J. Anat. (1981), **132**, 4, pp. 557–579
With 10 *figures*
Printed in Great Britain

Muscle development in the human fetus as exemplified by m. sartorius: a quantitative study

N. C. STICKLAND

Department of Anatomy, Royal (Dick) School of Veterinary Studies, Edinburgh EH9 1QH

(*Accepted* 26 *August* 1980)

INTRODUCTION

The size of an individual muscle is determined by the number and size of its constituent muscle fibres and, to a lesser extent, by the amount of connective tissue. It is known from several studies on both laboratory animals and agricultural animals that the size of muscle fibres can be affected by many factors such as age (Hammond & Appleton, 1932; Meara, 1947; Joubert, 1956*a*; Goldspink, 1962; Stickland & Goldspink, 1973), exercise (Goldspink, 1964) and level of nutrition (Joubert, 1956*a*; Stickland, Widdowson & Goldspink, 1975). The number of muscle fibres, on the other hand, appears to be genetically determined, showing no significant change after birth in most animals studied including the pig (Staun, 1963; Stickland & Goldspink, 1973) and certain laboratory animals (Eliot, Wigginton & Corbin, 1943; Rowe & Goldspink, 1969).

It is evident that the number of muscle fibres in a given muscle increases before birth until the genetically determined number is attained. The time during gestation when hyperplasia ceases has seemingly not been investigated in any detail except for a few studies including those on sheep by Joubert (1955, 1956*b*) and Swatland & Cassens (1973*a*), and on the ox by Stickland (1978). It is very often stated, however, that hyperplasia in human muscle is completed some time before birth. This statement is based on either the extensively quoted work of MacCallum (1898) who used muscle fibre counts from m. sartorius of each of only five fetuses and one full term baby, or the work of Montgomery (1962) who also used m. sartorius but from only two fetuses and one full term baby. It could well be misleading to try to draw too many conclusions from such limited data, especially when it is appreciated how large is the adult variation in fibre number in the muscles used, as shown by the results of these authors on several adult muscles.

Most work on human skeletal muscle has, in fact, been concerned with descriptions of the various muscular disorders (e.g. Adams, 1975). It is often stated by muscle pathologists that a large number of muscle diseases may be caused by various abnormalities of muscle development *in utero*. One example is myotubular myopathy in which, according to Spiro, Shy & Gonatas (1966), there is persistence of fetal myotubes in extrauterine life. Although myotubes appear in normal neonatal rat muscle (Ontell, 1977) they are an abnormal phenomenon in neonatal human muscle. This stresses one of the reasons why comparisons between animal and human muscle can often be misleading. Apart from studies on muscle diseases there have also been various descriptive or qualitative approaches to prenatal muscle development in the human, from the light microscopic study of Hewer (1927) to the ultrastructural

0021-8782/81/2828-1250 $02.00

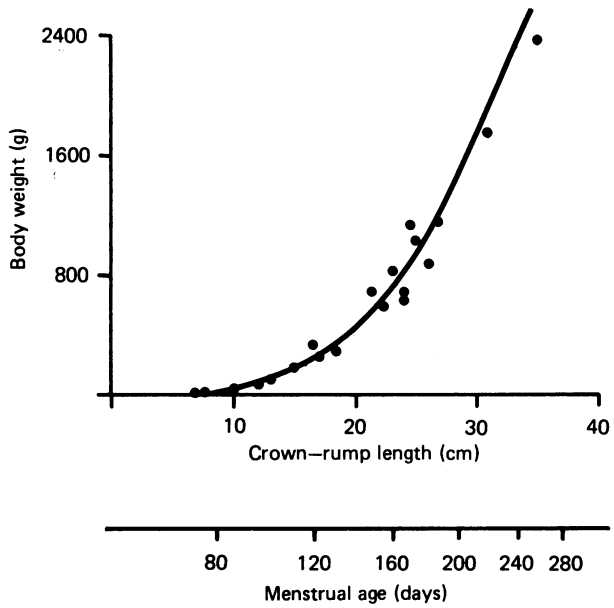

Fig. 1. The relationship between body weight and crown–rump length, and an indication of menstrual age (280 days = birth).

study of Tomanek & Colling-Saltin (1977). There has, however, been no comprehensive quantitative study of prenatal human muscle development based on an adequately large number of fetuses.

A study which defined, in quantitative terms, the pattern of normal muscle development in the human would be of interest to the pathologist studying developmental myopathies which could then be more clearly defined in quantitative terms.

The purpose of this investigation was, therefore, to quantify both the gross and cellular changes (including nuclei numbers, muscle cell size and numbers and myofibril proliferation) which take place in muscle development, but paying particular attention to the relative contributions of hyperplasia and hypertrophy to prenatal muscle growth.

MATERIALS AND METHODS

Muscle samples were removed from 21 fetuses which ranged in crown–rump length (CR) from 7·0 to 35·0 cm, which approximately corresponds to a range in menstrual age from 80 to 270 days (i.e. to about 10 days before birth: from the data cited by Langman, 1975). In order to overcome possible errors, it was decided to discuss the parameters investigated in relation to CR rather than an estimated age. CR may also be a more relevant parameter than age in this particular study as it is known that general growth is more related to the physiological age of an animal than the chronological age (Ragsdale, 1934). The weight of a fetus, although probably not so related to age as CR, may possibly be more closely related to some features of muscle development than CR and, therefore, another useful fetus parameter to consider in certain discussions. The fresh body weight (BW) and CR of each fetus used (together with an estimation of age) are shown graphically in Figure 1 which shows that, as one might expect, BW is approximately proportional to $(CR)^3$.

M. sartorius was removed entire from a hind limb of each fetus. The smaller fetuses had all been immersed in phosphate-buffered (pH 7·4) formalin solution so that the muscles could be removed fixed *in situ*. The muscles had to be removed fresh from the two largest fetuses and splinted at the *in situ* length (flexed limb position) before fixation. After this initial fixation, the length and weight of each muscle was noted. Complete transverse sections (2 mm thick) were then cut from each muscle at its mid-length level. These muscle sections (the larger ones cut into two or three segments longitudinally) were then placed in a phosphate-buffered (pH 7·4) paraformaldehyde–glutaraldehyde mixture (Karnovsky, 1965) for several hours before washing in phosphate buffer, post-fixation in 1 % osmium tetroxide and further washing. Dehydration was then carried out through a series of alcohols from 10 % to absolute, followed by clearing in propylene oxide and finally infiltration by, firstly, a mixture of propylene oxide and Araldite and then in Araldite alone, in which the tissue samples were also embedded. Transverse sections (1·5 μm thick) were cut from each block (with a few also cut longitudinally) on a Reichert OMU3 ultramicrotome using glass knives, and stained with 0·5 % toluidine blue.

Quantitative histology

The sections were first viewed under low power magnification and the image projected on to a screen so that the total section could be outlined thereby enabling total muscle cross sectional area to be measured. The sections were then viewed with a Leitz Ortholux microscope under × 40 and × 100 (oil immersion) objectives and several random photomicrographs obtained using a Leitz Orthomat camera attachment.

Numbers of cells and nuclei

The photomicrographs were used to estimate the numbers of muscle cells and nuclei per unit area. The area of the × 100 objective photomicrograph was 0·0054 mm^2 and this was taken as the unit area. The × 40 photomicrographs were used for the larger fetuses in order to cover a larger sampling area but not for the smaller fetuses, as cells in these were sometimes difficult to distinguish with this lower magnification. The results for the × 40 photomicrographs were always checked for accuracy by comparing counts from a few × 100 photomicrographs. On average, sufficient measurements were made so that cells in about 5 % of the total cross sectional area had been counted. The cells counted were classified as myotubes if myofibrils were peripheral with a clear area or nucleus in the centre, or myofibres if the myofibrils filled the cell and nuclei, if present, were peripheral. All nuclei were counted, no effort being made to distinguish satellite and other cell nuclei from muscle cell nuclei, as this was felt to be too difficult for accurate estimation at the light microscope level. However, there was the added advantage that direct comparisons could be made with biochemical estimations of DNA in developing muscle.

The total number of myofibres, myotubes and nuclei in the complete section of each muscle was estimated from the results of numbers per unit area and the total muscle cross sectional area measurements.

An estimation was made of the total number of nuclei in the complete m. sartorius of each fetus. This estimation was based upon the relationship derived by Abercrombie (1946), namely $N = (T/[T+D])\,n$, where N = the real number of nuclei whose centres are included in the section, n = the number of apparent nuclei

counted, T = the section thickness (1·5 μm), and D = the mean longitudinal dimension of the nucleus. The maximum nuclear length seen in the longitudinal sections which were made was found to be about 9·3 μm and was taken as the value of D. In this investigation, therefore, $N = 0{\cdot}1389\,n$. To obtain the total number of nuclei in each muscle this figure was then multiplied by $L/1{\cdot}5$, where L = muscle length (in μm) and 1·5 is the section thickness (in μm).

Size of cells

For each fetus the cross sectional area of 100 cells of each type (myotubes and myofibres) was measured using a planimeter (Clarkson's Zero-setting Compensating Planimeter) on the photomicrographs obtained with the × 100 objective. When the proportion of myotubes or myofibres was very small it was sometimes possible to measure only 50 cells. The mean cross sectional area of myotubes and myofibres for each fetus was then calculated. From these area measurements, diameters were estimated assuming the cross sectional areas to be circular, i.e. diameter = $\sqrt{([4 \times \text{Area}]/\pi)}$. These diameters were used to create histograms for some of the fetuses (CR 7·0, 7·5, 18·5, 23·0, 27·0 and 35·0 cm). Histograms of cell areas were not used as these give skewed distributions which obliterate many features of interest.

