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Data on the Distribution of Fibre Types in Thirty-six Human Muscles
An Autopsy Study

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INTRODUCTION

It is a well-established fact that mammalian skeletal muscle fibres can be classified into fibre types on the basis of their metabolic and electrophysiological characteristics. The application of histochemical techniques provides a convenient method of studying the distribution of these fibre types in tissue sections. The use of one technique in particular, namely the myofibrillar ATPase reaction, has proved to be of value since it most directly reflects the physiological difference between fast and slow twitch fibres.

In this study, a large number of human muscles have been examined with regard to the respective distribution of fibre types within them, with three main objectives in view. Firstly, we wished to obtain reliable data on the fibre type constitution of normal muscles, so that valid comparisons may be made with clinical material in which it is thought that there may be a significant alteration in fibre type proportions. The difficulties inherent in obtaining adequate normal material by means of biopsy are well known. As a result, recourse has often been made to material from subjects who could not necessarily be regarded as unequivocally normal clinically, otherwise biopsy would not, as a rule, have been performed. Moreover, the data thus acquired are limited almost exclusively to the findings in muscles which are commonly biopsied. Our second objective was to find out whether there was any correlation between fibre type constitution on the one hand and the well-recognised clinical phenomenon of selective sparing or affection of particular muscles in the various forms of muscular dystrophy on the other. In this aspect of the work it was necessary to study many more muscles than could be obtained by means of biopsy procedures. Thus, both from the point of view of the extent of the survey and because it was important to adopt the strictest possible criteria of normality, autopsy material provided the only alternative.

Our interest lay not only in the numerical distribution of muscle fibres of the two main histochemical types in the various muscles studied, but also in their spatial distribution. It has been shown that the fibres of the normal motor unit occupy a territory which overlaps with the territories of other motor units of different physiological types, giving rise to the characteristic mosaic pattern of normal muscle (Edstrom and Kugelberg 1968). In diseases of muscle of neurogenic origin, the successive processes of denervation and reinnervation may modify this "chequerboard" pattern and give rise to groups of fibres of uniform type (Engel 1970). In assessing the significance of such fibre type grouping in biopsy material, it is important to have available data on the extent to which such grouping may appear normally in the context of differing fibre type proportions. The compiling of such data comprises the third object of this study.

MATERIALS AND METHODS

Samples of muscle were obtained at autopsy from 6 male subjects aged between 17 and 30 years. These subjects had all died suddenly from head injury (3), asphyxia (1), acute ventricular failure (1) and severe internal abdominal haemorrhage (1). The survey was limited to subjects of one sex, within a restricted age range, in order to obtain a group of comparable individuals. Table 1 shows the ages, weights and heights of the 6 subjects.

TABLE 1

THE AGES, WEIGHTS AND HEIGHTS OF THE SIX MALE AUTOPSY SUBJECTS

P.M. number	*Age (years)*	*Weight (kg)*	*Height (cm)*
1	28	98	183
2	18	78	198
3	20	78	183
4	17	74	183
5	30	82	188
6	18	61	183

Muscle specimens were removed from one side of the body, within 24 hr of death. In some cases, both superficial and deep parts of a particular muscle were sampled (see Table 2), and in several instances specimens were taken from more than one head within a single muscle. Blocks measuring approximately 1 cm^3 were frozen in dichlorodifluoro-methane (Arcton 12) cooled to -150° C in liquid nitrogen. Cryostat sections were cut at a thickness of 10 μm and were stained to demonstrate the activity of myofibrillar ATPase at pH 9.5 (Hayashi and Freiman 1966). Sections were also stained with haematoxylin and eosin for histological examination.

Assessment of the muscle samples as regards fibre type proportions was made using the myofibrillar ATPase preparations. In each sample, three areas were selected at random and a total of 200 fibres were counted and classified as Type I or Type II. In this study, we did not differentiate between Type IIa and IIb (Brooke and Kaiser 1970) but classified them together as one type. The diameters of the same 200 fibres

TABLE 2

THE PERCENTAGES OF TYPE I FIBRES IN SAMPLES OF 36 HUMAN MUSCLES OBTAINED FROM 6 MALE ADULTS AT AUTOPSY

	Post-mortem No.					
	1	*2*	*3*	*4*	*5*	*6*
Abductor digiti minimi	N.T.	58.0	36.5	56.0	69.5	39.0
Abductor pollicis brevis	70.3	55.5	49.0	78.0	61.0	64.0
Adductor magnus (surface)	73.6	42.9	50.0	43.7	53.2	57.5
Adductor magnus (deep)	78.5	45.3	52.4	68.3	64.0	71.5
Adductor pollicis	86.0	69.5	72.0	92.5	83.0	79.1
Biceps brachii (surface)	53.3	34.4	34.4	49.0	45.0	37.4
Biceps brachii (deep)	61.0	50.5	37.4	57.9	56.0	40.5
Biceps femoris	80.5	73.0	54.3	73.4	56.7	63.6
Brachioradialis	59.8	32.7	27.8	43.5	45.2	29.5
Deltoid (superficial)	67.6	62.1	47.9	49.8	43.1	49.1
Deltoid (deep)	N.T.	64.5	43.9	76.8	58.0	61.8
1st Dorsal interosseus	N.T.	64.6	50.0	59.5	61.5	51.5
Erector spinae (surface)	100.0	57.9	26.7	62.7	50.5	52.6
Erector spinae (deep)	88.6	41.9	34.0	74.6	50.5	39.5
Extensor digitorum	49.0	42.2	48.4	45.5	56.2	42.5
Extensor digitorum brevis	74.5	46.0	32.5	37.5	45.0	36.5
Flexor digitorum brevis	N.T.	41.5	40.0	65.0	39.3	36.5
Flexor digitorum profundus	70.0	44.3	23.8	72.0	37.9	35.9
Frontalis	N.T.	N.T.	51.0	75.0	87.5	43.0
Gastrocnemius (lat. head surface)	46.0	45.5	35.2	46.0	37.7	50.7
Gastrocnemius (lat. head deep)	60.0	44.1	43.3	46.7	53.5	54.0
Gastrocnemius (medial head)	N.T.	47.6	49.3	53.4	46.9	56.9
Gluteus maximus	71.5	41.2	44.3	68.4	44.1	45.0
Iliopsoas	55.0	37.0	40.7	47.0	60.9	54.5
Infraspinatus	55.5	43.1	34.6	54.0	39.0	45.8
Latissimus dorsi	65.5	55.9	43.5	60.0	35.5	42.5
Orbicularis oculi	N.T.	21.0	14.0	23.5	18.0	0.5
Pectoralis major (clavic head)	57.3	39.3	31.9	46.5	45.5	33.0
Pectoralis major (sternal head)	60.7	29.2	27.4	57.5	44.6	39.5
Peroneus longus	76.4	57.5	49.0	63.0	69.8	59.5
Rectus abdominis	56.2	46.6	31.6	51.0	36.0	55.5
Rectus femoris (lat. head surface)	28.2	31.4	21.5	38.6	35.7	21.5
Rectus femoris (lat. head deep)	44.3	40.2	34.1	52.4	42.5	38.6
Rectus femoris (medial head)	N.T.	33.2	44.3	48.1	38.5	50.0
Rhomboid	61.9	38.5	43.3	47.5	45.0	31.2
Sartorius	59.5	48.3	38.5	51.9	60.5	39.0
Soleus (surface)	100.0	69.8	86.2	83.0	81.0	98.5
Soleus (deep)	100.0	87.5	90.6	77.0	83.0	96.0
Sternomastoid	46.5	37.0	30.1	25.8	32.4	39.2
Supraspinatus	72.0	46.0	41.4	85.0	64.5	46.9
Temporalis	N.T.	49.7	59.5	51.0	30.5	41.5
Tibialis anterior (surface)	80.5	79.0	57.6	70.0	85.6	67.6
Tibialis anterior (deep)	76.4	73.5	68.3	76.0	77.5	64.5
Trapezius	77.5	54.0	27.4	75.5	38.6	49.3
Triceps (surface)	58.2	31.0	20.0	37.5	34.0	14.5
Triceps (deep)	47.5	32.5	30.7	46.5	22.5	16.5
Vastus lateralis (surface)	45.5	33.8	41.4	49.8	23.3	32.9
Vastus lateralis (deep)	46.5	49.0	42.9	63.4	39.5	40.0
Vastus medialis (surface)	47.8	47.0	38.3	32.0	49.0	48.3
Vastus medialis (deep)	79.0	57.0	56.1	66.0	57.5	53.5

