Effects of Denervation on the Activities of some Tricarboxylic Acid-Cycle-Associated Dehydrogenases and Adenine-Metabolizing Enzymes in Rat Diaphragm Muscle

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1. The activity of several tricarboxylic acid-cycle-associated dehydrogenases, adenine-metabolizing enzymes and glutathione reductase and the content of myoglobin were measured in rat diaphragm muscle after unilateral nerve section. 2. Consistent with morphological disintegration of the mitochondria there was a rapid diminution in activity of NAD- and NADP-linked isocitrate dehydrogenase, malate dehydrogenase and glutamate dehydrogenase. 3. Creatine phosphokinase and adenylate kinase, by contrast, showed little change in activity; adenylylate deaminase and glutathione reductase activities increased during the hypertrophic phase. The concentration of myoglobin at first declined, then increased again. 4. The distribution of enzymes between the left and right hemidiaphragms was found not to be uniform. 5. Activities of adenine-metabolizing enzymes in the diaphragm were as great as in white muscle. It is suggested that their reputedly lower activities in red muscle properly refer to muscle containing a high proportion of intermediate fibres, which is not the case with diaphragm. 6. The possible causes of the transient hypertrophy after nerve section are discussed.

Denervation of muscle leads to fragmentation of the fibre mitochondria (Muscagetto & Patriarca, 1968). Concurrently, there is diminution in the extractable activity of characteristic enzymes (Romanul & Hogan, 1963) and depletion of functional mitochondrial activity. These changes may limit availability of energy within the cell and thus contribute to characteristic denervation atrophy (Carafoli et al., 1964).

Within 24 h of phrenic nerve section, mitochondria of rat diaphragm muscle show similar morphological changes (Miledi & Slater, 1969). The denervated hemidiaphragm, however, is peculiar in that nerve section results in an initial hypertrophy that occurs despite apparent diminution of mitochondrial function as judged by the electron-microscopic appearance and a decreased capacity for $^{14}$CO$_2$ formation from $[^14]$Cacetate (Kouvelas & Manchester, 1968). These observations contrast with the conclusions of Feng & Lu (1965) that hypertrophy is solely a function of the 'red' mitochondria-rich fibres, the 'white' fibres only undergoing atrophy. The hypertrophying tissue moreover is visibly paler than its innervated counterpart.

A more precise indication of the type of material resulting from the hypertrophy is gained by measurement of the activities of specific enzymes. We have thus investigated the extractable content of myoglobin and of some characteristic mitochondrial enzymes from diaphragm muscle for up to 15 days after denervation. Since it has been reported that creatine kinase, adenylylate kinase and adenylylate deaminase are normally present in higher specific activities in white than red muscle (Raggi et al., 1969), we have attempted to correlate the atrophy of the white fibres with changes in the activities of these enzymes. We were, however, unable to confirm the initial premise.

McCaman (1960, 1963) has suggested that a common feature of dystrophic and denervated muscles is a general elevation in the activities of NADP-linked dehydrogenases, whereas the activity of NAD-linked enzymes show a decrease. In common with other growing tissues (Gregory, 1939), the denervated hemidiaphragm has an enhanced concentration of GSH (40% greater than that of the control tissue, within 3 days of nerve section; L. V. Turner & K. L. Manchester, unpublished work). We have therefore included in the present paper measurements of the activity of glutathione reductase to complement the other dehydrogenase determinations.

Experimental

Materials

2-(N-2-Hydroxyethylpiperazin-N'-yl)ethanesulphonic acid (HEPES) A grade was obtained from Calbiochem, Los Angeles, Calif., U.S.A. α-Oxoglutarate and DL-isocitric acid lactone were obtained
from BDH Chemicals Ltd., Poole, Dorset, U.K. All other substrates, nucleotides and enzymes (hexokinase, 140 units/mg, 10 mg/ml; glucose 6-phosphate dehydrogenase, 140 units/mg, 5 mg/ml) were obtained from Boehringer Corp. (London) Ltd., London W.5, U.K.

Methods

The procedure for unilateral phrenicectomy and the general methods for the preparation and assay of the muscle extracts are described in the preceding paper (Turner & Manchester, 1972).

Enzyme assays. The specific conditions used for the extraction and assay of the individual enzymes in the muscle supernatant extracts were as follows.

