Effects of Denervation on the Glycogen Content and on the Activities of Enzymes of Glucose and Glycogen Metabolism in Rat Diaphragm Muscle

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1. Changes in the content and concentration of glycogen and in the activity of a number of enzymes involved in glucose and glycogen metabolism were studied in the rat hemidiaphragm after unilateral denervation. 2. After nerve section the tissue hypertrophies; this hypertrophy is said to be confined to the smaller red fibres and not to the white. 3. The total hexokinase activity increases, whereas that of total glycogen phosphorylase decreases. The specific activity of phosphorylase α , determined after Halothane anaesthesia, remains fairly constant. 4. In fed animals the denervated tissue stores less glycogen, but in the early stages its glycogen content does not fall on starvation. 5. The effect of denervation on the specific activities of several other characteristically white-fibre enzymes are not consistent with the response of glycogen phosphorylase; the increase in content of glyceraldehyde 3-phosphate dehydrogenase and lactate dehydrogenase is thought to be related to proliferation of the sarcoplasmic reticulum. 6. The ratio of lactate dehydrogenase M/H subunits increases at the height of the hypertrophy, but then declines as the mass of the tissue falls. 7. The chronology of these changes in enzyme activities suggests a multiplicity of distinct responses after nerve section not consistent with any one model, either specific fibre development or reversion to de-differentiated, foetal-type metabolism.

After section of its phrenic innervation the lateral region of the rat hemidiaphragm undergoes a pronounced transient hypertrophy (Martin & Sola, 1948; Feng & Lu, 1965), during which there is an increased rate of protein synthesis (Buse *et al.*, 1965), enhanced amino acid accumulation (Harris & Manchester, 1966) and a large increase in nucleic acid content (Manchester & Harris, 1968; Zak *et al.*, 1969). Various other metabolic changes have been recorded for the hypertrophying tissue, including a decreased response *in vitro* to insulin and growth hormone (Buse & Buse, 1959; Manchester, 1972). The mechanism by which the hypertrophic changes are brought about is as yet unknown; the passive rhythmic stretching induced by the contraction of the innervated contralateral portion, or an increase in the blood flow through the denervated tissue, are possibilities.

As well as displaying changes characteristic of hypertrophying tissue, the denervated rat hemidiaphragm also exhibits responses typical of denervated but atrophying muscles, e.g., an increased fibre sensitivity to acetylcholine (Elmqvist & Thesleff, 1960), a decreased membrane potential (Lullmann & Pracht, 1957), an apparent fragmentation of fibre

mitochondria (Miledi & Slater, 1969a) and increased rates of peptidase activity (Gutmann *et al.*, 1966). That a profound change takes place in the functional capacity of the denervated tissue is apparent in the dramatic alteration of the contractile properties of the muscle (Miledi & Slater, 1969b).

Rat diaphragm muscle is known to be composed of a mixture of 'red' and 'white' fibre types (Gauthier & Padykula, 1966) and histological examination of the hypertrophied tissue shows that only the smaller, mitochondria-rich fibres undergo the hypertrophy, the larger white fibres only atrophying after nerve section (Feng & Lu, 1965). In view of this fibre-specific response to denervation, we have examined various enzyme activities in the hypertrophied tissue with a view to correlating the metabolic changes with the reported change in proportion of fibre types. We have investigated the various parameters throughout the hypertrophic and into the atrophic phase of the response to nerve section.

Materials

Except for ATP, obtained from Kyowa Hakko Kogyo Co., Tokyo, Japan, all nucleotides and co-enzymes were obtained from Boehringer Corp. (London) Ltd., London W.5, U.K., as also were the coupling enzymes used in the assays: hexokinase,

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140 units/mg, 10mg/ml, glucose 6-phosphate dehydrogenase, 140 units/mg, 5mg/ml; 6-phosphogluconate dehydrogenase, 12 units/mg, 2mg/ml; phosphoglucomutase, 100 units/mg, 2mg/ml; 3-phosphoglycerate kinase, 400 units/mg, 10mg/ml.

2-(*N*-2-Hydroxyethyl)piperazin-*N'*-yl)ethanesulphonic acid (HEPES) and dithiothreitol were obtained from Calbiochem, Los Angeles, Calif., U.S.A.

Methods

Male albino rats, weighing about 100g at the time of slaughter, were used in this investigation. Left unilateral phrenicectomy, performed the stated number of days before death, was done under ether anaesthesia by means of a thoracotomy. As a routine benzylpenicillin was applied to the surfaces of the wound before suturing. Animals that failed to gain weight adequately or for any reason appeared unhealthy were discarded.

Rats were killed by decapitation and the hemidiaphragms were dissected out individually. After rinsing in ice-cold 0.14M-NaCl, they were blotted gently on filter paper (Whatman no. 40) and trimmed free of adipose and connective tissue before being weighed in air on a torsion balance. Homogenization in the appropriate ice-cold extraction buffer was carried out with a Silverson Laboratory Mixer-Emulsifier, the containing vessel being surrounded by ice during this process. To remove particulate matter from the muscle suspension, it was centrifuged as appropriate and the clear supernatant, diluted if necessary with homogenization medium, was used for the enzyme assays. All extracts were kept on ice and were assayed the day they were prepared. Enzyme activity in the supernatants was measured spectrophotometrically at 340nm with a Unicam SP.800 recording spectrophotometer with a constant-temperature cell housing, constant-wavelength and scale-expansion facilities and an accessory chart recorder. All assays, performed in duplicate, were made at 25°C and at a final pH of 6.9, the intracellular pH of rat muscle (Waddell & Bates, 1969). Preliminary experiments were done to ascertain (a) the optimum conditions for the extraction of enzyme activities, (b) that zero-order kinetics were being measured in the assay, and (c) that the concentrations of substrates, cofactors and subsidiary enzymes were optimum for the conditions employed. Unless stated otherwise, the reaction was initiated by the addition of the enzyme extract and was followed for up to 10min, as every reaction rate was linear for at least this period. In cases where a discrepancy between the activities of extracts from control and denervated muscles was apparent, random checks were made to ensure that the combined enzyme activities of both extracts were additive; in no case was the converse found to be true, presumably because the dilution of the enzyme in the assays

effectively removed activators or inhibitors that might have been present. Enzyme specific activities are expressed in terms of $\mu\text{mol/min}$ per mg wet wt. of tissue, measured at pH6.9 and 25°C.

