existence of one or more exchangeable cytoplasmic proteins that can firmly associate with pre-existing 50S ribosomes, as concluded by Warner (1966). In similar experiments there is no evidence for the existence of firmly bound but exchangeable 30S ribosomal proteins.

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Effects of Amino Acid Starvation on Ribosome Formation in HeLa Cells

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Balanced ribosome production requires co-ordinated synthesis and assembly of a large number of macromolecular components. Amino acid-starvation experiments should assist in identifying some of the interactions involved in this process in animal as well as in bacterial cells.

Recognized effects of amino acid starvation on ribosome formation in HeLa cells are as follows. (i) Methionine starvation results in complete arrest of formation of mature ribosomes, with deficient methylation of ribosomal precursor RNA (Vaughan, Soeiro, Warner & Darnell, 1967). Synthesis of ribosomal proteins continues at an appreciable rate during methionine starvation through protein turnover (Maden & Vaughan, 1968). (ii) Starvation for other essential amino acids, such as valine, results in slowdown of synthesis of 45S ribosomal precursor RNA and of its conversion into 32S RNA, but newly formed ribosomes continue to appear in the cytoplasm at an appreciable rate and to associate with polyribosomes (Maden, Vaughan, Warner & Darnell, 1969). (iii) Although both types of ribosomal subunit continue to be produced during valine starvation, production becomes imbalanced, fewer large than small subunits appearing in the cytoplasm. Lysine starvation exerts an opposite effect, fewer small than large ribosomal subunits appearing in the cytoplasm (Maden, 1969). The missing particles in each case cannot be found in the nucleus and are presumably selectively degraded. Each of these effects is reversible after several hours of amino acid starvation on restoration of the missing amino acid to the medium.

In the present work the analysis of imbalanced subunit production during amino acid starvation was extended. Starvation for any one of ten essential amino acids (arginine, histidine, isoleucine,

leucine, lysine, phenylalanine, threonine, tryptophan, tyrosine or valine) resulted in considerable slowdown of ribosome production. In most cases there was no major disturbance in the relative rates of formation of the two types of ribosomal subunit. Effects of leucine starvation resembled those of lysine starvation, but imbalance was less marked. The characteristic lysine- and valine-starvation effects were confirmed both in suspension and in monolayer HeLa-cell lines.

A possible explanation for the observed imbalance in ribosomal-subunit maturation during starvation for particular amino acids might be a disturbance in relative rates of synthesis of individual ribosomal proteins under these conditions. Results of experiments designed to test this possibility, by using polyacrylamide-gel electrophoresis of radioactively labelled ribosome proteins, will be presented.

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Methylation of Newly Synthesized Ribonucleic Acid by Isolated Rat Liver Nuclei: a Means of Characterizing Ribonucleic Acid Synthesized *in vitro*

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Ribosomal precursor RNA is the predominant species of RNA synthesized by nuclei *in vitro* in the presence of Mg^{2+} and *in vivo*. Ribosomal precursor RNA is methylated during synthesis in the cell and contains approx. 30 uridylylate residues/methyl group (Wagner, Penman & Ingram, 1967; Willems, Wagner, Laing & Penman, 1968). In this report an attempt is made to calculate the proportion of ribosomal precursor RNA synthesized by nuclei *in vitro* by comparing the incorporation of radioactivity into RNA from $[4-^{14}C]$ UTP and *S*-adenosyl-L- $[Me-^{14}C]$ methionine.

Radioactive RNA could be extracted with phenol from nuclei that had been incubated with labelled *S*-adenosyl-L-methionine and all four ribonucleoside triphosphates. The incorporation of radioactivity into RNA from UTP and *S*-adenosyl-L-methionine was measured in hot-trichloroacetic acid extracts of nuclei that after incubation had been washed with 0.4M-perchloric acid at 0°C and with lipid solvents