N.T. = Not taken.

in each sample were also measured; the data on fibre measurements forms the subject of a subsequent paper (Polgar, Johnson, Weightman and Appleton 1972).

In order to assess the amount of fibre type grouping in the samples, we adopted the method of Jennekens, Tomlinson and Walton (1971) who originated the idea of counting "enclosed" fibres in muscle specimens. "Enclosed" fibres are defined as those fibres which are completely surrounded by fibres of their own histochemical type. Since the packing of muscle fibres in fasciculi approximates to a hexagonal lattice array, it follows that on average 7 fibres of one type are required in order to give one "enclosed" fibre (see Fig. 1). If more fibres of the same type are added to such a group, it is clear that proportionally more fibres will be "enclosed" relative to the total number in the group. It is also clear that the number of "enclosed" fibres of any one type will tend to rise as the proportion of that fibre type in the sample rises. However, it has been shown recently, in rabbit and guinea pig muscle, that the fibres of some motor units are not randomly dispersed (James 1971). It was held to be possible, therefore, that the number of "enclosed" fibres might not be solely dependent upon the numerical proportion of that fibre type in the sample. Instead, it could be suggested that the spatial organisation of motor units might differ in two muscles which had the same fibre type proportions so that there was more uniform fibre type grouping and hence more "enclosed" fibres in one than in the other. In order to determine statistically whether there was any evidence of this sort of difference in organisation in the large number of muscles studied, a hypothetical model was devised for purposes of comparison. In the model situation, it was assumed that the arrangement of individual fibres conformed to a hexagonal lattice and that the spatial distribution of the fibres of the two histochemical types was random. On this basis, the expected number of "enclosed" fibres was calculated for a complete range of fibre type proportions. These data formed the basis for the assessment of any significant differences in the actual samples studied.

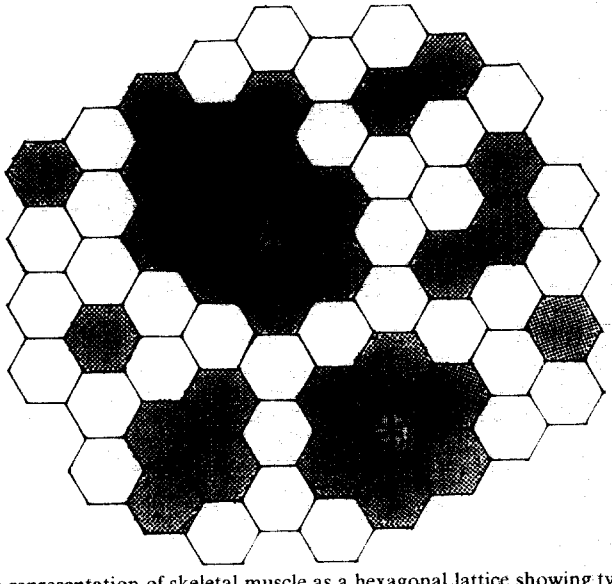

Fig. 1. A schematic representation of skeletal muscle as a hexagonal lattice showing two fibre types. "Enclosed" fibres (*) are those which are completely surrounded by fibres of their own fibre type.

