non-oxidative fibres from piglets of this age, other values in this figure being means of littermates.

After weaning there was a much lower percentage volume of mitochondria and lipid droplets than prior to weaning (represented by Figure 17). Between the littermates of 46 days (weaned at 41 days of age) and 84 days there appears to be little difference in the percentage volume of mitochondria and lipid droplets, which gave mean values of 10.6% and 3.6% for oxidative fibres, and 3.6% and 1.1% for non-oxidative fibres, respectively.

4.1.2. Comparison Of Myofibre Type Ultrastructure Between Littermates

Statistical analysis of the data showed that there was no significant difference in the myofibre percentage volume of either
Mitochondrial and lipid droplet percentage volumes of myofibres, expressed as an average value for fibres from large, small and runt littermates against age.

A Oxidative fibres, from 'deep' portion of m. semitendinosus.

B Non-oxidative fibres, from 'superficial' portion of m. semitendinosus.
Figure 17

Oxidative myofibres from the 'deep' portion of m. semitendinosus of littermates before and after weaning (x11000).

A  Before weaning  Large littermate, 33 days old
     Myofibrillar volume = 51.3%
     Mitochondrial volume = 15.0%
     Lipid droplet volume = 10.5%

B  After weaning  Large littermate, 46 days old (weaned at 41 days)
     Myofibrillar volume = 52.8%
     Mitochondrial volume = 7.6%
     Lipid droplet volume = 3.7%

L = Lipid droplets
Arrows indicate mitochondria
myofibrils, mitochondria or lipid droplets between large: runt, large: small and small: runt littermate categories, with one exception; overall the percentage myofibrillar volume of the oxidative fibres in the small littermates was higher ($P < 0.025$) than in the large littermates, by an average of 9.5%.

On consideration of individual littermates, however, there were specific instances of differences in the ultrastructural composition of non-oxidative fibres from the superficial portion of m. semitendinosus between littermates, namely in the myofibrillar percentage volume. Of the aforementioned severely runted littermates, of 9 and 15 days of age, which presented myofibrillar hypoplasia, the myofibrillar hypoplasia of the runt of 15 days' age was most marked (Figure 18A). This runt littermate had a myofibrillar percentage volume approximately 64% that of its littermates for its non-oxidative fibres (illustrated in Figures 18B and C). Also, while this runt's littermates had a myofibrillar percentage volume in their non-oxidative fibres about 143% that in their oxidative fibres, this value from the myofibres of the runt was 66%; reminiscent of the relative non-oxidative: oxidative myofibrillar percentage volumes of piglets less than 6 days of age. Although the myofibrillar hypoplasia of the 9 day old severe runt was not so considerable its non-oxidative myofibres still contained, as well as a reduced myofibrillar percentage volume with respect to its littermates, a far lower percentage volume of myofibrils compared to its oxidative fibres; the reverse being true of its littermates (and all pigs of a liveweight greater than 2200g). The mitochondrial and lipid droplet percentage volumes of these severely runted
Myofibrillar hypoplasia of the myofibres from the 'superficial' portion of m. semitendinosus of a severely runted piglet at 15 days postnatally.

A Low power electron micrograph (x4000) to demonstrate the consistent occurrence of myofibrillar hypoplasia in the myofibres of the severely runted piglet.

B High power (x11000) of a non-oxidative myofibre of the severely runted littermate (liveweight of 675g).

Myofibrillar volume = 38.8%
Mitochondrial volume = 6.6%
Lipid droplet volume = 1.4%

C High power (x11000) of a non-oxidative myofibre of the runt's small littermate (liveweight of 2750g) to illustrate myofibrillar content of a 'normal' fibre.

Myofibrillar volume = 60.8%
Mitochondrial volume = 6.7%
Lipid droplet volume = 3.5%

My = Myofibrils
M = Mitochondria
L = Lipid droplets
littermates did not appear to be abnormally different from those of their littermates.

4.4. DISCUSSION

4.4.1. Myofibrillar Development Of Myofibres

The proliferation of myofibrils within muscle fibres proceeds by way of splitting along the length of the myofibril with the eventual separation of the two fractions. The longitudinal myofibrillar splitting was considered by Goldspink (1970) to originate from the Z-disc but has since been thought by some workers to initiate at the I-band (Ashmore and Summers 1981). By studying the stretch-induced myofibrillar proliferation of a fast twitch muscle from the wings of 6 week old chickens, Ashmore and Summers (1981) deduced that the fraction of muscle cell volume occupied by myofibrils remained constant despite the muscle fibre hypertrophy. These workers deduced that the splitting of the myofibrils was initiated by growth in the myofibril diameter amounting to an increase of 25%. However, Stickland (1981) found that the number of myofibrils within cross sections of myofibres from prenatal human sartorius muscle did not quite keep pace with the TSA increase of the myofibre since the myofibrils sustained an increase in size.

In the present study the myofibrillar percentage volume was found to be different between muscle fibres from neonatal and relatively mature pigs. The two fibre types studied exhibited different rates of myofibrillar proliferation, the volume fraction occupied by myofibrils apparently remaining constant after 46 days of age.
The rate of increase in the myofibrillar percentage volume observed in the oxidative and non-oxidative fibres (evident in Figures 14 and 15) appeared to parallel measurements made on the TSAs of these two fibre types described in Chapter 3. The non-oxidative (FG) fibre type TSA increase after birth was greater than that of the oxidative (SO) fibre type in all littermates and likewise, the rate of myofibrillar proliferation in the non-oxidative fibres far exceeded that in the oxidative fibres. These results together accentuate the rather more dynamic nature of the postnatal growth of non-oxidative as opposed to oxidative fibres, which tends to suggest that non-oxidative fibres are at a relatively more immature stage of development in the neonatal pig. This does indeed appear to be the situation. The comparative lack of myofibrils evident in the non-oxidative fibres relative to the oxidative fibres from m. semitendinosus of piglets before 6 days of age makes it apparent that the development of oxidative fibres, with respect to myofibrillar protein content, proceeds that of the non-oxidative fibres before birth. The ultrastructural development of oxidative fibres prenatally therefore appears preferential to that of non-oxidative fibres in pig muscle and substantiates the apparent 'sparing' of their TSAs from the effects of growth retardation associated with low weight at birth, as illustrated in Table 10 (Chapter 3). Neonatal mice, however, fail to present a relative lack of myofibrils in either the soleus (a slow) muscle or the extensor digitorum longus (a fast) muscle; the myofibrillar percentage volume being greater than 60% in these muscles of the newborn mouse (Luff and Atwood 1971).
The apparent dynamic nature of the myofibrillar protein content of non-oxidative fibres of young pigs after birth would obviously make these fibres more prone to postnatal undernutrition. This was found by Oldfors et al. (1983) in the extensor digitorum longus muscle of protein deprived young rats. While the oxidative (SO and FOG) types failed to grow the non-oxidative (F6) fibres actually atrophied under this treatment. The results of the present study, therefore, appear to suggest that the comparative rate of myofibrillar development between oxidative and non-oxidative fibres might, in fact, be the basis for the differential nature of the response of these two fibre types to the effects of undernutrition. Unfortunately experiments carried out to ascertain the effects of postnatal undernutrition on the relative sizes of different fibre types appear to be limited to rats with no results available for such manipulations in mice and pigs. Specific work therefore needs to be done to obtain data on the relative rate of accumulation of myofibrils in different myofibre types together with their relative response (with respect to TSA) to postnatal undernutrition (in different species) to support or refute this hypothesis.

