Adv. Physiol. Sci. Vol. 5. Molecular and Cellular Aspects of Muscle Function
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THE CONTRACTILE PROTEINS OF RAT GASTROCNEMIUS DURING ITS REGENERATION AFTER MINCING

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Gastrocnemius muscles of rats weighing approximately 150g were minced and orthotopically autografted (1). The regenerating muscles were examined 1, 3, 6, 15, 30, 60, 120 and 240 days after surgery. The proteins soluble in 80 mM KCl-1 mM TES were removed and the precipitate was dissolved in SDS and electrophoresed (2). Twenty one bands were observed after staining with Coomassie blue. The gels were scanned with an automatic scanner and the areas of the bands were evaluated by numerical integration. The band corresponding to actin was identified. A calibration curve was obtained using purified actin run on the same slab gel electrophoresis. Analyses of 30 control muscles yielded an average content of 17 ± 2 mg of actin per 100 mg of total protein. The areas of the 20 other bands were computed using the area of the actin band as a reference set at 100%. In this way quantitative estimates were obtained for each of the 20 bands, together with estimates of the standard deviations of the means.

The electrophoretograms obtained from the precipitate of regenerating muscles were analysed in the same way as the control, the only difference being that there were only 4 to 6 experiments for each duration (instead of 30 controls). Means and their standard deviations were thus obtained for the 21 bands for each of the 8 periods after surgery.

Actin content (in mg per 100 mg of protein in the precipitate) decreased by about 30% at day 6 and 15; then it increased slowly, reaching the control value at day 120, and exceeding it by day 240. However the amount of precipitated protein in mg per gram wet weight muscle decreased from 100 mg/g (control) to less than 40 mg/g (at day 15). It increased then slowly thereafter. Thus the actin concentration per gram muscle of regenerates at day 240 was still half that of controls.

The largest changes were seen at day 15. There were large increases in the bands corresponding to myosin light chain (LC)1 (+70%), LC2 (+160%) and LC3 (+260%). Two dimensional isoelectric focusing-electrophoreses (3) demonstrated a large decrease in the myosin light chains but an increase of numerous proteins of approximately the same molecular weight. Tropomyosins did not show any changes. A 34.000 daltons unidentified component increased mainly at 15 days. A 53.000 daltons component increased by 150% at day 15 and returned slowly to normal by day 240. Two dimensional electrophoreses demonstrated that this component may well be vimentin. Bands corresponding to a protein of 72.000 daltons and protein C (140.000 daltons) (4) increased respectively by 100 and 50% a few days after surgery, and returned to normal at day 120 to 240.

In reference to actin, the areas of most of the bands were close to the control values, indicating that the relative proportions of the main proteins of the contractile apparatus are the same in the regenerate and in the control.

References

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