Enzyme assays

Hexokinase. Each hemidiaphragm was homogenized in 2ml of 0.3M-sucrose–20mM-HEPES–0.8M-KCl–1mM-EDTA, final pH adjusted with KOH to 6.9. The homogenate was left on ice for at least 1h before centrifugation at 30000g for 30min. The assay medium (3ml) consisted of 20mM-HEPES, 10mM-MgCl₂, 5mM-ATP, 5mM- β -mercaptoethanol, 25mM-glucose, 0.55mM-NADP⁺, and approx. 30 μg of glucose 6-phosphate dehydrogenase (final pH6.9). The reaction was initiated by the addition of the glucose. The centrifugation step was found to be necessary to remove components (presumably mitochondrial) that caused non-specific reduction of NADP⁺. With the high-ionic-strength medium used, the composition of which was suggested by the work of Hernandez & Crane (1966), some 15% more activity was solubilized than by a comparable medium lacking KCl.

Phosphoglucomutase. Each hemidiaphragm was homogenized in 2ml of 0.3M-sucrose–20mM-HEPES–1mM-EDTA, final pH6.9, and the homogenate was centrifuged at 30000g for 30min. The assay medium (3ml) consisted of 40mM-imidazole, 4mM-MgCl₂, 4mM-glucose 1-phosphate, 0.2mM-NADP⁺ and approx. 30 μg of glucose 6-phosphate dehydrogenase, final pH6.9. The use of glucose 1-phosphate containing 1% of glucose 1,6-diphosphate (BDH Chemicals Ltd., Poole, Dorset, U.K.) did not give any greater activity, as there was presumably enough of the cofactor in the extracts for catalytic activity.

Glucose phosphate isomerase. Each hemidiaphragm was homogenized in 2ml of 0.3M-sucrose–20mM-HEPES–1mM-EDTA, final pH adjusted with KOH to 6.9, and the homogenate was centrifuged for 30min at 30000g. The assay medium (3ml) consisted of 20mM-HEPES, 20mM-MgCl₂, 2mM-fructose 6-phosphate, 0.2mM-NADP⁺ and approx. 30 μg of glucose 6-phosphate dehydrogenase; final pH6.9.

Glucose 6-phosphate dehydrogenase. Each hemidiaphragm was homogenized in 2ml of 0.3M-sucrose–20mM-HEPES–1mM-EDTA, final pH6.9, and the homogenate was centrifuged at 30000g for 30min. The assay medium (3ml) contained 20mM-HEPES, 20mM-MgCl₂, 2mM-glucose 6-phosphate, 0.25mM-NADP⁺ and approx. 15 μg of 6-phosphogluconate dehydrogenase; final pH6.9.

6-Phosphogluconate dehydrogenase. Each hemidiaphragm was homogenized in 2ml of 0.3M-sucrose–20mM-HEPES–1mM-EDTA, final pH6.9, and the homogenate was centrifuged at 30000g for 30min.

The assay medium (3ml), final pH 6.9, contained 20mM-HEPES, 20mM-MgCl₂, 2mM-6-phosphogluconate and 0.25mM-NADP⁺.

Glyceraldehyde phosphate dehydrogenase. Each hemidiaphragm was homogenized in 1.5ml of 100mM-potassium phosphate-1mM-EDTA buffer, final pH 6.9. The homogenate was centrifuged at 2200g for 15min and the supernatant was diluted with 20mM-HEPES-1mM-EDTA-1mM-dithiothreitol, adjusted to pH 6.9 with KOH. The assay medium (3ml) contained 20mM-HEPES, 1mM-EDTA, 3.3mM-MgCl₂, 10mM-3-phosphoglycerate, 0.25mM-NADH, 1.5mM-ATP, 2.4mM-GSH, 75mM-semicarbazide hydrochloride, 100µg of 3-phosphoglycerate kinase, final pH adjusted with KOH to 6.9. The use of the special diluent was essential for the prevention of loss of activity; with it the enzyme remained stable for several hours if kept on ice, but the assay was always carried out as soon as possible after extraction.

Lactate dehydrogenase. Each hemidiaphragm was homogenized in 1.5ml of 100mM-potassium phosphate-1mM-EDTA buffer, final pH 6.9. The homogenate was centrifuged at 2200g for 15min. The assay medium (3ml) contained 100mM-potassium phosphate buffer, 10mM- or 0.33mM-pyruvate and 0.15mM-NADH, final pH 6.9.

Glycogen phosphorylase. With the rat diaphragm it was impracticable to use conventional freeze-clamping techniques for the preservation of activities of phosphorylase *a in vivo*, which might otherwise be increased owing to the stress experienced by the animal when the tissue was removed. The following procedure, utilizing the apparent ability of the gaseous anaesthetic Halothane to cause no stimulation of adrenaline secretion during anaesthesia (see, e.g., Price *et al.*, 1959; Li *et al.*, 1964), was devised in an attempt to overcome the difficulty. Each rat was removed as gently as possible from the keeping cage and placed in the anaesthetizing cage, which had been gassed for a few minutes previously with an oxygen-Halothane mixture produced by bubbling the oxygen through the liquid anaesthetic in a Dreschel bottle. The rat was quickly anaesthetized and, provided that adequate concentrations of the oxygen-Halothane mixture were present, would remain quiescent for long periods of time. Preliminary studies showed that about 10min of anaesthesia was adequate to obtain low activities of phosphorylase *a* [i.e. ratio of $a/(a+b)$ of 30%] but in practice a period of 20min was adopted, a check being maintained throughout of the rat's breathing rate. At the end of this period the rat was decapitated, the body cavity rapidly opened and the posterior surface of the diaphragm exposed. Ice-cold buffer (50mM-potassium phosphate-20mM-NaF-5mM-EDTA, final pH 6.9) was immediately rinsed over the surface of the muscle to cool it and to allow the NaF and EDTA inhibitors to take effect as soon as possible. A small hole was cut in the

diaphragm just below the xiphisternum and the pleural cavity was filled with more ice-cold buffer. The hemidiaphragms were then dissected out and soaked in the same buffer for about 5min before further manipulation. Homogenization was carried out in 2ml of medium containing 20mM-HEPES, 5mM-EDTA, 20mM-NaF, 5mM-cysteine-HCl, final pH adjusted to 6.9 with KOH; the homogenate was centrifuged at 30000g for 30min and the clear supernatant was diluted before assay with 50mM-potassium phosphate-1mM-EDTA-10mM-NaF, final pH 6.9. The assay medium (1ml) for determination of phosphorylase activity contained 50mM-potassium phosphate buffer, 1mM-EDTA, 10mM-NaF, 2.5mM-MgCl₂, 5mM-cysteine-HCl, 0.5% of rabbit muscle glycogen, 0.4mM-NADP⁺, 0.2mg of fructose 1,6-diphosphate/ml, ± 1 mM-AMP, approx. 10µg of phosphoglucomutase and 5µg of glucose 6-phosphate dehydrogenase; the final pH was 6.9. AMP was omitted from the assay for phosphorylase *a* determinations. EDTA and NaF were included in the various solutions to prevent interconversions of phosphorylase *a* and *b* in the extracts.

