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Postnatal growth and differentiation of muscle fibres in the mouse

I. A histochemical and morphometrical investigation of normal muscle

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INTRODUCTION

Histochemical classifications of striated muscle fibres now in use are based on the normal condition of mature muscles. Usually, three muscle fibre types are recognised, e.g. the classification of Peter *et al.* (1972), which distinguishes slow-twitch-oxidative (STO) and fast-twitch (FT) fibres that are further characterised as fast-twitch-oxidative-glycolytic (FTOG) and fast-twitch-glycolytic (FTG).

Ashmore, Tompkins & Doerr (1972) additionally described a population of fibres intermediate between 'red' and 'white' fibres. Several investigators have noted that these classifications, in fact, are an over-simplification (Romanul, 1964; Guth & Yellin, 1971; Khan, 1978; Pette & Spamer, 1979; Lowry *et al.* 1980). Swatland (1977) stated that "to a large extent, the boundaries between the different categories of fibres disappeared, once the need to categorize them subjectively was removed".

Especially with regard to developing muscle and pathological conditions the classifications that are commonly used appear too rigid. Here, they are of little assistance in assigning the fibre populations to different groups. Therefore, we have attempted the development of a classification that can be used to describe normal postnatal differentiation as well as changes that occur during the course of a muscular disease. The proposed classification is based on enzyme histochemical findings in postnatal murine muscle.

The activities of three enzymes (myosin ATPase, succinate-dehydrogenase (SDH) and α-glycerol-phosphate-dehydrogenase (GPOX)) have been applied in the construction and evaluation of graphics depicting the postnatal growth and differentiation of normal muscle fibres of three muscles of the lower leg of the mouse.

In another paper the development of dystrophic muscle will be described.

MATERIALS AND METHODS

Homozygous (Dy/Dy) mice of the Bar Harbor strain ReJ 129, in which muscle development is normal, were used. The mice were kept at room temperature and received food and water *ad libitum*. For light microscopic examination, three muscles of the lower hind leg (soleus, plantaris and gastrocnemius) were dissected as a

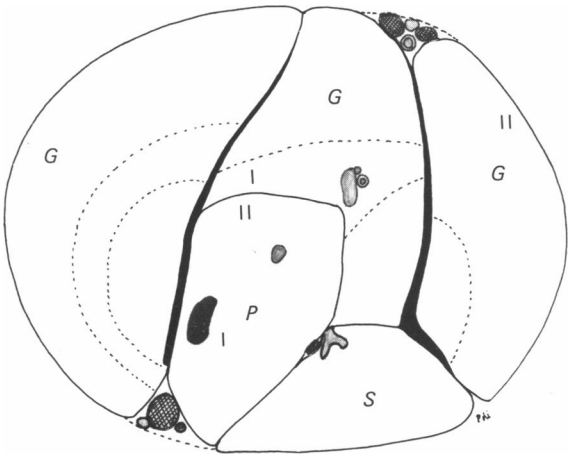


Fig. 1. Cross section through the mid-region of the muscle complex. *S*, soleus muscle; *P*, plantaris muscle; *G*, gastrocnemius muscle; solid black, tendons. In the plantaris muscle and the gastrocnemius muscle, regions I and II are indicated. Dashed lines in the gastrocnemius muscle delineate transitional zones between 'mixed' central and 'white' peripheral regions.

group, wrapped in a piece of skin of a 2 days old mouse to prevent disruption, and frozen in isopentane chilled with liquid nitrogen (−150 °C). The tissue was kept at −90 °C until use. The muscle complex was sectioned at 8–12 cross sectional levels, taken at regular intervals. At each level a number of serial transverse sections was cut at 10 μm in a Walter Dittes cryostat (−25 °C) and mounted on as many microscope slides as were needed to perform the following histochemical reactions: succinate dehydrogenase (SDH: EC 1.3.99.1, Nachlas *et al.* 1957), +phenazine methasulphate (PMS); α-glycerol-phosphate-dehydrogenase (GPOX: EC 1.1.99.5, Wattenberg & Leong, 1960, modified for GPOX, Jöbsis, 1971) and myofibrillar ATPase (ATPase: EC 3.6.1.3, Dubowitz & Brooke, 1973), pre-inc. pH 4·35. In addition, frozen sections were fixed in buffered 2% glutaraldehyde, stained with haematoxylin and eosin, and mounted in Euparal(R).

A microscopic image of the sections was projected on a sheet of white paper, using a drawing tube attached to a Zeiss microscope. A low magnification drawing of each of the cross sectional levels of each muscle was made, and the highest planimetric value of each individual muscle was considered to be representative of the size of that muscle. Also, at each cross sectional level, the total numbers of muscle fibres of the soleus and the plantaris muscles were counted, the highest value being chosen for further evaluation (this value does not necessarily represent the total number of fibres in the muscle).

Because pilot studies did not show systematic differences between the muscles of either leg, only one leg per animal was investigated, with no preference for left or right. The visual classification will be described in some detail: per muscle a sample of about 200 fibres was drawn (via projection). On serial sections, the density of the histochemical reaction product of each of the three enzymes tested was estimated for each fibre. Thus, for each fibre a code of three figures was devised, each code then being assigned a single letter. Figure 2 is, therefore, the result of a combination of three enzymes with three densities for SDH and ATPase and four densities for GPOX. This results in 36 possible combinations (= letters) of which 26 were

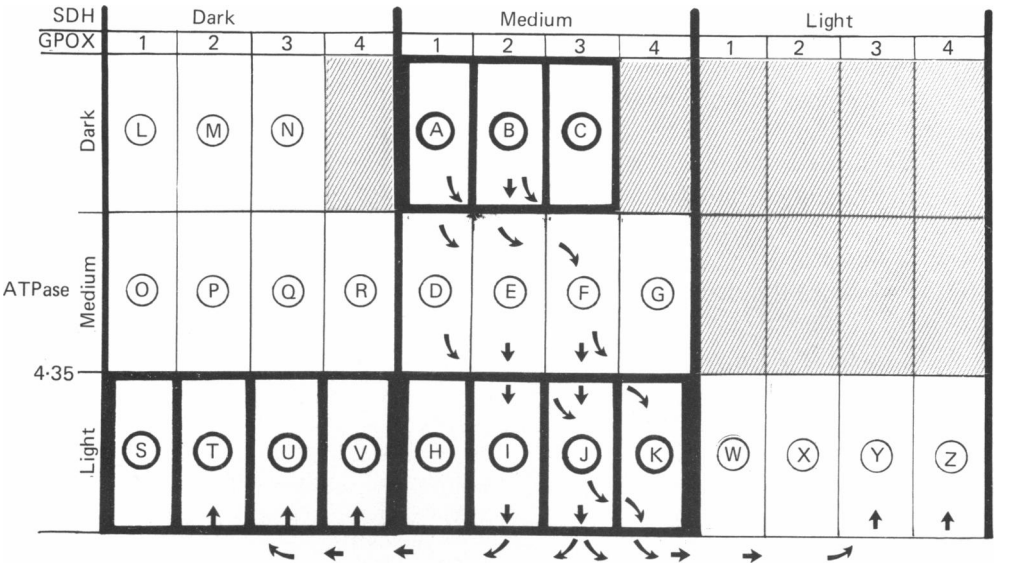


Fig. 2. Scheme of histochemical fibre types (A–Z). The SDH and ATPase reactions are in three gradations, and the GPOX in four (1, light; 4, dark staining). A–C, 'slow' fibres; D–G, 'transitional' fibres; L–N and O–R, 'rare' fibres; S–V, 'fast red' fibres; H–K, 'intermediate' fibres; W–Z, 'fast white' fibres. Combinations that were not found have been shaded. Arrows, pathways of probable development of adult 'fast' fibres.

actually found to occur in the present material and these have been named A–Z. It has been established, for most enzymes, that differences in staining densities between fibres reveal a gradual scale, rather than a separation into distinct classes. Only after the staining characteristics of different enzymes have been combined, clusters may develop (Spurway, 1980*a*, *b*). Each visual classification is therefore, by definition, at best an approximation of the real situation.