Intercellular space

The amount of intercellular space seen in the sections was calculated by multiplying the numbers of myotubes and myofibres per unit area (0·0054 mm^2) by their respective mean cross sectional areas. This gave the area in the cross section occupied by muscle cells (X mm^2), the percentage area being $(0{\cdot}0054 - X)/0{\cdot}0054 \times 100$. This figure subtracted from 100 % would, of course, give the percentage of intercellular space, thereby defining it here as that area not occupied by myotubes or myofibres.

Number of myofibrils

For each fetus the number of myofibrils in 10 myofibres and, when present, 10 myotubes was counted using the high power photomicrographs. The number of myofibrils per μm^2 was then calculated from these counts for each fetus and used to estimate the number of myofibrils in myofibres and, where applicable, myotubes of the mean cross sectional area already estimated (see above). Although, of course, the number of myofibrils per cell is very variable the number per μm^2 (the figure required) in any given muscle is fairly consistent, so that 10 was considered an adequate number of cells to measure. This was also the number used by Goldspink (1970).

Presentation of data

It was felt that, as the main aim of this investigation was to quantify the changes in various muscle parameters which take place during prenatal growth, the data could be most clearly presented in a graphical form. Most of the muscle parameters investigated were plotted against CR or BW for the reasons already discussed. An approximate indication of age can, however, be ascertained from Figure 1.

An attempt was made to define the plotted relationships by linear regression equations which frequently involved transforming one or both of the parameters, usually to the common logarithm. Various transformations were attempted for each plot until the best fit linear regression was found. When a linear regression did not appear to fit the relationship, however, an estimate curve was drawn, based upon

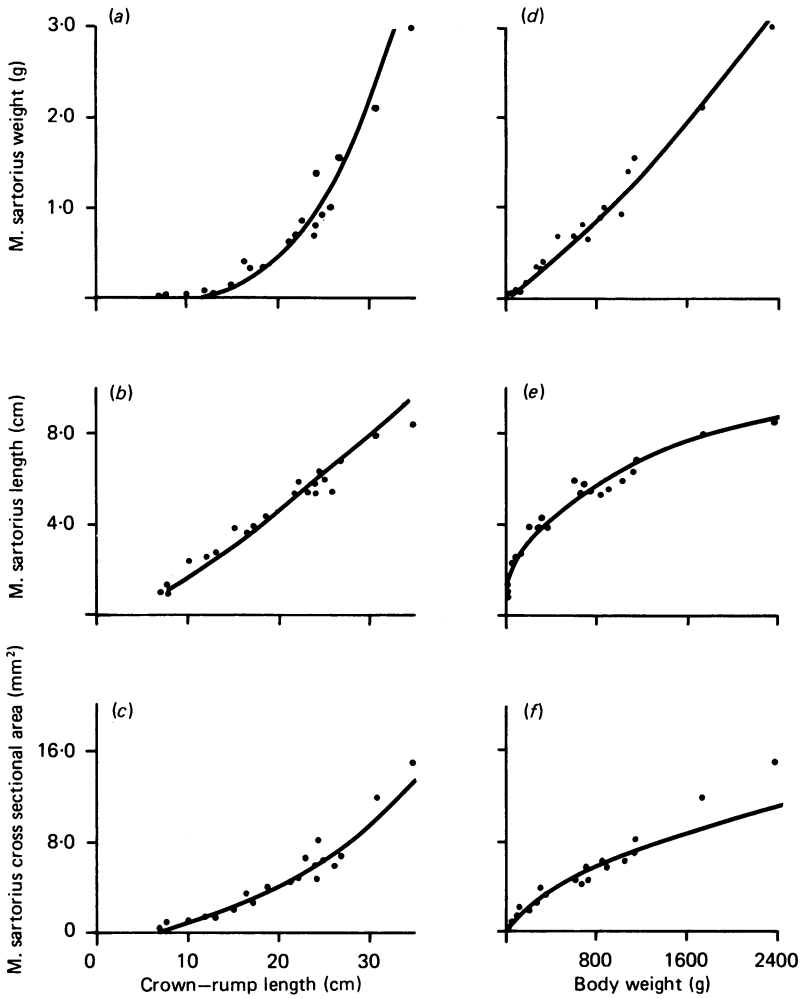

Fig. 2. The relationship between m. sartorius weight (*a*, *d*), length (*b*, *e*), cross sectional area (*c*, *f*) and (*a*, *b*, *c*) crown–rump length and (*d*, *e*, *f*) body weight.

previous computed lines, as described in the following section for the relationships concerned. Statistical methods were based upon Snedecor & Cochran (1967).

RESULTS

Gross parameters

The relationships between the weight, length and cross sectional area (at the mid-length level) of m. sartorius with both CR and BW are shown graphically in Figure 2, the equations of these curves being given in Table 1. This shows that the best fit curve for all these relationships is the power curve or 'allometric' relationship of the form $y = a.X^b$, where b is the differential growth ratio defined by Huxley (1924). This relationship is linear when log Y is plotted against log X (i.e. log Y = log a+b.log X). The value of b will be related to the dimensions of the parameters being investigated, e.g. when muscle weight (three dimensional parameter) is plotted

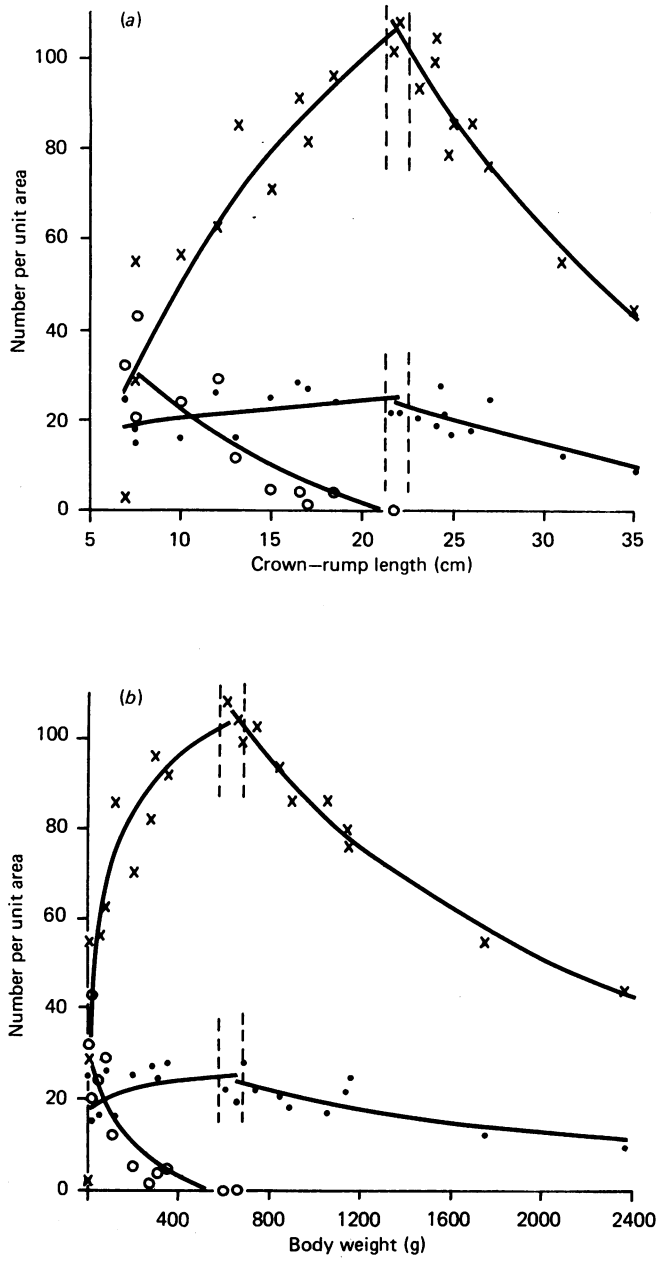

Fig. 3. The relationship between the number of myofibres (×), myotubes (○), and nuclei (●) per unit area and (*a*) crown–rump length, (*b*) body weight.

against CR (one dimensional), the value of *b* would be expected to approximate to 3. The fact that *b* is considerably higher ($P < 0{\cdot}001$) than this, with a value of 3·9 (Table 1), shows that m. sartorius weight has a higher growth rate than CR. Furthermore, the value of *b* in Figure 2*b* is significantly higher ($P < 0{\cdot}001$) than the 'expected' 1·00, whereas *b* in Figure 2*c* is not significantly different from the 'expected' 2·00. Taken as a whole, these results suggest that most of the higher rate