TABLE 3

VARIABILITY OF TYPE I FIBRE PROPORTIONS BETWEEN THE SIX AUTOPSY SAMPLES OF INDIVIDUAL SKELETAL MUSCLES

Muscle	χ^2	*Degrees of freedom*	*Degree of significance*
Abductor digiti minimi	61.47	4	* * *
Abductor pollicis brevis	45.98	5	* * *
Adductor magnus (surface)	52.57	5	* * *
Adductor magnus (deep)	66.66	5	* * *
Adductor pollicis	47.57	5	* * *
Biceps brachii (surface)	27.87	5	* * *
Biceps brachii (deep)	38.57	5	* * *
Biceps femoris	50.54	5	* * *
Brachioradialis	66.33	5	* * *
Deltoid (surface)	37.48	5	* * *
Deltoid (deep)	48.79	4	* * *
1st Dorsal interosseus	13.58	4	*
Erector spinae (surface)	238.66	5	* * *
Erector spinae (deep)	197.74	5	* * *
Extensor digitorum	11.31	5	*
Extensor digitorum brevis	93.24	5	* * *
Flexor digitorum brevis	43.79	5	* * *
Flexor digitorum profundis	156.46	5	* * *
Frontalis	111.50	3	* * *
Gastrocnemius (lat. head surface)	14.64	5	*
Gastrocnemius (lat. head deep)	17.79	5	* *
Gastrocnemius (medial head)	5.97	4	Not sig.
Gluteus maximus	77.28	5	* * *
Iliopsoas	34.60	5	* * *
Infraspinatus	27.74	5	* * *
Latissimus dorsi	55.28	5	* * *
Orbicularis oculi	50.26	4	* * *
Pectoralis major (clavic head)	39.56	5	* * *
Pectoralis major (sternal head)	82.74	5	* * *
Peroneus longus	43.28	5	* * *
Rectus abdominis	44.55	5	* * *
Rectus femoris (lat. head surface)	25.51	5	* * *
Rectus femoris (lat. head deep)	16.25	5	* *
Rectus femoris (medial head)	16.42	4	* *
Rhomboid	47.54	5	* * *
Sartorius	38.04	5	* * *
Soleus (surface)	113.35	5	* * *
Soleus (deep)	72.73	5	* * *
Sternomastoid	24.73	5	* * *
Supraspinatus	125.92	5	* * *
Temporalis	38.65	4	* * *
Tibialis anterior (surface)	55.95	5	* * *
Tibialis anterior (deep)	13.81	5	*
Trapezius	161.65	5	* * *
Triceps (surface)	112.65	5	* * *
Triceps (deep)	70.93	5	* * *
Vastus lateralis (surface)	41.85	5	* * *
Vastus lateralis (deep)	32.42	5	* * *
Vastus medialis (surface)	20.11	5	* *
Vastus medialis (deep)	38.68	5	* * *

Significance levels for 5 degrees of freedom:

p	0.05	0.01	0.001
χ^2	11.070	15.086	20.517
	*	* *	* * *

TABLE 4

THE 95% CONFIDENCE LIMITS FOR MEAN FIBRE TYPE PERCENTAGE IN YOUNG MALE ADULT SUBJECTS

	Mean % type I fibres	*Mean % type II fibres*	*With 95% confidence the true mean % will lie between*			
			for type I fibres		*for type II fibres*	
Abductor digiti minimi	51.8	48.2	34.6	72.0	31.0	65.4
Abductor pollicis brevis	63.0	37.0	52.1	73.9	26.2	47.9
Adductor magnus (surface)	53.5	46.5	41.6	65.4	34.6	58.4
Adductor magnus (deep)	63.3	36.7	50.3	76.3	23.7	49.7
Adductor pollicis	80.4	19.6	71.3	89.5	10.6	28.7
Biceps brachii (surface)	42.3	57.7	33.9	50.7	49.3	66.2
Biceps brachii (deep)	50.5	49.5	40.5	60.5	39.3	59.6
Biceps femoris	66.9	33.1	56.0	77.8	22.2	44.0
Brachioradialis	39.8	60.2	30.0	52.6	47.4	73.0
Deltoid (superficial)	53.3	46.7	43.4	63.2	36.8	56.7
Deltoid (deep)	61.0	39.0	46.2	75.7	24.2	53.8
1st Dorsal interosseus	57.4	42.6	51.4	63.4	36.6	48.6
Erector spinae (surface)	58.4	41.6	33.3	83.5	16.5	66.7
Erector spinae (deep)	54.9	45.1	32.0	77.8	22.2	68.1
Extensor digitorum	47.3	52.7	41.8	52.8	47.2	58.2
Extensor digitorum brevis	45.3	54.7	29.3	61.3	38.7	70.6
Flexor digitorum brevis	44.5	55.5	33.6	55.4	44.6	66.4
Flexor digitorum profundis	47.3	52.7	26.9	67.8	32.2	73.2
Frontalis	64.1	35.9	31.2	97.0	3.0	68.8
Gastrocnemius (lat. head surface)	43.5	56.5	37.4	49.6	50.4	62.6
Gastrocnemius (lat. head deep)	50.3	49.7	43.3	57.2	42.8	56.7
Gastrocnemius (medial head)	50.8	49.2	45.6	56.0	44.0	54.4
Gluteus maximus	52.4	47.6	36.1	66.8	33.2	61.9
Iliopsoas	49.2	50.8	39.5	58.8	41.2	60.5
Infraspinatus	45.3	54.7	36.7	54.0	46.0	63.3
Latissimus dorsi	50.5	49.5	38.2	62.8	37.3	61.8
Orbicularis oculi	15.4	84.6	4.1	26.7	73.3	95.9
Pectoralis major (clavic. head)	42.3	57.7	32.2	52.3	47.7	67.8
Pectoralis major (sternal head)	43.1	56.9	28.5	57.8	42.2	71.5
Peroneus longus	62.5	37.5	52.5	72.6	27.4	47.5
Rectus abdominis	46.1	53.9	35.4	56.9	43.1	64.6
Rectus femoris (lat. head surface)	29.5	70.5	22.0	37.0	63.0	78.0
Rectus femoris (lat. head deep)	42.0	58.0	35.6	48.5	51.5	64.4
Rectus femoris (medial head)	42.8	57.2	34.1	51.5	48.5	65.9
Rhomboid	44.6	55.4	33.7	55.3	44.7	66.2
Sartorius	49.6	50.4	39.6	59.7	40.3	60.4
Soleus (surface)	86.4	13.6	74.5	98.4	1.6	25.5
Soleus (deep)	89.0	11.0	80.2	97.9	2.1	19.8
Sternomastoid	35.2	64.8	27.5	42.8	57.2	72.5
Supraspinatus	59.3	40.7	41.1	77.5	22.5	58.9
Temporalis	46.5	53.5	32.9	60.1	39.9	67.1
Tibialis anterior (surface)	73.4	26.6	62.6	84.1	15.9	37.4
Tibialis anterior (deep)	72.7	27.3	67.2	78.1	21.9	32.8
Trapezius	53.7	46.2	32.8	74.6	25.4	67.2
Triceps (surface)	32.5	67.5	16.5	48.6	51.4	83.5
Triceps (deep)	32.7	19.6	45.8	54.2	54.2	80.4
Vastus lateralis (surface)	37.8	67.3	19.6	45.8	52.1	72.3
Vastus lateralis (deep)	46.9	53.1	37.5	56.2	43.8	62.5
Vastus medialis (surface)	43.7	56.3	36.4	51.1	48.9	63.6
Vastus medialis (deep)	61.5	38.5	51.5	71.5	28.5	48.5