First, fibres of adult animals were classified, and subsequently younger age groups were compared with the adults. Care was taken to minimise the variability in the observations: three to four sections per microscope slide were collected in order to examine the reproducibility of the thickness of the sections. The skin of a young mouse wrapped around the muscle complex served as a reference mark for the intensity of the histochemical reaction. To compare intensities between animals a 'comparing microscope' was used, while a low magnification of the muscle complex was used to compare intensities between fibres within the complex. Histochemical reactions were performed under standardised conditions.

After the histochemical profile of the muscle fibres in a sample had been assessed, the drawing was used to measure the size of individual fibres with the 'method of the closest circle'. This method was chosen because of its efficiency, since pilot experiments in which the method was compared with a computerized evaluation (on a 'bit pad') showed that mis-classifications were fewer than 4%. However, this method is only valid when true cross sections are used, which can be easily seen by examining the 'cytoarchitectural pattern' in the GPOX reaction: when the fibre is not truly cross sectioned, 'streaming' of this pattern results. Because the distribution of fibre types in the plantaris and gastrocnemius muscles is not homogeneous, two separate regions were evaluated (Fig. 1). In the plantaris muscle the average of both

regions (I and II) was estimated to give an acceptable mean of the muscle as a whole. All data were computerised for further analysis.

In outlining the most probable course of development of adult fibre types (Fig. 4), we assumed that any decrease in the total number of fibres occurred at random over the different fibre types present. Any increase in the number of fibres of a particular type (or types) is the result of an equal decrease in the number of fibres of another type (or types). Based on the percentages of the several fibre types at different ages and the general development assumed, a model was drawn that estimates what percentage of fibres of a certain type will change into fibres of another type. The most probable pathway that an adult fibre type has followed can be outlined by means of Bayes' formula:

The probability that an adult fibre *Rj* resulted from the development *Di* is:

$$P(Di/Rj) = \frac{P(Rj/Di)\,P(Di)}{\Sigma_1\,P(Rj/D_1)\,P(D_1)},$$

where *Di* is the certain development of fibres, *Rj* is the adult fibre type, $P(Rj/Di)$ is the probability that development *Di* results in *Rj* (= 0 or 1), $P(Di)$ is the probability of development *Di* and where the summation is over all possible developments.

Growth curves were computed, assuming an exponential growth model, to get an impression of the growth rate of the different muscles. The parameters in this model were estimated by the method of least squares.

Growth rates were calculated as follows:

$$\lambda(t) = A - Be^{-\alpha t}, \quad \gamma(t) = B\,(1-e^{\alpha t}), \quad \gamma'(t) = B\alpha e^{-\alpha t},$$

where $\lambda(t)$ is the muscle surface (mm^2) at age t, $\gamma(t)$ is the growth (mm^2) from birth to day t, B is total growth, $\tau_{\frac{1}{2}}$ is time (days) to half of total growth and $\gamma' t$ is the growth rate (mm^2/day) after t days.

	Soleus muscle	Plantaris muscle	Gastrocnemius muscle
$\lambda^{(t)}$	$1{\cdot}31-1{\cdot}18e^{-0{\cdot}043t}$	$1{\cdot}69-1{\cdot}61e^{-0{\cdot}035t}$	$14{\cdot}89-14.74e^{-0{\cdot}036t}$
$\gamma^{(t)}$	$1{\cdot}18\,(1-e^{-0{\cdot}043t})$	$1{\cdot}61\,(1-e^{-0{\cdot}035t})$	$14{\cdot}74\,(1-e^{-0{\cdot}036t})$
total growth	$1{\cdot}18\ mm^2$	$1{\cdot}61\ mm^2$	$14{\cdot}74\ mm^2$
$\tau_{\frac{1}{2}}$	*ca.* 16 days	*ca.* 20 days	*ca.* 19 days
$\gamma'(t)$	$0{\cdot}051e^{-0{\cdot}043t}$	$0{\cdot}056e^{-0{\cdot}035t}$	$0{\cdot}531e^{-0{\cdot}036t}$

RESULTS

Most information has been concentrated in the Tables and Figures. The results will be dealt with according to these.

Muscle growth and fibre number

Figure 3 shows the body weights of the animals. Planimetric values of the maximal cross sectional areas per muscle are also presented in Figure 3. The growth rate $\gamma'(t)$ of the gastrocnemius muscle (expressed in mm^2/day) was greater than that of the plantaris and soleus muscles which had about equal growth rates. The soleus, plantaris, and gastrocnemius muscles reached half of the total size after 16, 20, and 19 days, respectively. The fibre counts (Table 1) showed a tendency to increase between the age groups of 0–2 and 3–7 days. However, the variations between individual data were too large to make the differences statistically significant. In older animals, a slight decrease in the soleus muscle and a more pronounced decrease

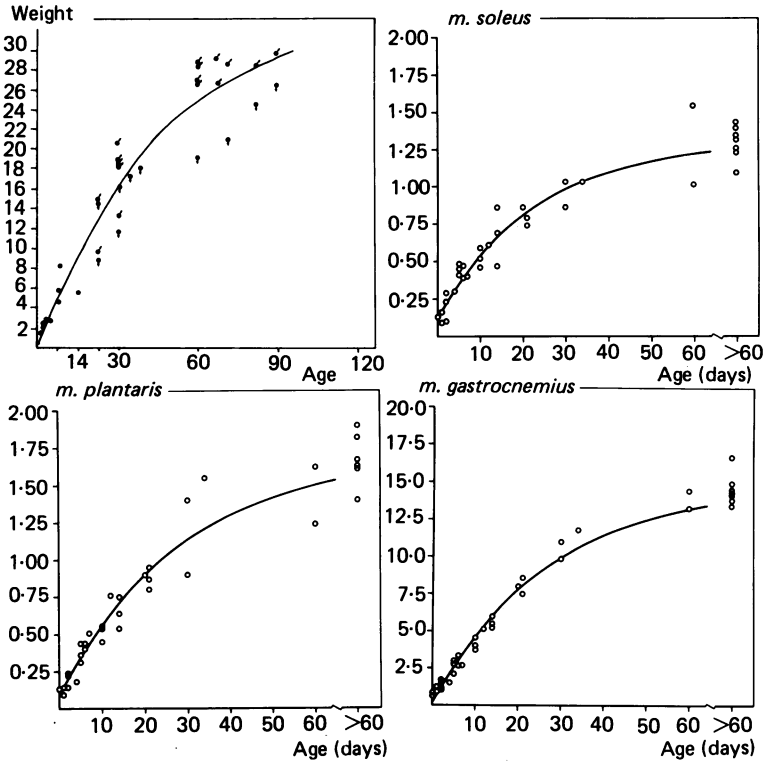


Fig. 3. Cross sectional area of soleus, plantaris and gastrocnemius muscles, expressed in mm^2. Drawn line, curve of least squares. Growth curve, animal weight in grams and age in days. Up to 3 weeks, each dot represents the average body weight of one litter. After 3 weeks, data for both sexes are presented separately (♂, ♀).