Table 1. *Equations of the computed regression lines shown in the Figures*

Figure	Parameters *X*	Parameters *Y*	Line	S_b
1	CR	BW	$Y = 3{\cdot}13.10^{-3}.X^{3{\cdot}20}$	0·042
2(*a*)	CR	Muscle weight	$Y = 3{\cdot}47.10^{-6}.X^{3{\cdot}91}$	0·162
2(*b*)	CR	Muscle length	$Y = 9{\cdot}21.10^{-1}.X^{1{\cdot}30}$	0·032
2(*c*)	CR	Muscle t.s. area	$Y = 8{\cdot}01.10^{-3}.X^{2{\cdot}07}$	0·064
2(*d*)	BW	Muscle weight	$Y = 2{\cdot}42.10^{-4}.X^{1{\cdot}22}$	0·076
2(*e*)	BW	Muscle length	$Y = 4{\cdot}12.10^{-1}.X^{0{\cdot}39}$	0·009
2(*f*)	BW	Muscle t.s. area	$Y = 9{\cdot}23.10^{-2}.X^{0{\cdot}62}$	0·024
3(*a*)	CR[a](7–22·5)	Fibres	$Y = 161.\log X - 110$	5·69
	CR (21–35)	Fibres (No. per unit area)	$Y = 515 - 306.\log X$	5·42
	CR (7–22·5)	Nuclei (No. per unit area)	$Y = 11{\cdot}8.\log X + 8{\cdot}96$	1·18
	CR (21–35)	Nuclei (No. per unit area)	$Y = 108 - 62{\cdot}9.\log X$	1·36
	CR	Tubes	$Y = 93{\cdot}7 - 70{\cdot}9.\log X$	4·15
3(*b*)	BW[b](0–680)	Fibres	$Y = 40{\cdot}4.\log X - 10{\cdot}6$	1·32
	BW (600–2400)	Fibres (No. per unit area)	$Y = 414 - 110.\log X$	2·70
	BW (0–680)	Nuclei (No. per unit area)	$Y = 4{\cdot}94.\log X + 10{\cdot}9$	0·70
	BW (600–2400)	Nuclei (No. per unit area)	$Y = 85{\cdot}1 - 21{\cdot}9.\log X$	0·67
	BW	Tubes	$Y = 61{\cdot}1 - 22{\cdot}5.\log X$	2·42
4(*b*)	BW	Fibres (Total no. per section)	$Y = 64685.\log X - 95932$	$2{\cdot}37.10^{3}$
	BW	Nuclei (Total no. per section)	$Y = 13649.\log X - 18462$	$0{\cdot}53.10^{3}$
5(*b*)	BW	Total no. nuclei in whole muscle	$Y = 9{\cdot}38.10^{7}.\log X - 1{\cdot}53.10^{8}$	$0{\cdot}42.10^{7}$
6	$(CR)^2$	Mean fibre area	$Y = 26{\cdot}18.1{\cdot}0009^{X}$	0·0027
	CR	Mean tube area	$Y = 47{\cdot}23.0{\cdot}9760^{X}$	0·0931
8	CR	% Cellular area	$Y = 58{\cdot}1.\log X - 10{\cdot}9$	1·47
10	Mean tube area	No. of myofibrils	$Y = 34{\cdot}4 - 0{\cdot}005.X$	0·054
	Mean fibre area	No. of myofibrils	$Y = 106{\cdot}5.\log X - 122{\cdot}0$	1·8

[a] Crown–rump length (cm). [b] Body weight (g).
S_b, Standard error of regression coefficient.

of muscle weight increase relative to CR is due to a higher rate of muscle length increase rather than cross sectional area increase. This situation also seems to apply for these parameters relative to BW (Fig. 2*d*, *e*, *f*).

Numbers of cells and nuclei

The numbers of myofibres, myotubes and nuclei per unit area (0·0054 mm²) of tissue section are plotted against CR in Figure 3*a* and BW in Figure 3*b*. At about 22 cm CR (Fig. 3*a*) and 650 g BW (Fig. 3*b*) there appears to be a very distinct change in the trends of the plotted points. For this reason the plots are divided into two segments at these particular CR and BW values. The equations for all the computed regression lines shown in Figure 3 are given in Table 1. The best curves to define these plots were all found to be logarithmic. Figure 3 shows that there is a considerable increase in the number of fibres per unit area up to about 22 cm CR or 650 g BW with a concomitant decrease in the numbers of myotubes to zero at these points. This means that there is initially a slightly higher proportion of myotubes to myofibres, but this is rapidly reversed. The number of nuclei per unit area increases slightly up to 21 cm CR or 550 g BW and then decreases. There appears to be no clear evidence for a better regression of any of these parameters on either CR or BW (Table 1). The slopes of all the lines shown in Figure 3, including all nuclei number lines, are significantly ($P < 0{\cdot}001$) different from the horizontal.

The total numbers of myofibres, myotubes and nuclei in the complete cross section of each muscle are plotted against CR in Figure 4*a* and BW in Figure 4*b*. The points in Figure 4*a* do not appear to follow a simple curve. It is possible that this curve is sigmoid, but in order to compute such a curve one must assume an upper asymptote which could be unjustified and, in any event, difficult to estimate. The plotted lines are, therefore, estimates based on the computed lines of Figures 2*c* and 3*a*. The points in Figure 4*b*, however, appear to fit reasonably well to a logarithmic curve (shown by broken lines), the equations of which are given in Table 1. The continuous lines shown in Figure 4*b* are lines estimated from Figures 2*f* and 3*b*, i.e. estimated in a similar way to the lines of Figure 4*a*. There seems to be fairly close agreement between computed and estimated lines in Figure 4*b* except for the divergence in myofibre number from 1200 g onwards. This divergence was converted to CR values and is indicated on Figure 4*a* by a broken line. This broken line probably improves the accuracy of the estimated line in Figure 4*a*, and further support for this adjustment is given later. Taken as a whole, the results in Figure 4 suggest that there is an initial slow increase in the number of myofibres up to about 12 cm CR (not identifiable in Figure 4*b*), followed by a very rapid increase up to about 22·5 cm CR or 700 g BW, after which the number increases at a declining rate. The number of myotubes remains relatively low until decreasing to zero at about 21 cm CR or 550 g BW. The nuclei number increases to about the 25 cm CR or 600 g BW stage and then appears to level off.

The total number of nuclei in whole muscles is plotted against CR in Figure 5*a* and against BW in Figure 5*b*. The lines shown are estimated from Figures 2*b* and 4*a* for Figure 5*a*, and from Figures 2*c* and 4*b* (estimated line) for Figure 5*b*. This is with the exception of the broken line in Figure 5*b* which is a logarithmic curve, whose equation is given in Table 1 and which roughly approximates to the estimated line. It can be seen from Figure 5*a* that there is an initial slow increase of nuclei up to about 15 cm CR, then a more rapid increase which probably, though not significantly, gradually slows down from about 30 cm CR. There would appear to be only slight evidence, therefore, of a slowing down of nuclear increase in later gestation as defined by CR. This is in contrast to Figure 5*b*, however, which shows an initial rapid increase in nuclei followed by a distinct slowing down of nuclear proliferation from about 400 and 800 g BW in computed and estimated curves respectively.

Size of cells

The average cross sectional areas of myotubes and myofibres at each CR are shown in Figure 6. Exponential curves, whose equations are given in Table 1, appear to best describe the decrease in myotube size and increase in myofibre size. It can be seen that at 7·5 cm CR the myotubes are more than 1½ times bigger in cross sectional area than the myofibres, but after this there is a decrease in myotube size and increase in myofibre size until about 15 cm CR when they are similar in size. The myotubes show a further slight decrease in size before they are no longer evident. The myofibres increase in size slowly at first and then more rapidly. The broken line in Figure 6 shows the overall mean size of cells at each CR. This line was estimated by using the computed lines in this figure and the proportions of myotubes and myofibres shown in Figure 3*a*. This shows that there is an initial decrease in overall cell cross sectional area from 7 to 10 cm CR, and from 10 cm onwards there is an initial slow rate of increase which continuously increases to a higher rate

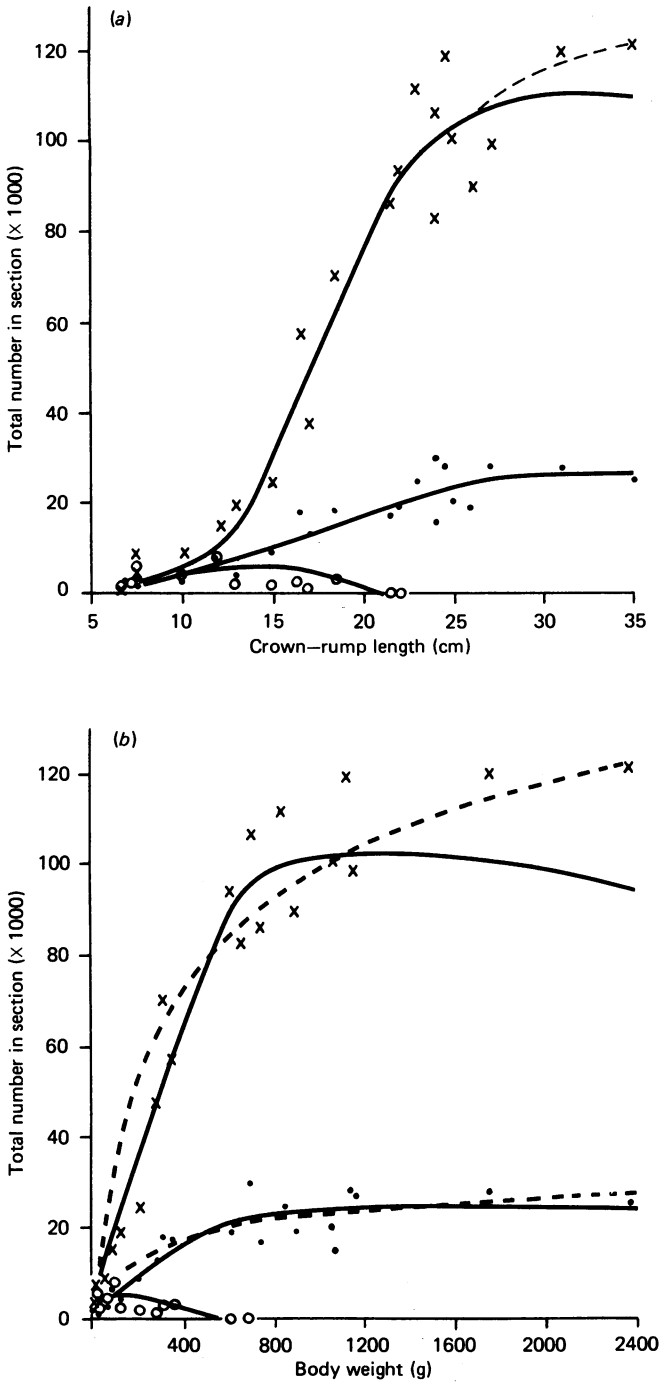

Fig. 4. The relationship between the total number of myofibres (×), myotubes (○) and nuclei (●) in a transverse section of m. sartorius and (*a*) crown–rump length, (*b*) body weight. The continuous lines (——) are estimated from other curves; the broken lines (– – –) are computed from the data points shown.

