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ISOLATION OF A CYTOPLASMATIC REGULATOR MEDIATING THE ACTION OF HORMONES ON MITOCHRONDRIA

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The activating action of small amounts of liver cytoplasm (the supernatant from the centrifugation of a rat liver homogenate at 30,000g) on the transport of calcium in mitochondria (increase in the calcium capacity and rate of active transport) is regulated by the administration of insulin or epinephrine to rats. The administration of insulin (1 unit/200 g body weight) to rats 45 minutes before their sacrifice almost doubles the activity of the cytoplasm. The administration of epinephrine (1 mg/kg body weight) 15 min before sacrifice causes the opposite effect. This is in harmony with the antagonism in the action of insulin and epinephrine on the liver metabolism.

The cytoplasmatic factor the activity of which is regulated by the level of the hormones was isolated and purified by successive heating at 95°C for 7 min, gel filtration through Sephadex G-25 (column equilibrated and eluted with 0.1 M KCl), chromatography on DEAE-cellulose, and electrophoresis in polyacrylamide gel. According to the results of gel filtration, the molecular weight of the regulator is about 5000 daltons. It was eluted from the DEAE-cellulose column with 0.35 M KCl. Before electrophoresis, the regulator was desalted on a column of Sephadex G-10. The hormone-dependent regulator from the cytoplasm acts on the mitochondria in very low concentrations, of the order of 10^{-8}. In many of its parameters, it differs from the nucleotide factor which we isolated previously from rabbit liver cytoplasm [1].

There are no nucleotides in purified preparations of the hormone-dependent regulator.

An intense band in the IR spectrum with a maximum at 1045 cm^{-1} may apparently be related to the skeletal vibrations of a P–O–C group, as is confirmed by elementary analysis for phosphorus. The presence of organic phosphorus in the molecule explains its high affinity for DEAE-cellulose. The presence of peptide bonds in the preparation was shown by the biuret method, and is also confirmed by the presence of characteristic bands in the IR spectra (amide I band with its maximum at 1670 cm^{-1}, and amide II band with its maximum at 1600 cm^{-1}). A considerable amount of sugar was found in a hydrolyzate of the preparation by the "Dubois" method, which shows the glycoprotein nature of the hormone-dependent regulator.

An investigation of the amino-acid composition of the glycoprotein showed the presence in it of a polypeptide consisting of 42 amino acids: aspartic acid 3; threonine 1; serine 7; glutamic acid 6; proline 4; glycine 6; alanine 3; valine 1; isoleucine 1; leucine 1; tyrosine 1; phenyl alanine 1; histidine 1; lysine 5; arginine 1.

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