

## Studies on the responses of different types of muscle fibre during surgically induced compensatory hypertrophy

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### INTRODUCTION

Numerous histological and physiological studies have been carried out on skeletal muscles that have been induced to undergo compensatory hypertrophy by the surgical removal of synergistic muscles (Rowe & Goldspink, 1968; Hall-Craggs, 1970, 1972; Hall-Craggs & Lawrence, 1970; Reitsma, 1969; Gutmann & Hajeki, 1971; Gutmann, Schiaffino & Hanzlikova, 1971; Schiaffino & Bormioli, 1973). However, most skeletal muscles are composed of heterogeneous populations of muscle fibres that can readily be distinguished in enzyme histochemical preparations. Some fibre types (Type I) possess a highly oxidative metabolism whilst others (Type II) are predominantly anaerobic (Dubowitz & Pearse, 1960). Intermediate types also occur frequently. Although some studies have been carried out on the adaptive responses of muscle fibre types to various types of stress, for example, exercise (Anderson & Henriksson, 1977; Faulkner, Maxwell & Lieberman, 1972; Gollnick *et al.* 1973; Guy & Snow, 1977; Kowalski, Gordon, Martinez & Adamek, 1969) and functional overload (Edgerton *et al.* 1972; Tomanek, 1975), there is relatively little data about the long term response to surgically induced compensatory muscle hypertrophy.

The present investigation was carried out to determine the responses of Type I, intermediate and Type II fibres in a muscle during compensatory hypertrophy. The extensor digitorum longus (EDL) muscle was used since it contains numerous fibres of all types (James, 1971, 1973) that can be readily identified by simple enzyme histochemical techniques. The muscle is also capable of maintaining hypertrophic features for relatively prolonged periods following removal of the tibialis anterior muscle (James, 1976).

### MATERIALS AND METHODS

#### *Operative techniques*

Adult male mice weighing 20–25 g were randomly selected and kept individually in cages for 1 week prior to operation. The cages measured 15 cm × 30 cm and possessed a smooth floor. Food and water were available *ad libitum*.

Under sterile conditions, and under ether anaesthesia, a mid-line longitudinal incision was made anterior to the ankle joint and prolonged proximally over the tibialis anterior muscle. The tendons of the tibialis anterior and extensor digitorum longus muscle were identified as they passed deep to the extensor retinaculum. The tendon of the tibialis anterior muscle was transected at the upper margin of the retinaculum, the proximal cut end of the tendon was reflected, and the nerves and arteries to the muscle interrupted. The deep proximal attachments of the muscle were severed and the muscle removed. Particular care was taken to ensure that,

as far as possible, the remaining leg structures were undisturbed and undamaged. The skin flaps were sutured with fine silk and the wound covered by Nobecutane®.

#### *Histochemical techniques*

The mice were killed 116 days after operation and EDL muscles from the operated and contralateral limbs removed for examination. Thin transverse slices were removed from the mid-belly of each EDL muscle and immediately frozen in Arcton chilled with liquid nitrogen. Sections 10  $\mu\text{m}$  thick were cut in a cryostat at  $-20^\circ\text{C}$  and stained to demonstrate succinate dehydrogenase (E.C.L.3.99.1) activity. The sections were incubated overnight in a medium consisting of 5 ml 0.1 M sodium succinate, 5 ml formylidimethylamide, 5 ml 0.1 M phosphate buffer at pH 7.6 and 10 mg nitro-BT tetrazolium salt (Davenport, 1964).