RESULTS

1. Fibre type constitution

The results shown in Table 2 list the percentages of Type I fibres in the 6 autopsy samples of each of the 36 muscles studied. Because more than one site was sampled in some cases, a total of 50 sample sites was arrived at, though in a few isolated instances, particular muscle samples were not obtainable. A chi-square test (Brandt–Snedecor) was used to compare the post-mortem samples in terms of fibre type proportion. The results of these tests are given in Table 3, together with the corresponding significance levels. It can readily be seen that in 41 out of the 50 sets of muscle sample there is a highly significant difference between samples taken from the different subjects. It is apparent from Table 2 that the difference cannot be attributed to the fact that any one subject is aberrant when compared with the others. Rather, the differences arise as a result of an inherently wide variation in the fibre type proportions

TABLE 5

COMPARISON OF FIBRE TYPE PROPORTIONS IN SUPERFICIAL AND DEEP AREAS OF CERTAIN SKELETAL MUSCLES

	No. of post-mortem subject					
	1	*2*	*3*	*4*	*5*	*6*
Adductor magnus	n.s.	n.s.	n.s.	*** S > D	* S > D	** S > D
Biceps brachii	n.s.	*** S > D	n.s.	n.s.	* S > D	n.s.
Deltoid	n.s.	n.s.	n.s.	*** S > D	** S > D	** S > D
Erector spinae	*** D > S	** D > S	n.s.	** S > D	n.s.	** D > S
Gastrocnemius (lat. head)	** S > D	n.s.	n.s.	n.s.	** S > D	n.s.
Rectus femoris (lat. head)	*** S > D	n.s.	** S > D	** S > D	n.s.	*** S > D
Soleus	n.s.	*** S > D	n.s.	n.s.	n.s.	n.s.
Tibialis anterior	n.s.	n.s.	* S > D	n.s.	* D > S	n.s.
Triceps	* D > S	n.s.	* S > D	n.s.	* D > S	n.s.
Vastus lateralis	n.s.	** S > D	n.s.	** S > D	*** S > D	n.s.
Vastus medialis	*** S > D	* S > D	*** S > D	*** S > D	n.s.	n.s.

S > D = Significantly more Type II fibres in surface area than in deep area of muscle.
D > S = Significantly more Type II fibres in deep area than in surface area of muscle.
n.s. = No significant difference between surface and deep areas.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

within the same muscle in a group of subjects whom we would expect to be comparable. In this small group it was impossible to draw any conclusions as to the influence of either age or physical build on the fibre type constitution of the skeletal muscles. Clearly, it would be of interest to investigate the possibility that fibre type proportions may vary with age or as a result of differences in occupation. It is also possible that there may be a significant difference in fibre type proportions between male and female subjects, since Brooke and Engel (1969) have shown a sex difference in the relative sizes of the two main fibre types. Until these questions have been answered, we would consider it safer to use data on mean fibre type proportions to compare only subjects matched for age and sex.

Table 4 sets out the approximate 95 % confidence limits for the mean fibre type percentages in the case of both Type I and Type II fibres. This treatment of the data provides a convenient basis of comparison of routine biopsy material. It can be seen that the variance in fibre type proportions of the individual muscles differs widely. Several reasons for this may be suggested. Firstly, in large muscles with complex physiological functions, the problem of sampling error will be greater than in smaller and less complex muscles. Also, in the case of muscles receiving their innervation from different segmental nerves, the chance of significantly different fibre type proportions occurring in different areas of the same muscle will be increased. In this study, every effort was made to minimise sampling error by taking muscle specimens from exactly the same site at each autopsy.

In several muscles, samples were taken from both superficial and deep sites, and in the case of gastrocnemius, pectoralis major and rectus femoris, samples were taken from both heads of the muscle. The specimens thus obtained have been compared in order to give some indication of the variation to be expected within individual muscles. The results of these comparisons are given in Tables 5 and 6. It can be seen that a significant difference in fibre type proportion between deep and superficial areas of the muscles thus sampled is not a constant finding, but where it does occur it is most frequently due to the fact that the superficial area contains a higher proportion of

TABLE 6

COMPARISON OF FIBRE TYPE PROPORTIONS IN DIFFERENT HEADS OF 3 SKELETAL MUSCLES

	No. of post-mortem subject					
	1	*2*	*3*	*4*	*5*	*6*
Gastrocnemius (lateral head *vs.* medial head)	n.s.	n.s.	* * L > M	n.s.	n.s.	n.s.
Pectoralis major (clavicular head *vs.* sternal head)	n.s.	* C > S	n.s.	* C > S	n.s.	n.s.
Rectus femoris (lateral head *vs.* medial head)	* * L > M	n.s.	* * * L > M	* L > M	n.s.	* * * L > M

All muscle samples in this table were taken from the surface of the muscle.
L > M = Significantly more Type II fibres in lateral head than in medial head.
C > S = Significantly more Type II fibres in clavicular head than in sternal head.
n.s. = not statistically significant.

Type II fibres (see Table 5). Similarly, there was no consistently significant difference in the fibre type proportions of samples taken from different heads of gastrocnemius, pectoralis major or rectus femoris (see Table 6).

2. Fibre type grouping

In the theoretical model of muscle fibre distribution, it was assumed that the fibres of each fibre type were randomly arranged. Thus the probability that a fibre at a given locus will be, say, a Type I fibre, can be expressed as a function of p, the proportion of Type I fibres in the sample. It follows, therefore, that the probability of a Type II fibre occurring at a given locus in the same sample will be $1\text{-}p$. The probability of a Type I being "enclosed" by 6 other Type I fibres will be p^6, so that in a sample of N fibres the expected number of Type I "enclosed" fibres will be Np^7. For small values of p a Poisson approximation can be used to calculate the expected number of "enclosed" fibres and its standard deviation; for larger values of p the Normal approximation may be used. As the number of "enclosed" fibres is binomially distributed, the standard deviation is $Np^7(1\text{-}p^6)$.