Table 1. *Number of fibres in the soleus and plantaris muscles*

	0–2	3–7	8–14	15–21	22–34	⩾ 60
Soleus muscle						
Median	713	849	810	850	850	784
Min.–max.	568, 948	756, 916	794, 856	771, 863	749, 908	719, 878
No. of animals	7	6	7	4	3	9
Plantaris muscle						
Median	906	1263	1139	1197	1028	940
Min.–max.	388, 1341	1236, 1414	1016, 1272	1094, 1319	946, 1163	793, 996
No. of animals	6	5	7	4	3	9

in the plantaris muscle were observed. Similar observations have been described by Betz, Caldwell & Ribchester, 1979; Ontell & Dunn, 1978; Ihemelandu, 1980; Layman, Hegarty & Swan, 1980.

Histochemical differentiation and fibre growth

Based on the classification shown in Figure 2, theoretically 36 combinations of enzyme activities of a single fibre are possible, but in the muscles of mice of different ages only 26 combinations (A–Z) were found. In Figure 5(*a*, *b*) the frequencies of seven fibre classes are plotted against age groups. This results in the visualisation

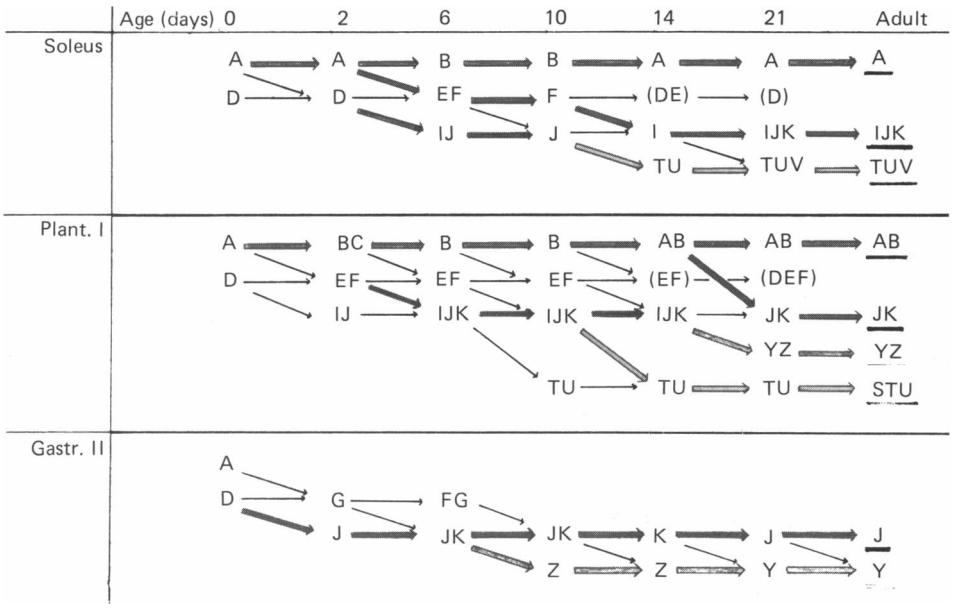


Fig. 4. Most probable pathways of development of adult fibre types in three regions of the complex: soleus muscle, plantaris muscle region I and gastrocnemius muscle region II. Broad arrows indicate the most probable pathways.

of shifts within the fibre population during postnatal development. Besides the frequently occurring types, 'rare' types were also found (L–N, O–R), especially in regions with a mixed fibre population, and during certain periods of development. In the adult, the main types were A–C (resembling the STO fibres of Peter *et al.* 1972), the S–V fibres (id. FTOG), and the W–Z fibres (id. FTG).

Soleus muscle (Fig. 5*a*)

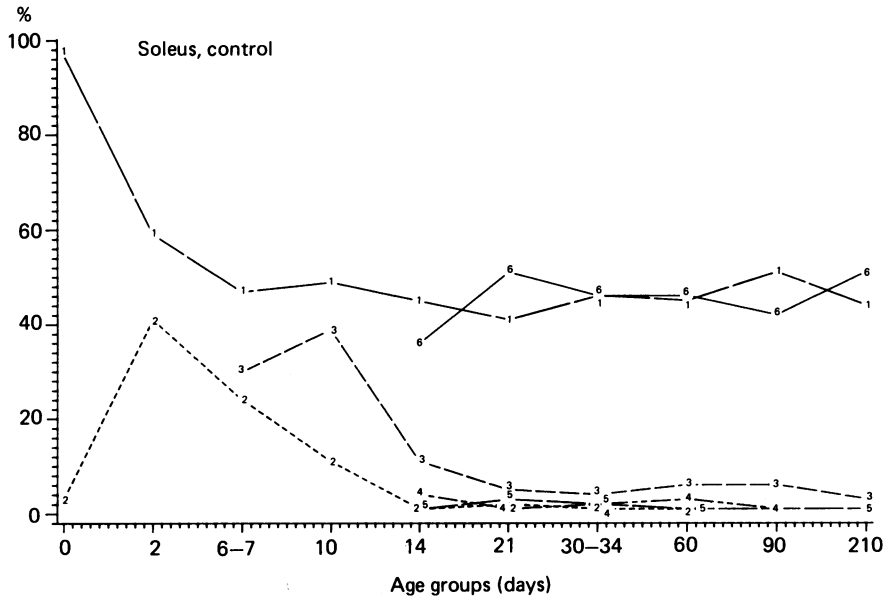
The adult muscle is mainly composed of the following types: A (±44%), I–J (±4%) and T–V (±48%). At 2 days after birth, about half of the fibres were of type D. At one week, I–K fibres appeared in the sections, and, slightly later, S–V fibres were observed. In animals of 14 days and older, L–N and O–R fibres were found with low frequency.

Plantaris muscle (Fig. 5*a*)

The adult muscle was found to be composed of A–B (±2%), I–K (±18%), S–V (±40%) and Y–Z (±40%) fibre types. At birth, about one third of the fibres was of the D type. I–J fibres were observed already at day 2, and at day 10 the first T–V fibres were found, followed by Z fibres at two weeks of age. The percentage of A–C fibres continued to decrease, so that, in the adult, only a few A fibres remained. I–K fibres remained as a substantial part of the population, bridging the gap between T–V and Y–Z fibres. 'Rare' types were observed during the period between 14 and 30 days of age.

Gastrocnemius muscle (Fig. 5*b*)

Because of local variations in fibre type composition, two regions (I and II) were evaluated. In the adult muscles, A–C fibres were missing in both regions. The