The existence of a comparatively low percentage of myofibrils in the myofibres of neonatal piglets, younger than 6 days, is substantiated by the results of Bradley et al. (1980). These workers observed the ultrastructure of fibres from m. adductor and m. sartorius of normal and Splayleg pigs; m. adductor was chosen by virtue of its reduced function in Splayleg pigs which prevents the hindlimbs of the affected piglet from maintaining a normal stance.
Bradley et al. (1980) discovered that the myofibrillar hypoplasia of both m. adductor and m. sartorius was not confined to piglets exhibiting Splayleg symptoms but was a normal occurrence in piglets after birth until one week of age. Although a quantitative assessment of the degree of myofibrillar hypoplasia was not established there appear (from the electron micrographs presented) to be no profound differences between the myofibrillar content of the muscle fibres from normal and Splayleg piglets.

Swatland (1976b), from observations on the longissimus muscle of pigs of a liveweight range from 24 - 139 kg, found that the number of myofibrils within cross sections of 'red' fibres, staining positively for reduced diphosphopyridine nucleotide tetrazolium reductase, (DPNH - TR positive; indicative of oxidative capacity) was greater, and more highly correlated with liveweight \( r = 0.90 \), than the number within 'white', DPNH - TR negative (non-oxidative) fibres \( r = 0.66 \). These observations appear to contradict the results of the present study that established the myofibrillar percentage volume as being highest in the non-oxidative (white) fibres than in the oxidative (red) fibre types. This might be explained by a divergence in the TSA of myofibrils between the two fibre types; oxidative fibres may possess more, but smaller, myofibrils amounting to a lower percentage volume than found in the non-oxidative fibres. Although measurements of myofibrillar TSA were neither presented in the results of Swatland's (1976b) work nor undertaken in the present study observations suggest, as illustrated in Figure 13 (C and D), that oxidative fibres do, indeed,
tend to possess smaller diameter myofibrils.

The high correlation between myofibrillar percentage volume of non-oxidative fibres and liveweight \( (r = 0.727) \) was emphasised by the myofibres of the two severely runted piglets \( (9 \text{ and } 15 \text{ days old}) \) in this study \( (\text{as mentioned in the Results}) \). The high degree of myofibrillar hypoplasia in this myofibre type of these piglets was reminiscent of piglets less than 6 days old, which weighed less than 2200g \( (\text{an apparently critical weight associated with the attainment of a myofibrillar content equivalent to that within oxidative fibre types}) \). This demonstrates the relative dependence of non-oxidative fibre myofibrillar proliferation on the growth of the animal postnatally and illustrates the fact that myofibrillar hypoplasia is not a pathological condition but a consequence of the stage of muscle development; its existence in piglets over 6 days of age was a reflection of hindered muscular development in association with general body growth retardation.

The existence of a significantly greater \( (P < 0.025) \) correlation between the non-oxidative myofibrillar percentage volume and age \( (r = 0.924) \) than liveweight \( (r = 0.727) \) is indicative of the developmental nature of the process of the acquisition of myofibrils in this fibre type relative to cell volume; this strongly suggests that there is an age-related factor in the attainment of myofibrillar percentage volume in the non-oxidative fibres. The accumulation of myofibrillar mass is probably more related to the animal's liveweight than the percentage volume of myofibres in these non-oxidative fibres. This phenomenon would explain the finding of
equivalent percentage volumes of myofibrils between littermates despite their liveweight differences.

4.4.2 Birthweight and Myofibrillar Development

Helander and Thulin (1962) found that there was a direct relationship between the maximal isometric tension in the skeletal muscles of cat and rabbit limbs and their total myofilament TSA (determined biochemically). This relationship was further substantiated by the decrease in maximum tetanus tension produced in the muscles of mice after partial starvation for 7 days; the decrease in strength being proportional to the decrease in size and number of the myofibrils (Goldspink 1965). Such a relationship indicates that the muscles of young (or exceptionally growth retarded) piglets are relatively weaker than the muscles of older pigs (that are showing liveweight gain) on a per-unit-TSA basis due to their comparatively lower myofibrillar percentage volume. Maximum muscle strength is realised when myofibrillar percentage volume has reached an upper limit and remains constant with growth (over 46 days of age was suggested by the present study).

The failure of the myofibres of the low birthweight littermates (that were exhibiting normal growth rates) to present significant deficiencies in their percentage myofibrillar volumes made it apparent, in view of the reputed relationship between myofibrillar TSA and muscle strength, that there was no apparent effect on the strength per unit TSA of the muscle of light, relative to heavy, birthweight pigs. However, when severe prenatal growth retardation was associated with a lack of postnatal liveweight gain
the muscles of the affected piglet presented a lower degree of myo-
fibrillar proliferation than was characteristic for their age and,
therefore, they presumably possessed muscles that were unable to
exert an equivalent force to those of their large littermates.

The significantly greater (P < 0.025) percentage volume of
myofibrils in the oxidative myofibres of the small, relative to
the large, littermates may be due to the fact that the myofibrillar
content of oxidative fibres does not appear to be very highly
correlated with age (r = 0.445) or live weight (r = 0.450). The myo-
fibrillar percentage volume of this myofibre type might, perhaps,
be a reflection of the pig's relative growth rate (during the first
growth phase, 0 - 12 days, the small pigs did indeed have a
greater, although not significantly greater, growth rate than the
large and runt littermates: Figure 1) which may be an indication of
the rate of protein synthesis and the pig's nutritional status.

4.4.2. Occurrence Of Glycogen Granules

The granular material observed in the non-oxidative myofibres
of neonatal piglets (Figure 13B), due to the density and large size
of the granules, was considered to be glycogen (Deutsch and Done
1971; Bradley et al. 1980), in abundance from 35 days' gestation
(Campion et al. 1981). A compliment of ribosomes, smaller in size
than glycogen granules and forming a rosette-type pattern within
myofibres (Deutsch and Done 1971), probably comprises a part of the
granular material; although small granules were detected, their
characteristic conformation was obscured by the high glycogen con-
tent. However, in non-oxidative myofibres of the extreme runts (9
and 15 days old) the granular material was less dense (illustrated in Figures 18A and B) than in neonates and lacked large granules, the distribution of this material corresponding to that of ribosomes as described by Deutsch and Done (1971).