NAD-linked isocitrate dehydrogenase. Each hemi-diaphragm was homogenized in 1.5 ml of 100 mM potassium phosphate–1 mM-EDTA buffer, final pH 6.9, and the homogenate was centrifuged at 2200 g for 15 min. The assay medium (3 ml) contained 1 mM-EDTA, 2 mM-ADP, 5 mM-DL-isocitrate, 1.5 mM-MnCl₂, 0.6 mM-NAD⁺, 0.375 g of Triton X-100/litre and 20 mM-HEPES buffer, final pH 6.9. The reaction was initiated by the addition of the NAD⁺. ADP stabilizes the enzyme and the Triton X-100 eliminates NADH oxidase activity.

NADP-linked isocitrate dehydrogenase. Muscle extract was prepared as for the NAD-linked enzyme. The assay medium (3 ml) contained 1 mM-EDTA, 10 mM-MgCl₂, 2.6 mM-DL-isocitrate, 0.3 mM-NADP⁺, 0.375 g of Triton X-100/litre and 20 mM-HEPES buffer, final pH 6.9. Triton X-100 eliminates non-specific NADP⁺ reductase activity.

Malate dehydrogenase. Muscle extracts were prepared as for NAD-linked isocitrate dehydrogenase. The assay medium (3 ml) contained 1 mM-EDTA, freshly neutralized 0.2 mM-oxaloacetate, 0.5 mM-NADH and 50 mM-potassium phosphate buffer, final pH 6.9. At the dilution of the extract used in the assay NADH oxidase activity was negligible.

Glutamate dehydrogenase. Muscle extract was prepared as for NAD-linked isocitrate dehydrogenase. The assay medium (3 ml) contained 1 mM-EDTA, 60 mM-NH₄Cl, 3 mM-oxoglutarate, 0.25 mM-NADH, 0.03 g of Triton X-100/litre and 50 mM-potassium phosphate buffer, final pH 6.9. Reagent blanks containing enzyme but no oxoglutarate were used.

Creatine phosphokinase. Each hemidiaphragm was homogenized in 2 ml of 0.3 M sucrose–20 mM-HEPES–1 mM-EDTA medium, final pH 6.9, and the homogenate was centrifuged at 30000 g for 30 min. The assay medium (1 ml) contained 6 mM-MgCl₂, 20 mM-glucose, 5 mM-cysteine hydrochloride, 1 mM-ADP, 10 mM-AMP, 0.8 mM-NADP⁺, 10 mM-creatine phosphate, approx. 10 μg each of hexokinase and glucose 6-phosphate dehydrogenase and 20 mM-HEPES buffer, final pH 6.9. The AMP included in the assay mixture inhibits adenylate kinase activity.

Adenylate kinase. Muscle extract was prepared as for creatine kinase. The assay medium (3 ml) contained 6 mM-MgCl₂, 1 mM-ADP, 20 mM-glucose, 0.8 mM-NADP⁺, 5 mM-cysteine hydrochloride, approx. 30 μg each of hexokinase and glucose 6-phosphate dehydrogenase and 20 mM-HEPES buffer, final pH 6.9.

Adenylate deaminase. Each hemidiaphragm was homogenized in 2.0 ml of 0.3 M sucrose–20 mM-HEPES–1 mM-EDTA–0.8 M-KCl medium, final pH 6.9; the high ionic strength was required to prevent adsorption of the enzyme to myofibrils. The homogenate was centrifuged for 30 min at 30000 g. The assay medium (3 ml) contained 100 mM-KCl, 2 mM-AMP and 20 mM-HEPES buffer, final pH 6.9. The KCl in the assay medium activates the enzyme. The production of inosinic acid was followed at 285 nm (Raggi et al., 1969).

Glutathione reductase. Muscle extracts were prepared as for NAD-linked isocitrate dehydrogenase. The assay medium (3 ml) contained 1 mM-EDTA, 2.6 mM-GSSG, 0.2 mM-NADPH and 50 mM-potassium phosphate buffer, final pH 6.9.

Myoglobin assay. The extraction and determination of myoglobin was carried out essentially by the method of Reynafarje (1963).

Statistical treatment of results. The values listed in the tables are the means ± S.E. of the activities, either units/g wet wt. (specific activity) or units/hemidiaphragm (total activity), of the particular parameter measured at the stated number of days after denervation. Statistical significance of the paired differences of values, indicated if P was less than 0.05, was evaluated by Student’s t test.