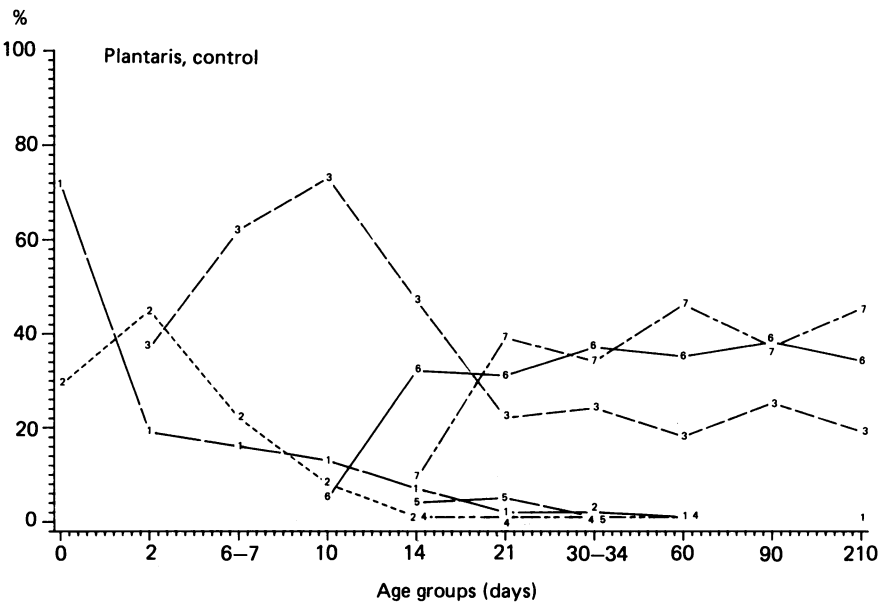
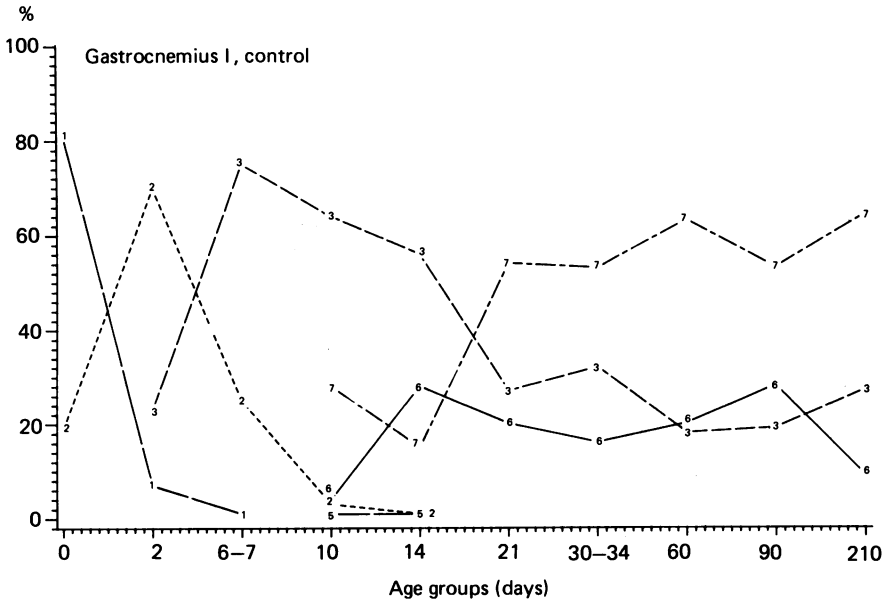


Fig. 5(*a*)

Fig. 5 (*a–b*). Frequencies of seven fibre classes (see Fig. 2) plotted against the age groups for the soleus and plantaris muscles and gastrocnemius muscle regions I and II. (1) Class A–C; (2) class D–G; (3) class H–K; (4) class L–N; (5) class O–R; (6) class S–V; (7) class W–Z. During postnatal development, shifts in fibre types occur. They often show up as chiasmata in the graphs. Number of animals, 24.



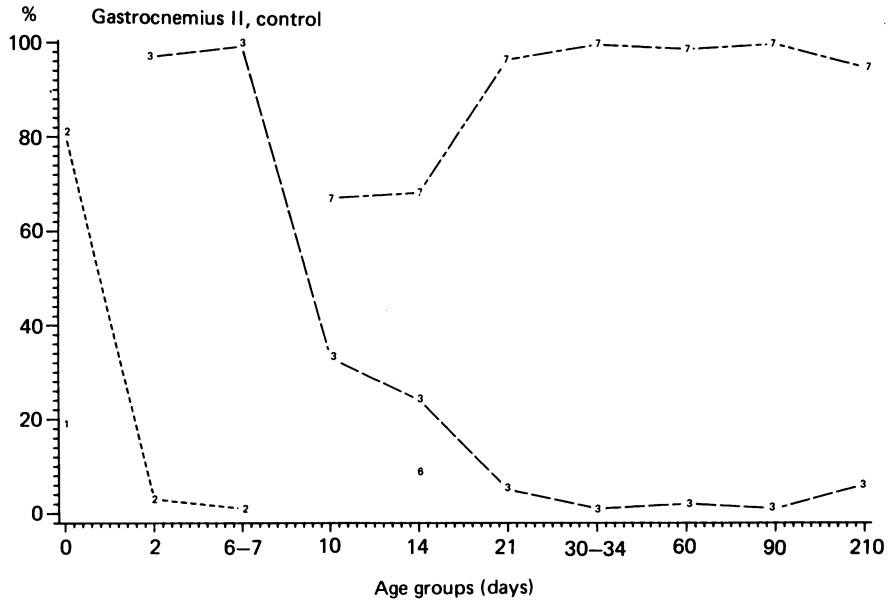


Fig. 5(*b*)

gastrocnemius muscle region I was composed of T–V (± 15 %), J–K (± 25 %) and Y–Z (± 60 %) fibre types. In the gastrocnemius muscle region II, the majority of fibres were of the Y (± 95 %) type, while some (5 %) J type fibres were found. At birth, about 80 % of the fibres were of the D type in the peripheral region, but, at day 2, nearly all fibres were of the J type. At day 10, more than 50 % of the fibres were of the Z type.

In Figure 6(*a–d*), the relation between fibre type and fibre size is plotted against age. For each fibre type the average size is given within each age group. Figures 8–10 are representative for this development.

It should be noted that a considerable variation in the percentages of different types occurs because fibre types appear to develop or disappear in the process of postnatal differentiation. Some letters in Figure 6, therefore, represent only one or a few fibres. However, it appears that each fibre type has a characteristic size, and combining the different age groups results in a characteristic growth pattern. This indicates a strong relation between histochemical activity pattern and fibre size (growth). When the different regions in the complex are compared, it becomes clear that this type/size relation is not fixed, but depends on the location of the fibre in the muscle complex. In Figure 7 an example is given of the range in fibre sizes within the types. Generally, the data indicate a normal distribution, which makes the presentation of average sizes in Figure 6 acceptable.

DISCUSSION

Histochemical classifications may be based on metabolic enzymes (Dubowitz & Pearse, 1960), on ATPase isoenzymes (Brooke & Kaiser, 1970*a*), or a combination of both (Peter *et al.* 1972). The choice depends on the aim of the investigation and the preference of the investigator, but this often makes it difficult, if not impossible, to compare the results (Nemeth, Hofer & Pette, 1979). Most classifications are based on three fibre types. These classifications have been found too rigid and restrictive for a precise analysis of the course of events during postnatal maturation and muscular diseases.

Moreover, electrophysiological investigations have shown that the variety of contractile characteristics of individual motor units is much more extensive than could have been expected on the basis of the usual histochemical classifications (Burke *et al.* 1971). Investigations using immunofluorescence of the myofibrillar apparatus of striated muscle have demonstrated that shifts and alterations in the protein profile occur during normal postnatal development (Pool, 1980; Rubinstein & Kelly, 1980; Whalen, 1980) as well as during pathological processes (Kelly & Rubinstein, 1980).