Determination of glycogen

Rats were anaesthetized with Halothane and, as soon as the pinch/withdrawal reflex of the hind foot was found to be absent, the animals were decapitated. The tissues were rinsed and weighed as described above and then rapidly immersed in 30% (w/v) KOH. The tissue was digested by placing the tubes in a boiling-water bath for a few minutes. Glycogen was assayed by the method of Good *et al.* (1933), the glucose produced from the acid hydrolysis of the polysaccharide being assayed by the *o*-tolidine-linked glucose oxidase method of Marks (1959). Glycogen concentrations are expressed as µmol/g wet wt.

Statistical treatment of results

The values in the tables are the means $\pm$ S.E.M. of the enzyme activity (either units/g wet wt. or units/hemidiaphragm) of the particular parameter measured at the stated number of days after denervation. The statistical significance of the paired differences of values obtained for control and denervated tissues from the same rat was tested by Student's *t* test. Statistically significant differences are indicated if $P < 0.05$.

Results

Change of tissue weight and of glycogen content

Tissue-weight relationships. Table 1 shows the changes in weight of control and denervated tissues after denervation of the left hemidiaphragm for up

Table 1. *Weight relationships of the denervated rat diaphragm*

For details see the text. Each value is the mean $\pm$ S.E.M. of the number of observations indicated in parentheses. The *P* value for the paired differences was <0.001 in each case except that marked *.

Time elapsed since phrenicectomy (days)	Wet wt. of the hemidiaphragms (mg)		Denervated (percentage of control value)
	Innervated controls	Paired differences	
0	88.7 $\pm$ 1.2	-9.1 $\pm$ 0.8 (71)	90
1	92.2 $\pm$ 1.1	-3.8 $\pm$ 0.9 (71)	96
2	98.5 $\pm$ 1.8	8.7 $\pm$ 3.7 (8)*	109
3	89.5 $\pm$ 1.1	13.7 $\pm$ 0.9 (66)	116
5	89.8 $\pm$ 1.2	26.2 $\pm$ 1.5 (52)	130
7	91.5 $\pm$ 1.4	21.8 $\pm$ 1.2 (62)	123
10	90.5 $\pm$ 1.6	17.1 $\pm$ 1.2 (82)	120
15	92.4 $\pm$ 2.2	-18.0 $\pm$ 1.7 (59)	80

to 15 days after nerve section. The use in this study of such large numbers of rats has, because of the difficulty in obtaining them all at identical weights, permitted various statistical observations to be made on the weight relationships of the rat diaphragm. First, the wet wt. of the right-hand control hemidiaphragm is linearly related to the wet wt. of the rat carcass by the relationship:

$$\text{Wet wt. of control hemidiaphragm (mg)} = 16.7 + 0.796(\text{carcass wt. in g})$$

The correlation coefficient, $r = 0.741$, for 466 observations was highly significant ($P < 0.001$). Left unilateral phrenicectomy caused no significant difference in the slope of the regression line. A compensatory hypertrophy of the control hemidiaphragm after operation is thus unlikely. Secondly, regression analysis indicated in unoperated rats a linear relationship between the weight of the left hemidiaphragm with respect to that of the right, the expression being of the form:

$$\text{Wt. of left hemidiaphragm (mg)} = 23.8 + 0.628(\text{wt. of right hemidiaphragm in mg})$$

The correlation coefficient, $r = 0.776$, was highly significant ($P < 0.001$; $n = 71$). The finite value of the intercept in this equation demonstrates that the relative proportions of the two hemidiaphragms change with respect to the weight of the rat. In 100 g rats the wet wt. of the left hemidiaphragm is roughly 90% of the tissue of the right (first line of Table 1). With the exception of the value obtained for rats 2 days after denervation, when the number of samples was exceptionally small, denervation of the left hemidiaphragm did not significantly change the slope of the regression line, but the value of the intercept nearly doubled. Thus smaller rats demonstrate a greater hypertrophy after denervation, as was also concluded by Stewart (1968).

Glycogen content. Maintenance of glycogen reserves is an important feature of the metabolism of active muscle. In rats allowed free access to food before death, the effect of nerve section was to cause by the third day a decrease of glycogen concentration, which persisted (Table 2). In terms of tissue content the decrease was less marked until the 15th day, by which time the hypertrophic phase had passed.

Overnight starvation before death decreased the glycogen concentration of diaphragm muscle by some 60% (see first lines of Tables 2 and 3). In rats denervated for up to 7 days such starvation did not lead to lowering of the concentration of glycogen in the paralysed tissue (compare Tables 2 and 3). Only at 10 days after denervation was the concentration of glycogen in the paralysed tissue of starved rats significantly lower ($P < 0.05$) than in the fed rats. Glycogen content in tissue of starved rats is therefore markedly greater in the first days after nerve section.

Enzyme activities after denervation

Metabolism of white muscle is more orientated towards glucose and glycogen usage and glycogenolysis than is that of red. If the hypertrophy after nerve section is mainly of the red fibres a gradual decrease in the activity of some of these enzymes might be expected. Therefore the activities of the following enzymes were measured.

Hexokinase. Hexokinase activity was that extracted at high ionic strength. Activity per unit wt. changed little for at least a week after nerve section; the tissue total activity therefore increased during the hypertrophy (Table 4). When atrophy of the tissue became apparent both the specific activity and the total activity of hexokinase fell to values below that of the innervated tissue.

Phosphoglucumutase. In contrast to hexokinase, the

Table 2. *Effect of unilateral phrenicectomy on the concentration of glycogen in the hemidiaphragms of fed rats*
For details see the text. Each value is the mean $\pm$ S.E.M. of the number of observations shown in parentheses.