The loss of glycogen granules, coinciding with the attainment of a high myofibrillar protein content (also observed by Bradley et al. 1980) was probably associated with a change in cellular metabolism after birth; in particular GPase, which utilises glycogen, was demonstrated histochemically between 6 and 19 days of age (at a liveweight of approximately 2.5 kg) in the myofibres of m. semitendinosus in this study (see Chapter 3). Dalrymple et al. (1973) found that biochemically determined glycogen levels of porcine semimembranosus and trapezius muscles dropped from a high level at birth to 'adult' levels by one week of age which coincided with an increase in the GPase activity above a value of 20 μM Pi/minute/g of tissue, this enzyme being shown, biochemically, to be active at birth while it was not demonstrated histochemically (Chapter 3). Although the severe runts were of a liveweight below that apparently associated with the histochemical demonstration of myofibre GPase activity (2.5 kg) the apparent lack of glycogen granules probably indicated that GPase was active in their myofibres, although was not of a sufficient level to be manifest by histochemical techniques.

4.4.4. Mitochondrial And Lipid Droplet Content Of Myofibres

Both the oxidative and non-oxidative fibres exhibited a considerable rise in mitochondrial percentage volume within a few days
after birth; the mitochondrial percentage volume at birth, and the increase thereafter, was highest in the oxidative fibres (as previously established: Gauthier 1968; Salmons et al. 1978; Galvas et al. 1982). This phenomenon is indicative of an enhanced mitochondrial proliferation which, proceeding by fission, was more or less sustained in proportion with fibre TSA (ie mitochondrial percentage volume remained approximately constant) between about 4 and 33 days postnatally in both fibre types (Figure 16). Between 33 and 46 days the rate of mitochondrial proliferation appeared to decline in relation to fibre TSA, to be maintained in proportion with fibre TSA thereafter, a factor indicated by the apparent lack of a marked alteration in mitochondrial percentage volume after 46 days.

The mitochondrial percentage volume in both oxidative and non-oxidative fibres appeared to decline just before weaning which is suggestive of a change in cellular metabolism related to dietary adjustments; the high-fat milk diet consumed from birth was gradually replaced by the voluntary consumption of creep feed (see Materials and Methods, Chapter 1) prior to weaning to allow a gradual adjustment to the solid diet after weaning.

In the oxidative fibres the mitochondrial percentage volume changes were paralleled by the percentage volume changes in lipid droplets. The close structural association between mitochondria and lipid droplets (Gauthier and Padykula 1966) is due to the use, by enzymes localised within mitochondria, of lipid substrate for the oxidative reactions performed by these enzymes to provide energy (in the form of ATP) for cellular metabolic processes. A relation-
ship between plasma lipid values and muscle oxidative capacity is suggested by the work of Kraeling et al. (1978) who found that blood triglyceride bore a direct relationship with muscle SDHase activity in pig foetuses. The percentage volume of lipid droplets in the oxidative fibres exhibited a considerable rise between birth and weaning which was probably a reflection of the high-fat milk diet, and therefore higher plasma lipid content, of the suckling piglets. Bradley et al. (1980) also found intrafibre lipid droplets to increase after one day of age. Moody et al. (1978) demonstrated a dramatic rise in the chloroform-extracted muscle lipid concentration from 1 - 3 days and thence, until 28 days, it remained constant; with increasing age there was a reduction in the proportion of fibres staining positively for intrafibre lipid (demonstrated by Oil Red O) and a gradual increase in the amount of interfascicular lipid.

Suzuki (1974) suggested that the oxidative fibres (exhibiting a positive reaction for β-hydroxybutyrate dehydrogenase) of starved sheep possessed the ability to include fatty droplets, mobilised from body stores, to compensate for the lack of dietary energy intake. The apparent reflection (in the present study and that of Suzuki, 1974) of high plasma fat content within the oxidative fibres alone might be due to the relatively higher capillary density and blood flow of oxidative (SO) fibres than non-oxidative (FG) fibre types (Mackie and Terjung 1983), or the capacity of the fibre type to utilise lipids, as suggested by Suzuki (1974).

The results of the present study tend to suggest that the
ultrastructural diversification of fibre types may not parallel their acquisition of histochemical differentiation. Oxidative fibres from the deep portion of m. semitendinosus inevitably maintained their positive SDHase staining throughout the course of this study (see Chapter 3) but the non-oxidative fibres of the superficial portion of this muscle were shown, histochemically, to exhibit a capacity for oxidative metabolism at birth, eventually losing it between 6 and 19 days of age. These observations do not correspond with the mitochondrial percentage volume estimations determined for non-oxidative fibres which presented values at birth, when these fibres were SDHase positive, equal to values at 84 days when SDHase staining was negligible. It would therefore appear that the SDHase activity of a myofibre was not necessarily reflected by mitochondrial content.

One of the most interesting points to come out of this work was the discovery that the low birthweight pigs (exhibiting postnatal growth) had muscles of equivalent ultrastructural composition to those of their heavier littermates. This indicates that the muscles of low birthweight piglets develop and probably function biochemically and physiologically as well as those of heavier birthweight pigs, the quality of individual myofibre type subcellular structure evidently being unimpaired by growth restriction in utero.
5. OBSERVATIONS ON GIANT FIBRES

5.1. INTRODUCTION

During the course of observations made on the porcine muscle investigated in this study, myofibres were noted, both with the light and electron microscope, that resembled previously reported 'giant' fibres (Cassens et al. 1969; Hendricks et al. 1971; Bader 1982). In mature pig muscle, these fibres are characteristically transverse circular in section with a larger diameter than surrounding myofibres. Fibres with these specifications were discerned in the foetal muscle of humans by Fenichel (1963), and Wohlfart (1937) who classified them as '8' fibres, although there are no reports of the presence of such fibres in foetal pig muscle.

There is a reported association between giant fibres and PSE (pale, soft and exudative) muscle from stress-susceptible pigs. Cassens et al. (1969), Cooper et al. (1969) and Dutson et al. (1978) found that giant fibres, although they occurred inconsistently in pork exhibiting PSE characteristics, were never found in normal muscle. Giant fibres bear a marked resemblance to some myofibres of dystrophic animals of various species as discussed by Dutson et al. (1978). In particular, muscle from subjects suffering from Duchenne and Becker-type dystrophy possesses dense, round fibres larger than adjacent fibres (Schmalbruch 1982) that are segmentally contracted and in the initial stage of segmented necrosis. It therefore seems feasible to suggest that giant fibres might represent some pathological change within the muscle.
As giant fibres were present in the pig muscle of the present study they were considered an interesting anomaly to investigate further and so extend the limited, and often contradictory, literature available on their histochemical and ultrastructural characteristics and so, possibly, elucidate an explanation for the existence of these myofibres with their 'peculiar' staining properties and structural abnormalities.

5.2. MATERIALS AND METHODS

Material, from which giant fibres were recognised, came from the semitendinosus and trapezius muscle samples of the Large Whites in this study that had been prepared for both light and electron microscopy as described in Chapters 2, 3, and 4.

It should be noted that none of the pigs, whose muscle contributed to this study, exhibited symptoms of stress-susceptibility or were the progeny of PSE muscled pigs. PSE muscle had, in the past, been identified in pigs of this Large White herd on meat inspection at a commercial abattoir and these animals had been eradicated from the herd.