Results

Tricarboxylic acid-cycle-associated enzymes

Because of the technical difficulties associated with the fractional extraction procedure for enzyme distribution studies described by Pette (1966) when applied to the small quantity of tissue furnished by a single rat hemidiaphragm, no attempt has been made to determine the intracellular distribution of the tricarboxylic acid-cycle-associated dehydrogenases. Both the NADP-linked isocitrate dehydrogenase and malate dehydrogenase occur in appreciable quantities both outside and inside mitochondria.

Unilateral phrenicectomy results in a rapid decrease in content of tricarboxylic acid-cycle dehydrogenases. The total activity of NADP-linked isocitrate dehydrogenase is decreased by 15% in 24 h and decreases for a further 2 days to about 50% of the control value (Table 1). At 10 days after denervation a further decrease occurs, but this is probably no
Table 1. Effect of unilateral phrenectomy on the total activity of various dehydrogenases of rat diaphragm

Each value is the mean ± S.E.M. of six observations except for † where there were 10 observations. *P < 0.05, **P < 0.01, ***P < 0.001. Specific activities (nmol/min per mg wet wt. of tissue) of the innervated controls averaged 1.93 for NAD-linked isocitrate dehydrogenase, 13.5 for NADP-linked isocitrate dehydrogenase, 830 for malate dehydrogenase and 0.86 for glutamate dehydrogenase. The relation of the specific activity of the denervated tissue as a percentage of the innervated control is indicated by the numbers in parentheses.

<table>
<thead>
<tr>
<th>Time elapsed since phrenectomy (days)</th>
<th>NAD-linked isocitrate dehydrogenase</th>
<th>NADP-linked isocitrate dehydrogenase</th>
<th>Malate dehydrogenase</th>
<th>Glutamate dehydrogenase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Denervated (μmol/min per hemidiaphragm)</td>
<td>Denervated (μmol/min per hemidiaphragm)</td>
<td>Denervated (μmol/min per hemidiaphragm)</td>
<td>Denervated (μmol/min per hemidiaphragm)</td>
</tr>
<tr>
<td></td>
<td>(μmol/min per hemidiaphragm)</td>
<td>Denervated (percentage of control value)</td>
<td>(μmol/min per hemidiaphragm)</td>
<td>Denervated (percentage of control value)</td>
</tr>
<tr>
<td></td>
<td>Innervated controls</td>
<td>Paired differences</td>
<td>Innervated controls</td>
<td>Paired differences</td>
</tr>
<tr>
<td>0</td>
<td>147 ± 8</td>
<td>-5 ± 7</td>
<td>97 (116)</td>
<td>1.35 ± 0.004</td>
</tr>
<tr>
<td>1</td>
<td>144 ± 9</td>
<td>-29 ± 12</td>
<td>82 (93)</td>
<td>0.91 ± 0.005</td>
</tr>
<tr>
<td>3</td>
<td>155 ± 6*</td>
<td>71.6 ± 6*</td>
<td>54 (50)</td>
<td>1.25 ± 0.003</td>
</tr>
<tr>
<td>5</td>
<td>208 ± 11</td>
<td>-103 ± 13***</td>
<td>54 (44)</td>
<td>1.22 ± 0.005</td>
</tr>
<tr>
<td>7</td>
<td>256 ± 18</td>
<td>-113 ± 7***</td>
<td>50 (42)</td>
<td>1.40 ± 0.005</td>
</tr>
<tr>
<td>10</td>
<td>234 ± 16</td>
<td>-123 ± 14***</td>
<td>47 (40)</td>
<td>1.55 ± 0.11</td>
</tr>
<tr>
<td>15</td>
<td>191 ± 9</td>
<td>-145 ± 8***</td>
<td>24 (37)</td>
<td>1.17 ± 0.04</td>
</tr>
</tbody>
</table>

Table 2. Effect of unilateral phrenectomy on the total activity of creatine phosphokinase, adenylate kinase, adenylate deaminase and glutathione reductase of rat diaphragm

Each value is the mean ± S.E.M. of six observations except for adenylate kinase, where for † there were 11 observations or for ‡‡ there were 12 observations. Specific activities (nmol/min per mg wet wt. of tissue) of the innervated controls averaged 1130 for creatine phosphokinase, 164 for adenylate kinase, 71 for adenylate deaminase and 690 for glutathione reductase. The relation of the specific activity of the denervated tissue as a percentage of the innervated control is indicated by the figures in parentheses.