TABLE 7

THE RELATIONSHIP BETWEEN THE OBSERVED NUMBER OF MUSCLE FIBRES OF A PARTICULAR FIBRE TYPE AND THE EXPECTED NUMBER OF "ENCLOSED" FIBRES OF THE SAME TYPE

Number of fibre type observed	*Number of "enclosed" fibres of that type expected*	*99.9% ± Confidence limits*
0	0.0	0.0
20	0.0	0.0
40	0.0	0.3
60	0.0	0.7
80	0.3	2.0
100	1.6	4.0
120	5.6	7.6
140	16.5	12.5
160	41.9	18.4
170	64.1	20.7
180	95.7	22.1
190	139.7	20.1
195	167.5	16.1
200	200.0	0.0

Table 7 sets out the expected number of "enclosed" fibres for a complete range of fibre type proportions, taking $N = 200$, the actual number of fibres examined. From this table it is apparent that comparatively small numbers of enclosed fibres of a given fibre type are to be expected until the percentage constitution of that fibre type rises above 70%. From this point onwards, there is a steep rise in the expected number of "enclosed" fibres until, of course, this reaches 100% in a muscle sample consisting solely of that particular fibre type.

The actual number of "enclosed" fibres observed in any one sample was compared with the number expected in a hypothetical sample of identical fibre type constitution

TABLE 8

COMPARISON OF OBSERVED NUMBER OF "ENCLOSED" FIBRES WITH THE EXPECTED NUMBER OF "ENCLOSED" FIBRES

number	*Type I*						*Type II*				
	1	*2*	*3*	*4*	*5*	*6*	*1*	*2*	*3*	*4*	*5*
ıctor digiti minimi	NT						NT				
ıctor pollicis brevis	+ + +		+								+
ıctor magnus (surface)								–			
ıctor magnus (deep)				– –	–	–					
ıctor pollicis			– –								
ıs brachii (surface)								– –	– –		
ıs brachii (deep)				–							
ıs femoris	+				–	–					
ıioradialis								–	–		
ıid (surface)		–									
ıid (deep)	NT					–	NT				
ıorsal interosseus	NT		+	–	+ + +		NT		+ +		+ +
ıor spinae (surface)											
ıor spinae (deep)									– –		
ısor digitorum											
ısor digitorum brevis	+ + +		+ +		+ + +	+	+ + +	+ + +		+ +	+ + + +
ır digitorum brevis	NT						NT				
ır digitorum profundis			+ +	+					+		
talis	NT	NT		+ + +	– – –		NT	NT			
rocnemius lat. head ırface)											
rocnemius lat. head ep)						+ +	+ +				
rocnemius medial ad	NT						NT				
eus maximus					+	–					
soas								– –			
spinatus							+ + +			–	
ısimus dorsi											
cularis oculi	NT						NT	– –	– – –	+ +	– – –
oralis major ıvicular head								– –		+	
oralis major sternal ad								–			
ıneus longus					+						

(*continued*)

BLE 8 (*contd.*)

. number	*Type I*						*Type II*				
	1	*2*	*3*	*4*	*5*	*6*	*1*	*2*	*3*	*4*	*5*
us abdominis											
tus femoris t. head (surface)											
tus femoris lat. head eep)										–	
tus femoris edial head	NT						NT				
mboid	–										–
orius	+ + +										
us (surface)		+	– –			– – –					
us (deep)				+	+						
nomastoid			+						– –		
raspinatus				+					–		
poralis											– –
alis anterior (surface)			+	–	– –						
alis anterior (deep)							+ +	– –			
pezius									– –		– –
eps (surface)	–										
eps (deep)											+
tus lateralis (surface)											
tus lateralis (deep)											–
tus medialis (surface)										– – –	+ +
tus medialis (deep)					–						

= observed number enclosed fibres lower than expected $p < 0.05$.
= observed number enclosed fibres lower than expected $p < 0.01$.
– – = observed number enclosed fibres lower than expected $p < 0.001$.
= observed number enclosed fibres higher than expected $p < 0.05$.
= observed number enclosed fibres higher than expected $p < 0.01$.
+ + = observed number enclosed fibres higher than expected $p < 0.001$.
= sample not taken.

as the sample under scrutiny. Figs. 2 and 3 express graphically the results of the comparison of observed and expected "enclosed" fibre numbers which are set out in detail in Table 8. In those muscle samples where significantly high numbers of "enclosed" fibres occur, it can be concluded that the degree of grouping of fibres of uniform fibre type is greater than would occur in a randomly distributed population. One muscle in particular stands out in this respect, namely, extensor digitorum brevis. This muscle contained a significant amount of uniform fibre type grouping in samples from all the subjects ($p < 0.001$ in 4 out of the 6 subjects). In some cases, both fibre types showed evidence of grouping; in others, only one fibre type was involved. Abductor pollicis

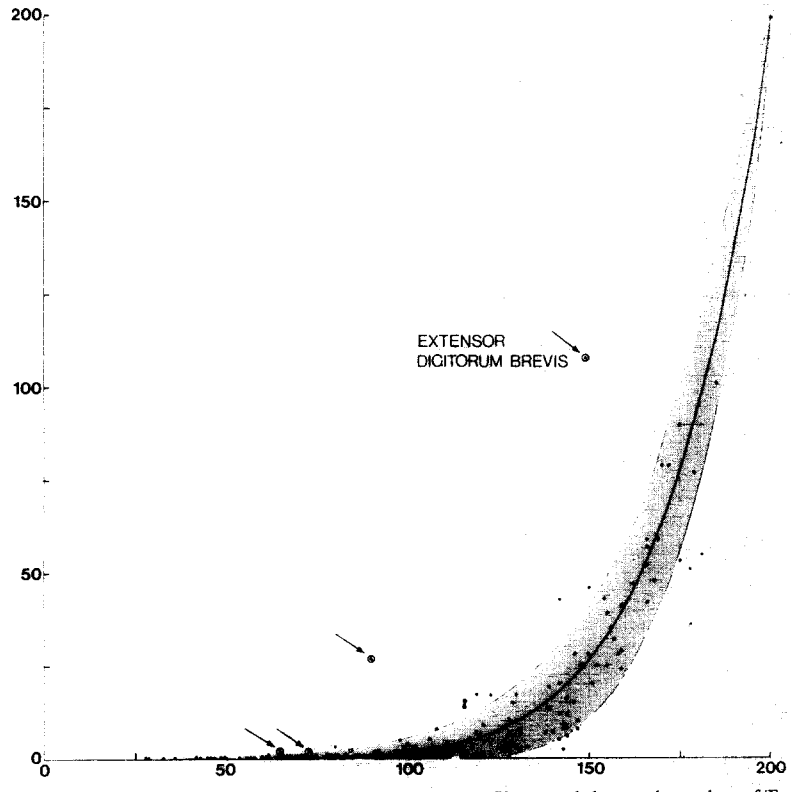

Fig. 2. The relationship between the number of enclosed Type I fibres and the total number of Type I fibres observed. The shaded area shows the formal 99.9% limits about the mean expected enclosed fibres. The only muscle which is above the limit more than once is extensor digitorum brevis which appears 4 times out of 6 post-mortem specimens.

brevis, 1st dorsal interosseus and flexor digitorum profundus showed less consistent evidence of fibre type grouping. In the other muscles, it was either extremely sporadic or non-existent. There was no suggestion that any individual subject showed significantly more instances of uniform fibre grouping than any other.