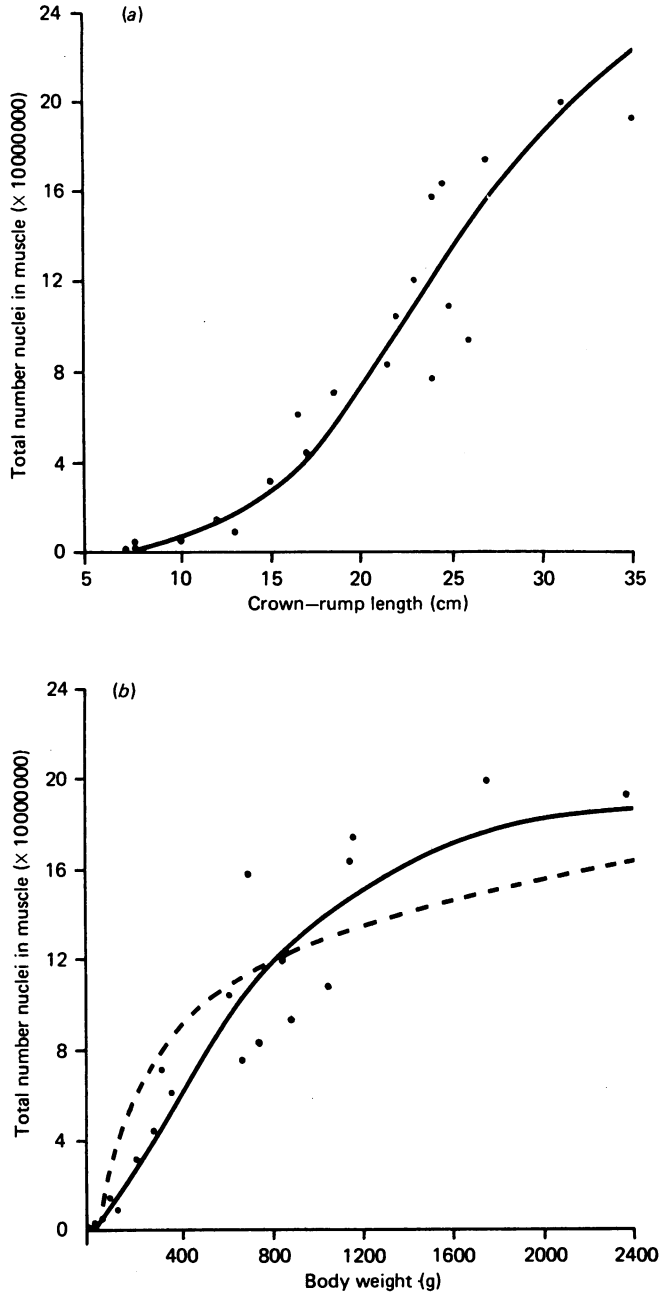

Fig. 5. The relationship between the total number of nuclei in whole m. sartorius and (*a*) crown–rump length, (*b*) body weight. The continuous lines (——) are estimated from other curves; the broken lines (– – –) are computed from the data points shown.

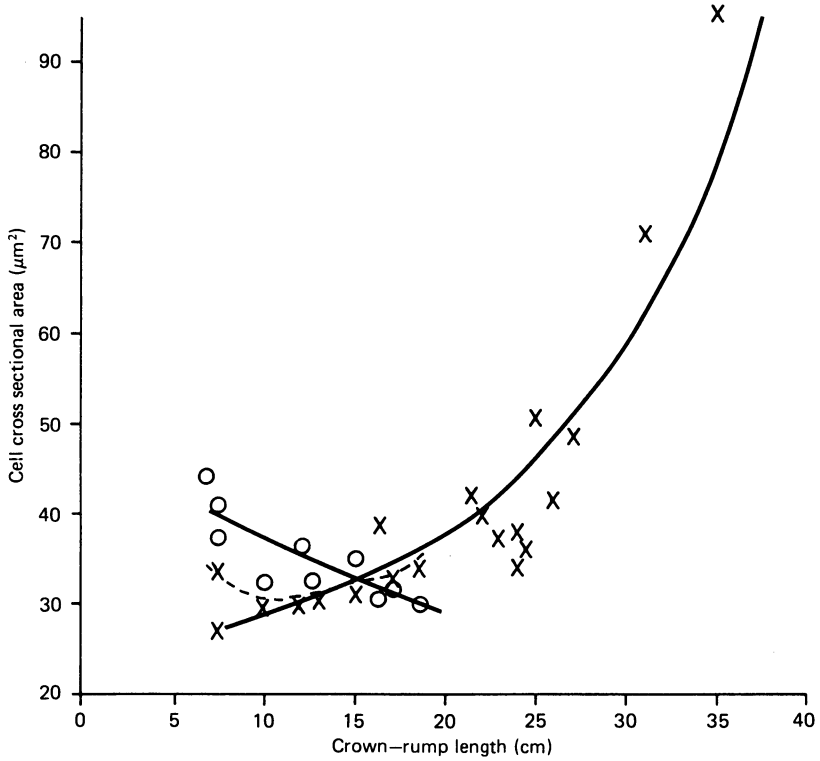

Fig. 6. The relationship between mean cross sectional area of myofibres (×) and myotubes (○) and crown–rump length. The broken line shows the change in overall mean cell cross sectional area.

in later gestation. The change in rate is so marked that, whereas from 10 cm CR to 20 cm CR cross sectional area only increases by about one-fifth, from 10 cm CR to 35 cm CR the area is almost tripled.

Fibre diameter frequency distributions are shown in Figure 7 for the muscles of six fetuses. Histograms such as these were made for all 21 fetuses, but only these six are necessary to demonstrate the general trends. The total proportion of myofibres to myotubes seen in this Figure was based on the cell number counts already mentioned, so that the percentage of these proportions in each size class could be used in the histograms. Figure 7 shows that originally (7·0 cm CR), there is a wide range in the diameter of myotubes from about 6 to 11 μm. There is then (illustrated here by the 7·5 cm CR histogram) a decrease in the frequency of the larger diameter myotubes although the overall range of myotube diameters is similar. At this same stage there is an appearance of myofibres ranging from about 2 to 11 μm in diameter. Later (18·5 cm CR) only a few small diameter myotubes remain (about 6 to 7 μm), although the distribution of myofibres is similar except that there are no myofibres in the smallest size class, i.e. about 2 to 3 μm. The next stage (23·0 cm CR) shows a similar range of myofibre diameters from about 3 to 11 μm, with apparently only the medium diameter fibres increasing in size from the previous (18·5 cm CR) stage. There then (27·0 cm CR) appears to be an increase in size of small, medium and large diameter fibres with an extended upper limit of about 14 μm, although the lower limit remains at about 3 μm. The trend of a general increase is continued

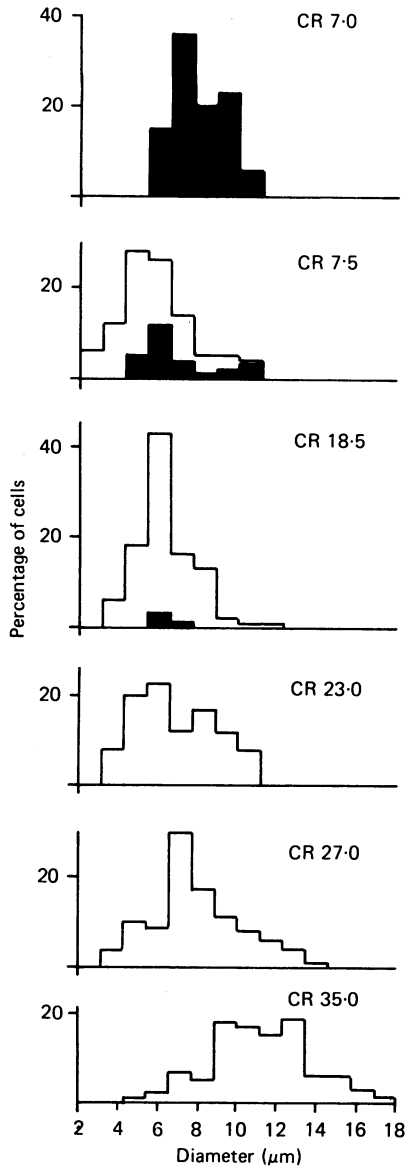

Fig. 7. Histograms showing the distribution of myotube and myofibre diameters at various crown–rump lengths (CR) from 7·0 cm to 35·0 cm.

