

## CHANGES IN MYOSIN LIGHT CHAINS IN THE RAT SOLEUS AFTER THYROIDECTOMY

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### 1. Introduction

The occurrence of physiological and structural changes in skeletal muscle in hypothyroidism is well-documented [1–3]. Studies in man and in experimental animals have shown prolongation of both the time taken to reach peak tension and the time to half relaxation during evoked muscle contractions [2–5]. The mechanism of this slowing in muscle contractile properties is uncertain but histochemical studies suggest that it may be related to changes in muscle fibre types. Selective atrophy and loss of type 2 (fast-twitch) fibres has been found in patients with long-standing hypothyroidism [6]. In the rat soleus, thyroidectomy leads to an increase in the proportion of type 1 (slow-twitch) fibres, consistent with the increase observed in alkali-lability of the myosin ATPase extracted from the muscle [7].

The aim of this study was to determine whether changes in the physiological and histochemical properties of soleus muscles in thyroidectomised rats could be correlated with the myosin phenotypes expressed. Myosins from fast-twitch and slow-twitch muscles can be distinguished by gel electrophoresis of their light chains in the presence of sodium dodecyl sulphate [8]. Their electrophoretic mobilities are such that the light chains can be identified when myofibril preparations are analysed in this way. However, we have also purified myosins from these myofibrils to confirm the assignments and compared ATPase activities of these different myosin preparations. In addition, in an attempt to determine whether the changes induced

by the hypothyroid state result from a direct effect on the muscle or a neurally-mediated one, we have studied the influence of denervation on the fibre type composition of the soleus in thyroidectomised animals.

### 2. Materials and methods

A total thyroidectomy was performed in three groups of 8-week-old male Sprague-Dawley albino rats under sodium pentobarbitone anaesthesia (50 mg/kg). These animals were subsequently examined 12 weeks after thyroidectomy. At that time the terminal serum thyroxine levels in the thyroidectomised animals were ~25% of those in control animals, while serum calcium levels were not significantly different in the two groups. Thyroidectomised animals failed to grow and their mean weight at the time of the terminal experiment was just >50% of that of control animals. The weight of the soleus muscle was lower in thyroidectomised animals than in controls but was not reduced relative to total body weight.

In the first group of (12) animals the isometric twitch characteristics and histochemical fibre type profiles of the soleus were studied. Studies of the contractile properties were performed *in vivo* using the standard techniques in [9]. For histochemistry the soleus was removed *in toto* and frozen in dichlorodifluoromethane (Arcton 12 ICI). Transverse sections (10  $\mu$ m) of the muscles were prepared to demonstrate the activity of myofibrillar ATPase at pH 9.5 [10] and after acid preincubation at pH 4.6 and 4.3 [11].

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In the second group of (6) animals, the left soleus muscle was denervated at the time of thyroidectomy by resecting a 1 cm segment of its nerve just before it entered the muscle and then suturing the proximal stump into an adjacent muscle. After 12 weeks the hypothyroid status of these animals was confirmed by serum thyroxine estimation and histochemical fibre profiles of both innervated and denervated soleus muscles were compared.

The third group of (6) animals was used for studying the myosin light chain patterns in the soleus muscles. Muscles were weighed, and after a transverse segment had been taken from one muscle of each animal for histochemical analysis, the remaining muscles were frozen in liquid nitrogen and stored at  $-20^{\circ}\text{C}$ . Myofibrils were prepared from groups of muscles pooled from two or more animals as in [8]. In parallel, groups of extensor digitorum longus muscles (EDL) were used to prepare myofibrils of fast-twitch fibres as a control. Gel electrophoresis was carried out on SDS-12.5% polyacrylamide gels using a discontinuous Tris-glycine buffer system and gels were stained with Coomassie brilliant blue. This method gave a good separation of the myosin light chains and those originating from fast-twitch and slow-twitch fibres could be readily distinguished. The gels were densitometered using a Camag flat bed scanner and peak areas estimated by using an Evans and Sutherland plotter and PDP 11 computer. Calculation of the molar ratios of the different light chains was based on known molecular weight values and the

assumption that there was no difference in dye uptake between the various light chains. Since the light chains in different fibre types are chemically related, this assumption is probably valid within the accuracy required for these experiments.