Through the histochemical demonstration of both acid and alkaline preincubated ATPase in fresh frozen sections the characteristic size and shape of giant fibres, as described in the literature, enabled their identification after which their staining properties and occurrence were ascertained. For EM observations giant fibres were first identified in 1µm thick plastic sections cut on a Reichert OMU3 microtome from muscle samples, prepared and mounted
for transverse sectioning as described in Chapter 4. These sections were dried onto slides with double distilled water, stained with 1% toluidine blue for 2 to 3 minutes, rinsed and then viewed under a light microscope; giant fibres were once again distinguished by their size and shape. Sections containing giant fibres were cut for EM, stained with uranyl acetate and lead citrate, and then viewed and photographed on a Philips 400 EM. On the electron micrographs qualitative and quantitative observations were made of the giant fibre ultrastructure as for normal myofibres (as described in Chapter 4). A few selected blocks from which these transverse sections originated were then trimmed and reorientated 90 degrees to enable the preparation of longitudinal sections (LS) from the same fibres observed in TS. This method eliminated the difficulty in recognising giant fibres in LS under the EM, a procedure that has rarely been reported.

5.3. RESULTS

5.3.1. Observations On Occurrence

Giant fibres were histochemically discernible in the muscle of animals of 12 days of age or older. Prior to 12 days the staining was not intense enough within fibre types to permit the distinction of giant fibres. Also at this young age giant fibres did not exhibit TSAs of sufficient disparity to enable identification from adjacent fibres on this basis, nor did they present the clearly defined circumferences of mature giant fibres (characteristics evident in Figures 19 and 20). The latter feature was, in the older animal, associated with the close packing of fibres in muscle
sections in which case giant fibres appeared to be separated from adjacent fibres; in the young animal myofibres are loosely packed so this phenomenon was not exhibited.

Giant fibres were observed in m. semitendinosus from 17 of the 20 animals (85%) old enough to present giant fibres under the light microscope, and in m. trapezius 9 of the 20 animals (45%). In both muscles under 1% (0.18 ± 0.39: mean ± SD) of the total myofibre population were classified as 'giant' in all but one littermate (small, 46 days) that contained 2.5% of its m. semitendinosus myofibre population as giant fibres; this muscle bore traits of PSE muscle, i.e. it was of abnormally pale colouration, was flabby and watery.

5.2.2. Observations On Histochemical Staining Properties

All recognised giant fibres possessed the capacity for oxidative metabolism, as demonstrated by staining for SDHase, but appeared to exhibit a negligible propensity for glycolytic metabolism, none being found that reacted positively for GPase (Figure 19). Giant fibres were classified as either alkaline ATPase-positive or negative (Figure 20) although the staining of giant fibres was of a greater intensity than adjacent normal fibres (Figure 19). Of the giant fibres distinguished in m. semitendinosus 64% exhibited 'slow' fibre histochemical staining properties while in m. trapezius this value was 53%.

Alkaline ATPase readily distinguished giant from normal myofibres (in animals of 12 days of age onwards) by making evident a
Histochemical staining characteristics of an 'SO'-type giant fibre (6).

The fresh frozen sections came from the deep portion of m. semitendinosus of the small, 84 day old, littermate (liveweight of 21750g) and were stained for the demonstration of:

A  Alkaline ATPase
B  Acid ATPase
C  GPase
clear ring around the giant fibres (Figure 20), a phenomenon not exhibited to such a marked extent through the demonstration of acid ATPase, SDHase or GPase activity.

Staining with hematoxylin and eosin (H and E) revealed that, whereas normal myofibres were generally pale pink, the giant fibres exhibited a deep pink colouration dotted with many blue particles.

5.3.3. Observations On Ultrastructure

Observing transverse sections of muscle under the EM revealed the presence of giant fibres in the muscle of neonatal animals (they were not detected at this age under the light microscope) when they were characterised primarily by a greater myofibrillar staining intensity (indicative of a higher protein concentration: Figure 21) than surrounding fibres.

Table 11 displays the percentage volume occupied by organelles of some giant fibres identified from m. semitendinosus of a few littermates, and the composition of the average myofibre from the same portion (deep or superficial) of the muscle of the littermate concerned. A comparison of these results revealed that, on average, giant fibres possessed a greater percentage volume of mitochondria (53% more), lipid droplets (256% more) and myofibrils (26% more) than the normal myofibre, while possessing about 51% less sarcoplasmic reticulum and 61% less remaining sarcoplasm (demonstrated visually in Figure 22A). This figure also shows the greater staining intensity observed in the nuclei of giant fibres as opposed to those of normal myofibres. With a higher magnification (x 60,000)
Figure 20

Staining properties of two giant fibres (G) with respect to the histochemical demonstration of alkaline ATPase.

This muscle sample came from the deep portion of m. semitendinosus of the small littermate of 46 days of age (liveweight of 8000g).

\[ G_1 = \text{Alkaline ATPase positive} \]
\[ G_2 = \text{Alkaline ATPase negative} \]

Figure 21

Early appearance of giant fibre as identified under the EM (x6000).

The muscle sample was from the deep portion of m. semitendinosus of the neonatal runt littermate (liveweight of 900g).
Electron micrograph to demonstrate the ultrastructural properties of a 'giant' myofibre and an adjacent 'normal' myofibre.

These muscle fibres were from the deep portion of m. semitendinosus of the large littermate of 84 days of age (liveweight of 26750g).

A  TS  x6500

B  LS  x8000

Note abundant mitochondria (M) and lipid droplets (L), and greater electron density of nucleus (N) and myofibrils (My) of the giant fibre.
it was evident that giant fibres failed to exhibit the hexagonal array of actin and myosin myofilaments seen in normal fibres preventing the identification of these filaments (Figure 23).

Table 11 - The Percentage Organelle Composition Of Giant And Normal Fibres From M. Semitendinosus Of A Few Selected Littermates.

<table>
<thead>
<tr>
<th>Littermate and Age</th>
<th>Fibre Type</th>
<th>Mitochondria</th>
<th>Lipid Droplets</th>
<th>Myofibrils</th>
<th>Sarcoplasmic Reticulum</th>
<th>Remaining Cytoplasm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Runt 0 days</td>
<td>Normal (n=10)</td>
<td>10.67</td>
<td>3.47</td>
<td>43.23</td>
<td>5.10</td>
<td>38.22</td>
</tr>
<tr>
<td></td>
<td>Giant (n=1)</td>
<td>21.99</td>
<td>7.76</td>
<td>56.03</td>
<td>3.02</td>
<td>11.20</td>
</tr>
<tr>
<td>Small 4 days</td>
<td>Normal (n=10)</td>
<td>8.11</td>
<td>2.93</td>
<td>39.62</td>
<td>7.06</td>
<td>42.28</td>
</tr>
<tr>
<td></td>
<td>Giant (n=1)</td>
<td>11.23</td>
<td>7.25</td>
<td>68.12</td>
<td>3.99</td>
<td>9.41</td>
</tr>
<tr>
<td>Large 27 days</td>
<td>Normal (n=10)</td>
<td>19.32</td>
<td>15.31</td>
<td>48.02</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Giant (n=2)</td>
<td>15.59</td>
<td>7.81</td>
<td>61.25</td>
<td>2.59</td>
<td>12.78</td>
</tr>
<tr>
<td>Runt 33 days</td>
<td>Normal (n=10)</td>
<td>4.12</td>
<td>1.94</td>
<td>72.45</td>
<td>10.31</td>
<td>11.18</td>
</tr>
<tr>
<td></td>
<td>Giant (n=3)</td>
<td>13.08</td>
<td>10.37</td>
<td>65.41</td>
<td>4.85</td>
<td>6.29</td>
</tr>
<tr>
<td>Large 84 days</td>
<td>Normal (n=10)</td>
<td>11.27</td>
<td>1.84</td>
<td>53.68</td>
<td>7.87</td>
<td>25.34</td>
</tr>
<tr>
<td></td>
<td>Giant (n=3)</td>
<td>13.64</td>
<td>13.31</td>
<td>58.09</td>
<td>2.77</td>
<td>12.19</td>
</tr>
</tbody>
</table>