In those muscle samples where significantly low numbers of "enclosed" fibres were recorded, it would seem reasonable to conclude that the degree of dispersion of fibres of that fibre type is greater than would occur if the population were randomly distributed. However, the method employed is less suitable for detecting evidence of this than for detecting fibre type grouping. In the vast majority of cases it was found that there was no significant difference between the observed and the expected number of "enclosed" fibres, which would strongly suggest that in most normal muscles the two main fibre types are in fact randomly dispersed.

3. Fibre type constitution of muscle selectively affected in the muscular dystrophies

One of the objectives of this study was to examine the possibility that the extent to which individual muscles are affected in dystrophic processes might in some way be influenced by their fibre type composition. For example, there is evidence that myo-

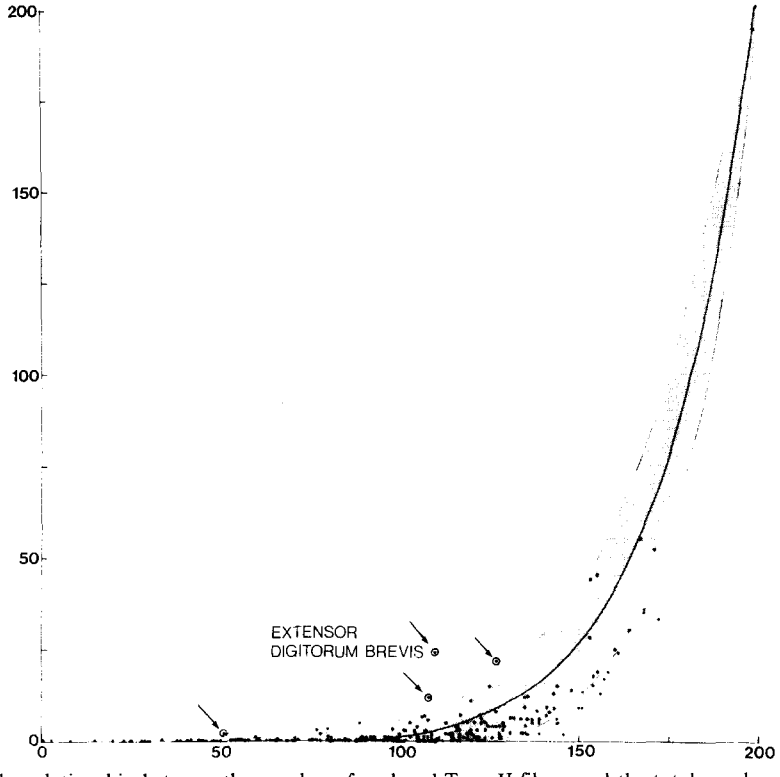

Fig. 3. The relationship between the number of enclosed Type II fibres and the total number of Type II fibres observed. The shaded area shows the formal 99.9 % limits about the mean expected enclosed fibres. The only muscle which is above the limit more than once is extensor digitorum brevis which appears 4 times out of 6 post-mortem specimens.

tonic dystrophy involves a selective atrophy of Type I fibres (Engel 1970), and for this reason it could be argued that muscles with a high proportion of Type I fibres might be most severely affected. In Duchenne dystrophy, however, there is no such evidence of the particular involvement of one fibre type, yet the phenomenon of selective sparing of muscles is just as apparent as in myotonic dystrophy.

Table 9 lists those muscles which are usually selectively affected or spared in Duchenne dystrophy and myotonic dystrophy, together with their respective percentage constitutions of Type I fibres as measured in this study. In general, the order in which the muscles are listed reflects the order in which they are affected in the disease, though naturally there is some variation between individual cases.

From this Table it is immediately obvious that there is no simple correlation between the fibre type composition of individual skeletal muscles and their being affected or spared in either Duchenne dystrophy or myotonic dystrophy. There is some suggestion that the overall tendency is for muscles with high Type II fibre percentages to be more severely affected in Duchenne dystrophy, but tibialis anterior, for instance, with its very low percentage of Type II fibres, is an obvious exception to this. In spite of its apparent predilection for Type I fibres, there was no evidence to show that myotonic dystrophy selectively affects muscles with a high proportion of that fibre type.

TABLE 9

THE RESPECTIVE PERCENTAGES OF TYPE I FIBRES FOUND IN THOSE MUSCLES SELECTIVELY INVOLVED EARLY (ACCORDING TO CLINICAL EXAMINATION) IN DUCHENNE DYSTROPHY AND MYOTONIC DYSTROPHY, AND IN THOSE RELATIVELY SPARED

Affected early	*Type I* %	*Relatively spared*	*Type I* %
Duchenne dystrophy			
(Upper limb)		(Upper limb)	
Pectoralis major	42.3	Deltoid–surface	53.3
Biceps brachii–surface	42.3	–deep	60.9
–deep	50.5	Infraspinatus	45.3
Brachioradialis	39.8	Adductor digiti minimi	51.8
Triceps–surface	32.5	Adductor pollicis brevis	62.9
–deep	32.7	1st Dorsal interosseus	57.4
(Lower limb)		(Lower limb)	
Vastus medialis–surface	43.7	Gastrocnemius–lat. head surface	43.5
–deep	61.5	–lat. head surface	50.3
Vastus lateralis–surface	37.8	–medial head	50.8
–deep	46.9	Soleus–surface	86.4
Tibialis anterior	73.4	–deep	89.0
Peroneus longus	62.5		
Adductor magnus–surface	53.5		
–deep	63.3		
Myotonic dystrophy			
Temporalis	46.5	Extensor digitorum brevis	45.3
Sternomastoid	35.2	Flexor digitorum brevis	44.5
Flexor digitorum profundus	47.3	Adductor digiti minimi	51.8
Extensor digitorum	47.3	Adductor pollicis brevis	62.9
Tibialis anterior–surface	73.4	1st Dorsal interosseus	57.4
–deep	72.7		
Peroneus longus	62.5		
Gastrocnemius–lat. head surface	43.5		
–lat. head deep	50.3		
–medial head	50.8		