Our proposed classification based on the usual visual division into three (four) densities, distinguishes 26 fibre types. They roughly correspond with fibre types described by other investigators: the STO group (A–C), the FTOG group (S–V), the FTG group (W–Z), the intermediate group (H–K) and the transitional group (D–G). Recently, Spurway (1980*a*, *b*), Nemeth *et al.* (1979) and Nemeth & Pette (1980) have published evidence of the occurrence of FO (fast-oxidative) fibres in adult mouse and rat muscle. Moreover, Nemeth *et al.* (1979) and Nemeth & Pette (1980) described SOG (slow-oxidative-glycolytic) fibres. In our classification, fibres with these characteristics would be classed as S (T) plus H (I) for the FO type, and N (M) plus C (B) for the SOG type. The 'slow twitch' group can be characterised rather well with regard to the 'fast twitch' group by the myosin ATPase (at pH 4·35), which renders the 'slow twitch' fibres dark brown (Ashmore *et al.* 1972; Spurway, 1980*a*). It is accepted that this enzyme is somehow related to the intrinsic speed of a muscle fibre (Barany, 1967). SDH and GPOX were used to separate subgroups, mainly in the 'fast' population. The SDH reaction can give information about the resistance to 'fatigue' (Edström & Kugelberg, 1968; Kugelberg & Lindegren, 1979; Burke *et al.*

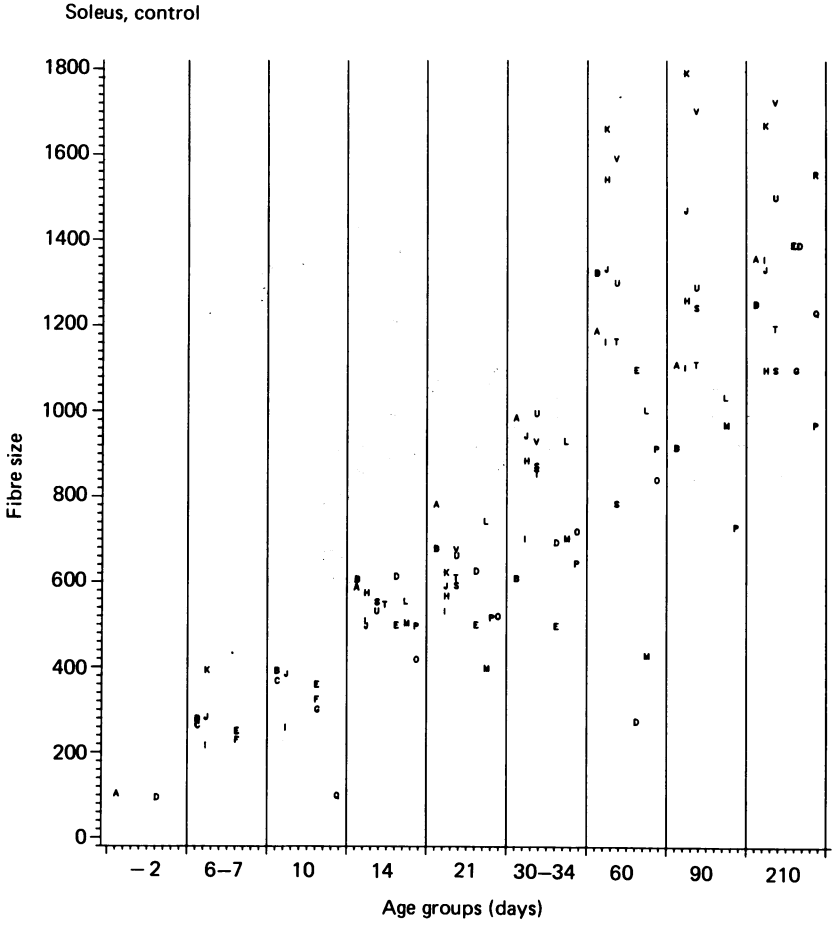

Soleus, control
Fibre size
1800
1600
1400
1200
1000
800
600
400
200
0
−2
6–7
10
14
21
30–34
60
90
210
Age groups (days)

Fig. 6(*a*)

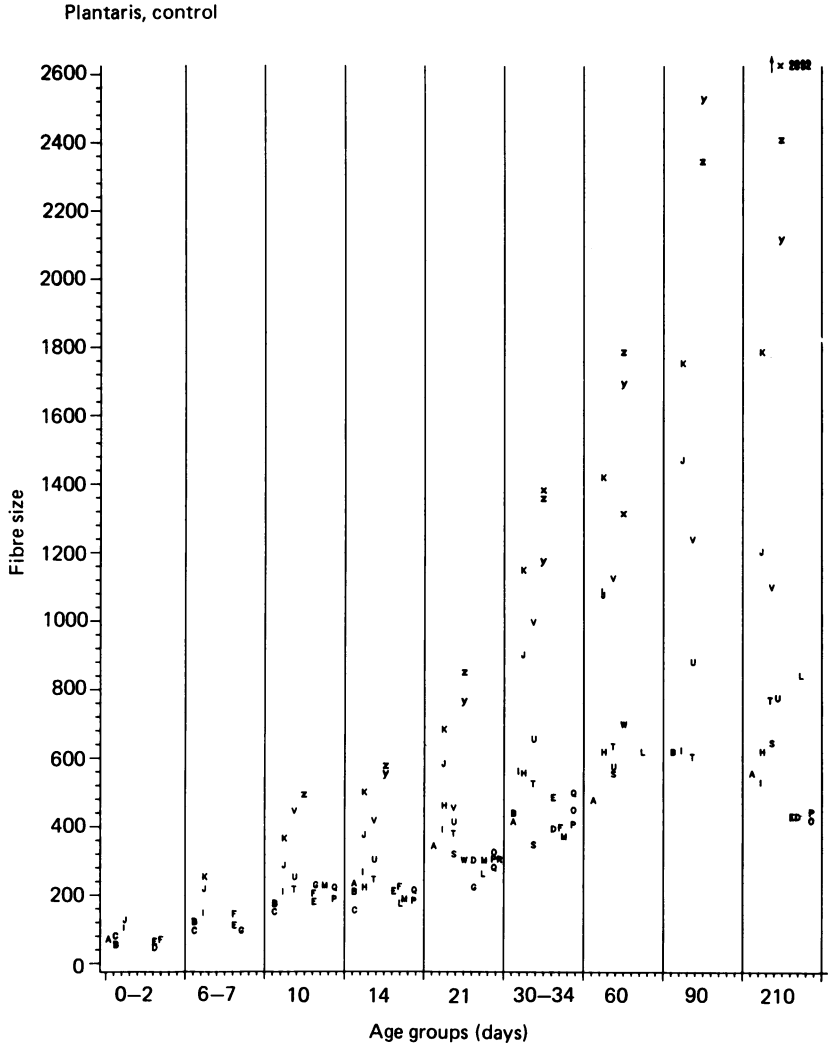

Plantaris, control
Fibre size
2600
2400
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
0–2
6–7
10
14
21
30–34
60
90
210
Age groups (days)

Fig. 6(*b*)