Time elapsed since phrenicectomy (days)	Glycogen concentration (μ mol of glucose/g wet wt.)		Denervated (percentage of control value)
	Innervated controls	Paired differences	
0	32.1 $\pm$ 2.7	0.2 $\pm$ 0.5 (17)	101
1	30.8 $\pm$ 2.2	-1.6 $\pm$ 1.9 (13)	98
3	31.7 $\pm$ 3.8	-16.4 $\pm$ 3.9 (7) $P < 0.01$	52
7	32.3 $\pm$ 2.0	-12.2 $\pm$ 1.6 (7) $P < 0.001$	63
10	32.4 $\pm$ 2.1	-11.1 $\pm$ 2.1 (13) $P < 0.001$	69
15	34.6 $\pm$ 2.3	-16.1 $\pm$ 2.6 (7) $P < 0.001$	55

Table 3. *Effect of unilateral phrenicectomy on the concentration of glycogen in the hemidiaphragms of starved rats*
For details see the text. Each value is the mean $\pm$ S.E.M. of the number of observations shown in parentheses.

Time elapsed since phrenicectomy (days)	Glycogen concentration (μ mol of glucose/g wet wt.)		Denervated (percentage of control value)
	Innervated controls	Paired differences	
0	13.4 $\pm$ 1.2	0.2 $\pm$ 0.5 (8)	102
1	14.4 $\pm$ 0.9	14.8 $\pm$ 1.6 (7) $P < 0.001$	206
3	11.9 $\pm$ 1.2	3.1 $\pm$ 1.1 (8) $P < 0.05$	133
7	16.8 $\pm$ 1.6	1.9 $\pm$ 1.0 (8)	115
10	14.1 $\pm$ 1.6	1.5 $\pm$ 1.6 (12)	124
15	13.0 $\pm$ 2.4	1.2 $\pm$ 1.9 (7)	126

specific activity of phosphoglucumutase declined during hypertrophy, reaching the lowest value by 5 days (Table 5); thereafter the specific activity increased. In terms of total activity the normal value was maintained until well into the atrophic phase.

Glycogen phosphorylase. Phosphorylase activity is expressed in two ways, that of phosphorylase *a* and that measured in the presence of 1 mM-AMP, i.e. total (*a* + *b*) phosphorylase. Specific activity of phosphorylase *a* did not change markedly during the period of study until 15 days, but in terms of total phosphorylase *a* activity there was an increase, reaching a maximum about the fifth day (Table 6). For total (i.e. *a* + *b*) phosphorylase activity there was a significant decrease 1 day after nerve section, but 1 day later activity was normal (Table 7). Thereafter the total activity of this enzyme decreased to very low values. The ratio of phosphorylase *a* to total phosphorylase thus rises sharply from 31% initially, to double that by the fifth day, at which value it stayed.

Glucose phosphate isomerase. Because of the initial hypertrophy the specific activity of the enzyme in the

denervated hemidiaphragm showed a steady decline after nerve section (Table 8). The total activity did not alter appreciably until 7 days, after which there was a sharp fall.

Glyceraldehyde 3-phosphate dehydrogenase. Like glucose phosphate isomerase the total activity of this enzyme remained unchanged during the first 5 days, but by 15 days it was much decreased (Table 9). Specific activity steadily declined.

Lactate dehydrogenase. The response of lactate dehydrogenase was similar to that of glyceraldehyde phosphate dehydrogenase (Table 10).

To obtain an indication of the relative proportions of the H- and M-type isoenzyme subunits in the extracts, activity of the enzyme was assayed at two different concentrations of the pyruvate substrate, 0.33 mM and 10 mM. The ratio of lactate dehydrogenase activity at low pyruvate concentration to that at high pyruvate concentration, which increases linearly the greater the proportion of the H-type subunits (Wilson *et al.*, 1963), was found in preliminary studies to be 4.15 for rat heart, 1.30 for gastrocnemius

Table 4. *Effect of unilateral phrenicectomy on the extractable activity of hexokinase in the rat hemidiaphragm expressed in terms of total and specific activity*

For details see the text. Each value is the mean $\pm$ s.e.m. of the number of observations shown in parentheses.

Time elapsed since phrenicectomy (days)	Sp. activity of hexokinase (nmol/min per mg wet wt.)		Denervated (percentage of control value)	Total activity of hexokinase (nmol/min per hemidiaphragm)		Denervated (percentage of control value)
	Innervated controls	Paired differences		Innervated controls	Paired differences	
0	4.34 $\pm$ 0.21	0.07 $\pm$ 0.06 (12)	102	397 $\pm$ 18	-52 $\pm$ 6 (12) $P < 0.001$	87
1	5.04 $\pm$ 0.22	-0.38 $\pm$ 0.06 (12) $P < 0.001$	93	435 $\pm$ 19	-60 $\pm$ 9 (12) $P < 0.001$	87
3	4.52 $\pm$ 0.16	-0.06 $\pm$ 0.11 (12)	99	406 $\pm$ 24	35 $\pm$ 11 (12) $P = 0.01$	110
5	4.57 $\pm$ 0.17	-0.12 $\pm$ 0.08 (11)	99	393 $\pm$ 22	125 $\pm$ 9 (11) $P < 0.001$	133
7	5.42 $\pm$ 0.22	-0.32 $\pm$ 0.19 (11)	97	479 $\pm$ 35	80 $\pm$ 25 (11) $P < 0.01$	118
10	4.53 $\pm$ 0.11	-0.80 $\pm$ 0.06 (12) $P < 0.001$	82	460 $\pm$ 11	-43 $\pm$ 11 (12) $P < 0.01$	91
15	4.71 $\pm$ 0.17	-1.07 $\pm$ 0.17 (12) $P < 0.001$	79	509 $\pm$ 27	-167 $\pm$ 23 (12) $P < 0.001$	68

Table 5. *Effect of unilateral phrenicectomy on the total and specific activity of phosphoglucomutase in the rat hemidiaphragm*

For details see the text. Each value is the mean $\pm$ s.e.m. of the number of observations shown in parentheses.