Figure 228 shows a giant fibre in LS adjacent to a normal myofibre and dramatically emphasises the higher percentage volume of
Figure 23

High power electron micrograph (x130000) of transversely sectioned myofilaments of

A Normal fibre

B Giant myofibre

The myofilaments of the giant fibre fail to exhibit the characteristic hexagonal array evident in the normal myofibre.
mitochondria and lipid droplets quantified for the giant fibres. This figure also enables a comparison of the myofibrillar protein banding pattern in these two contracted fibres. The characteristic bands evident in the normal fibre are absent in the giant fibre and they appear to exhibit only one type of broad, kinked band, the distance between which is about 1/3 the length of the sarcomeres (Z-band to Z-band) in normal fibres. In LS giant fibres could be seen in association with the evidently normal fibres (ie electron density of myofibrils the same as other surrounding normal fibres) that also appeared to exhibit enhanced contraction, evident by the reduced sarcomere length and apparent obliteration of normal banding pattern in places (Figure 24).

5.4. DISCUSSION

5.4.1. Occurrence

Although giant fibres have been exclusively associated with the muscle of low quality (PSE) or stress-susceptible pigs by some workers (Cooper et al. 1969; Dutson et al. 1978) they have been observed in the muscle of pigs not exhibiting such conditions (Cassens et al. 1969; Hendricks et al. 1971) although no workers have reported such a high incidence of giant fibres in the muscles of normal pigs as determined in the present study. In the results presented here 85% of animals more than 12 days old had giant fibres in their muscles as opposed to previous reports of 8% (Cassens et al. 1969) and 30% (Hendricks et al. 1971).

The breed of pig investigated by Cassens et al. (1969) was
Figure 24

LS of super-contrated giant fibre (central position) and adjacent myofibres (x 3000). A portion of one of the 'normal' fibres is also super-contrated (S) but fails to show the intense electron density of the myofibrils of the giant fibre.

Note the high concentration of mitochondria (M) throughout the giant fibre.
not specified while Hendricks et al. (1971) looked at Poland China pigs, an American breed. This suggests that the incidence of giant fibres is possibly a breed-specific trait enhanced in purebred pigs, as PSE pork is, which might suggest that pedigree Large Whites are either a particularly susceptible breed or that they have undergone a high degree of inbreeding.

Both Dutson et al. (1978) and Cassens et al. (1969) also found that when giant fibres occurred they represented less than 1% of the total myofibre population. However, the former authors obtained this value from low quality, PSE Yorkshire (American term for 'Large White') pig muscle alone whereas the muscle in the present study and that of Cassens et al. (1969) was not known to be of poor quality. When, as in one case, the semitendinosus muscle was found to exhibit PSE symptoms the incidence of giant fibres was much greater, occupying 2.5% of the total myofibre population.

5.4.2. Histochemical Staining Properties

Some previous researchers have found giant fibres to be exclusively alkaline ATPase-positive (Cassens et al. 1969) and peripherally located within fascicles (Hendricks et al. 1971), the characteristic location of histochemically defined fast fibres in pig muscle, although Bader (1982) observed a minority to exhibit negative alkaline ATPase staining. The findings of the present work did not suggest that giant fibres are predominantly of fast histochemical staining characteristics since in both m. semitendinosus and m. trapezius slightly more than 50% were classified as 'slow' fibres. Although giant fibres of the present study were found to
exhibit high oxidative (and non-glycolytic) staining; the majority have previously been defined as of intermediate oxidative capacity (Cassens et al. 1969; Dutson et al. 1978) and even those exhibiting a near negative oxidative staining reaction have been observed (Cassens et al. 1969). The glycolytic capacity of giant fibres has only been defined in the literature by Cassens et al. (1969) whose work supports the view that these fibres exhibit a negligible propensity for glycolytic metabolism. This concept does not tally with the reported association of giant fibres and PSE pork which is characterised by the presence of a high proportion of 'intermedi- ate' (Cooper et al. 1969) or 'white' (Swatland and Cassens 1973b) fibres that stain positively for GPase.

The darker H and E staining, together with blue particles, observed in the giant fibres is indicative of basophilic inclusions, a result reflected by the giant fibres in the muscle studied by Dutson et al. (1978), a pattern of staining that might represent a large RNA content within the fibres (Adams et al. 1962).

5.4.3. Ultrastructure

Many of the ultrastructural anomalies exhibited by the giant fibres of the present study have also been observed in Large White pig muscle by Dutson et al. (1978). These workers also found that nuclei of giant fibres presented denser staining than those of normal muscle fibres which suggested that the nucleoprotein of these fibres' chromatin was tightly coiled and was not in the process of genetic information transcription. Hence giant fibres would appear
to possess a reduced synthesising capacity associated with a lowered cellular activity. This phenomenon does not correspond with the apparently high RNA content, as mentioned above, which is indicative of a heightened cellular activity. Altogether the productivity status of giant fibres cannot be clearly defined and appears contradictory which in itself is an indication of deranged cellular activity.

The disarray of myofilaments seen in both transverse (Figure 23) and longitudinal (Figure 24) sections of giant fibres under the EM is suggestive of the super-contracted myofibres observed in unfrozen and prerigor frozen ovine semitendinosus muscle due to various postmortem incubation temperatures by Cook and Wright (1966). Super-contraction would cause the myosin filaments to exert considerable strain on the Z-discs, either rupturing these discs or causing the myosin filaments to fold up in the area of the Z-discs. This would, as Dutson et al. (1978) proposed, explain the lack of characteristic myofibrillar banding, the thickness and kinking of the bands evident in LS, and the absence of the hexagonal orientation of myofilaments in TS together with the increased myofibrillar packing as observed by Bader (1982).

Bradley et al. (1980) observed occasional myofibres in the periphery of sections of muscle from one day old piglets (normal and Splayleg) containing electron dense myofibrils whose internal structure was uncharacteristic of normal myofibres. These fibres, which appear to identify with myofibres defined as giant fibres in the neonatal muscle of the present study, were interpreted by Brad-
ley et al. (1980) as super-contracted fibres that were merely an artefact of the preparation procedure. If the fibres observed by Bradley et al. (1980) are indeed consistent with the 'giant' fibres of the present study they cannot be assumed to be artefacts due to their increased percentage volume of mitochondria and lipid droplets (yet to be discussed).