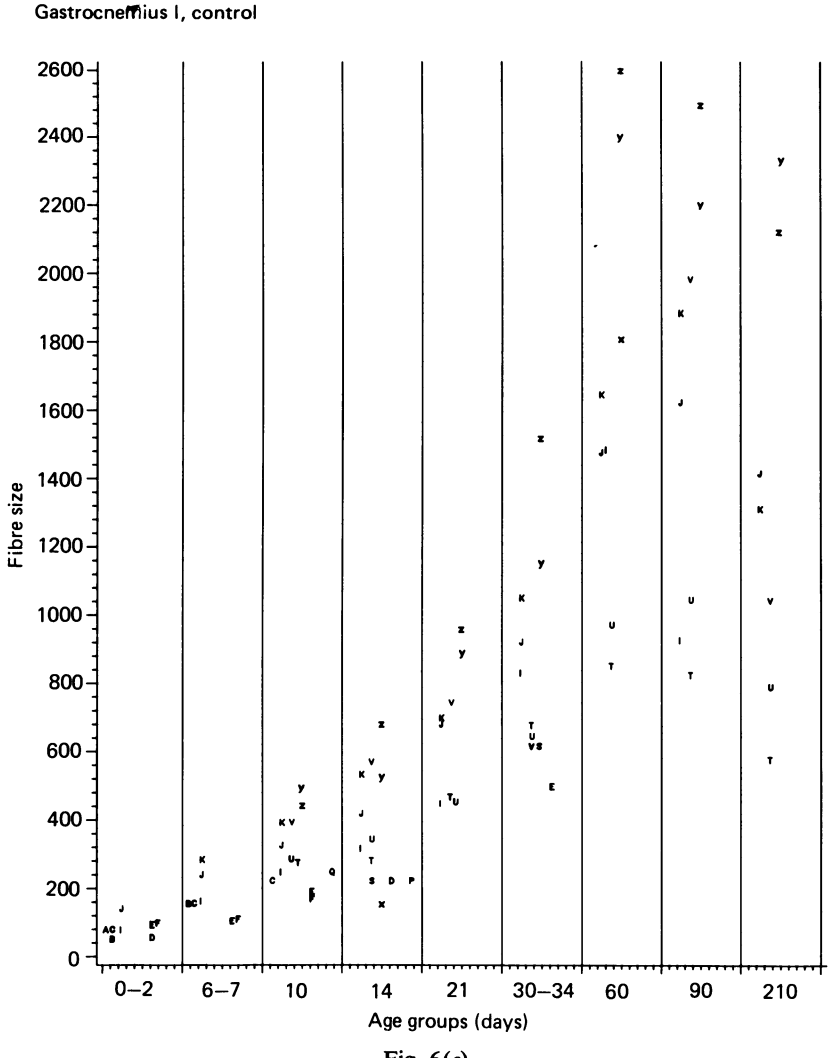


Fig. 6(*c*)

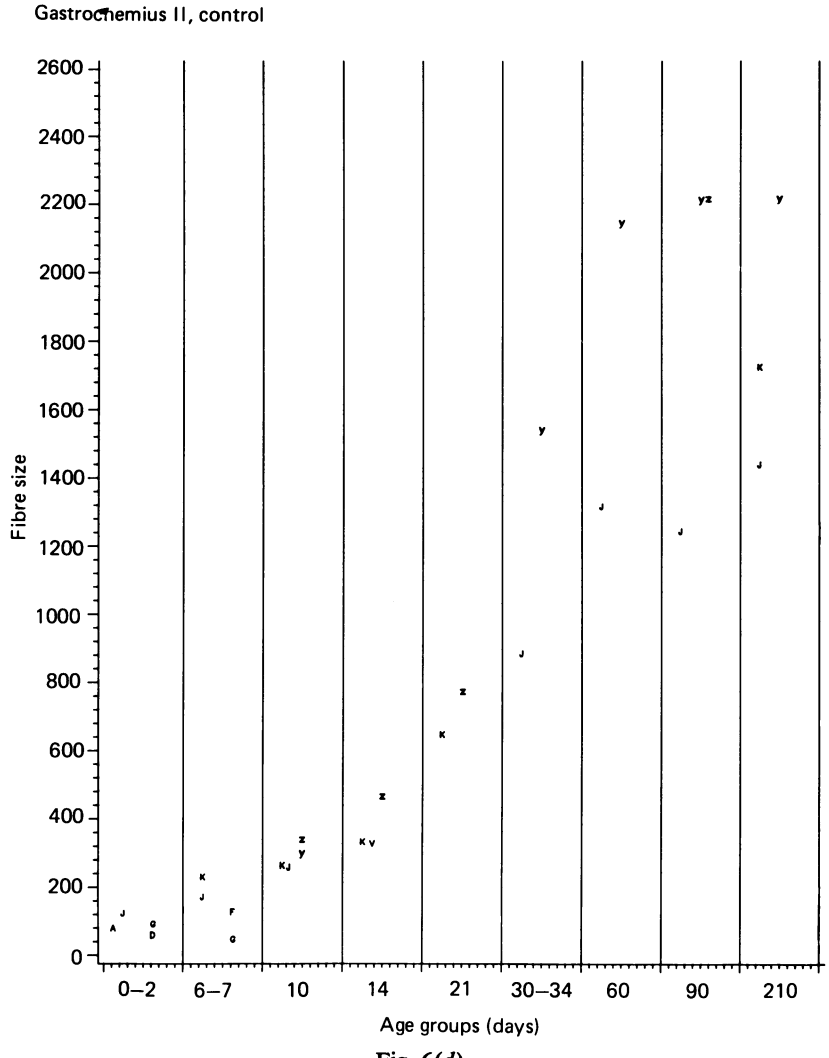


Fig. 6(*d*)

Fig. 6 (*a–d*). Relation between fibre type and size (in μm^2). Each letter represents the average size within the age group. Note that a fibre type appears to follow a specific growth pattern during postnatal development, implying a certain relation between (enzyme) activity and size.

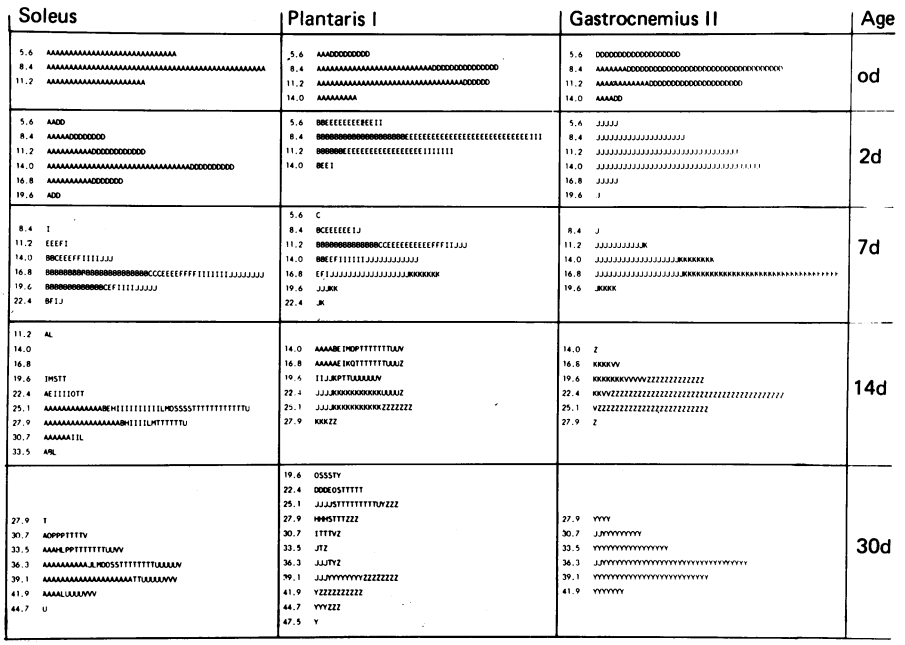


Fig. 7. Histograms of muscle fibre samples, revealing the distribution of a fibre type over size classes during development. Note that, in general, a normal distribution is indicated for a fibre type. One animal each was chosen to represent an age group. Each letter indicates 1% of the total sample for that fibre type. Fibre size classes are in arbitrary units.

1971). α-glycerol-phosphate-dehydrogenase is a mitochondrial enzyme and is proportional to the glycolytic activity in muscle (Pette & Spamer, 1979). Myosin ATPase, SDH and GPOX together visualize important characteristics of muscle fibres. While it could thus appear that our proposed classification merely replaces the existing ones, it has nevertheless enabled us to trace the probable course taken during the maturation of the (3 basic) adult fibre types.

