Studies on the Dissociation of Tissue Factor (Tissue Thromboplastin) on Treatment with Butan-1-ol

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Studies on the bovine lung and brain tissue factors have shown that thromboplastin activity is due to the intact lipoprotein complex. The lipoprotein complex can be dissociated by treatment with butan-1-ol under mild conditions, resulting in the partial dissociation of the phospholipid moiety, with a subsequent loss in the thromboplastin activity, as revealed by one-stage and two-stage thromboplastin assay procedures. The tissue-factor activity can be regenerated almost quantitatively on incubation of the protein moiety with aqueous phospholipid emulsion. The phospholipid fractions from brain, lung and soya bean can meet the requirement of the lipid moiety. Investigation of the influence of the concentration of phospholipids on the protein moiety has shown that with the increase in the concentration of phospholipids there is a gradual increase in thromboplastin activity to a maximum, followed by a sharp fall. It appears that phospholipids may play a regulatory role in the activation of prothrombin *in vivo*.

Protein Synthesis in *Mycobacterium tuberculosis* H₃₇R_v and the Effect of Streptomycin

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Streptomycin inhibits the incorporation of ¹⁴C-labelled amino acids into proteins by whole cells of *Mycobacterium tuberculosis* H₃₇R_v that are sensitive to streptomycin (more than 80% inhibition), whereas in a single-step streptomycin-resistant mutant the drug has no effect on the incorporation.

An efficient amino acid-incorporating system *in vitro* from *M. tuberculosis* H₃₇R_v has been standardized. Significant expression of the poly(U)-dependent synthesis of polyphenylalanine was observed when S-20 extracts from 12-day-old cells were used. Maximum incorporation of [¹⁴C]phenylalanine into protein was obtained at an Mg²⁺ concentration of 15mM, and a high concentration of poly(U) (100 µg/0.1 ml) was necessary. The system showed dependence on added tRNA. Mycobacterial aminoacyl-tRNA synthetases can charge both homologous tRNA (from *M. tuberculosis* H₃₇R_v) and heterologous tRNA (from *Escherichia coli*) with equal efficiency;

charging is optimal at a tRNA concentration of 100 µg/0.1 ml).

Streptomycin at very low concentrations inhibits the poly(U)-dependent incorporation of [¹⁴C]-phenylalanine into protein, i.e. at as low a concentration as 0.5nM 32% inhibition was observed. The mycobacterial protein-synthesizing system is 100-fold more sensitive to streptomycin inhibition than is the *E. coli* system. Thus the inhibition *in vitro* agrees well with the sensitivity of the tubercle bacilli to the antibiotic *in vivo*.

Protein Biosynthesis in Magnesium-Deficient Rats

By SHANTA AMARLAL and C. R. KRISHNA MURTI (*Division of Biochemistry, Central Drug Research Institute, Lucknow, India*)

Symptoms such as diminished growth, neuromuscular and central-nervous-system changes and electrocardiographic changes have been reported in magnesium deficiency. Energy production was also impaired in terms of lowered P/O ratios in the tissues of experimental animals in which dietary magnesium deficiency had been produced. The object of the present work was to elucidate the possible role of magnesium in protein biosynthesis *in vivo*.

Magnesium deficiency in weanling albino rats was produced by feeding the animals on a low-magnesium (2-4mg/100g) synthetic diet and quadruple-glass-distilled water.

One month after the onset of deficiency the animals were injected with [1-¹⁴C]valine and after 2h the extent of incorporation into the proteins of homogenates and mitochondria from heart, brain, kidney, muscle and liver was determined in magnesium-deficient and -supplemented rats.

In homogenate protein synthesis was decreased to 40-60% of that of controls after 1 month of deficiency production. Mitochondrial protein synthesis was decreased to 60-80% of the control value. The adverse effect on protein synthesis was dependent on the extent of magnesium deficiency: the longer the animals were on the magnesium-deficient diet the less was the extent of incorporation of precursor amino acid into proteins.

Supplementation of the diet with Mg²⁺ for 3 weeks partially reversed the inhibition of protein synthesis.