#### *Quantitative techniques*

An image of each section was superimposed at a known magnification on to a regular point counting grid formed by a series of intersecting horizontal and vertical lines. The regular lattice of points formed by the line intersections was used as in a typical morphometrical analysis (Underwood, 1970) to determine the cross sectional area ( $A$ ) of each fibre examined using the relation

$$A = n.d^2$$

where  $n$  is the number of intersections falling on each fibre. Values for the grid constant ' $d$ ' were obtained by dividing the distance between the grid lines by the image magnification. Individual cross sectional areas of at least 100 contiguous fibres lying in the central region of each muscle section were measured. It has previously been shown that 100 fibres can be regarded as a sufficiently large sample for the characterization of most fibre size distributions in skeletal muscle (Joubert, 1956). Following the measurement of each muscle fibre its identity as a Type I, intermediate or Type II fibre was established. In the normal EDL muscle of the mouse the fibre type can readily be identified by its succinate dehydrogenase activity (James, 1973). Type I fibres are relatively small, lie centrally within fasciculi (James, 1971), and stain heavily for succinate dehydrogenase activity (Fig. 1). Large subsarcolemmal accumulations of formazan-stained mitochondria abound in these fibres. Intermediate fibres are larger than Type I fibres: they lie both centrally and peripherally within fasciculi, and show moderate staining for succinate dehydrogenase activity in their peripheral regions. Their central regions are much less strongly stained. Type II fibres are the largest fibres in the muscle: they tend to lie peripherally within fasciculi, and exhibit relatively little staining for succinate dehydrogenase activity.

In any study on changes of muscle fibre cross sectional areas particular care must be taken to ensure that comparisons are made at similar sarcomere length. One section from each control and hypertrophic muscle was squashed between slide and coverslip using finger and thumb pressure to re-orientate cut fibres so that their relative sarcomere lengths would be examined by polarized light. No significant differences were found between the sarcomere lengths of control and hypertrophic fibres.

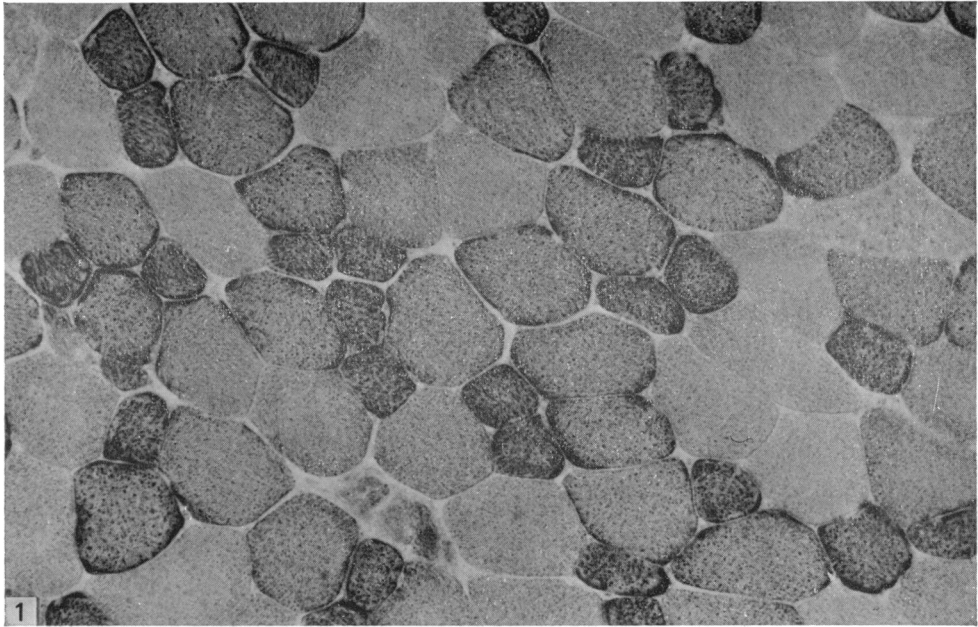


Fig. 1. Transverse section of normal EDL muscle stained for succinate dehydrogenase activity.  $\times 310$ .