Growth and differentiation of muscle fibres during postnatal development

At birth, skeletal muscles in rat and mouse are in fact underdeveloped, compared with other animals and man (Dubowitz, 1965). During the postnatal period, muscle fibres not only hypertrophy, but their (iso-) enzymic pattern also changes drastically. The contractile elements, too, may pass through a differentiation process which has been visualized by immunocytochemical techniques (Gauthier, 1980; Rubinstein & Kelly, 1980; Pool, 1980; Whalen, 1980). Metabolic parameters may be very flexible, because the half-life of these enzymes is about one day (Pette & Dölken, 1975). Changes in the myofibrillar system may take longer to achieve (Wirtz, unpublished data). An important event during the early postnatal period is the elimination of polyneural innervation. It is not known what influence this has on muscle fibre type, nor how these processes are connected with postnatal behaviour, e.g. directed motor activity. It is supposed that in the rat the majority of fibres change from polyneural innervation between days 10 and 16 postnatally (Betz *et al.* 1979; Thompson, Kuffler & Jansen, 1979; Brown, Jansen & van Essen, 1976). Lapointe & Nosal (1979) examined the primitive twitch activities in rats, and observed that these spontaneous twitches disappeared when locomotor activity developed around day 17. Muscle

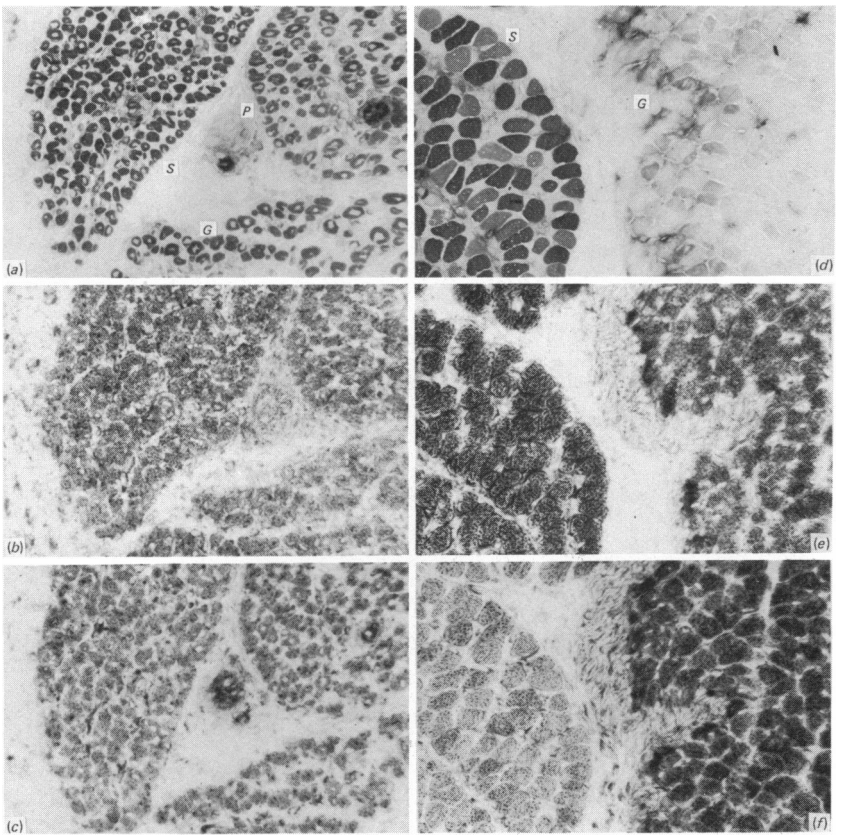


Fig. 8

Figs. 8–10. Growth and differentiation of muscle fibres. All photographs have the same magnification. *S*, soleus muscle; *P*, plantaris muscle; *G*, gastrocnemius muscle. (*a*) and (*d*): ATPase; (*b*) and (*e*): SDH; (*c*) and (*f*): GPOX. (Serial sections per age). Fig. 8(*a–c*) 0 days of age; (*d–f*) 7 days of age. Fig. 9(*a–c*) 10 days of age; (*d–f*) 14 days of age. Fig. 10(*a–c*) 1 month of age; (*d–f*) 7 months of age. Magn. ±120×.

fibres within one motor unit are supposed to be homogeneous for certain metabolic enzymes (Nemeth, Pette & Vrbová, 1980). Changes in fibre type may be partly explained by a switch in innervation from one motor neuron to another by individual fibres (Betz *et al.* 1979), mainly in young muscle, or by changes in the control exerted by the motor neuron. In the latter case it might be expected that all fibres in the unit would be involved in the change of pattern (Edström & Kugelberg, 1968). Welt, Scheller, Schippel & Schippel (1978) observed that histochemical differentiation of muscle fibres became visible in the rat when the animals started walking (i.e. 3 weeks postnatally), while Curless & Nelson (1976) and Haltia, Berlin, Schucht & Sourander (1978) reported the shift from IIC fibres into 'fast twitch' (IIA) fibres in the rat soleus and extensor digitorum longus muscles, respectively, at an age of about 18 days. Hughes (1966) stated that, in the mouse, most muscle fibres were mononeurally innervated after day 10. It is interesting to note that, according to the present classification, during this period (10–14 days) adult 'fast' types appeared. Haltia *et al.* (1978) discerned three phases in the development of the extensor digitorum longus and anterior tibialis muscles of rats. During the first 5 days, fibres (called F-type and

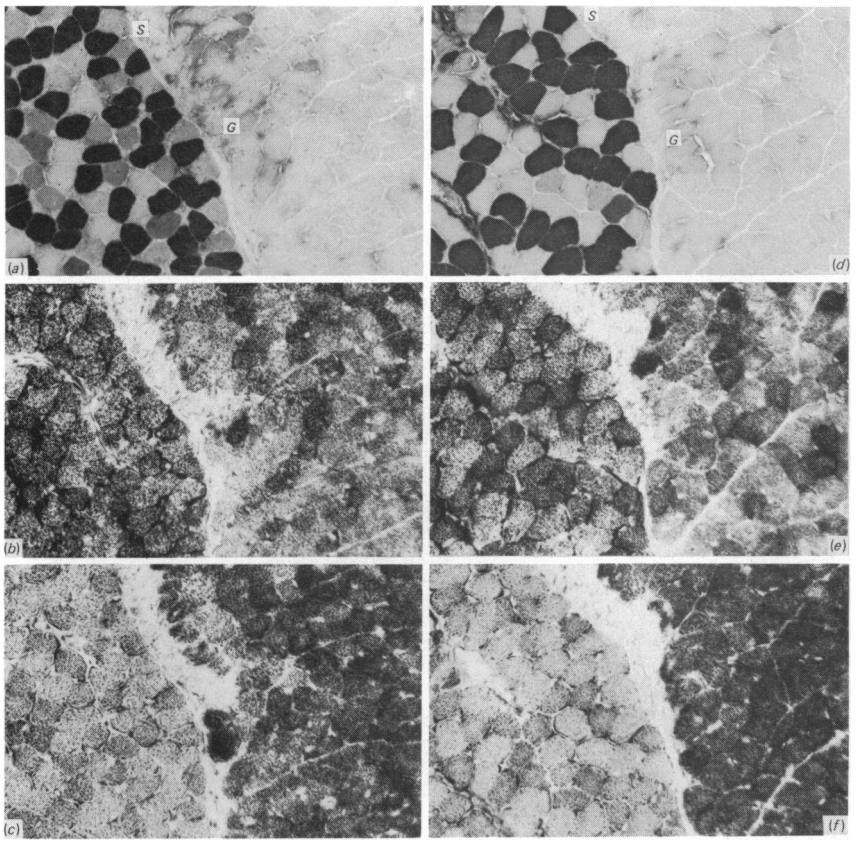


Fig. 9

resembling IIC fibres of Brooke & Kaiser, 1970*b*), changed into IIB. Between 5 and 15 days, no spectacular changes were noted. Between 15 and 20 days, IIA fibres developed from F fibres. From 20 to 60 days, the number of type I fibres increased. In the hamster, Johnson & Pearse (1971) found changes in the ATPase, phosphorylase and diaphorase patterns 1 day, 5 days and 15 days after birth, respectively. α-glycerol-phosphate-dehydrogenase differentiation was found not earlier than 30 days postnatally. Goldspink & Ward (1979) were able to distinguish (in mouse and hamster) three adult fibre types 2 weeks postnatally, using metabolic enzymes, and at 3 weeks with myosin ATPase. Dubowitz (1965) found no differentiation in diaphorase in neonatal rats. The 'chess board' pattern developed gradually during the first two weeks.