(35·0 cm CR) with a complete shift in the range of fibre diameters to between 5 and 18 μm.

Intercellular space

The amount of cross sectional area occupied by myotubes and myofibres in each muscle section is plotted abainst CR in Figure 8. This shows that there is an increase (the rate of which steadily decreases) in total cellular area from about 38% at

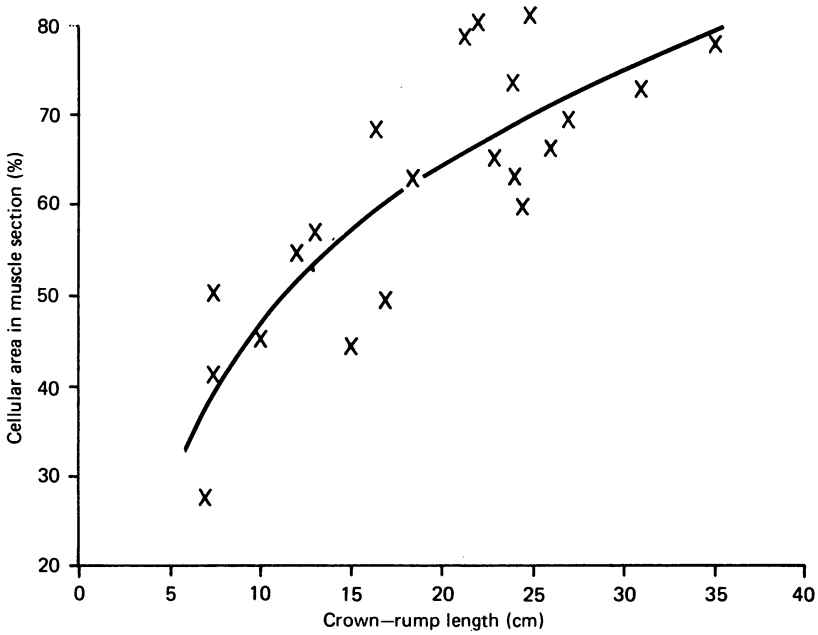

Fig. 8. The relationship between the percentage cellular area in transverse sections of m. sartorius and crown–rump length.

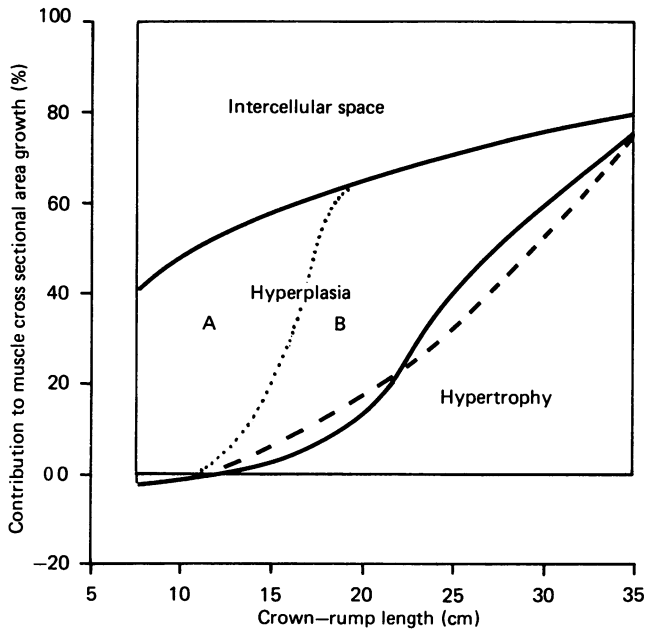

Fig. 9. The change in percentage contributions of hyperplasia and hypertrophy to muscle cross sectional area growth with increasing crown–rump length. The broken line (– – –) and dotted line (.....) are discussed in the text. A is real hyperplasia; B is apparent hyperplasia or longitudinal hypertrophy.

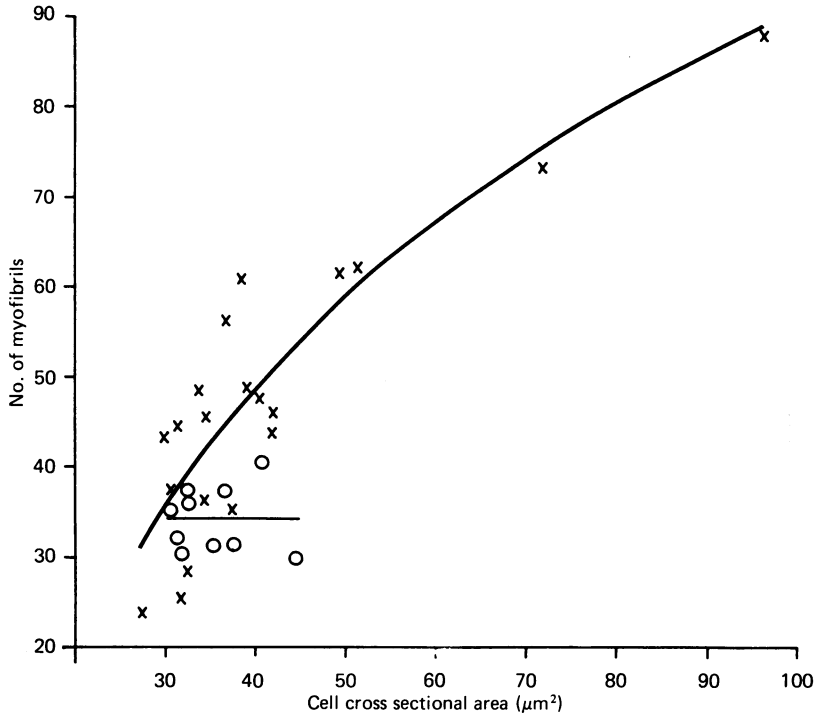

Fig. 10. The relationship between the number of myofibrils and the cross sectional area of myofibres (×) and myotubes (○).

7 cm CR to about 79 % at 35·0 cm CR, i.e. the cellular area doubles between 7·0 and 35·0 cm CR. Conversely, the amount of 'intercellular' space decreases by two thirds, from about 62 % at 7·0 cm CR to about 21 % at 35·0 cm CR. The equation for the computed line in Figure 8 is logarithmic and is given in Table 1.

Relative contributions of hyperplasia and hypertrophy to muscle growth

Figure 9 is an attempt to estimate the relative contributions of hyperplasia and hypertrophy to prenatal muscle cross sectional area growth.

The proportion of intercellular space decreases during the period studied (Fig. 8), which means that the increase in actual cellular cross sectional area is relatively larger than the total muscle cross sectional area. The relative contributions of hyperplasia and hypertrophy to this cellular area increase were estimated by measuring the increase in fibre number (From Fig. 4*a* – including broken line) and mean cell cross sectional area (from Fig. 6) respectively, over defined 2·5 cm CR stages. One type of increase was then expressed as a percentage of the two combined increases and plotted as shown in Figure 9. As a check on the accuracy of the fibre number curves shown in Figure 4*a*, it was decided to use the increases in mean cell cross sectional area and the increases in total cellular area (estimated from a combination of Figures 2*c* and 8) in order to estimate the contribution of hyperplasia by an indirect method. Using these estimated hyperplasia values and the actual hypertrophy values produces the broken line shown in Figure 9. This shows fairly close approximation to the actual relative contributions of hyperplasia and hypertrophy to muscle cross sectional area growth. The results of Figure 9 show very clearly that

there is a marked change from hyperplasia as the only positive contribution to muscle cellular cross sectional area growth early on, to a 50:50 contribution with hypertrophy at about 23·5 cm CR, and then to only about a 6% contribution (hypertrophy 94%) at 35·0 cm CR. Figure 9 is discussed at greater length later.

Number of myofibrils

The numbers of myofibrils in mean-sized myotubes and myofibres for each fetus are plotted against cell cross sectional area in Figure 10. The equations for each line are given in Table 1. The curve for myofibres is logarithmic, indicating that the number of myofibrils does not quite keep pace with increase in cross sectional area of the myofibre. There was no evidence, however, for a relationship between size of myotubes and myofibril number, and in fact there was no significant difference from the horizontal for the regression line shown.

DISCUSSION

Gross aspects

The relationship between CR and BW is within the normal range exhibited by the human fetus according to the figures of Langman (1975), although BW would appear to be in the lower part of the normal range for the fetuses above 30 cm CR. The cubic power relationship between BW and CR means that at the end of the first half of gestation CR is about half that of the full term, but BW is only about one-seventh of the full term.

As far as the gross muscle parameters are concerned (Fig. 2), there are some discrepancies between these results and the results of MacCallum (1898). MacCallum found m. sartorius in five fetuses to be all slightly longer than those at similar CR shown here, e.g. at 20 cm CR he gave 5·2 cm as the length (contrast the 4·6 cm given here – Fig. 2*b*). The main discrepancies, however, are in the cross sectional areas which MacCallum gave as 5·8 mm^2 at 17 cm CR and 8·4 mm^2 at 20 cm CR, both much higher than values shown here (Fig. 2*c*) although, conversely, his values at 7·4, 10·2 and 13·0 cm CR were lower than those given here. It is difficult to explain these discrepancies as there is no indication in MacCallum's paper as to how and in what position the muscles were fixed and subsequently treated.