Dutson et al. (1978) found no recognisable mitochondria or sarcoplasmic reticulum in the giant fibres of the muscle they studied but numerous vacuolar structures. It is possible that giant fibres are more susceptible to postmortem lysis (a consequence of their deranged properties) and the 24 hour lapse between death and muscle fixation in the study of Dutson et al. (1978) might have been sufficient to execute this breakdown. Evidence for the concept that the subcellular components of abnormal muscle fibres are more susceptible to postmortem disruption is provided by the observations made by Cloke et al. (1981) on the effects of severe freeze-thaw contraction on muscle samples from normal and PSE porcine muscle; the membrane-bound organelles of PSE muscle were markedly disrupted after this treatment indicating their relatively enhanced fragility.

The giant fibres in the present study (which were fixed within 1/2 an hour of death) clearly exhibited an excessive number of well preserved mitochondria and sarcoplasmic reticulum (Figure 22). The enhanced oxidative capacity of the giant fibres, as recognised by the greater percentage volume of mitochondria and lipid droplets than in normal fast or slow myofibres, is consistent with the
changes seen within muscle fibres subjected to prolonged contraction, and cannot merely be explained as a preparation artefact. The fast myofibres of genetically spastic mice, a condition that subjects the muscles to increased nervous activity, are seen to adapt to the almost continual stimulation by an increased capacity for oxidative metabolism. The increased activity of muscle induced by exercise, as reviewed by Salmons and Henriksson (1981), has been shown to be associated with an increased mitochondrial volume, significantly above that of control fast and slow muscles, and also dramatically reduced sarcoplasmic reticulum, which considerably affects calcium ion uptake from the myofibre and consequently muscle relaxation.

Intense physical work to the point of fatigue has been found, in the anterior tibialis muscle of rats (Schmerling et al. 1981), to disturb the myofibrillar organisation and cause the destruction of mitochondria (which might be related to the apparent absence of mitochondria in the giant fibres observed by Dutson et al. 1978). This supports the concept, previously mentioned, that functionally stressed myofibres are more prone to postmortem lysis. The enhanced metabolism of the fibres themselves was probably not responsible for the disruption of the mitochondria in view of the perfect preservation of mitochondria (evident in Figure 22) in the obviously functionally over-loaded giant fibres observed in this work; the EM procedure employed clearly afforded optimal conditions to combat this effect on organelle lysis.
5.4.4. Possible Cause Of Giant Fibre Abnormalities

Although giant fibres have been compared with myofibres of certain muscular dystrophies (Dutson et al. 1978) such myopathies are invariably associated with degenerative changes (Shafiq et al. 1969; Schmulbruch 1982), no signs of which were observed in the giant fibres of the present study.

The observations of metabolic changes associated with combating the effects of fatigue support the concept of an active functional abnormality in giant fibres inducing sustained, high-intensity contraction. The super-contraction of giant fibres was occasionally observed to elicit a similar hyper-contractile effect in adjacent fibres (Figure 24) which, due to its inconsistency along the length of the myofibre, suggests that it was possibly a passive effect generated by physical contact with the giant fibre. Super-contracted portions of normal fibres did not possess myofibrillar electron density as intense as that seen in giant fibres. This phenomenon, together with the abnormally high ATPase (alkaline stable or labile) activity of the giant fibres suggests that the extent of super-contraction within giant fibres was sufficiently chronic to induce compensatory myofibrillar proliferation and enhanced ATPase activity. It is therefore proposed that giant fibres are a phenomenon of normal pig muscle, perhaps resulting from some structural defect, such as an inadequate amount of sarcoplasmic reticulum, eliciting hyper-contractile activity and structural anomalies within the fibre.
5. EFFECT OF BREEDING FOR LOW AND HIGH BODY WEIGHT ON PRENATAL MUSCLE DEVELOPMENT IN THE MOUSE - A PRELIMINARY INVESTIGATION

This pilot study was performed between April and May of 1983 with facilities made available at the Department Of Anatomy and Cellular Biology, Tufts University, Boston, Massachusetts, USA. The material, prepared for EM, was supplied by Professor G Goldspink, Department of Zoology, Hull University.

5.1. INTRODUCTION

Selection for body weight, as discussed in Chapter 2, produces concomitant changes in muscle bulk in mice (Luff and Goldspink 1967; Hooper 1982), and in pigs (Miller et al. 1975), which these workers found to be primarily attributable to changes in the total myofibre number within the muscles.

Luff and Goldspink (1987) found that mice bred for a low body weight at 6 weeks postnatally (QS strain) had up to 30% fewer fibres in their biceps brachii, anterior tibialis and extensor digitorum longus muscles than those bred for a higher body weight (QL strain) at this age. Thus, a genetic effect on muscle fibre number development, expressed in utero, is apparent in the adult mouse. As alluded to in Chapter 2, there is a marked effect on prenatal muscle development resulting in a reduced muscle fibre number (approximately 17 - 19%) in the nutritionally deprived animal (rat: Bedi et al. 1982; pig: Wigmore and Stickland 1983). Wigmore and Stickland (1983) concluded that this apparent nutritional effect on muscle fibre number was caused by a reduced secondary: primary
fibre number ratio in the smaller pig, the primary fibre number remaining unaffected; this hypothesis was further confirmed by the results discussed in Chapter 2. The smaller diameter primary fibres of the undernourished prenatal pig have a reduced surface area (Wigmore and Stickland 1983) on which the myoblasts can align and fuse to form secondary myofibres and, thus, fewer fibres are able to develop, in contrast to the situation in its heavier littermates which possessed larger diameter primary fibres.

The question raised by this is, “Do the genetic and nutritional factors bring about their effects on total muscle fibre number by the same mechanism?”. The aim of this preliminary investigation was to elucidate the mechanism of the reduced fibre number seen in the QS, relative to the QL, strain of mice.

6.2. MATERIALS AND METHODS

Limbs, which provided the muscle material for this investigation, were from foetal mice of the QS and QL strains bred at the Zoology Department, Hull University. The progenitors of these two strains of mice were initially bred by Falconer (1960) from the same original stock by selection for size between 3 and 6 weeks of age; the mature body weight of the large (QL) strain was approximately twice that of the small (QS) strain. The limbs were fixed and embedded for EM at Hull University. Material was available from mice of 14, 16, 18, and 20 days' gestation, plus neonates.

An average of five limbs were available from each strain of each age. Each limb was sectioned from the proximal end and sec-
tions of 1 μm thickness, were stained with 1% toluidine blue for light microscopy. The sections were viewed with an oil immersion lens (x63) to identify the primary and secondary myofibre populations (see Introduction of Chapter 2 for the relevance of these two myofibre populations). The two fibre populations were distinguished morphologically, primary fibres having the greater diameter and, often, centrally located nuclei or a myofibrillar-free region, as seen in foetal pig muscle by Wigmore and Stickland (1983).