Myosins were prepared from the various myofibrillar preparations as in [8], and further characterised both by gel electrophoresis and ATPase activity measurements. The conditions for the ATPase activity measurements using a pH-stat have been reported [12].

### 3. Results

#### 3.1. Physiological and histochemical studies

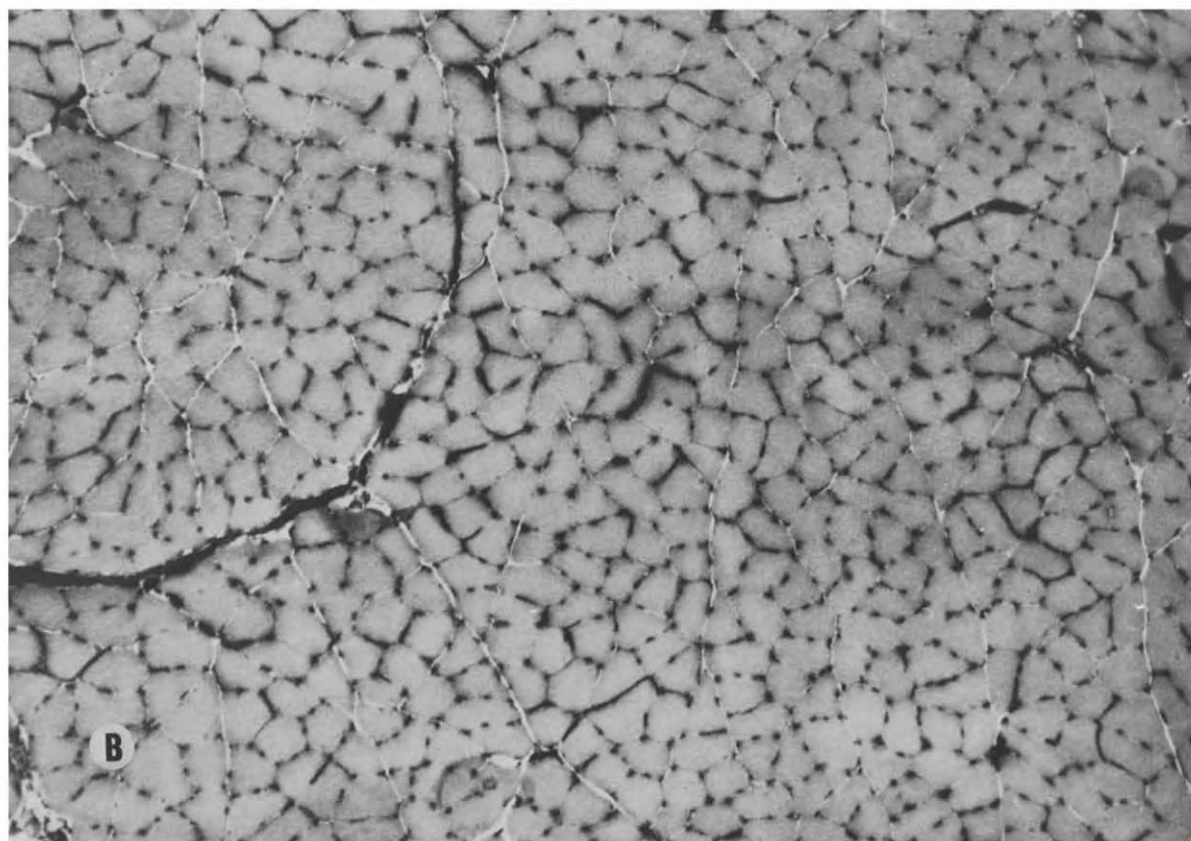
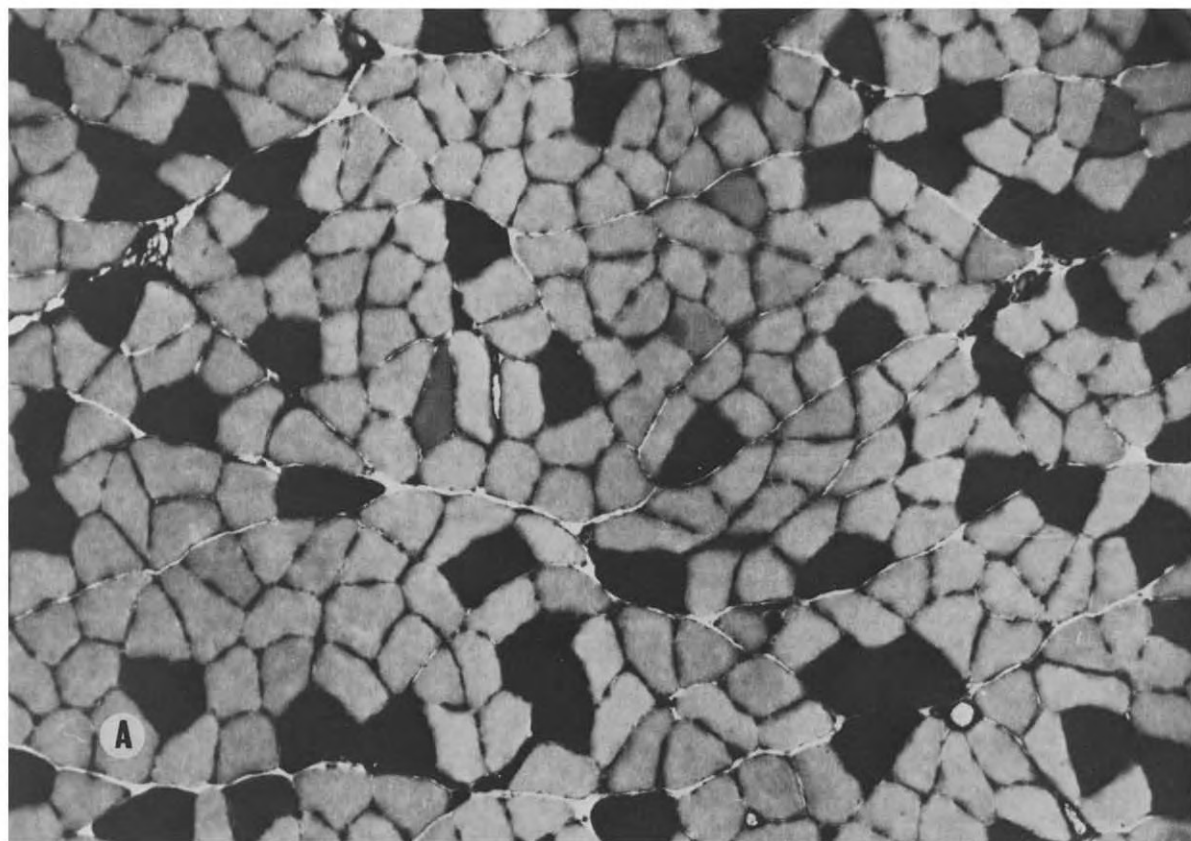
In thyroidectomised animals there was marked slowing of the contraction and relaxation phases of the soleus isometric twitch and a reduction in the maximum rate of development of tension during a tetanus, with no change in the twitch-tetanus ratio (table 1). The ability of these muscles to follow high frequency stimulation (50–500 Hz) was preserved, indicating normal neuromuscular transmission. The changes in twitch properties were similar to those that occur after cross reinnervation of a fast-twitch muscle with the nerve originally innervating a slow-twitch muscle [13].