There appears to be little other data available on gross parameters of individual muscles in prenatal development except for muscle weights in 36 bovine fetuses (Stickland, 1978), weights of various muscles in 11 porcine fetuses (Swatland, 1973) and muscle weights, lengths and widths in 40 ovine fetuses (Joubert, 1956*b*). In general, the results of these studies do not disagree with the trends shown in Figure 2. In particular, the fact that muscle length increase is the major factor contributing to muscle mass increase is in agreement with Joubert (1956*b*). It should be noted, however, that both Joubert (1956*b*) and Swatland (1973) found that rates of prenatal growth varied in muscles from different anatomical locations. Hence, the results shown in Figure 2 for m. sartorius may not be the same, in absolute terms, as the trends seen in other human muscles.

Effects of fixation

Before proceeding to the next section it is worth considering the effect of fixation on the various parameters investigated. The effect of formalin fixation on the weight of prenatal porcine muscle has been investigated in this laboratory and an increase in weight of 10–15% after fixation (no further processing) has been shown to occur.

Body weight, however, only increases by up to about 5 %, presumably due to other tissues being affected less than muscle. Joubert (1956*b*) also found little change in ovine fetus weights after fixation at all stages of gestation.

The effect of fixation and subsequent processing (as used in this present investigation) on cellular parameters has been investigated in this laboratory by Wigmore (personal communication) who has shown that both myotube and myofibre diameters in Araldite sections of porcine muscle are about 63 % of their diameters in frozen sections in early gestational stages and, at later stages, processed myofibres are about 70 % of the frozen ones. It is interesting to note that diameters of muscle fibres fixed in glutaraldehyde and embedded in Epon were also found to be about 70 % of the fresh fibre diameter (Eisenberg & Mobley, 1975) as were fibres fixed in either glutaraldehyde or formalin and embedded in wax (Stickland, 1975). The relative proportion of 'intercellular' space does not appear to be significantly affected at any gestational stage by the processing used here.

The effects of tissue processing do not alter the significance of any of the relative trends in muscle development which are concluded from the results. Even the fact that cell diameters are affected to a greater extent in early gestation than in late does not significantly affect the relative trends, owing to the fact that the difference is only 7 %. In other words, although the ordinate in some of the figures would be shifted up or down if allowance is made for fixation effects, the general shape of the curves and relative changes would not be affected. It was therefore decided not to make any corrections for fixation and processing effects but to discuss the results actually obtained from the processed tissue. It is useful, however, to be aware of these processing effects in any discussion of absolute rather than relative values.

Number and size of cells

The results of this investigation have shown that, in prenatal human muscle development, there is initially a double population of muscle cells, with the proportions of myotubes to myofibres being high at 7 cm CR, but rapidly decreasing to zero at about 21 cm CR or 550 g BW (Fig. 3), although the total number of myotubes shows little significant change over most of this period (Fig. 4). This double population of cells in developing muscle was noted in early work on human muscle by both MacCallum (1898) and Hewer (1927). MacCallum stated that myotube disappearance was lost by 17 cm CR whereas Hewer believed this was achieved later, by the 26th week (i.e. 25 cm CR from Fig. 1). The results of this investigation suggest that 21 cm CR is the time, i.e. between these two estimates, but this is quite different again from the 16th week (14 cm CR) given by Cuajunco (1942), but in closer agreement with the 20th week (about 19 cm CR) noted by Webb (1972). It is important to realise that some of the variation in these results may be due to different muscles being used, as it is known that different muscles develop at varying rates at the cellular level (e.g. Joubert 1956*b*).

The significance of the two populations of muscle cells in early development was probably first realized by Wohlfart (1937) who put forward a biphasic theory of muscle development, calling the larger myotubes B-fibres and the smaller myofibres A-fibres. This biphasic development has been shown in muscles of many animals, including the pig (Ashmore, Addis & Doerr, 1973; Swatland & Cassens, 1973*b*), lamb (Ashmore, Robinson, Rattray & Doerr, 1972), ox (Stickland, 1978), rat (Kelly & Zacks, 1969) and chick (Kikuchi, 1971). It is interesting to note that myotube appearance is lost by about mid-gestation in all the agricultural animals

mentioned, and this also appears to be the case in this present investigation on human fetuses. Various histochemical studies (e.g. Ashmore *et al.* 1972; Ashmore, Addis & Doerr, 1973; Fenichel, 1963) have shown that the large myotubes or B-fibres which develop first are destined to become slow-twitch fibres of adult muscle, whereas the smaller A-fibres (the large majority of which do not go through a distinct myotube stage) are destined to become fast-twitch fibres, although it is known (e.g. Davies, 1972) that some of these latter fibre types will convert to slow-twitch fibres during further development. It should be pointed out, however, that, unless certain histochemical tests are carried out, this double population of cells with different destinies can only be appreciated in early development, i.e. before the large myotubes have transformed into myofibres. It should also be noted that recently Beermann, Cassens & Hausman (1978) showed that, in completely fast portions of muscles, the large myotubes do not show slow-twitch histochemical properties, even in early development.

It can be seen from Figure 3 that, as well as myotube appearance being lost at about 21 cm CR (or 550 g BW), the number of myofibres per unit area changes from an increasing trend to a marked decrease at this same time. This distinct change must be due partly to a slowing down in the decrease of intercellular space (Fig. 8) and, perhaps more significantly, to the commencement of a more rapid fibre hypertrophy phase (Fig. 6). The decrease in intercellular space was noted in human muscle development by Dickerson & Widdowson (1960) and Widdowson (1969), and the significance of the chemical changes associated with these decreases is discussed by the same authors.

As far as cell size is concerned, there appears to be very little significant hypertrophy until about 22 cm CR and, in fact, there is an initial decrease in average cell size (due to the initially large myotubes decreasing in size) (Fig. 6) in agreement with Thurley (1972). The fact that myofibre hypertrophy is greatest in later gestation has also been shown quite clearly in sheep (Joubert, 1956*b*), pig (Thurley, 1972) and ox (Stickland, 1978). The data on cell cross sectional area in human sartorius muscle given by MacCallum (1898) show marked differences from the measurements given here. MacCallum gives 59 μm^2 at 7·4 cm CR, 23 μm^2 at 13 cm CR and 56 μm^2 at 20 cm CR (contrast Fig. 6). Part of the discrepancy, especially for the larger values from the smaller fetuses, may be due to the fact that MacCallum estimated fibre area by dividing a unit area by the number of fibres in that unit area, i.e. no provision for intercellular space was apparently made. Cuajunco (1940) gave results of fibre diameter measurements in m. sartorius at various stages and these are in close agreement with the results here (Fig. 7).

The histograms of Figure 7 show that the distribution width of myofibre diameters is the same from 7·5 cm CR to 23·0 cm CR. The slight increase in mean myofibre diameter (Fig. 6) is due to the smaller fibres increasing in size. It is not until 27·0 cm CR that the largest fibres start to hypertrophy. This is analogous to the situation in neonatal rat muscle (Ontell & Dunn, 1978) which shows no change in distribution width for fibre diameters in the first week post partum although the average size of fibre does increase. Rat muscle is particularly immature at birth, with fibre number increasing quite markedly after birth (Rayne & Crawford, 1975), and therefore more comparable, perhaps, with later gestational stages in the human.

The results shown here (Fig. 4) indicate that the rate of myofibre number increase, as seen in a complete muscle cross section, starts to slow down at about 22 cm CR, i.e. when hypertrophy starts to become more significant, but does not stop even up

to the time near birth. This is in contrast to MacCallum's (1898) results, which indicated that the number of fibres stopped increasing at 17 cm CR when the number in a sartorius section was 128×10^3 and, although the number was 152×10^3 at 20 cm CR, the number was 117×10^3 at birth. These three values do, however, stress the degree of individual variation in human sartorius muscle, which is why the results of Montgomery (1962) are difficult to interpret. Montgomery found that there were 101×10^3 fibres in a sartorius section at birth and 134×10^3 at four months of age. He rightly concluded that, from his results, it was difficult to say when fibre number had stopped increasing.

From Figure 9, also, it can be seen that hyperplasia, as measured by the number of myofibres in a complete muscle section, does not completely stop before birth. The contribution of hyperplasia to muscle cross sectional area increase is, however, greatly reduced to only about 6 % near birth, with a concomitant sharp increase in the contribution of hypertrophy over the period studied. The initial 'negative hypertrophy' between 7·5 and 10 cm CR is due to the initial decrease in average cell size (Fig. 6). At this time hyperplasia accounts for all muscle cross sectional area increase. The contribution of hypertrophy shows a rapid increase at about 22 cm CR such that, at about 23·5 cm CR, there is an equal contribution by hyperplasia and hypertrophy. Near birth, as already mentioned, nearly all the muscle area increase is due to hypertrophy, with no more than a 6 % contribution from hyperplasia. Therefore, although one cannot say that this hyperplasia does not stop before birth, its importance to muscle cross sectional area growth is not very significant near birth and, although there may be some hyperplasia after birth, its effect postnatally will be even less significant.