A clear distinction between the two fibre populations was only possible in the foetal mouse muscle of 16 days’ gestation. From the limbs of this age the only muscle that could be readily identified in more than one limb was m. extensor carpi radialis, which was recognised in two limbs of each strain. Total myofibre counts were not attempted as the position of sectioning relative to the muscle length could not be ascertained. Therefore, differences in the secondary: primary fibre number ratios only, between the two strains, were estimated.

Sections were obtained from the relevant muscle blocks and stained for EM (as described in Materials and Methods, Chapter 4). Electron micrographs of randomly selected areas were taken (x 2400) with a Philips 200 EM. The secondary and primary fibre numbers were estimated from the electron micrographs (total magnification x 6000) together with measurements of the "lesser fibre diameters" (Brooke 1970) of these two fibre populations. The "lesser fibre diameter" measures the maximum diameter across the aspect of the fibre perpendicular to the longest axis. This measurement is
designed specifically to allow for the distortion which occurs when myofibres are cut obliquely, and is particularly applicable to small samples where the plane of sectioning, relative to the fibre length, cannot easily be controlled.

The significance of differences between parameters were evaluated using Student's 't'-test.

6.1. RESULTS

The results of secondary: primary fibre number ratios, determined by light and electron microscopy, for the two strains of mice at 16 days are displayed in Table 12. There were no significant differences \( (P > 0.25) \) between the secondary: primary fibre number ratios of the two strains of mice as determined by either microscope. Although the secondary: primary fibre number ratio, as determined by EM, was not significantly less \( (P > 0.05) \) than that determined by light microscopy in the QS strain, the difference was significant \( (P < 0.01) \) in the QL strain. The EM probably gave the most correct evaluation of the absolute ratio as the high magnification enabled myofibres to be distinguished from non-muscle cells (such as fibroblasts) which may well be counted as secondary myofibres under the light microscope. For comparative work, however, those values determined by light microscopy were adequate; although the light microscopy appeared to introduce an over-estimation of the ratio, it was consistent.
Table 12 - Secondary: Primary Fibre Number Ratios In The QS And QL Strains At 16 Days

<table>
<thead>
<tr>
<th>Secondary: Primary Fibre Number Ratio Determined By:</th>
<th>Limb No.</th>
<th>QS</th>
<th>QL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light microscopy</td>
<td>1</td>
<td>2.64 (n=80)</td>
<td>2.64 (n=94)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2.01 (n=98)</td>
<td>2.21 (n=126)</td>
</tr>
<tr>
<td>(Total mean ± SD)</td>
<td></td>
<td>2.29 ± 1.11</td>
<td>2.40 ± 1.07</td>
</tr>
<tr>
<td>Electron microscopy</td>
<td>1.94 ± 1.14 (n=48)</td>
<td>1.80 ± 1.21 (n=30)</td>
<td></td>
</tr>
</tbody>
</table>

The results of the fibre diameter measurements of secondary and primary fibres are shown in Table 13. At this age (16 days) the primary fibres were significantly greater (P < 0.001) in mean diameter than the secondary fibres in both the QL and QS strains. While the mean secondary fibre diameter of the QS strain was significantly greater (P < 0.05) than that determined for the QL strain, the primary fibres were significantly greater (P < 0.01) in diameter in the QL than in the QS strain. The primary: secondary fibre diameter ratios were thus 1.4 and 2.0 for the QS and QL strains respectively.
Table 13 - Mean Fibre Diameters Of Secondary And Primary Fibres From The QS And QL Strains

<table>
<thead>
<tr>
<th>Strain Of Mouse</th>
<th>Primary Fibre Diameter (µm)</th>
<th>Secondary Fibre Diameter (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>QS</td>
<td>4.80 ± 1.06 (n=28)</td>
<td>3.41 ± 0.85 (n=33)</td>
</tr>
<tr>
<td>QL</td>
<td>6.18 ± 1.16 (n=19)</td>
<td>3.02 ± 0.72 (n=21)</td>
</tr>
</tbody>
</table>

6.4. DISCUSSION

Due to the limited amount of material used in this pilot study the results must be considered somewhat tentatively although the indications have important implications to studies of prenatal muscle development.

This investigation suggests that the average difference of approximately 27% discovered in the total muscle fibre number between QS and QL strains by Luff and Goldspink (1967) in the biceps brachii, anterior tibialis and extensor digitorum longus muscles did not result from a difference in the secondary: primary fibre number ratio between the two strains. It would, therefore, appear that genetic factors act to cause the disparity in total muscle fibre number between different strains of a breed, as shown between strains of mice selected for divergent body weight (Luff and Goldspink 1967; Hanrahan et al. 1973) via the total number of primaries developing in the prenatal muscle. Thus a strain of
mouse, that has a higher mature body weight, has bulkier muscles containing more fibres (Hooper 1982) due solely to their inherently greater primary fibre number.

A confusing factor was the significantly greater \((P < 0.05)\) mean secondary fibre diameter of the QS strain as opposed to that determined for the QL strain, while the primary fibres of the latter group were significantly larger \((P < 0.01)\) than those of the former group (Table 13); this resulted in a greater diversity between the size of the two fibre populations in the QL strain. This meant that primary fibre size was greater in the QL than in the QS strain by a factor of 1.3, which was equal to the relative size difference found between the primaries of small, growth retarded, and large pig foetuses (of 82 days' gestation) by Wigmore and Stickland (1983). However, in the present study, the larger template bestowed by the primary fibres of the QL strain was not matched by a greater production of secondary fibres. This appears to contradict the hypothesis of Wigmore and Stickland (1983) that the relatively larger surface area of the primaries of well, as opposed to undernourished, pig foetuses enabled the formation of a greater population of secondaries. It is probable, however, that the maximum secondary: primary fibre number ratio of the m. extensor carpi radialis of the mouse was fulfilled in the QS strain, and was impossible to exceed in the QL strain.

The secondary: primary fibre number ratio as estimated by light microscopy of foetal mouse muscle of 16 days' gestation averaged at approximately 2.3 which was much lower than that of foetal
pig muscle of an equivalent gestational age, when it was estimated by Wigmore and Stickland (1983) to be 17.5. The secondary fibres in foetal mammalian muscle, once formed, are displaced from the primaries by successive generations of secondaries (Lamb: Ashmore et al. 1972b; Pig: Swatland and Cassens 1973a). Obviously the development of muscle in mice and pigs is not totally comparable since a mouse foetus spends much less of its time in utero (approximately 19 days; Evans and Sack 1973) than does a pig foetus (approximately 114 days: Perry 1956) and, therefore, its muscle development must occur during a shorter period of time when far fewer secondaries are formed.