In the normal animal, the soleus contains ~20% fast-twitch oxidative/glycolytic (type 2a) fibres and the remaining fibres are slow-twitch oxidative (type 1) (table 1, fig. 1a). In the thyroidectomised animals there

Table 1  
Changes in contractile properties, fibre type composition and myosin properties in the soleus muscle after thyroidectomy

	Control	Thyroidectomised
Contraction time (ms)		
(time to peak tension)	34.5 $\pm$ 0.99 (8)	62.7 $\pm$ 2.87 (8) <sup>a</sup>
Time to half relaxation (ms)	52.5 $\pm$ 3.18 (8)	82.5 $\pm$ 1.88 (8) <sup>a</sup>
Maximum rate of rise of tetanic tension (% max. tetanic tension ms <sup>-1</sup> )	3.03 $\pm$ 0.29 (8)	1.98 $\pm$ 0.16 (8) <sup>a</sup>
Total fibre number	2465 $\pm$ 102.2 (10)	2419 $\pm$ 92.1 (10)
% Type 1 fibres, slow-twitch oxidative	78.3 $\pm$ 3.0 (10)	98.7 $\pm$ 0.78 (10) <sup>a</sup>
% Type 2a fibres, fast-twitch oxidative-glycolytic	20.6 $\pm$ 3.02 (10)	1.1 $\pm$ 0.69 (10) <sup>a</sup>
% Type 2c fibres	1.10 $\pm$ 0.21 (10)	0.2 $\pm$ 0.09 (10) <sup>a</sup>
% Fast-twitch myosin	18.7	2.1
	16.0	0

<sup>a</sup> Statistically significant at the  $p = 0.05$  level using Student's *t*-test