Time elapsed since phrenicectomy (days)	Sp. activity of phosphoglucomutase (nmol/min per mg wet wt.)		Denervated (percentage of control value)	Total activity of phosphoglucomutase (μ mol/min per hemidiaphragm)		Denervated (percentage of control value)
	Innervated controls	Paired differences		Innervated controls	Paired differences	
0	115 $\pm$ 3	-4 $\pm$ 2 (6)	96	10.6 $\pm$ 0.6	-1.4 $\pm$ 0.5 (6) $P < 0.05$	87
1	126 $\pm$ 3	-11 $\pm$ 4 (6) $P < 0.05$	92	11.8 $\pm$ 0.6	-1.4 $\pm$ 0.5 (6) $P < 0.05$	87
3	116 $\pm$ 4	-19 $\pm$ 4 (6) $P < 0.01$	84	10.1 $\pm$ 0.7	-0.7 $\pm$ 0.3 (6)	95
5	128 $\pm$ 4	-26 $\pm$ 3 (9) $P < 0.001$	80	11.6 $\pm$ 0.6	0.1 $\pm$ 0.3 (9)	102
7	123 $\pm$ 5	-21 $\pm$ 3 (9) $P < 0.001$	83	11.4 $\pm$ 0.5	0.7 $\pm$ 0.5 (9)	108
10	111 $\pm$ 3	-4 $\pm$ 2 (6)	96	9.7 $\pm$ 0.9	1.4 $\pm$ 0.6 (6)	118
15	131 $\pm$ 4	-0.1 $\pm$ 4 (6)	100	11.8 $\pm$ 0.6	-3.5 $\pm$ 0.5 (6) $P < 0.001$	70

Table 6. *Effect of unilateral phrenicectomy on the total and specific activity of phosphorylase a of rat hemidiaphragm*

For details see the text. Each value is the mean $\pm$ S.E.M. of the number of observations shown in parentheses.

Time elapsed since phrenicectomy (days)	Sp. activity of phosphorylase a (nmol/min per mg wet wt.)		Total activity of phosphorylase a (nmol/min per hemidiaphragm)		Denerivated (percentage of control value)
	Innervated controls	Paired differences	Innervated controls	Paired differences	
0	1.88 $\pm$ 0.19	0.03 $\pm$ 0.12 (6)	117 $\pm$ 22	-14 $\pm$ 10 (6)	92
1	1.68 $\pm$ 0.09	-0.04 $\pm$ 0.08 (9)	160 $\pm$ 11	-18 $\pm$ 7 (9)	90
2	1.79 $\pm$ 0.14	0.11 $\pm$ 0.07 (8)	177 $\pm$ 14	27 $\pm$ 9 (8)	116
3	1.70 $\pm$ 0.19	0.10 $\pm$ 0.11 (7)	157 $\pm$ 17	45 $\pm$ 13 (7)	127
5	1.88 $\pm$ 0.24	0.20 $\pm$ 0.06 (6)	176 $\pm$ 24	106 $\pm$ 13 (6)	161
7	1.45 $\pm$ 0.09	0.15 $\pm$ 0.10 (6)	146 $\pm$ 12	56 $\pm$ 16 (6)	142
10	1.72 $\pm$ 0.15	-0.18 $\pm$ 0.10 (6)	171 $\pm$ 8	12 $\pm$ 12 (6)	109
15	1.63 $\pm$ 0.08	-0.47 $\pm$ 0.06 (5)	153 $\pm$ 8	-48 $\pm$ 7 (5)	68
		$P < 0.01$		$P < 0.01$	

Table 7. *Effect of unilateral phrenicectomy on the specific and total activity of total (i.e. a + b) glycogen phosphorylase in the rat hemidiaphragm*

For details see the text. Each value is the mean $\pm$ S.E.M. of the number of observations shown in parentheses.

Time elapsed since phrenicectomy (days)	Sp. activity of total phosphorylase (nmol/min per mg wet wt.)		Total activity of total phosphorylase (nmol/min per hemidiaphragm)		Denerivated (percentage of control value)
	Innervated controls	Paired differences	Innervated controls	Paired differences	
0	6.34 $\pm$ 0.61	-0.37 $\pm$ 0.37 (6)	596 $\pm$ 66	-78 $\pm$ 25 (6)	87
1	5.82 $\pm$ 0.45	-1.46 $\pm$ 0.26 (9)	554 $\pm$ 49	-178 $\pm$ 29 (9)	69
2	5.92 $\pm$ 0.52	-0.23 $\pm$ 0.22 (8)	589 $\pm$ 59	22 $\pm$ 29 (8)	108
3	5.47 $\pm$ 0.44	-1.08 $\pm$ 0.36 (7)	506 $\pm$ 41	9 $\pm$ 32 (7)	99
5	6.05 $\pm$ 0.35	-2.84 $\pm$ 0.29 (6)	562 $\pm$ 31	-126 $\pm$ 25 (6)	78
7	7.01 $\pm$ 0.37	-4.07 $\pm$ 0.35 (6)	701 $\pm$ 41	-328 $\pm$ 42 (6)	54
10	6.92 $\pm$ 0.27	-4.50 $\pm$ 0.24 (6)	702 $\pm$ 57	-413 $\pm$ 42 (6)	42
15	5.54 $\pm$ 0.52	-4.08 $\pm$ 0.56 (5)	504 $\pm$ 47	-372 $\pm$ 53 (5)	28
		$P < 0.01$		$P < 0.01$	

Table 8. *Effect of unilateral phrenicectomy on the total and specific activity of glucose phosphate isomerase in the rat hemidiaphragm*

For details see the text. Each value is the mean $\pm$ S.E.M. of the number of observations indicated in parentheses.

Time elapsed since phrenicotomy (days)	Sp. activity of glucose phosphate isomerase (nmol/min per mg wet wt.)		Total activity of glucose phosphate isomerase (μ mol/min per hemidiaphragm)		Denervated (percentage of control value)
	Innervated controls	Paired differences	Innervated controls	Paired differences	
0	88.8 $\pm$ 5.2	0.0 $\pm$ 1.0 (6)	8.17 $\pm$ 0.40	-0.80 $\pm$ 0.27 (6) <i>P</i> < 0.05	90
1	84.3 $\pm$ 3.1	-5.1 $\pm$ 2.4 (6)	7.86 $\pm$ 0.48	-0.82 $\pm$ 0.32 (6)	91
3	80.4 $\pm$ 2.7	-16.9 $\pm$ 2.7 (6) <i>P</i> < 0.01	7.04 $\pm$ 0.54	-0.80 $\pm$ 0.27 (6) <i>P</i> < 0.05	90
5	76.0 $\pm$ 2.6	-18.7 $\pm$ 1.9 (8) <i>P</i> < 0.001	6.80 $\pm$ 0.26	-0.25 $\pm$ 0.11 (8)	97
7	79.1 $\pm$ 1.6	-23.9 $\pm$ 1.5 (9) <i>P</i> < 0.001	7.33 $\pm$ 0.24	-0.83 $\pm$ 0.23 (9) <i>P</i> < 0.01	90
10	82.2 $\pm$ 3.3	-28.4 $\pm$ 3.0 (6) <i>P</i> < 0.001	8.29 $\pm$ 0.68	-1.95 $\pm$ 0.38 (6) <i>P</i> < 0.01	77
15	69.4 $\pm$ 2.1	-30.5 $\pm$ 1.9 (6) <i>P</i> < 0.001	6.20 $\pm$ 0.32	-3.77 $\pm$ 0.33 (6) <i>P</i> < 0.001	40