Table 1. Cross sectional areas of muscle fibre types in control and hypertrophic EDL muscles

Animal no.	Type I fibres ( $\mu\text{m}^2 \pm \text{s.e.}$ )	Intermediate fibres ( $\mu\text{m}^2 \pm \text{s.e.}$ )	Type II fibres ( $\mu\text{m}^2 \pm \text{s.e.}$ )	Type I fibres ( $\mu\text{m}^2 \pm \text{s.e.}$ )	Intermediate fibres ( $\mu\text{m}^2 \pm \text{s.e.}$ )	Type II fibres ( $\mu\text{m}^2 \pm \text{s.e.}$ )
1	723.1 $\pm$ 54.3	1549.1 $\pm$ 50.5	1778.4 $\pm$ 38.7	781.6 $\pm$ 30.2	2265.1 $\pm$ 97.3	2761.2 $\pm$ 84.9
2	444.6 $\pm$ 27.4	1303.4 $\pm$ 65.0	1949.2 $\pm$ 77.6	697.3 $\pm$ 43.3	1670.8 $\pm$ 87.8	2295.5 $\pm$ 81.2
3	610.7 $\pm$ 35.4	1624.0 $\pm$ 53.0	2204.3 $\pm$ 61.8	870.5 $\pm$ 62.0	2323.6 $\pm$ 210.1	3582.5 $\pm$ 218.3
4	547.6 $\pm$ 27.4	1223.8 $\pm$ 67.3	1638.0 $\pm$ 40.0	491.4 $\pm$ 37.4	1310.4 $\pm$ 94.1	2111 $\pm$ 108.1
5	542.9 $\pm$ 31.4	1258.9 $\pm$ 47.6	1488.2 $\pm$ 43.3	575.6 $\pm$ 34.9	1432.1 $\pm$ 67.6	1773.7 $\pm$ 80.3
6	556.9 $\pm$ 43.9	1258.9 $\pm$ 62.3	2021.8 $\pm$ 48.8	1010.9 $\pm$ 66.7	2150.5 $\pm$ 158.2	3830.6 $\pm$ 146.0
	Control			Hypertrophic		

Values for the mean and standard error of each population of control and hypertrophic fibres were  $1256.6 \mu\text{m}^2 \pm 27.4$  and  $1680.8 \mu\text{m}^2 \pm 54.1$  respectively. The differences between means statistically highly significant ( $P < 0.001$ ).

## RESULTS

In all the animals examined the EDL muscles were larger and redder than their contralateral control muscles.

A full analysis of the size of Type I, intermediate and Type II fibres and their frequencies of occurrence in the hypertrophic and control EDL muscles was carried out. The muscle fibres of the hypertrophic muscles were found to be larger than in control muscles (Fig. 2). The frequency of occurrence of each of the fibre types did not differ following hypertrophy. It was found that the fibre types increased their cross sectional areas by differing amounts. Hypertrophic Type II fibres were found to have increased in area ( $\sim 40\%$ ) relatively more than intermediate ( $\sim 30\%$ ) or Type I fibres ( $\sim 18\%$ ). Statistical analysis, using the standard error of means test, showed such differences to be highly significant ( $P < 0.001$ ).

Table 2. *Properties of muscle fibre type population in control and hypertrophic muscle*

	Type I	Intermediate	Type II
Size distribution properties of control fibres:			
(i) mean	585.0 ± 18.9 μm <sup>2</sup>	1364.0 ± 26.9 μm <sup>2</sup>	1804.1 ± 27.1 μm <sup>2</sup>
(ii) skewness	1.67 ± 0.19	0.17 ± 0.20	0.39 ± 0.19
(iii) kurtosis	4.22 ± 0.38	-0.67 ± 0.40	-0.05 ± 0.37
Size distribution properties of hypertrophic fibres:			
(i) mean	692.6 ± 23.2 μm <sup>2</sup>	1766.7 ± 57.3 μm <sup>2</sup>	2550.6 ± 83.3 μm <sup>2</sup>
(ii) skewness	0.61 ± 0.24	0.64 ± 0.22	0.82 ± 0.23
(iii) kurtosis	0.63 ± 0.47	0.72 ± 0.45	0.50 ± 0.47
Frequency of occurrence in control EDL (%)	33.9	30.7	35.4
Frequency of occurrence in hypertrophic EDL (%)	31.9	35.6	32.5
Percent increase in mean cross section area in hypertrophic EDL	~ 18%	~ 29.5%	~ 41%

Standard errors of skewness and kurtosis were calculated using the formula  $\sqrt{\frac{6}{n}}$  and  $\sqrt{\frac{24}{n}}$  respectively, where  $n$  = sample size.