<table>
<thead>
<tr>
<th>Time elapsed since phrenectomy (days)</th>
<th>Creatine phosphokinase</th>
<th>Adenylate kinase</th>
<th>Adenylate deaminase</th>
<th>Glutathione reductase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(μmol/min per hemidiaphragm)</td>
<td>Denervated (percentage of control value)</td>
<td>(μmol/min per hemidiaphragm)</td>
<td>Denervated (percentage of control value)</td>
</tr>
<tr>
<td></td>
<td>Innervated controls</td>
<td>Paired differences</td>
<td>Innervated controls</td>
<td>Paired differences</td>
</tr>
<tr>
<td>0</td>
<td>108 ± 7</td>
<td>-15 ± 4*</td>
<td>86 (94)</td>
<td>14.4 ± 2.9</td>
</tr>
<tr>
<td>1</td>
<td>111 ± 7</td>
<td>-13 ± 4*</td>
<td>87 (90)</td>
<td>13.8 ± 2.4</td>
</tr>
<tr>
<td>3</td>
<td>100 ± 2</td>
<td>-15 ± 2***</td>
<td>85 (72)</td>
<td>19.6 ± 1.4</td>
</tr>
<tr>
<td>5</td>
<td>99 ± 4</td>
<td>-16 ± 2***</td>
<td>84 (70)</td>
<td>15.0 ± 2.1</td>
</tr>
<tr>
<td>7</td>
<td>110 ± 4</td>
<td>-26 ± 6**</td>
<td>77 (58)</td>
<td>13.8 ± 1.5</td>
</tr>
<tr>
<td>10</td>
<td>119 ± 6</td>
<td>-45 ± 6***</td>
<td>63 (54)</td>
<td>16.8 ± 1.8</td>
</tr>
<tr>
<td>15</td>
<td>96 ± 6</td>
<td>-65 ± 7***</td>
<td>33 (47)</td>
<td>12.9 ± 1.9</td>
</tr>
</tbody>
</table>
more rapid than that of other proteins, since the specific activity of the enzyme remains unchanged. A similar response is shown by the NADP-linked enzyme and by malate dehydrogenase.

Optimum activity of malate dehydrogenase of the total tissue extract was found with an oxaloacetate concentration of 0.2 mm. Under these conditions the mitochondrial enzyme is likely to be under significant substrate inhibition (Siegel & England, 1960; England et al., 1960). Activity was similar to that recorded for the extramitochondrial enzyme of rat heart (Shonk & Boxer, 1964). By contrast with isocitrate dehydrogenase and malate dehydrogenase, glutamate dehydrogenase showed only a small decrease in total activity for the first 5 days (Table 1). After 10 days its specific activity may have increased.

No evidence for any increase in either total or specific activity of the above tricarboxylic acid-cycle-associated enzymes was thus observed during the phase of hypertrophy.

**Adenine-nucleotide-metabolizing enzymes**

The total tissue activity of creatine phosphokinase remains fairly constant for the first 5 days after nerve section (Table 2) and then declines by day 15 to roughly one-third of the value for the innervated control. By contrast, total activity of both adenylate kinase and particularly adenylate deaminase appears to increase in the first few days after nerve section, i.e., during the hypertrophy phase. Thereafter total activity of adenylate kinase declines similarly to that of creatine phosphokinase.

The decline in activity of these enzymes by 15 days could suggest increasing 'redness' of the muscle, but the early increases do not support red-fibre hypertrophy. However, a survey of the extractable activities of these enzymes in a number of rat muscles (Table 3) suggests that diaphragm muscle has activities of these enzymes comparable with those of white muscles. As well as being inconsistent with the scheme for fibre types proposed by Raggi et al. (1969), these results suggest, like those of Goldspink et al. (1970), that adenylate kinase/creatine kinase ratios in rat muscle do not, as proposed by Golische et al. (1970), correlate with the contractile properties of the individual muscles.

**Myoglobin**

Our values for myoglobin concentration are similar to those of Burleigh & Schimke (1969). Nerve section results in little change in pigment content for the first 5 days or so (Table 4); the concentration of myoglobin thus decreases. Thereafter the pigment concentration increases and eventually exceeds the control value.