Table 9. *Effect of unilateral phrenicectomy on the total and specific activity of glyceraldehyde 3-phosphate dehydrogenase in the rat hemidiaphragm*

For details see the text. Each value is the mean $\pm$ S.E.M. of the number of observations shown in parentheses.

Time elapsed since phrenicectomy (days)	Sp. activity of glyceraldehyde 3-phosphate dehydrogenase (nmol/min per mg wet wt.)		Total activity of glyceraldehyde 3-phosphate dehydrogenase (μ mol/min per hemidiaphragm)		Denervated (percentage of control value)
	Innervated controls	Paired differences	Innervated controls	Paired differences	
0	205 $\pm$ 8	5 $\pm$ 4 (12)	18.9 $\pm$ 0.8	-2.9 $\pm$ 0.6 (12) <i>P</i> < 0.001	86
1	175 $\pm$ 7	11 $\pm$ 6 (12)	16.5 $\pm$ 1.0	-0.7 $\pm$ 0.4 (12)	96
3	210 $\pm$ 13	-26 $\pm$ 5 (12) <i>P</i> < 0.001	18.6 $\pm$ 1.5	-0.6 $\pm$ 0.5 (12)	98
5	207 $\pm$ 7	-33 $\pm$ 7 (12) <i>P</i> < 0.001	18.7 $\pm$ 0.7	1.3 $\pm$ 0.6 (12)	107
7	222 $\pm$ 10	-44 $\pm$ 6 (12) <i>P</i> < 0.001	21.9 $\pm$ 1.0	-1.9 $\pm$ 0.8 (12) <i>P</i> < 0.05	92
10	190 $\pm$ 9	-42 $\pm$ 6 (12) <i>P</i> < 0.001	18.6 $\pm$ 1.2	-2.1 $\pm$ 0.9 (12) <i>P</i> < 0.05	90
15	249 $\pm$ 6	-95 $\pm$ 5 (6) <i>P</i> < 0.001	23.4 $\pm$ 1.5	-13.7 $\pm$ 1.0 (6) <i>P</i> < 0.001	41

Table 10. Effect of unilateral phrenicectomy on the specific and total activity of lactate dehydrogenase of rat hemidiaphragm measured at low (0.33 mM) pyruvate concentration

Time elapsed since phrenicectomy (days)	For details see the text. Each value is the mean $\pm$ S.E.M. of the number of observations shown in parentheses.					
	Sp. activity of lactate dehydrogenase (nmol/min per mg wet wt.)		Total activity of lactate dehydrogenase (μ mol/min per hemidiaphragm)		Denervated (percentage of control value)	
	Innervated controls	Paired differences	Innervated controls	Paired differences	Denervated	Denervated (percentage of control value)
0	239 $\pm$ 2	4 $\pm$ 10 (6)	21.7 $\pm$ 0.3	-2.9 $\pm$ 0.8 (6)	102	88
1	204 $\pm$ 7	1 $\pm$ 9 (6)	18.4 $\pm$ 0.7	-1.9 $\pm$ 0.7 (6)	101	90
3	260 $\pm$ 8	-45 $\pm$ 7 (6)	21.0 $\pm$ 0.9	-0.9 $\pm$ 0.4 (6)	83	96
5	218 $\pm$ 6	-35 $\pm$ 7 (6)	20.4 $\pm$ 0.9	1.3 $\pm$ 0.6 (6)	84	107
7	257 $\pm$ 12	-42 $\pm$ 12 (6)	26.4 $\pm$ 1.8	-2.3 $\pm$ 1.9 (6)	85	94
10	254 $\pm$ 12	-53 $\pm$ 11 (6)	24.6 $\pm$ 1.8	-2.8 $\pm$ 1.4 (6)	80	90
15	263 $\pm$ 16	-67 $\pm$ 17 (5)	23.5 $\pm$ 1.8	-9.0 $\pm$ 1.4 (5)	75	62

and 1.94 for liver. Unilateral phrenicectomy produced little change in the ratio of the activities for the first 3 days (Table 11). By 5 days there was a significant decrease, indicating a relative decrease in H-type subunit. At 7 days, the difference was less and at 10 days it was not apparent, but by 15 days, when the total activity of the enzyme was much decreased, a diminution in the ratio was again found (Table 11).

Pentose-shunt dehydrogenases. Activities of glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase in rat diaphragm are very low, less than one-hundredth that of hexose phosphate isomerase. Nevertheless, the effect of unilateral phrenicectomy on the activity of these enzymes is the most marked of those measured. A massive increase in activity, expressed both in terms of total and specific activity, was found soon after denervation, reaching a maximum between 3 and 7 days after nerve section (Tables 12 and 13). In the later stages of the hypertrophy the specific activity of the enzymes declined and by 15 days the total activity was no greater than in the controls. An increase in the activity of these enzymes may accompany fibrosis at a later stage of the atrophic phase (Garcia-Buñuel & Garcia-Buñuel, 1967).

Discussion

Methodology

Use of pH 6.9. To simplify procedures and comparisons all extractions and assays were done at pH 6.9, which is the best available estimate of the intracellular pH of muscle (Waddell & Bates, 1969). Because of changes of metabolite and effector concentrations as well as dilution of the enzymes the activities recorded may be different from those attainable *in vivo*. At this particular pH it is noteworthy that the apparent order of activities is fairly similar to that presented elsewhere (von Fellenberg *et al.*, 1962). Total phosphorylase measured at pH 6.9 is substantially less than at 7.5 (Manchester *et al.*, 1970) and the ratio of glucose phosphate isomerase to hexokinase is greater than reported by von Fellenberg *et al.* (1962) both because of a lower value for hexokinase and a higher one for the isomerase.