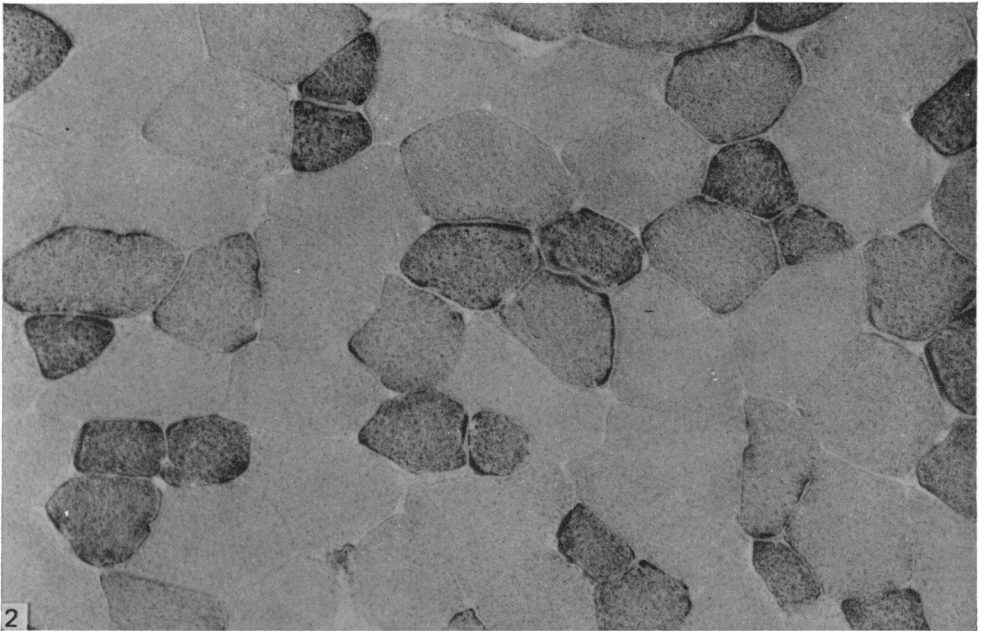


Fig. 2. Transverse section of hypertrophic EDL muscle stained for succinate dehydrogenase activity. Note the presence of fewer fibres in the field as compared with Fig. 1 because of the increased sizes of Type I, intermediate and Type II fibres. × 310.

## DISCUSSION

The results clearly indicate that Type II fibres increase in size, as measured by their cross sectional areas, relatively more than Type I fibres during surgically induced compensatory hypertrophy. In the present study the hypertrophy was maintained for a longer period than in most other studies, which have reported either an identical response by the fibres or a preferential increase in size and number of Type I fibres, particularly when the hypertrophy was induced in early life (Schiaffino & Bormioli, 1973). The results in the present study also differ from many of those previously reported in relation to muscle adaptation following training and exercise in that the increases in fibre diameter during surgically induced hypertrophy were found to be larger and to affect Type II fibres especially. Some investigators, however, have reported large size increases of up to 38 % for intermediate fibres and 92 % for Type I fibres (Maxwell, Faulkner & Lieberman, 1973). The fibre size increases found in the present study were smaller than those previously reported for compensatory hypertrophy in the EDL muscle (James, 1976). However, the tissues were prepared for examination using completely different methods, and neither in the previous study nor in the present have attempts been made to correct for the shrinkage or swelling of tissue. For example, linear changes in fresh frozen sections can be significantly large (Branemark & Ekholm, 1969; Eriksson & Myrhage, 1972) and areal changes are correspondingly larger; this could easily account for differences reported in the present study from those given in earlier studies (James, 1976).