**Glutathione reductase**

In conjunction with the concentrations of GSH, the total tissue activity of glutathione reductase increased in the first few days after nerve section (Table 2). Thereafter the total activity changed little, though the specific activity after the fifth day rose steadily.

**Discussion**

In rats of the weight used in this study the wet weight of the left hemidiaphragm is only 90% of that of the right (Turner & Manchester, 1972). Yet the total activity of the mitochondria-associated dehydrogenases is equal in the two hemidiaphragms,
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Table 4. Effect of unilateral phrenectomy on the content of myoglobin in the rat hemidiaphragm

Each value is the mean ± S.E.M. of the number of observations shown in parentheses. *P <0.05. The concentration of myoglobin in the innervated tissue averaged 1.82 mg/g wet wt. of tissue. The relation of the concentration in the denervated tissue as a percentage of the innervated control is indicated by the figures in square brackets.

<table>
<thead>
<tr>
<th>Time elapsed since phrenectomy (days)</th>
<th>Myoglobin content (µg/hemidiaphragm)</th>
<th>Denervated (percentage of control value)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Innervated controls</td>
<td>Paired differences</td>
</tr>
<tr>
<td>0</td>
<td>156 ± 5</td>
<td>-10 ± 5 (11)</td>
</tr>
<tr>
<td>1</td>
<td>157 ± 9</td>
<td>-18 ± 8* (11)</td>
</tr>
<tr>
<td>3</td>
<td>150 ± 9</td>
<td>-14 ± 7 (11)</td>
</tr>
<tr>
<td>5</td>
<td>167 ± 8</td>
<td>-13 ± 11 (8)</td>
</tr>
<tr>
<td>7</td>
<td>156 ± 12</td>
<td>3 ± 11 (10)</td>
</tr>
<tr>
<td>10</td>
<td>143 ± 8</td>
<td>20 ± 10 (9)</td>
</tr>
<tr>
<td>13</td>
<td>247 ± 19</td>
<td>1 ± 16 (8)</td>
</tr>
<tr>
<td>15</td>
<td>184 ± 16</td>
<td>-15 ± 16 (11)</td>
</tr>
</tbody>
</table>

with the result that the specific activity in the left is greater. By contrast cytoplasmic enzymes appear to occur in similar specific activities and thus lower total activities in the left portion. The anatomical or physiological significance of these differences is not clear.

The decrease in activity of the tricarboxylic acid-cycle-associated dehydrogenases is consistent with the disintegration of the mitochondria and the decreased capacity for 14CO2 formation from acetate (Kouvelas & Manchester, 1968). However, it is noteworthy that decline in total activity of NADP-linked isocitrate dehydrogenase and of malate dehydrogenase, which are both approx. 50% extramitochondrial in rat muscle, also occurs at the same time. It is also significant that degradation of mitochondria, which are found principally within the red fibres, should be occurring at a time when other structures in the fibres are proliferating. Both L-ascorbate and GSH increase in concentration after denervation (Graff et al., 1965; L. V. Turner & K. L. Manchester, unpublished work), and both cause mitochondrial swelling (Lehninger, 1965). It is possible to speculate that either or both of these compounds or other similar materials may be the cause of mitochondrial lysis. If the decrease in activity of the tricarboxylic acid-cycle-associated dehydrogenases results from cessation of synthesis without enhancement of breakdown, the results of Table 1 would indicate a turnover time at least as high as 3–5 days, a figure as rapid as other estimates for muscle proteins (Waterlow & Stephen, 1968). The lower activity of glutamate dehydrogenase is also consistent with decreased concentrations of ammonia in the tissue (L. V. Turner & K. L. Manchester, unpublished work) and may be indicative of an initial overall decrease in the rate of protein catabolism, which would in part explain increased concentrations of several free amino acids (L. V. Turner & K. L. Manchester, unpublished work) as well as facilitating protein synthesis.

McCaman (1960, 1963) has suggested that a common enzymic pattern in atrophic muscles, whether dystrophic or denervated, is a decrease in NAD-linked dehydrogenases and increase in the NADP-linked enzymes. Increases in the activity/unit weight of tissue for the pentose-shunt dehydrogenases and glutathione reductase, and a decrease in glyceraldehyde phosphate dehydrogenase and lactate dehydrogenase specific activities in the denervated hemidiaphragm (Turner & Manchester, 1972), as well as a decrease for the NAD-linked dehydrogenases noted in the present paper, are consistent with this view. The decrease in NADP-linked isocitrate dehydrogenase is, however, an exception.