Use of Halothane. The minimal disturbance of catecholamine release (Price *et al.*, 1959; Li *et al.*, 1964) coupled with the lack of depressant effect on neuromuscular transmission (Shimoji *et al.*, 1968) makes the use of Halothane anaesthesia advantageous in the study of glycogen metabolism in tissues such as diaphragm that are accessible only relatively slowly. The glycogen content of hemidiaphragms from rats killed by decapitation without anaesthesia was 25 μ mol of glucose/g wet wt., some 20% lower than the values quoted in Table 2, which are relatively high

Table 11. *Effect of left unilateral phrenicectomy on the ratio of activity of lactate dehydrogenase isoenzymes in rat hemidiaphragm*

For details see the text. The ratio is determined as the activity measured at 0.33mm-pyruvate divided by that measured at 10mm-pyruvate. Each value is the mean $\pm$ S.E.M. of the number of observations shown in parentheses.

Time elapsed since phrenicectomy (days)	Lactate dehydrogenase activity ratio		
	Innervated controls	Denervated hemidiaphragms	Paired differences
0	3.02 $\pm$ 0.08	3.01 $\pm$ 0.08	-0.01 $\pm$ 0.07 (6)
1	3.38 $\pm$ 0.09	3.40 $\pm$ 0.12	0.02 $\pm$ 0.06 (6)
3	3.24 $\pm$ 0.07	3.38 $\pm$ 0.12	0.13 $\pm$ 0.17 (6)
5	4.31 $\pm$ 0.11	3.87 $\pm$ 0.06	-0.44 $\pm$ 0.05 (6)
		$P < 0.01$	$P < 0.001$
7	3.04 $\pm$ 0.04	2.82 $\pm$ 0.07	-0.22 $\pm$ 0.06 (6)
		$P < 0.05$	$P < 0.02$
10	3.00 $\pm$ 0.05	2.91 $\pm$ 0.04	-0.09 $\pm$ 0.04 (6)
15	4.71 $\pm$ 0.05	4.27 $\pm$ 0.11	-0.44 $\pm$ 0.08 (5)
		$P < 0.01$	$P < 0.01$

for what is considered to be a tissue containing predominantly red fibres (compare, for example, with the value for rat gastrocnemius reported by Graff *et al.*, 1965).

Piras & Staneloni (1969) observed that after activation phosphorylase *a* took approx. 5–10 min to return to normal. This is the rationale of keeping the animals under anaesthesia for long enough to allow the decay of circulating endogenous catecholamines secreted in response to initial handling. The proportion of phosphorylase *a* found here in innervated tissues ($29 \pm 1\%$ for 59 observations) compares favourably with values for other muscle preparations with intact nerve and blood supplies (Cori, 1956; Stubbs & Blanchaer, 1964; Adrouny, 1969; Drummond *et al.*, 1969).

Effects of denervation

After nerve section there is a rapid increase in the protein content of the tissue. A large proportion of this is of contractile protein (Stewart, 1955), but it is important to determine which of the various metabolic enzymes also showed a net increase in total activity. Clearly hexokinase and the pentose-shunt dehydrogenases show increases, whereas smaller rises in glyceraldehyde 3-phosphate dehydrogenase, lactate dehydrogenase and phosphoglucomutase activities are also indicated, though each enzyme displays a distinctly different chronology. By comparison total phosphorylase activity shows a steady decline to low values.

The increase in hexokinase is consistent with a hypertrophy of the red fibres as suggested by Feng & Lu (1965). The increase is in keeping with the maintained (Buse & Buse, 1959) or even increased

(Manchester, 1972) glucose uptake and suggests that diminished glycogen stores are unlikely to result from inadequate phosphorylation of entering glucose.

A high total activity of phosphorylase is usually considered as being evidence for the presence of a high proportion of white fibres within the tissue (Bass *et al.*, 1969). The converse, however, may not necessarily be true, for a decrease in the activity of the enzyme has been reported for a number of conditions, for example, after exercise (Edgerton *et al.*, 1970), in various pathological states (Smith, 1965), and as seen here, after denervation. The changes in the two enzymes result in a rise in the hexokinase/phosphorylase ratio, which is again consistent with hypertrophy of the red fibres, but the variety of factors that appear to affect phosphorylase activity may vitiate the validity of this parameter as an index of fibre type; this point is discussed further by Turner & Manchester (1972) with respect to creatine phosphokinase and adenine nucleotide-metabolizing enzymes.

Evidence that control over glycogen mobilization still remains in the hypertrophying muscle is provided by the fact that phosphorylase *a* activity is maintained despite the decreasing total enzyme activity. Taking into account the hypertrophy, the total content of glycogen in the 7-day-denervated tissue is identical with that of the normal tissue. The synthesis of the polysaccharide is thus still a significant process after a week of denervation.

The transitory decrease of total phosphorylase activity 1 day after nerve section was totally unexpected. However, it offers a possible explanation for the rapid increase in glycogen concentration occasionally reported for rat muscle 1 day after denervation. An examination of the literature reveals

Table 12. *Effect of unilateral phrenicectomy on the specific and total activity of glucose 6-phosphate dehydrogenase in the rat hemidiaphragm*

For details see the text. Each value is the mean $\pm$ s.e.m. of the number of observations shown in parentheses.

Time elapsed since phrenicectomy (days)	Sp. activity of glucose 6-phosphate dehydrogenase (nmol/min per g wet wt.)			Denervated (percentage of control value)	Total activity of glucose 6-phosphate dehydrogenase (nmol/min per hemidiaphragm)			Denervated (percentage of control value)
	Innervated controls	Paired differences			Innervated controls	Paired differences		
0	228 ± 22	4 ± 16 (6)		101	21.4 ± 2.8	-2.0 ± 2.1 (6)	91	
1	353 ± 9	213 ± 39 (6)		160	33.0 ± 1.9	18.1 ± 3.6 (6)	154	
		<i>P</i> < 0.01				<i>P</i> < 0.01		
3	303 ± 33	319 ± 38 (5)		206	28.1 ± 3.9	37.9 ± 5.7 (5)	235	
		<i>P</i> < 0.01				<i>P</i> < 0.01		
5	342 ± 25	155 ± 16 (9)		148	29.3 ± 1.8	26.5 ± 2.6 (9)	194	
		<i>P</i> < 0.001				<i>P</i> < 0.001		
7	326 ± 47	49 ± 16 (9)		120	30.2 ± 4.3	14.1 ± 2.5 (9)	157	
		<i>P</i> < 0.05				<i>P</i> < 0.001		
10	258 ± 15	22 ± 11 (6)		109	25.8 ± 2.0	7.5 ± 1.5 (6)	129	
						<i>P</i> < 0.01		
15	228 ± 17	63 ± 8 (6)		128	20.8 ± 2.2	-1.6 ± 2.1 (6)	96	
		<i>P</i> < 0.001						

Table 13. *Effect of unilateral phrenicectomy on the specific and total activity of 6-phosphogluconate dehydrogenase of rat hemidiaphragm*

For details see the text. Each value is the mean $\pm$ s.e.m. of the number of observations shown in parentheses.