The size changes measured in the present study were calculated for a system in which the proportion of fibre types had not apparently become altered during adaptation. In studies on some muscles, alterations in the proportions of their fibre types seem to have taken place (Edgerton, Gerchman & Carrow, 1969; Maxwell *et al.* 1973; Faulkner *et al.* 1972; Guy & Snow, 1977; Gonyea & Bonde-Petersen, 1978; Costill *et al.* 1976; Guth & Wells, 1972; Yellin, 1974; Jaweed, Herbison & Ditunno, 1977) whilst in other muscles the proportions seem not to have been altered (Guy & Snow, 1977; Edgerton *et al.* 1969; Suominen, Heikkinen & Parkatti, 1977). However, insufficient care seems to have been taken over the definitions of a change in fibre type in many of these studies. For example, studies in which only succinate dehydrogenase activity has been recorded have often interpreted simple increases in staining intensity for that enzyme as indicative of a transformation of Type II or intermediate fibres to intermediate or Type I (Faulkner *et al.* 1972; Baldwin *et al.* 1972).

The results obtained in the present study indicate a differential response of the fibre types. Any study attempting to measure alterations in fibre cross sectional areas following an experimental procedure must take into account the possible effects of an alteration in the number of sarcomeres within the fibres. For example, during compensatory hypertrophy of EDL muscle additional sarcomeres could have been added to the ends of the fibres (Williams & Goldspink, 1973). Consequently, fixing control and experimental fibres at identical lengths, but with more sarcomeres present in the experimental fibres, would spuriously increase their cross sectional area. The failure to find different sarcomere lengths in Type I and Type II fibres would seem to exclude this possibility. It does not necessarily follow that the preferential increase in the size of Type II fibres is due to any intrinsic properties of the fibres: hypertrophy would simply be limited by metabolic needs and oxygen supply. Hill (1965) has predicted on theoretical grounds that the maximum size of a cylindrical muscle fibre held at an external partial pressure of oxygen 'y' is given by  $2(k.y/Q)$  where  $Q$  is the

metabolic rate of the fibre and  $k$  the diffusion constant of oxygen in muscle tissue. Consequently, in an heterogeneous population of muscle fibres the maximum diameter of a muscle fibre should be inversely proportional to the square root of its metabolic rate. Type II fibres, in possessing a lower metabolic rate, might therefore be expected to attain a much larger diameter under the conditions of the present experiment.

Some workers have previously found difficulty in achieving muscular hypertrophy in laboratory animals. Although hypertrophy has been induced by a wide variety of exercise regimes (Gordon, Kowalski & Fritts, 1967), some of such studies have been questioned because a change in performance was not demonstrated (Gutmann & Hajek, 1971; Mackova & Hnik, 1973). Other investigators have used compensatory hypertrophy as their *modus operandi* (Denny Brown, 1960; Bass, Mackova & Vitek, 1973; Tomanek & Woo, 1970), assuming that the elimination of muscles by tenotomy or myectomy functionally overloads the remaining synergists which react by undergoing rapid hypertrophy.

Investigators have suggested that compensatory hypertrophy is triggered by mechanical stretching (Sola, Christensen & Martin, 1973), for example, that due to the action of antagonist muscles (Mackova & Hnik, 1973; Yellin, 1974). Though the precise mechanism of surgically induced compensatory hypertrophy has yet to be elucidated, nevertheless it remains a valuable experimental procedure since the results are reproducible and sufficiently large to be quantitated readily. Moreover contralateral muscles can be used as controls without having to take account of between-animal variance. In addition, greater specificity in the choice of hypertrophic muscle can be achieved compared with many exercise regimes, particularly methods involving the swimming of artificially weighted rodents and the training of laboratory cats (Gonyea & Ericson, 1976). These latter techniques are also ethically unacceptable to some investigators. The changes found in surgically induced compensatory hypertrophy may well differ fundamentally from those occurring during adaptation to exercise, and this would not be inconsistent with the variations in response to different types of human exercise. For example, endurance training in long-distance runners produces relatively little visible muscle hypertrophy, but isometric training in weight-lifters induces a markedly visible and widespread hypertrophy.

#### SUMMARY

The sizes of the different types of muscle fibre in the extensor digitorum longus (EDL) muscles of mice have been measured, EDL muscles showing compensatory hypertrophy following the removal of the tibialis anterior muscle 116 days previously being compared with normal contralateral controls. Contrary to previous findings, the hypertrophy was well maintained after 116 days and Type II fibres were enlarged preferentially.

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