That hemidiaphragm has as much creatine kinase, adenylyl kinase and adenylyl deaminase activity as the white muscle of the hind limb of the rat (Table 3) is surprising in view of the observations of Raggi et al. (1969). A possible explanation is that the ‘intermediate’ type of fibre is that with the lowest concentrations of these enzymes. About 70% of the fibres of the rat soleus are intermediate (Stein & Padykula, 1962), whereas only some 16% of this type of fibre is found in the plantaris muscle (Edgerton et al., 1969). The intermediate type fibres of the rat diaphragm is about 20% (Gauthier & Padykula, 1966). Direct comparisons such as these may, however, be unreliable, since the fibre proportions in a muscle may change with age (Bertalanffy & Pyroznyski, 1953; Lewis, 1968). That the red and white fibres should have comparable activities of these
enzymes would correlate with recent findings that both red and white fibres may be of the fast-twitch type, whereas only the intermediate fibre is slow-twitch (Edgerton & Simpson, 1969; Barnard et al., 1971).

The activities of the three adenine-nucleotide-metabolizing enzymes change at different rates. These results do not therefore support the concept of an 'operon-like type of control' for the three enzymes (Kendrick-Jones & Perry, 1967), though lack of knowledge of their rates of turnover (Schimke, 1969) may preclude definitive conclusion on this point.

The precise cause of the hypertrophy of the red fibres of the hemidiaphragm after nerve section remains unknown. The passive rhythmic stretch to which the tissue is subjected by the innervated control hemidiaphragm has been suggested as a cause (Feng & Lu, 1965), but it fails to explain the fibre specificity, and the experiments of Stewart (1968) provide no support. In small mammals such as the rat, blood flow through muscle is reported to be the rate-limiting step in O₂ consumption (Honig et al., 1971). An initial increase in net blood flow through the diaphragm after denervation has been shown by Anrep et al. (1934) and is expected, since section of the phrenic nerve will have the effect of isolating the vasomotor neurones involved in the control of blood flow through the tissue. The experiments of Buse & Buse (1959, 1961) have also suggested an increased blood flow through the denervated hemidiaphragm, the effect being apparent for at least 3 days after nerve section. Coupled with the cessation of work after nerve section, the increase in blood flow would have the effect of raising the oxygen partial pressure in the tissue. Since changes in the proportion of the lactate dehydrogenase iso-enzymes have been shown to result from alterations of the oxygen partial pressure of the cellular environment (Goodfriend & Kaplan, 1963; Cahn, 1964; Hellung-Larson & Anderson, 1970), the slight increase in proportion of the H-type subunits in the first 3 days after nerve section (Turner & Manchester, 1972) may reflect this greater oxygen availability, as also may the increase in glutathione reductase activity, presumably to counteract fluctuations in the redox state of the muscle (compare the chronology of the paired differences for the lactate dehydrogenase isoenzyme ratio with those for glutathione reductase activity). This is also consistent with the fact that, although the red fibres are reported to hypertrophy, the myoglobin content of the tissue does not increase until after the peak of the hypertrophy. Reis & Wooten (1970) have demonstrated a direct correlation between the rate of blood flow through muscle and its myoglobin content. The relationship, however, appears to be under neural control, and thus does not conflict with the situation of the denervated hemidiaphragm.

Increase in oxygen partial pressure in the perfused working rat heart has the effect of raising the rate of protein synthesis (Schreiber et al., 1966, 1967). Assuming that a similar cause is responsible for the hypertrophy of the denervated hemidiaphragm, the fibres that would benefit most from any enhanced capillary blood flow would be the 'red' fibres, since Romanul (1964) has shown that in mixed skeletal muscle the capillary density around the mitochondria-rich fibres is appreciably greater than that around the white fibres. By the fifth day after nerve section, the tissue hypertrophy has reached its peak, and the predominance of the increased rate of protein synthesis has declined. At this time a relative increase in the proportion of the M-type subunits of lactate dehydrogenase is indicated (Turner & Manchester, 1972), but over the next 5 days this difference diminishes, and concurrently the concentration of myoglobin begins to rise. It therefore seems unlikely that the proposed increase in oxygen partial pressure within the tissue should persist for more than a few days after nerve section, and its decline may well account for the eventual atrophy of the tissue.

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