Subcellular Protein Movement in Rat Uterus and Liver

By V. J. BEARD and R. J. B. KING. (*Department of Hormone Biochemistry, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX, U.K.*)

Oestradiol is transported from cytoplasm to nucleus by a protein, but all experiments have used labelled steroid to detect these events; it is also known that proteins enter the nucleus and affect nuclear function (Gurdon & Woodland, 1968).

To study the movement of protein in uterus and liver we isolated nuclei and a 10^6g supernatant fraction from these tissues, labelled *in vitro* with [3H]tryptophan and [^{14}C]lysine; unlabelled fractions were also isolated. The free amino acids were removed by dialysis, and the nuclei and soluble fractions were mixed in various combinations and then reincubated for 1h. After reisolation the fractions were analysed for 3H and ^{14}C . Entry of radioactivity from labelled supernatant to unlabelled nuclei and loss of radioactivity from labelled nuclei to unlabelled supernatant was measured.

Preliminary experiments with uterus showed that 1mM-ATP doubled nuclear uptake from supernatant, but neither *N*-ethylmaleimide nor dithiothreitol (1mM) affected transfer. At 37°C transfer was slightly greater than at 4°C. In all cases nuclei preferentially incorporated ^{14}C from the supernatant.

In studies on radioactivity transfer between fractions from mature uterus and liver, with $^3H/^{14}C$ -labelled supernatant as donor, uterine supernatant was better than liver supernatant in transferring 3H and ^{14}C to both uterine and liver nuclei. In the uptake by uterine nuclei from uterine supernatant the $^{14}C: ^3H$ ratio was 6:1, whereas with uterine nuclei plus liver supernatant the ratio was 1:1. Uterus supernatant preferentially transferred ^{14}C in all cases. With heterologous fractions uterine supernatant was still better at transferring ^{14}C than 3H , but this did not apply with liver supernatant. Liver nuclei were better than uterine nuclei at accepting ^{14}C from liver supernatant and uterus nuclei were better than liver nuclei at accepting ^{14}C from uterine supernatant, indicating some nuclear specificity with the exception that uterus supernatant was always better than liver. There was no specificity with 3H transfer.

In studies with $^3H/^{14}C$ -labelled nuclei as donor, because of low radioactivity counts these results were inconclusive.

In studies on the effect of 7-day ovariectomy on uterine transfer, no detectable effect on amounts of radioactivity transferred was obtained and the $^{14}C: ^3H$ ratio (6:1) was not affected by ovariectomy.

If the radioactivity measured indicates transfer of protein, preferential incorporation of basic protein is thus specific to uterus compared with liver.

Gurdon, J. B. & Woodland, H. R. (1968). *Biol. Rev.* **43**, 233.

Proteolipid Metabolism in Denervated Muscle

By G. G. LUNT, E. DE ROBERTIS and E. STEFANI. (*Instituto de Anatomía General y Embriología, Facultad de Medicina, Universidad de Buenos Aires, Buenos Aires, Argentina*)

Denervation of a muscle results in an increase in the area of the muscle membrane that is sensitive to acetylcholine (Miledi, 1960). This may be explained by activation of dormant receptors or by synthesis of new receptors in the membrane. Previous work has shown the presence of special proteolipids in nerve-ending membranes of cerebral cortex and electric organs having many of the properties of receptors (De Robertis, Fiszer & Soto, 1967; La Torre, Lunt & De Robertis, 1970). We have now studied the proteolipids of muscle and the effect of denervation on their metabolism.

The left phrenic nerve of adult Wistar rats was cut in the pleural cavity and 10 μCi of [$G-^3H$]leucine (220 mCi/mol; The Radiochemical Centre, Amersham, Bucks., U.K.) were injected intraperitoneally. Groups of five rats were killed at distinct times and a membrane fraction was prepared (Holland & Perry, 1969) from the excised hemidiaphragms. Lipids were extracted (Folch, Lees & Sloane-Stanley, 1957) and a total proteolipid fraction prepared by treatment of the extract with diethyl ether (Soto, Pasquini, Placido & La Torre, 1969). The incorporation of leucine into the proteolipids of denervated muscle membranes increased more rapidly with time than in the corresponding normal muscles. Thus 22 days after denervation the denervated membrane proteolipids had a specific radioactivity of 2360 d.p.m./mg compared with 910 d.p.m./mg for the proteolipids from normal muscles. At 31 days the specific radioactivity of the proteolipids from denervated muscle had decayed to the same value as those from normal muscle.

Other animals similarly denervated were given 10 μCi of [$G-^3H$]leucine intraperitoneally 20 days after denervation and were killed 30h later. Column chromatography of the lipid extracts of the whole muscles on Sephadex LH-20 (Soto *et al.* 1969) gave three main peaks of proteolipid. The specific radioactivity of the proteolipids from denervated muscle was generally higher than in normal muscle and the difference was particularly

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.

De Robertis, E., Fiszer, S. & Soto, E. F. (1967). *Science, N.Y.*, **165**, 716.

Folch, J., Lees, M. & Sloane-Stanley, G. H. (1957). *J. biol. Chem.* **226**, 497.

Holland, D. & Perry, S. V. (1969). *Biochem. J.* **114**, 161.

La Torre, J., Lunt, G. G. & De Robertis, E. (1970). *Proc. natn. Acad. Sci. U.S.A.* **65**, 716.

Miledi, R. (1960). *J. Physiol., Lond.*, **151**, 1.

Soto, E. F., Pasquini, J. M., Placido, R. & La Torre, J. (1969). *J. Chromat.* **41**, 400.

Enzyme Changes Indicative of Fibre Type in the Denervated Rat Hemidiaphragm

By L. V. TURNER and K. L. MANCHESTER. (*Department of Biochemistry, University College London, Gower Street, London W.C.1, U.K.*)

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.

Edgerton, V. R. & Simpson, D. R. (1969). *J. Histochem. Cytochem.* **17**, 828.

Feng, T. P. & Lu, D. X. (1965). *Scientia sin.* **14**, 1772.

Miledi, R. & Slater, C. R. (1968). *J. Cell Sci.* **3**, 49.

Miledi, R. & Slater, C. R. (1969). *Proc. R. Soc. B*, **174**, 253.

Padykula, H. A. & Gauthier, G. F. (1970). *J. Cell Biol.* **46**, 27.

Pette, D. (1966). In *Regulation of Metabolic Processes in Mitochondria*, *Biochim. biophys. Acta Library Vol. no. 7*, p. 28. Ed. by J. M. Tager, S. Papa, E. Quagliariello & E. C. Slater. Amsterdam: Elsevier Publishing Co.