DISCUSSION

Although there have been several recent studies concerning the relative sizes of the two main fibre types in human skeletal muscle, comparatively little attention has been directed towards the assessment of the numerical ratios of these fibre types. Certainly, no previous study has had as its main objective a systematic analysis of this parameter in a wide range of human muscles. From the wealth of data thus accumulated, it was thought that some correlation between fibre type constitution and function might emerge, as has been the case in studies of the fast and slow twitch muscles of small mammals. However, from the outset one is only too well aware of the complexity of human musculature, and the fact that anatomical complexity is seldom fortuitous but develops in response to a functional demand. Thus in very few instances can one simple function be assigned to a particular muscle. Far more frequently, muscles fulfil

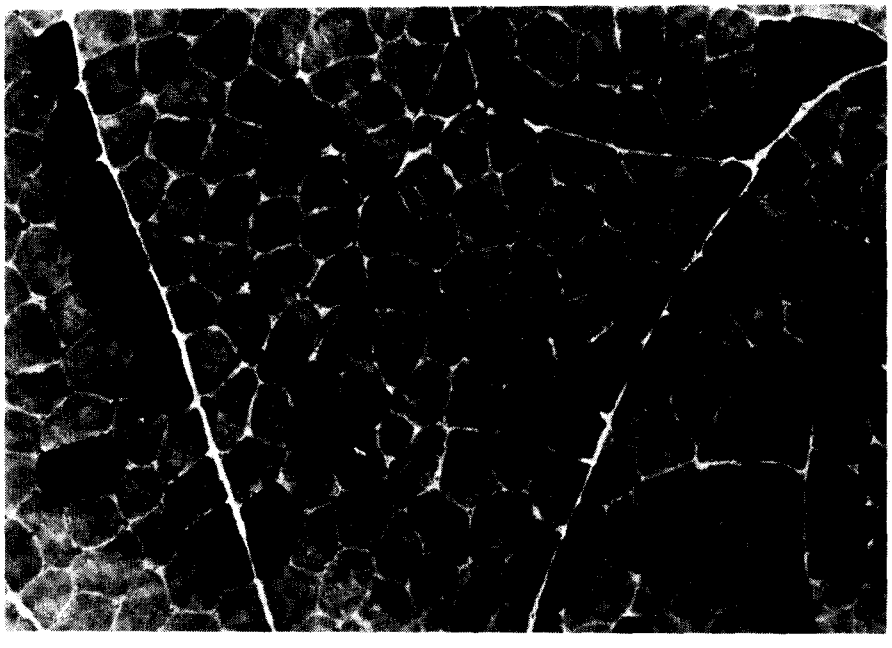

Fig. 4. Adductor pollicis from post-mortem No. 4 (myofibrillar ATPase preparation, × 150). Note the large number of enclosed Type I fibres, which is nevertheless within normal limits for a muscle with *c.* 80% Type I fibres.

both a postural function involving tonic activity and also participate in movements involving phasic activity.

However, it has been found that muscles with an almost exclusively postural function, such as soleus and, to a lesser extent, tibialis anterior, have particularly high proportions of Type I fibres (*c.* 87% and 73% respectively) which was in accordance with expectation. The similarly high percentage (*c.* 80%) of Type I fibres in adductor pollicis (see Fig. 4) may well be explained on the basis of its function of sustained contraction in the act of gripping.

At the other end of the scale, as far as fibre type constitution is concerned, are found the orbicularis oculi, sterno-mastoid, triceps and the lateral head of rectus femoris. Since the act of blinking is perhaps the best example of a phasic activity, the high proportion of Type II fibres in orbicularis oculi was to be expected. The function of the triceps in the predominantly phasic activity of extending the forearm is well enough established and the high proportion (67.5%) of Type II fibres was to be expected in this muscle also. Similarly, the sterno-mastoid, controlling the more delicate and precise of the movements of the head, has little postural function and its high Type II fibre constitution reflects this. More surprisingly, an even higher proportion (*c.* 70%) of Type II fibres was found in the superficial region of the lateral head of the rectus femoris (see Fig. 5). However, it is likely that the function of assisting the iliopsoas to support the pelvis on the femur is performed mainly by the medial sector of this muscle, and the lateral sector is primarily involved in the flexion of the femur and extension of the knee.

Fig. 5. Rectus femoris (lateral head) from post-mortem No. 2. (myofibrillar ATPase preparation, × 150). Note the close adherence to a hexagonal lattice array in some areas of the section. The mean Type II fibre percentage is *c.* 70% for this muscle.

The vast majority of the muscle samples had between 40% and 70% of Type I fibres, and in comparatively few muscles was there a striking predominance of either fibre type.

The large variation in fibre type percentages between different autopsy samples of individual muscles has already been discussed. Brooke and Engel (1969) found an even wider variation in their study of biceps brachii and vastus lateralis, perhaps because their series included a wider range of ages and some subjects who had previous histories of neuromuscular disease. Nevertheless, in the case of male subjects, the mean percentage of Type I fibres for both vastus lateralis and biceps brachii approximated to 37.5% (ratio of Type I : Type II fibres = 0.6). This agrees quite closely with our values of *c.* 38% for vastus lateralis and *c.* 42% for biceps brachii. The samples obtained by Brooke and Engel were all from biopsy material and hence have been assumed to be most directly comparable with our samples of the superficial areas of these muscles. In the case of the female subjects studied by Brooke and Engel, there was a definitely greater proportion of Type I fibres in biceps brachii as compared with the male subjects, whereas female vastus lateralis tended to have a smaller proportion of Type I fibres than in the male. The classification of their subjects into groups of similar clinical status rather than into similar age groups precluded any comment as to the possible influence of age on fibre type proportions, but re-examination of their data with this point in mind failed to detect any such influence in their series. However, it has been shown recently (Moore, Rebeiz and Holden 1971) that the average fibre diameter in a range of muscles underwent a gradual but steady increase until the late third or early

fourth decade, thereafter slowly diminishing in size through middle and old age. Until reliable data exist to prove or disprove such an effect of age on fibre type constitution, we consider it safer to use data on mean fibre type for age and sex.