Literature concerning muscle fibre differentiation as well as growth during postnatal development is scarce. Johnson & Pearse (1971) observed that, in the quadriceps muscle of the hamster, the STO fibres were the smallest, FTG fibres the largest and FTOG fibres were intermediate in size. The size pattern developed between 25 and 40 days postnatally. Curless & Nelson (1976) observed no difference in size between II C and II A fibres in rat soleus muscles, whereas type I fibres tended to be larger than type II fibres. The data presented by Haltia *et al.* (1978), concerning the extensor digitorum longus muscle in rats, show that there was an increase in the average size when F fibres switched into II fibres, 5 days postnatally. The IIB fibres

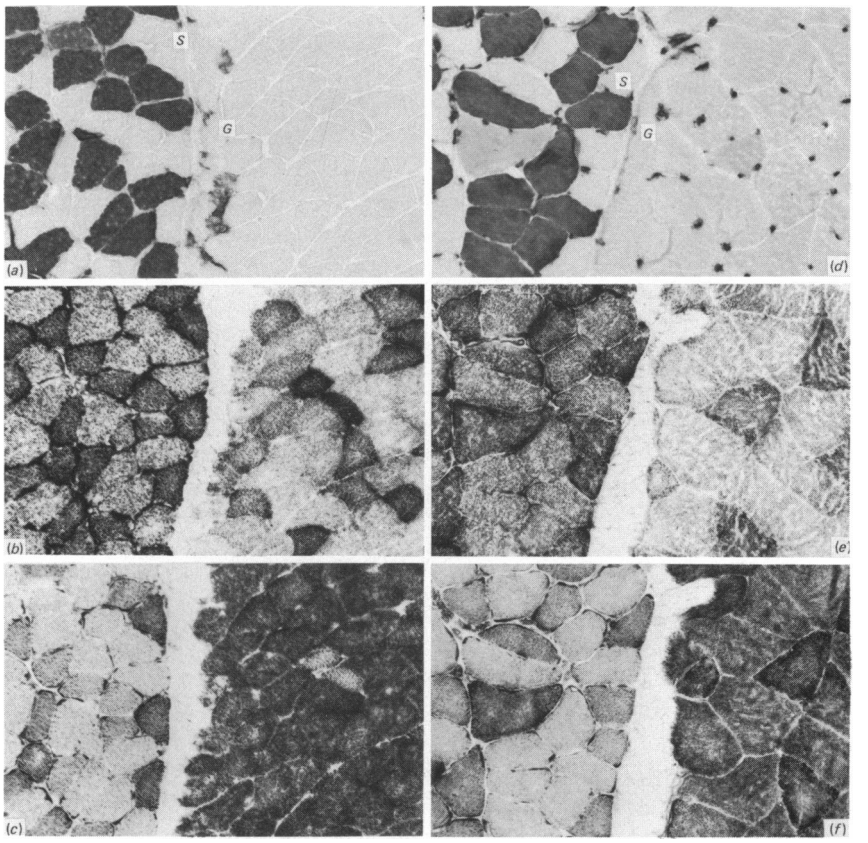


Fig. 10

were intermediate in size between the small fibres and type I fibres. With our approach, postnatal growth and differentiation are visualized in detail per muscle fibre type. In certain regions the pathways of development can be followed quite easily (e.g. gastrocnemius II), but in other regions the situation may be more complex (e.g. plantaris muscle).

The following developmental pathways are proposed: according to the classification used, the future 'slow' A–C fibres do not change histochemically. These findings are in agreement with those of Curless & Nelson (1976), who found that type I fibres remained consistent after birth. From the perinatal period on, the future 'fast' fibres pass through a process of differentiation that lasts about 2–3 weeks. The pathways are roughly indicated by arrows in Figure 2. Starting from A–C fibres they develop into D–G ('transitional') and subsequently into H–K ('intermediate') fibres. Then an apparent bifurcation leads either to the adult S–V and/or W–Z fibres. A varying percentage remains as H–K fibres. Transitional fibres have been mentioned by Ringqvist (1973), Davies (1972) and Khan (1978), while intermediate fibres are also generally accepted. Transitional fibres may be characterised as fibres in the process of differentiation from 'slow' to 'fast', or *vice versa*, whereas the intermediate fibres are histochemically and morphometrically intermediate between both the 'fast' FTOG (S–V) and FTG (W–Z) fibres. The most probable pathways leading to the adult situation are presented in Figure 4 for the 'slow' soleus muscle, the 'mixed'

plantaris muscle and the 'fast' gastrocnemius muscle region II, because the muscles (regions) showed differences in their development (see Materials and Methods). It appears that there is a strong relationship between the histochemical profile and the size of a fibre in all age groups, indicating that each fibre type follows a characteristic growth pattern already from birth on. This can be clearly seen in the plantaris muscle (Fig. 6*b*).

If the developmental pattern, as proposed above, is accepted, it will be noted that a change in the histochemical make-up of a fibre is sometimes accompanied by a rather sudden change in size. In the plantaris muscle, for example, the I–K fibres that developed from the E–G fibres between day 0–2 and 6–7 are much larger than the E–G fibres were at day 6–7. Thereafter, the members of both groups follow their own growth pattern. The same is true for Z fibres that developed from H–K fibres about day 10. In contrast, the T fibres do not increase in size compared with H and I fibres. The ultimate size of a fibre appears to depend also on its location in the muscle complex: the relation between type and size is not fixed, but depends on the location in the complex and, presumably, on muscle function.

SUMMARY

A histochemical classification of muscle fibres based on three enzymes (ATPase, pre-inc. pH 4·35; succinic dehydrogenase and α-glycerol-phosphate-dehydrogenase) was used to describe postnatal growth and differentiation of muscle fibres. The m. soleus, m. plantaris and m. gastrocnemius were examined in normal mice from birth to the young adult stage. At birth, differentiation of the gastrocnemius muscle was in a more advanced stage than that of the plantaris and the soleus muscles, while the last of these was the least developed. During growth, as well as in the (young) adult, there was a distinct relation between fibre type and size, which, however, differed per muscle (region). The development of muscle fibres was a gradual process, rather than a succession of distinct stages, although a change in fibre type was often accompanied by a change in size. Differentiation of fibres already occurred perinatally, and the 'adult fast' fibre types appeared during the second week postnatally, varying with the muscle region. During development, a percentage of fibres remained as a population that was histochemically and morphometrically intermediate between the fast-oxidative-glycolytic and fast-glycolytic adult fibres. A model is presented in which the most probable pathways of development are depicted.

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