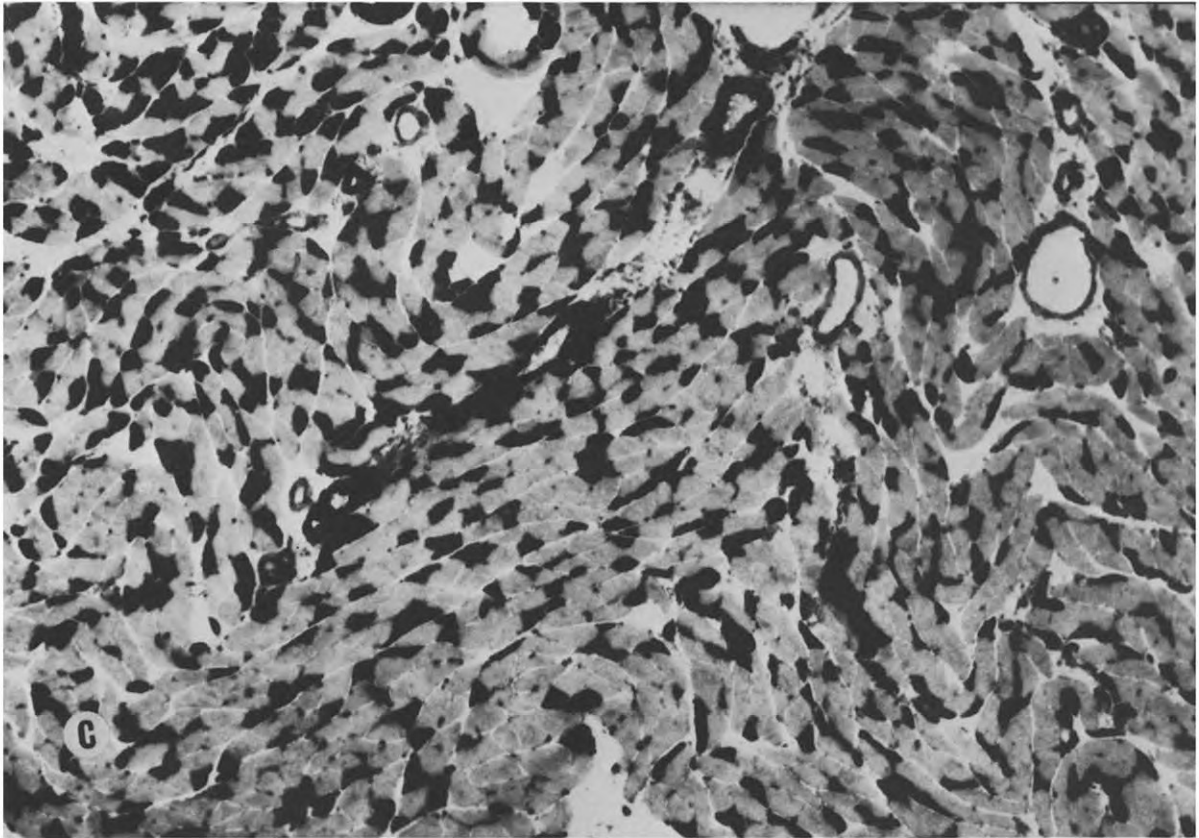


Fig.1.(A) Normal rat SOL muscle showing the admixture of light type 1 (slow oxidative) and dark type 2a (fast oxidative/glycolytic) fibres. (B) Hypothyroid rat SOL muscle consisting solely of light type 1 fibres. (C) Denervated hypothyroid rat SOL muscle from contralateral limb of animal shown in 1B. Note the preservation of the mosaic of type 1 and 2a fibres. Myofibrillar ATPase (pH 9.5). Original magnification  $\times 117$ .

was an almost total conversion of type 2a fibres into type 1 fibres (table 1, fig.1b). There was a close correlation between the degree of slowing of the muscle twitch and the fibre type transformation in the muscle.

There was a marked contrast in the fibre type profiles of the 2 solei in the animals in which 1 muscle had been denervated. Whereas the innervated muscles was composed almost entirely of type 1 fibres, the denervated muscle still showed a normal admixture of type 1 and type 2 fibres although, as expected, both fibre types had undergone a considerable degree of atrophy (fig.1c). In other words, the conversion of type 2a to type 1 fibres which occurred in the innervated soleus had not occurred in the denervated muscle.

### 3.2. Myosin light chains

Comparison of gels of SOL myofibrils from control and hypothyroid rats showed the presence of a band of mobility identical to LC2<sub>f</sub> of EDL (the Nbs<sub>2</sub> light chain [8]) in the control samples, which was largely absent from the hypothyroid animals. Although this band was much weaker than the LC2<sub>s</sub> light chain, it could be distinguished from both this and other myofibrillar components including troponin subunits. Further confirmation that this band was LC2<sub>f</sub> was obtained by gel electrophoresis in two dimensions using the O'Farrell procedure [14]. (We are grateful to Dr R. Whalen for running these gels for us.) The existence of fast-twitch myosin light chains in the control preparations is also supported by the presence of the shoulder on the LC1<sub>s</sub> peak, which corresponds to the LC1<sub>f</sub> (alkali 1) light chain. (2 D