In the part of the present study which was concerned with the spatial distribution of the two main fibre types, we hoped to gain greater insight into the organisation underlying the familiar "chequerboard" appearance of muscle sections. In order to do this, the actual muscle samples have been compared with a mathematical model to test the hypothesis that the distribution of the fibre types was random. In the great majority of cases this was in fact found to be so, since there was no significant difference in the number of "enclosed" fibres actually observed and the numbers predicted on the basis of the "hexagonal lattice" model.

There are many difficulties in formulating a mathematical model of a biological situation. In this particular instance it is evident that the model is not a strictly accurate one since the arrangement of the muscle fibres themselves inevitably sometimes departs from the hexagonal lattice of the model. Also, it was necessary to count fibres outside the region whose fibre type proportion was calculated, in order to determine whether a fibre on the edge of the region is enclosed. Thus the possibility arises that the fibres involved may have a slightly different fibre type proportion p from that noted which may materially alter the expected number of "enclosed" fibres. If these extra fibres have a proportion of only 0.1 different from the ones counted, the difference in expectation of the number of "enclosed" fibres is of the same order as the standard deviation. These inaccuracies make the technique slightly over-sensitive. However, it has the virtue of providing a simple and objective method of assessing whether the fibre type distribution in any muscle sample is random or not. In this respect, it has proved to have some predictive power, since it singled out extensor digitorum brevis as being the one instance of a muscle with a consistently non-random spatial distribution of fibre types.

In a study of this particular muscle in subjects whose ages ranged from 2 months to over 80 years, it has been shown that while a normal mosaic pattern apparently exists in early childhood, definite instances of uniform fibre type groupings have been recorded within the first decade of life (Jennekens, Tomlinson and Walton 1972). The occurrence in apparently normal older subjects of the characteristic features of denervation atrophy in this muscle has led to the adoption of the view that the muscle is particularly prone to recurrent cycles of denervation and re-innervation from a comparatively early stage of life. In the present study, unequivocal evidence of grouped denervation atrophy was noted in the 2 oldest subjects. Since groups of fibres of uniform type were seen in all the subjects, it is likely that the process of re-innervation can keep pace with that of denervation in younger persons. Isolated instances of grouped fibre atrophy were also noted in abductor pollicis brevis and 1st dorsal interosseus, two muscles in which there was also an excess of uniform fibre type grouping as reflected in the abnormally high number of "enclosed" fibres in some samples. The mathematical model thus seems to provide a means of quantitating the histological consequences of denervating processes.

In young adults, the spatial distribution of the different fibre types appears to be random in the great majority of muscles. The special case of extensor digitorum brevis and, to a lesser extent, abductor pollicis brevis, however, leads one to question whether

increasing age might not similarly affect other muscles. It might be expected that distal muscles would be particularly susceptible to sub-clinical neuropathic conditions of the "dying-back" variety. However, in this study it is of interest to note the lack of fibre type grouping in flexor digitorum brevis as compared with extensor digitorum brevis, and in adductor pollicis as compared with abductor pollicis brevis, which are pairs of muscles situated approximately equidistant from the segment of the spinal cord supplying their nerves.

The question of sub-clinical pressure or entrapment neuropathies operating in the same way to modify the spatial distribution of fibre types is a possibility in the case of abductor pollicis brevis since it is supplied by the terminal branch of the median nerve which is affected in the carpal tunnel syndrome. Compression of the ulnar nerve is also common, especially at the elbow, and this could affect the 1st dorsal interosseus muscle. Similarly, it was suggested by Jennekens *et al.* (1972) that the lateral terminal branch of the deep peroneal nerve supplying extensor digitorum brevis might be compressed intermittently at its entrance to the muscle by the pressure of ill-fitting shoes.

It is perhaps unwise to regard the occurrence of re-innervation and the occurrence of groups of fibres of uniform fibre type as inseparable factors. This study has provided evidence that a random mosaic pattern of fibre distribution can be considered to be the norm for a wide range of muscles. Even where uniform fibre type grouping occurs, it is likely that this is secondary to an arrangement of fibre types which was originally random. However, it is impossible to exclude the converse situation in which denervation and re-innervation take place but fail to produce groups of fibres of uniform type. For uniform fibre grouping to occur, at least two conditions must be satisfied. Firstly the denervated fibres must occupy a fairly compact territory, which pre-supposes the involvement of more than one motor unit, since the fibres of any one unit are scattered (Edstrom and Kugelberg 1968). Secondly, the process of re-innervation must be effected either by a single motor unit or by more than one unit of the same type. If the first condition does not obtain, the collateral re-innervation of disseminated atrophied fibres is unlikely to produce uniform fibre type grouping, though repetition of the same chain of events might eventually have this effect. If the second condition is not fulfilled and two motor units of different physiological type participate in the re-innervation process, it is likely that a mosaic pattern will result in much the same way as it does in the initial innervation process. Thus it is quite possible that the successive phases of denervation and re-innervation may be more common than is generally supposed since they will remain undetected unless the process gives rise to physiological or histological anomalies. Situations may also be envisaged in which the process of re-innervation leads to the fibre type constitution of a particular muscle becoming significantly different from normal, while at the same time the number of enclosed fibres is merely consistent with the new fibre type constitution, and would give no indication that re-innervation had taken place. The examination of biopsy material for evidence of previous denervation should therefore take into account not only the number of enclosed fibres observed, but also the proportion of the respective fibre types which the muscle examined contains.

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SUMMARY

Samples of skeletal muscle were taken from 50 sites in each of 6 previously normal male autopsy subjects aged between 17 and 30 years. The respective percentages of Type I and Type II fibres were calculated and showed that there was a wide variation in fibre type proportions between the 6 samples in almost all the muscles studied. Examination of the mean fibre type proportions of each muscle revealed that predominantly tonic muscles had a high percentage of Type I fibres and predominantly phasic muscles had a high percentage of Type II fibres. Most of the muscles studied were known to fulfil both tonic and phasic functions, however, and showed no striking preponderance of either fibre type.

The spatial distribution of the fibre types was examined in order to determine whether this was random or not. The number of "enclosed" fibres observed in the actual samples was compared statistically with the number expected to occur in a hexagonal lattice model, assuming a random distribution. In the great majority of muscles, the distribution of the fibre types was in fact random, though isolated instances of grouping of fibres of uniform type were noted in some distal muscles and more regularly in extensor digitorum brevis.

The methods used in the quantitative assessment of the proportions and spatial distribution of the respective fibre types in normal muscle have obvious applications in the study of neuromuscular disease.

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