It is important to realise that the hyperplasia so far discussed has been that inferred from fibre counts made in one cross section at the mid-length level in m. sartorius, although counts made near the proximal or distal end would have yielded similar results according to Montgomery (1962). This measured hyperplasia is really a measure of 'apparent hyperplasia' in that increases in fibre length can cause an apparent hyperplasia by fibres growing into the level of the section. This phenomenon was probably first appreciated by MacCallum (1898) and has since been reiterated by many authors. It is difficult, however, from one section of each muscle, to estimate when real hyperplasia has ceased. Serial sections could be employed and then studied to obtain an estimate but this method would be extremely laborious. A possible alternative is to estimate, from histograms such as Figure 7, when there are no more fibres in the smallest size category. This probably then indicates when no new myofibres are forming. This latter method has been used by several authors to estimate the cessation of real hyperplasia and has been estimated to be by about two thirds of the gestation period in sheep (Joubert, 1956*b*; Swatland & Cassens, 1973*a*), pig (Swatland, 1973) and ox (Stickland, 1978). It has been mentioned in this investigation that 18·5 cm CR is the first stage when no myofibres in the smallest size class are present. There was, however, a frequency of two in this smallest class at 21·5 cm CR but none in this class thereafter. It should be mentioned that some of these very small fibres may be the tapering ends of larger fibres and not newly formed fibres. However, the tapering ends of fibres in developing porcine muscle appear very short in teased single fibre preparations, such that the tapering portion is seldom longer than about 10 μm (Wigmore, personal communication). An approximate estimate, based on the histogram results, would be 18 to 22 cm CR as the probable end of real hyperplasia but to avoid overestimation, due to the

possibility of sectioned tapering fibre terminations, it is probably better to take the lower end of this range. This rough estimate therefore indicates that real hyperplasia has ceased by about mid-gestation, which means that the hyperplasia phase of Figure 9 can be divided into a real hyperplasia phase (A) and an apparent hyperplasia phase (B). This latter phase is, of course, a result of longitudinal hypertrophy with the 'hypertrophy' phase being fibre cross sectional area hypertrophy.

Number of nuclei

There is a significant decline in the number of nuclei per unit area from about 22 cm CR (600 g BW) onwards (Fig. 3). This decline appears to continue postnatally as Stickland *et al.* (1975) working on pigs and Montgomery, Dickerson & McCance (1964) working on the fowl found that concentration of nuclei was highest in animals with a low body weight.

The results on total numbers of nuclei show that the increase in nuclei numbers in both whole sections (Fig. 4) and whole muscles (Fig. 5), though less so in the latter, slows down in later gestation. Montgomery's (1962) results on sartorius muscle nuclei showed a steady increase both in sections and whole muscles. His results were, however, based on counts of subsarcolemmal nuclei only, whereas this study included counts on all nuclei. As the source of muscle nuclei is from satellite cells, which are probably undifferentiated myoblasts (Stromer *et al.* 1974), it is conceivable that subsarcolemmal nuclei could increase at a greater rate than total nuclei. This indicates, however, that in later gestation satellite cell nuclei are incorporated into myofibres at a greater rate than they are dividing. As all nuclei were counted in this investigation it is possible to compare directly the results here with the biochemical results of Widdowson, Crabb & Milner (1972), whose estimates of total DNA content in human gastrocnemius muscle showed a slower rate of increase in later gestation, comparable with Figure 5. Protein/DNA ratios also increased markedly in later gestation, thereby indicating a decline in DNA concentration which also agrees with the results of Figure 3.

All the results on the numbers of myofibres, myotubes and nuclei so far discussed (Figs. 3 to 5; Table 1) have not shown that there is a closer relationship of these cellular parameters with either CR or BW. There is some evidence, however (Widdowson *et al.* 1972) that some muscle parameters (including total DNA) are lower in small-for-dates fetuses as compared to normal. All the fetuses in this investigation were, however, in the normal range of BW for given CR values. It would be interesting to repeat this sort of quantitative work on fetuses from both normal and small-for-dates populations and thereby investigate which parameters, if any, relate better to BW and which to CR, the latter probably being more related to chronological age.

Number of myofibrils

The results of Figure 10 show that, at any given cross sectional area, myofibres have more myofibrils than myotubes, which is understandable by virtue of the fact that myofibres are uniformly filled with myofibrils whereas myotubes, by definition, contain only a peripheral ring of myofibrils.

It has been shown here that, during development, myotubes decrease in cross sectional area (Fig. 6). It would seem that this decrease in size is not associated with any change in the number of myofibrils (Fig. 10) during the period studied. Fidziańska (1971), however, observed an increase in the number of myofibrils in myo-

tubes in early human embryos between the 9th and 16th week, i.e. up to about 14 cm CR. Taken as a whole, the results available suggest that, although there must be some initial increase in myofibril number in myotubes, there is probably no significant change in the latter half of the myotube's life, remaining at about 35 myofibrils per myotube. It would seem that, when the myotube reaches about 30 μm^2 in cross sectional area, it loses its myotube appearance and becomes a myofibre, and it is not until this stage that the number of myofibrils start to significantly increase (Fig. 10). It is interesting to note that the relationship between myofibril number and cell size is not a linear one; myofibril number does not keep pace with size increase in later gestation. This type of curve is also seen postnatally in the mouse (Goldspink, 1970). In the present investigation a 50% increase in cell size from 30 μm^2 to 45 μm^2 corresponds to a 50% increase in the number of myofibrils. In later gestation, however, a 50% increase in cell size from 60 μm^2 to 90 μm^2 corresponds to only a 20% increase in myofibril number. This phenomenon may be partly due to the fact that myofibrils also increase in size during growth, although it is known that longitudinal splitting of myofibrils may occur when myofibril diameter is twice the normal, and this in fact seems to be the mechanism whereby myofibrils increase in number during growth (Goldspink, 1970; Shear, 1974).

CONCLUSIONS

It has not been the aim of this investigation to elaborate the mechanisms of prenatal muscle development, but to quantify the cellular changes which take place and so build up an accurate time-sequence of cellular events. The most important results of this investigation are probably those summarised in Figure 9 describing the rate of changeover from hyperplasia to hypertrophy during gestation.

As was stated in the Introduction to this investigation, muscle size is determined by muscle fibre size and number and intercellular 'space'. Although there may be some genetic control over maximum fibre size, this is a very variable parameter and, from the many studies already mentioned, it appears that fibre number is far more genetically determined. There is some evidence, from experimental work on animals, that malnutrition during pregnancy affects ultimate cell number in the tissues of the offspring (Robinson, 1969; Widdowson, 1971). Winick & Noble (1966) also found that cell number in various tissues was affected by malnutrition in rats from birth to weaning, which could recover when subsequently adequately fed. As rats are relatively immature at birth this may be analogous to prenatal malnutrition effects in other animals. From the results of this investigation it would seem that malnutrition *in utero* could affect muscle cell number (as estimated from a complete transverse section) in two ways. Firstly, malnutrition in the first half of gestation could affect real hyperplasia irreversibly. Secondly, malnutrition in the second half of gestation could reduce the apparent hyperplasia giving only an apparent affect of low cell number at birth. It is possible, however, that in this latter case adequate nutrition could allow recovery to take place by longitudinal hypertrophy of existing muscle fibres causing an increase in the fibre number apparent in a transverse section of muscle. As far as cell cross sectional area is concerned, malnutrition is likely to have a much more profound effect on this parameter in later gestation but, as with longitudinal hypertrophy, it is probable that this effect could be largely overcome by subsequent adequate nutrition.

The continuous change and rates of change in various cellular parameters shown

here have stressed the importance of describing muscle development in quantitative terms such as the graphical representations given here. It would be interesting to make a quantitative study of muscle development of, as already suggested, small-for-dates fetuses or of fetuses at risk for muscular dystrophy. These studies could then be compared with the present investigation and any significant differences more clearly defined in numerical terms.

SUMMARY

M. sartorius was removed from 21 human fetuses ranging from 7·0 to 35·0 cm crown–rump length (CR). Various gross and cellular changes (as seen in a transverse section) which take place in developing human skeletal muscle were quantified. The weights, lengths and cross sectional areas (at mid-length level) of m. sartorius were found to exhibit allometric relationships with CR and body weight (BW).

Initially (7·5 cm CR) myotubes were more numerous and larger (40 μm^2 cross sectional area) than the myofibres (about 26 μm^2). This situation was soon reversed, however, so that at about 19 cm CR myotubes were only a very small proportion of the total muscle cell population and somewhat smaller than the myofibres in cross sectional area. At about 21 cm CR all myotube appearance was lost, whilst the total number of myofibres increased rapidly up to about 22·5 cm CR, and thereafter the rate slowed down. This stage (22·5 cm CR) seemed, in fact, to be about the time when hypertrophy of myofibres started to markedly replace hyperplasia as the main factor contributing to total muscle cross sectional area increase, although there was still a 6% contribution from hyperplasia at 35 cm CR. At 18 to 22 cm CR there were no more myofibres in the smallest size class (2–3 μm diameter). This may be an indication that real hyperplasia had ceased at this point so that beyond this the hyperplasia seen was only apparent and represented longitudinal growth of existing myofibres.

Throughout the period studied the amount of intercellular space decreased (at a declining rate) from about 62% at 7 cm CR to about 21% at 35 cm CR. Results on counts of nuclei suggested that total muscle nuclear proliferation slowed down in later gestation. Myofibril number was not related to myotube size but increased, though at a declining rate, with myofibre size. All the muscle parameters mentioned were plotted against CR, and sometimes BW, and regression equations given wherever possible.

Sincere thanks are due to Dr I. Smith of the Royal Hospital for Sick Children, Edinburgh, for making the muscle samples and relevant fetus data available, and for useful discussions. For technical assistance I am indebted to Messrs G. Goodall, S. Mitchell, D. Penman and I. Sneddon.

REFERENCES

ABERCROMBIE, M. (1946). Estimation of nuclear population from microtome sections. *Anatomical Record* **94**, 239–247.

ADAMS, R. D. (1975). *Diseases of Muscle. A Study in Pathology*. 3rd ed. New York: Harper & Row.