Time elapsed since phrenicectomy (days)	Sp. activity of 6-phosphogluconate dehydrogenase (nmol/min per wet wt.)			Denervated (percentage of control value)	Total activity of 6-phosphogluconate dehydrogenase (nmol/min per hemidiaphragm)			Denervated (percentage of control value)
	Innervated controls	Paired differences			Innervated controls	Paired differences		
0	223 ± 14	-14 ± 16 (6)	94	20.9 ± 2.2	-3.6 ± 1.6 (6)	85		
1	327 ± 22	46 ± 20 (6)	115	30.6 ± 2.6	3.4 ± 2.5 (6)	112		
3	280 ± 11	146 ± 11 (6)	153	24.5 ± 2.0	18.1 ± 2.2 (6)	174		
		<i>P</i> < 0.001			<i>P</i> < 0.001			
5	287 ± 13	89 ± 16 (9)	132	24.7 ± 0.9	18.0 ± 2.7 (9)	172		
		<i>P</i> < 0.001			<i>P</i> < 0.001			
7	282 ± 30	39 ± 8 (9)	117	26.1 ± 2.8	11.6 ± 1.6 (9)	152		
		<i>P</i> < 0.01			<i>P</i> < 0.001			
10	291 ± 15	6 ± 11 (6)	103	29.4 ± 2.5	5.9 ± 1.2 (6)	121		
					<i>P</i> < 0.01			
15	210 ± 14	14 ± 16 (6)	108	19.2 ± 2.3	-5.3 ± 1.8 (6)	76		
					<i>P</i> < 0.05			

that, of those cases where the method of killing was specifically stated, no transitory increase of glycogen concentration was observed if an anaesthetic had been used before removal of the tissue for analysis (Lazere *et al.*, 1943; Hines & Knowlton, 1935; the present paper), whereas if decapitation was employed the increase in glycogen was found (Martinek & Mikulas, 1954; Carafoli, 1964; Graff *et al.*, 1965). If, as we found, the concentration of glycogen in the denervated tissue is essentially identical 1 day after nerve section with that in the control muscle, an impairment of the capacity to mobilize the polysaccharide as a response to stress would result in a higher concentration of glycogen being found in the denervated tissue compared with the contralateral control muscle. Administration of adrenaline to rats denervated 1 day previously results in a glycogen concentration in the control hemidiaphragm consistently lower than in the denervated tissue (L. V. Turner, unpublished work). Changes in denervated muscles reported as a percentage of the contralateral control value rather than in terms of the absolute quantity may obscure the relatively smaller decrease in the denervated tissue which otherwise appears as an increase in glycogen.

Total activities of glyceraldehyde 3-phosphate dehydrogenase and lactate dehydrogenase, both found at high activities in white muscle, might have been expected to decline after nerve section. As these enzymes are localized in the sarcoplasmic reticulum (Fahimi & Karnovsky, 1966), the increase in total activity, concurrent with the peak of the hypertrophy, may be related to the proliferation of the tubular system observed in the hypertrophying tissue (Hajek *et al.*, 1967). Changes in the proportions of lactate dehydrogenase isoenzymes have been shown to result from alterations in the partial pressure of oxygen in the cellular environment (Goodfriend & Kaplan, 1963; Cahn, 1964; Hellung-Larson & Anderson, 1970). Diaphragm is a highly aerobic tissue, as indicated by the high proportion of H-type subunits, and is well supplied by capillary vessels. The temporary decrease in the proportion of the H-type subunits (Table 11) at the peak of the hypertrophy may indicate a decreased oxygen partial pressure in the tissue, though why this should be so is not clear; local overproduction of a metabolite with vasoconstrictive properties (Heddy & Scott, 1968) is one possibility that may explain fluctuations in the proportions of the isoenzyme subunits.

Garcia-Buñuel & Garcia-Buñuel (1967) concluded that an increase in the activity of pentose-shunt dehydrogenases 2 weeks after nerve section was related to the progressive increase in collagen of the atrophic tissue. In the present study we have found a rise in enzyme activities at a much earlier stage before the proportion of connective tissue increases (Stewart, 1955). It seems as likely, therefore, that the increase

in shunt metabolism is related to the production of pentose to support the substantial rise in nucleic acid content of the hypertrophying tissue (Manchester & Harris, 1968) and/or an increased need for NADPH for synthetic purposes.

A frequent interpretation of the changes of enzyme activities in muscle after nerve section has been in terms of a de-differentiation or reversion to a foetal type of metabolism. The diversity of the responses to phrenicectomy that are reported here for the rat hemidiaphragm [the increase in hexokinase content, the proliferation of the sarcoplasmic reticulum and of its characteristic enzymes, the transitory nature of the activity changes of the pentose-shunt dehydrogenases and of the proportions of the lactate dehydrogenase isoenzyme subunits, as well as the alterations in enzyme activities reported by Turner & Manchester (1972)] make it highly improbable that the tissue is experiencing a reversion to a foetal type of metabolism, at least during the hypertrophy phase. The response of the tissue is clearly complex. The similarity of some of the metabolic properties of the denervated hemidiaphragm to those induced in the normal tissue by insulin, i.e. increased protein synthesis and uptake of certain amino acids, is noteworthy and may indicate a common mechanism. Turner & Manchester (1972) report the changes in activity of some mitochondrial enzymes and of the adenine nucleotide-metabolizing enzymes that are of importance in maintenance of the ATP concentrations for muscle contraction and advance a possible explanation for the fibre-specific hypertrophy of the tissue.

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