The diversity in primary fibre size between the two strains of mice can be explained by the fact that, since the QL strain have heavier mature body weights than the QS strain they presumably carry larger foetuses; this is indicated by the results of Hammond (1944) which suggested that maternal size was the major determinant of offspring size in horses. Muscle fibre TSA has been directly related to liveweight by many workers, including Davies (1972). Indeed it was shown by Luff and Goldspink (1967) that the fibres of the heavier QL strain of mature mice were larger than those of the lighter QS strain by a factor of 1.2, a similar difference to that seen between the primary fibres of the two strains in the foetal mice of the present study. The secondary fibres, that develop after 14 days' gestation, have probably had insufficient time by 16 days to grow and develop the size difference manifest by this age between the primaries of these two strains.
It is probable that the primary fibre size was not a limiting factor in prenatal muscle development in the genetically small mice of this study. Presumably a genetic effect determines the maximum secondary: primary fibre number ratio possible for the muscle within any species and this ratio is manifest under normal circumstances, cases such as undernutrition in utero being a deviation below the norm.
GENERAL DISCUSSION

Considered as a whole the results of the various techniques employed in this study expressed one general and extremely important theme, namely, that the differential growth rates of pig littermates prior to birth failed to affect the rate of relative to liveweight, growth and development of the body and its component organs, including skeletal muscle, after birth. The equivalent rates of muscle growth and development among littermates at the gross level (Chapter 1) were not only reflected at the cellular, but also at the subcellular level; demonstrated by the attainment of myofibre type TSAs (Chapter 3) and the ultrastructural modifications that occurred with growth (Chapter 4). It was further demonstrated that the rate of changes occurring in the histochemically determined myofibre type profiles (Chapter 3) and therefore, presumably, the rate of changes in the physiological properties of the muscle were, interestingly, also similar between littermates of disparate birthweight.

The apparent absence of differential rates of growth and development among littermates after birth meant that the effects of prenatal growth restriction, evident in the low birthweight piglets, persisted throughout the duration of this study. This phenomenon also applies to the cellularity of the muscle (Chapter 2) which, since fibre number remains constant after birth, meant that the lower fibre number evident in the runt littermates was permanent. These results on the cellularity of the muscle are of most significance to the meat industry. The reduced m. semitendi-
nosus fibre number of the low birthweight pig is indicative of a lowered total muscle fibre number in the whole carcass (Stickland and Goldspink 1975). This lower fibre number suggests a reduced potential for meat production in the low birthweight pig relative to its heavier birthweight siblings, as exemplified most markedly by the two extremely runted piglets of the present study.

The two exceptionally runted animals suffered a very severely reduced total muscle fibre number that combined diminished secondary and primary fibre populations; fibre number reductions associated with low birthweight were usually executed through an affected secondary fibre population alone. This profound effect on the muscle cellularity of these animals appeared to be related to their lack of weight gain. From the observations made on the 'runts' of this study it was concluded that the exceptionally low absolute and relative birthweight of these two extremely runted piglets constituted what would best define a true 'runt', ie an animal exhibiting a comparatively reduced rate of growth and development; none of the other 'runts' satisfied this qualification. The definition of a runt is, therefore, proposed as an animal of a birthweight less than 50% that of its largest male littermate and more than 2.5 SD below its mean litterweight, stipulations far harsher than those previously defining a runt.

The relationship between muscle cellularity and general body growth is to be considered in a follow-up research project through the investigation of the relative muscle cellularity of low birthweight piglets exhibiting catch-up growth.
The differences in liveweight that persisted between littermates (Chapter 1) appeared responsible for the disparities in the body weight of various parameters, such as percentage occupied by organs, and fibre type percentages and TSAs, considered between siblings on either an age- or weight-constant basis. The littermates of disparate birthweight chosen for this study were of tremendous importance as they helped to reveal age, in association with the well documented liveweight effects on the development of some of the measured parameters. Liveweight gain can be kept minimal by dietary manipulations (Stickland et al. 1975) but one cannot halt the progression of biological aging. It would be of considerable interest to keep pigs at a relatively constant liveweight and compare, in particular, their muscle growth and development with that of well-nourished animals of the same age. In this way the developmental, as opposed to the growth aspects of skeletal muscle parameters, which are associated with the attainment of liveweight, could be defined more conclusively.
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<table>
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<tr>
<th>AGE (DAYS)</th>
<th>LITTERMATE</th>
<th>BIRTHWEIGHT (g)</th>
<th>LIVEWEIGHT (g)</th>
<th>M. SEMITENDINOSUS (g)</th>
<th>M. TRAPEZIUS (g)</th>
<th>TOTAL BRAIN (g)</th>
<th>CEREBELLUM (g)</th>
<th>LIVER (g)</th>
<th>KIDNEYS (g)</th>
<th>ADRENALS (g)</th>
<th>HEART (g)</th>
<th>CROWN-RUMP LENGTH (cm)</th>
<th>BACK-FAT THICKNESS (mm)</th>
<th>HUMERUS LENGTH (cm)</th>
<th>FEMUR LENGTH (cm)</th>
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### Appendix 2

**Total And Primary Fibre Number Counts For M. Semitendinosus And Estimated Secondary To Primary Fibre Number Ratio For Both M. Semitendinosus And M. Trapezius For All Animals**

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>Littermate</th>
<th>M. Semitendinosus</th>
<th>M. Trapezius</th>
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<tbody>
<tr>
<td></td>
<td>Total Fibre Number</td>
<td>Primary Fibre Number</td>
<td>Secondary: Primary Fibre Number Ratio</td>
</tr>
<tr>
<td>0</td>
<td>Large</td>
<td>4268912</td>
<td>15750</td>
</tr>
<tr>
<td></td>
<td>Small</td>
<td>405859</td>
<td>15635</td>
</tr>
<tr>
<td></td>
<td>Runt</td>
<td>391970</td>
<td>14867</td>
</tr>
<tr>
<td>1</td>
<td>Large</td>
<td>4668405</td>
<td>21384</td>
</tr>
<tr>
<td></td>
<td>Runt</td>
<td>4988935</td>
<td>18017</td>
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<tr>
<td></td>
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<td>19884</td>
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<tr>
<td>2</td>
<td>Large</td>
<td>565767</td>
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### Appendix 3A

**Percentages Of Fibre Types In The Deep Portion Of M. Semitendinosus**

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## Appendix 38

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* Littermates less than 9 days of age had 100% FO fibres.
## Appendix 4

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- FO/FG

And

Fibre Types Of

andosus

- FO/FG

Semitendinosus

- FG

FO/FG

FG

FG

FO/FG

FG

FO/FG

FG

FO/FG
Oxidative Myofibres From Littermates Of Ages 0 – 9 Days: Organelle Percentage Volume

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Appendix 6B

Non-Oxidative Myofibres From Littermates Of Ages 0 - 9 Days:
Organelle Percentage Volume

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<td>66.0 (3.0)</td>
<td>5.8 (1.4)</td>
<td>1.2 (0.4)</td>
</tr>
<tr>
<td>84</td>
<td>Large</td>
<td>63.5 (1.5)</td>
<td>4.8 (0.8)</td>
<td>2.1 (0.2)</td>
</tr>
<tr>
<td></td>
<td>Small</td>
<td>71.1 (1.3)</td>
<td>1.4 (0.2)</td>
<td>0.4 (0.2)</td>
</tr>
<tr>
<td></td>
<td>Runt</td>
<td>67.3 (1.7)</td>
<td>1.9 (0.4)</td>
<td>1.4 (0.3)</td>
</tr>
</tbody>
</table>