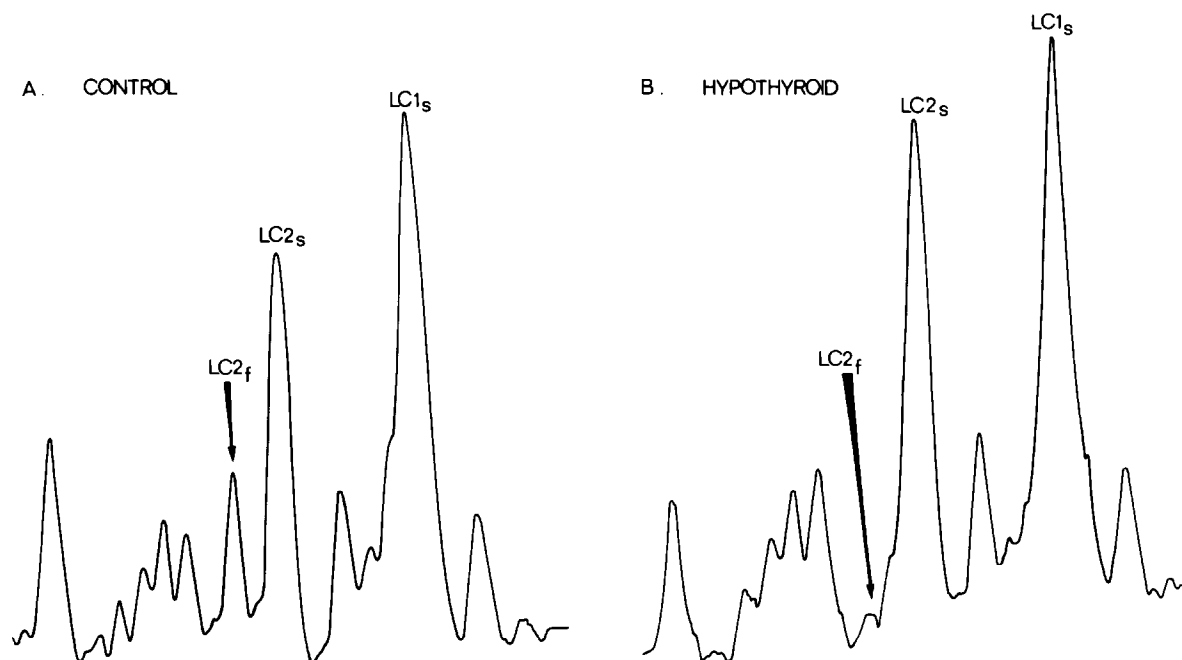


Fig.2. Densitometer traces of SDS-polyacrylamide gels of myofibrils from soleus muscles of control and hypothyroid rats. Positions of myosin light chains from slow-twitch and fast-twitch fibres are indicated as LC1<sub>s</sub>, LC2<sub>s</sub> and LC2<sub>f</sub>, respectively.

gel analysis again showed this more clearly). Molar ratios of the marked light chains were calculated from the densitometer peak areas. The mean ratio of LC1<sub>s</sub> : LC2<sub>s</sub> was  $1.05 \pm 0.07$  for all myofibril samples scanned. The % fast-twitch myosin was calculated from the molar ratio of LC2<sub>f</sub> and the mean value of LC1<sub>s</sub> and LC2<sub>s</sub>. In two preparations of myofibrils we obtained values of 18.7% and 16.0% for control preparations and 2.1% and 0% for corresponding hypothyroid preparations. These results indicated that thyroidectomy had virtually eliminated the fast-twitch myosin from soleus muscles and confirmed the histochemical observations both qualitatively and quantitatively.

Further support for this conclusion was obtained after purification of the myosins from these preparations. Gels again showed the presence of LC2<sub>f</sub> in control soleus preparations which was largely absent from the hypothyroid samples. Densitometry proved more difficult with the myosin preparations because of fluctuations in the base line. The amplitude of these fluctuations was significant when compared with the height of the minor LC2<sub>f</sub> component. Nevertheless, our results suggested that the control preparations contained  $\geq 10\%$  more myosin of the

fast-twitch phenotype, and this was confirmed in the ATPase assays. Enzymic activities were measured both in the absence of divalent cations (0.6 M KCl and 1 mM EDTA) and in the presence of actin and in both cases the activities for control soleus were significantly higher than those for the corresponding samples from hypothyroid animals. For example, for 1 preparation, the EDTA ATPase for control soleus was  $0.556 \mu\text{mol P}_i \cdot \text{mg}^{-1} \cdot \text{min}^{-1}$ , while corresponding values for hypothyroid soleus and control EDL were 0.365 and 1.19, respectively. This value is consistent with the presence of  $\sim 16\%$  fast-twitch myosin in the control soleus preparation. We consider these results only supportive since we do not know whether there is any selectivity in the extraction of different myosin isoenzymes and the absolute activities obtained with myosins prepared from frozen muscles were lower than those from fresh muscles.

#### 4. Discussion

Our findings indicate that the slowing in contractile properties and changes in fibre type composition of the soleus muscle after thyroidectomy are accom-

panied by an almost total loss of fast-twitch myosin from the muscle. The reduction in fast light chain content paralleled closely the conversion of fast-twitch (type 2a) into slow-twitch (type 1) fibres in the muscle during the 3 months after thyroidectomy.

The question that arises is whether the loss of fast light chains and accompanying changes in myofibrillar ATPase properties result from removal of a direct effect of thyroid hormone on gene expression in the muscle fibre, or from changes in the pattern of neural activation of the muscle due to the thyroid deficiency state. The finding that the fibre type transformation induced by thyroidectomy can be prevented by denervation of the soleus muscle at the time of thyroidectomy suggests that the latter is more likely, although it is theoretically possible that the denervated muscle does not react in the same way as the innervated muscle to the effects of thyroid hormone deficiency. Further studies are required to determine whether the changes after thyroidectomy are due to a primary effect on muscle, on the lower motor neurone, or to a combined effect on muscle and nerve.

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