marked in the second peak of proteolipid, which showed a fivefold increase in specific radioactivity. Other experiments showed that only this peak had the capacity to bind labelled acetylcholine and methylhexamethonium.

These findings demonstrate that denervation promotes an increased turnover of particular proteolipids of the muscle having receptor properties. We suggest that such metabolic changes could be related to the increased cholinergic sensitivity of the muscle that follows denervation.

This work was supported by Grant no. 456-D10 of the Multiple Sclerosis Society, U.S.A. G.G.L. thanks The Wellcome Trust for the award of a Fellowship.

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Enzyme Changes Indicative of Fibre Type in the Denervated Rat Hemidiaphragm

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Feng & Lu (1965) on the basis of limited histological studies reported that the hypertrophy of the denervated rat hemidiaphragm was a function solely of the 'red' fibres of the tissue. Similarly, we have observed a decreased glycogenolytic capacity in the hypertrophied tissue indicative of a decreasing proportion of 'white'-fibre metabolism (Turner & Manchester, 1969). Rat diaphragm, however, is known to contain a significant proportion of 'intermediate'-type fibres (Padykula & Gauthier, 1970), a type which it has been suggested (Edgerton & Simpson, 1969) might be identical with the 'slow-twitch' fibre. Since within a few days of denervation there is an appreciable decrease in the speed of contraction of the hypertrophied tissue (Miledi & Slater, 1969) and since the histological technique to discriminate between 'red' and 'intermediate' fibres was not then available to Feng & Lu (1965), there is a possibility that the hypertrophy might involve the 'intermediate'-type fibre, either exclusively or else in conjunction with the 'red'-fibre population. Such a concept might help to explain the following observations, which appear anomalous in terms of a 'red'-fibre hypertrophy.

A significant decrease in the concentration of myoglobin is observed in the denervated tissue within 3 days of nerve section. After 5 days, however, the concentration rises such that by the 10th day after denervation normal values are regained and by the 15th day a 20% increase is found. In terms of myoglobin content, though there is only a very gradual rise in the denervated tissue, reaching a peak of 120% of control values at 10 days after nerve section and thereafter declining. Net production of myoglobin thus lags appreciably behind synthesis of other muscle proteins; maximal protein content of the denervated hemidiaphragm occurs 5 days after phrenicectomy.

A major distinction between 'red' and 'intermediate' fibre types is the relatively lower mitochondrial content of the latter (Padykula & Gauthier, 1970). Fragmentation of the normally tortuous and elongated mitochondria of the 'non-white' fibres of the rat diaphragm was observed by Miledi & Slater (1968) within 24h of nerve section, but they were unable to decide whether the total content of mitochondria was altered. Content of NAD-specific isocitrate dehydrogenase (EC 1.1.1.41), which in muscle is located exclusively in the mitochondrion (Pette, 1966), is found to fall rapidly after phrenicectomy: 1 day after nerve section a statistically significant decrease in the content of the enzyme was found in the denervated tissue. By 5 days, the enzyme concentration had reached a plateau at a value approx. 40% of that of the control tissue; this value is maintained for up to 15 days after nerve section. NADP-specific isocitrate dehydrogenase (EC 1.1.1.42) and malate dehydrogenase (EC 1.1.1.37) also show similar decreases in content after denervation. In contrast, glutamate dehydrogenase (EC 1.4.1.3), although decreasing in concentration to 60% of control values at the peak of the hypertrophy, actually increases in concentration as the tissue begins to lose weight. Possibly this response, absent for the other enzymes, is indicative of the role the enzyme plays in feeding products of protein catabolism into the tricarboxylic acid cycle.

L. V. T. is grateful to the Medical Research Council for a Studentship.

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Induction of δ -Aminolaevulate Synthetase Activity in Cultured Chick-Embryo Liver Cells by Ribonucleic Acid

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AIA* is porphyrinogenic in experimental animals (Goldberg & Rimington, 1954), and specifically induces δ -aminolaevulate synthetase, the initial and rate-limiting enzyme of porphyrin biosynthesis (Granick, 1966).

Hickman, Saunders, Dowdle & Eales (1967) found that high-molecular-weight RNA isolated from the livers of rats rendered porphyric by administration of AIA increased the porphyrin content of segments of chick-embryo liver grown in organ culture. Repetition of these experiments in this laboratory has given inconclusive results. In the present study, however, we have measured aminolaevulate synthetase activity by the method of Dowdle, Mustard & Eales (1967) in cultured chick-embryo liver cell monolayers (Skea & Nemeth, 1969).

Rats were rendered porphyric by intraperitoneal injection of AIA and RNA was prepared from their livers and from the livers of normal rats by the method of Parish & Kirby (1966). Incubation of cell monolayer cultures of 14–15-day-old chick embryo liver with a solution of RNA from porphyric rat liver (0.2mg of RNA/ml of Eagle's minimum essential medium) caused a two- to threefold increase in aminolaevulate synthetase activity within 4h, the enzyme activity returning to the control value by 8h. No increase of aminolaevulate synthetase activity was found in cultures incubated with normal rat liver RNA. This effect is apparently RNA-dependent, since prior incubation of porphyric rat liver RNA with ribonuclease abolished it. Since RNA isolated from normal rat liver homogenate deliberately contaminated with AIA did not increase aminolaevulate synthetase activity in the cultures, the effect was presumably not due to contaminating traces of AIA.

Preliminary fractionation of porphyric-rat liver RNA has been carried out on Sephadex G-100 by the method of Kay & Cooper (1969). By this method 90% of the RNA was excluded from the column, the rest being eluted later in two shallow peaks. The RNA from the three fractions was reprecipitated with ethanol and tested separately at a concentration of 0.2mg of RNA/ml for ability to increase aminolaevulate synthetase activity in chick-embryo liver cultures. All three fractions showed significant inducing activity, but the two lower-molecular-weight fractions were two to three times as active as the Sephadex-excluded fraction. Similar fractions of normal rat liver RNA showed no significant inducing activity.

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Binding of Polylysine and Toluidine Blue to Deoxyribonucleoprotein

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The accessibility of the DNA component of dNP* to molecules of various sizes was studied by measuring its binding to the dye Toluidine Blue, and to poly-L-lysine hydrobromide of three different molecular weights. Mixtures with dNP were made at concentrations such that all the dNP precipitated. Estimates were then made of the excess of unbound dye or polylysine over a range of dNP concentrations.

With Toluidine Blue, about 60% of the DNA phosphate groups in dNP were available for binding. This high value was not attributable to dissociation of the DNA from the protein, nor to any substantial binding to the protein acidic groups.

With polylysine, estimates were made of both completely unused polylysine molecules and of lysyl residues in molecules only partly bound to dNP, i.e. of unused ('wasted') residues. 'Titration' values of available DNA phosphate groups were 46, 38 and approx. 44% respectively for polylysine preparations of mol.wt. 1250, 7790 and 62000 (PL1, PL2 and PL3). In DNA, 100% of the phosphate groups were available. Near the point of mutual charge neutralization with dNP and DNA no lysyl residues were 'wasted', at least for preparations PL1 and PL2. This result and the

* Abbreviation: AIA, allylisopropylacetamide.

* Abbreviation: dNP, deoxyribonucleoprotein.