for 22h with insulin in medium 199 the activities of glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase increase as they do in mammary tissue of the living mouse at parturition. From the effects of inhibitors of RNA, DNA and protein synthesis we have formed the hypothesis to explain these increases in enzyme activity, that in organ culture, and in the living mouse at parturition, the rate of uptake of glucose by the mammary tissue is stimulated. This results in an increased concentration of a metabolic product of glucose within the tissue, and this increase in concentration stimulates the synthesis of mRNA for the two enzymes. About 10h later these messengers are translated into protein and so increase the rate of enzyme synthesis.

Our present results lend further support to this hypothesis. They are based on the finding that the complex medium 199 can be replaced by a medium containing only insulin, glucose, amino acids and inorganic salts. Withdrawal and replacement of the components of this medium has enabled us to distinguish between substrates that do and do not induce the synthesis of the RNA that is required for the increases in enzyme activity. Synthesis of this RNA is initiated by the addition of glucose to the medium and lasts for 4h. Glucose may be replaced by the closely related metabolites mannose and fructose but not by ribose, glycerol or pyruvate, although these compounds will supply the tissue with energy for the 18h of culture that remains after essential RNA synthesis is complete. Although amino acids are required they may be added after RNA synthesis is inhibited at 4h with actinomycin D. Insulin is not required if the glucose or fructose concentration is raised, and at normal glucose concentrations it can be replaced by 1mM-cysteine (see Lavis & Williams, 1970).

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Biosynthesis *in vitro* of Tryptophanase with Polyribosomes from *Escherichia coli*

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The only protein of *Escherichia coli* whose biosynthesis *in vitro* has been well characterized to date is β -galactosidase, for which a DNA-dependent system is available (Zubay & Chambers, 1969). We have studied the biosynthesis of tryptophanase and have established that free polyribosomes will make the enzyme *in vitro*. Tryptophanase is inducible by tryptophan and is repressible by catabolites.

Polyribosomes were isolated by the method of Parish (1969) from induced and repressed cultures of *E. coli* M.R.E. 600 and from *E. coli* 54.23, a tryptophanase—strain derived from strain M.R.E. 600. The polyribosomes were incubated under conditions of protein biosynthesis. Supernatant was made from either repressed strain M.R.E. 600 or strain 54.23. Radioactive leucine was used as label. A small, but statistically significant, increase in the amount of tryptophanase associated with induced strain M.R.E. 600 polyribosomes was found after protein synthesis. The incorporation of radioactive leucine into tryptophanase was measured by stopping protein synthesis, adding unlabelled tryptophanase and purifying the preparation for tryptophanase by using a modification of the method of Burns & De Moss (1962). The final step in purification is DEAE-cellulose chromatography. A peak of radioactivity, coincident with the peak of tryptophanase activity, was found when induced strain M.R.E. 600 polyribosomes were used. The incorporation was shown to be energy-dependent by omitting ATP and GTP from the reaction mixture, in which case there was a decrease in the amount of synthesis. Negative controls were obtained for zero-time incubations and with polyribosomes from repressed strain M.R.E. 600 or strain 54.23.

The labelled tryptophanase eluted from the DEAE-cellulose was analysed by polyacrylamide-gel electrophoresis and sucrose-density gradient centrifugation and was shown to be homogeneous. Samples of labelled tryptophanase from 5min and 40min incubations were digested with carboxypeptidase by the technique of Davies (1969). Comparison of the initial rates of release of labelled leucine established that after the short-time incubation the radioactivity was largely near the C-terminus of the peptide chains and that after prolonged (40min) incubation longer stretches of the chains are labelled.

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