ASHMORE, C. R., ADDIS, P. B. & DOERR, L. (1973). Development of muscle fibers in the fetal pig. *Journal of Animal Science* **36**, 1088–1093.

ASHMORE, C. R., ROBINSON, D. W., RATTRAY, P. & DOERR, L. (1972). Biphasic development of muscle fibers in the fetal lamb. *Experimental Neurology* **37**, 241–255.

BEERMANN, D. H., CASSENS, R. G. & HAUSMAN, G. J. (1978). A second look at fiber type differentiation in porcine skeletal muscle. *Journal of Animal Science* **46**, 125–132.

CUAJUNCO, F. (1940). Development of the neuromuscular spindle in human fetuses. *Carnegie Institute, Washington Publication* 518. *Contributions to Embryology* **28**, 95–128.

CUAJUNCO, F. (1942). Development of the human motor end plate. *Carnegie Institute, Washington Publication* 541. *Contributions to Embryology* **30**, 127–152.

DAVIES, A. S. (1972). Postnatal changes in the histochemical fibre types of porcine skeletal muscle. *Journal of Anatomy* **113**, 213–240.

DICKERSON, J. W. T. & WIDDOWSON, E. M. (1960). Chemical changes in skeletal muscle during development. *Biochemical Journal* **74**, 247–257.

EISENBERG, B. R. & MOBLEY, B. A. (1975). Size changes in single muscle fibers during fixation and embedding. *Tissue and Cell* **7**, 383–387.

ELIOT, T. S., WIGGINTON, R. C. & CORBIN, K. B. (1943). The number and size of muscle fibers in the rat soleus in relation to age, sex and exercise. *Anatomical Record* **85**, 307–308.

FENICHEL, G. M. (1963). The B fiber of human fetal skeletal muscle. A study of fiber diameter size. *Neurology* **13**, 219–226.

FIDZIANSKA, A. (1971). Electron microscopic study of the development of human foetal muscle, motor end-plate and nerve. *Acta neuropathologica* **17**, 234–247.

GOLDSPINK, G. (1962). Studies on postembryonic growth and development of skeletal muscle. *Proceedings of the Royal Irish Academy* B **62**, 135–150.

GOLDSPINK, G. (1964). The combined effects of exercise and reduced food intake on skeletal muscle fibres. *Journal of Cellular and Comparative Physiology* **63**, 209–216.

GOLDSPINK, G. (1970). The proliferation of myofibrils during muscle fibre growth. *Journal of Cell Science* **6**, 593–604.

HAMMOND, J. & APPLETON, A. B. (1932). *Study of the Leg of Mutton. Part V. Growth and Development of Mutton Qualities in the Sheep.* London: Oliver & Boyd.

HEWER, E. E. (1927). The development of muscle in the human foetus. *Journal of Anatomy* **62**, 72–78.

HUXLEY, J. S. (1924). Constant differential growth ratios and their significance. *Nature* **114**, 895–896.

JOUBERT, D. M. (1955). Growth of muscle fibre in the foetal sheep. *Nature* **175**, 936–937.

JOUBERT, D. M. (1956*a*). An analysis of factors influencing post-natal growth and development of the muscle fibre. *Journal of Agricultural Science* **47**, 59–102.

JOUBERT, D. M. (1956*b*). A study of pre-natal growth and development in the sheep. *Journal of Agricultural Science* **47**, 382–428.

KARNOVSKY, M. J. (1965). A formaldehyde–glutaraldehyde fixative of high osmolarity for use in electron microscopy. *Journal of Cell Biology* **27**, 137 A.

KELLY, A. M. & ZACKS, S. I. (1969). The histogenesis of rat intercostal muscle. *Journal of Cell Biology* **42**, 135–154.

KIKUCHI, T. (1971). Studies on the development and differentiation of muscle. III. Especially on the mode of increase in the number of cells. *Tohuku Journal of Agricultural Research* **22**, 1–15.

LANGMAN, J. (1975). *Medical Embryology*. 3rd ed. Baltimore: Williams & Wilkins.

MACCALLUM, J. B. (1898). On the histogenesis of the striated muscle fibre and the growth of the human sartorius muscle. *Johns Hopkins Hospital Bulletin* **9**, 208–215.

MEARA, P. J. (1947). Post-natal growth and development of muscle, as exemplified by the gastrocnemius and psoas muscles of the rabbit. *Onderstepoort Journal of Veterinary Science* **21**, 329–466.

MONTGOMERY, R. D. (1962). Growth of human striated muscle. *Nature* **195**, 194–195.

MONTGOMERY, R. D., DICKERSON, J. W. T. & MCCANCE, R. A. (1964). Severe undernutrition in growing and adult animals. 13. The morphology and chemistry of development and undernutrition in the sartorius muscle of the fowl. *British Journal of Nutrition* **18**, 587–593.

ONTELL, M. (1977). Neonatal muscle: an electron microscopic study. *Anatomical Record* **189**, 669–690.

ONTELL, M. & DUNN, R. F. (1978). Neonatal muscle: a quantitative study. *American Journal of Anatomy* **152**, 539–555.

RAGSDALE, A. C. (1934). Growth standards for dairy cattle. *University of Missouri: Agricultural Experiment Station, Research Bulletin* **336**, 1–12.

RAYNE, J. & CRAWFORD, G. N. C. (1975). Increase in fibre numbers of the rat pterygoid muscles during postnatal growth. *Journal of Anatomy* **119**, 347–357.

ROBINSON, D. W. (1969). The cellular response of porcine skeletal muscle to prenatal and neonatal nutritional stress. *Growth* **33**, 231–240.

ROWE, R. W. D. & GOLDSPINK, G. (1969). Muscle fibre growth in five different muscles in both sexes of mice. I. Normal mice. *Journal of Anatomy* **104**, 519–530.

SHEAR, C. R. (1974). Myofibril proliferation in developing skeletal muscles. In *Recent Advances in Myology* (ed. W. G. Bradley, D. Gardner-Medwin & J. N. Walton), pp. 364–373. Amsterdam: Elsevier.

SNEDECOR, G. W. & COCHRAN, W. G. (1967). *Statistical Methods*. 6th ed. Ames, Iowa: The Iowa State University Press.

SPIRO, A. J., SHY, G. M. & GONATAS, N. K. (1966). Myotubular myopathy: persistence of fetal muscle in an adolescent boy. *Archives of Neurology* **14**, 1–14.

STAUN, H. (1963). Various factors affecting number and size of muscle fibres in the pig. *Acta agriculturae scandinavica* **13**, 293–322.

STICKLAND, N. C. (1975). A detailed analysis of the effects of various fixatives on animal tissue with particular reference to muscle tissue. *Stain Technology* **50**, 255–264.

STICKLAND, N. C. (1978). A quantitative study of muscle development in the bovine foetus (*Bos indicus*). *Anatomia Histologia Embryologia* **7**, 193–205.

STICKLAND, N. C. & GOLDSPINK, G. (1973). A possible indicator muscle for the fibre content and growth characteristics of porcine muscle. *Animal Production* **16**, 135–146.

STICKLAND, N. C., WIDDOWSON, E. M. & GOLDSPINK, G. (1975). Effects of severe energy and protein deficiencies on the fibres and nuclei in skeletal muscle of pigs. *British Journal of Nutrition* **34**, 421–428.

STROMER, M. H., GOLL, D. E., YOUNG, R. B., ROBSON, R. M. & PARRISH Jr, F. C. (1974). Ultrastructural features of skeletal muscle differentiation and development. *Journal of Animal Science* **38**, 1111–1141.

SWATLAND, H. J. (1973). Muscle growth in the fetal and neonatal pig. *Journal of Animal Science* **37**, 536–545.

SWATLAND, H. J. & CASSENS, R. G. (1973*a*). Inhibition of muscle growth in foetal sheep. *Journal of Agricultural Science* **80**, 503–510.

SWATLAND, H. J. & CASSENS, R. G. (1973*b*). Prenatal development, histochemistry and innervation of porcine muscle. *Journal of Animal Science* **36**, 343–354.

THURLEY, D. C. (1972). Increase in diameter of muscle fibres in the foetal pig. *British Veterinary Journal* **128**, 355–358.

TOMANEK, R. J. & COLLING-SALTIN, A.-S. (1977). Cytological differentiation of human fetal skeletal muscle. *American Journal of Anatomy* **149**, 227–246.

WEBB, J. N. (1972). The development of human skeletal muscle with particular reference to muscle cell death. *Journal of Pathology* **106**, 221–228.

WIDDOWSON, E. M. (1969). Changes in the extracellular compartment of muscle and skin during normal and retarded development. *Nutritio et Dieta* **13**, 60–68.

WIDDOWSON, E. M. (1971). Intra-uterine growth retardation in the pig. I. Organ size and cellular development at birth and after growth to maturity. *Biology of the Neonate* **19**, 329–340.

WIDDOWSON, E. M., CRABB, D. E. & MILNER, R. D. G. (1972). Cellular development of some human organs before birth. *Archives of Disease in Childhood* **47**, 652–655.

WINICK, M. & NOBLE, A. (1966). Cellular responses in rats during malnutrition at various ages. *Journal of Nutrition* **89**, 300–306.

WOHLFART, G. (1937). Über das Vorkommen verschiedener Arten von Muskelfasern in der Skelettmuskulatur des Menschen und einiger Säugetiere. *Acta psychiatrica et neurologica Suppl.* **12**, 1–119.