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

at 37°C. More than 95% of the acid-precipitable radioactivity incorporated from UTP and approx. 15% of that incorporated from *S*-adenosyl-L-methionine was present in the hot-trichloroacetic acid-soluble fraction. When nuclei (1mg of DNA) were incubated for 5min at 37°C with labelled *S*-adenosyl-L-methionine and all four nucleoside triphosphates the hot-trichloroacetic acid-soluble fraction contained 53pmol of methyl group, but only 36pmol of methyl group when the nucleoside triphosphates were omitted or when actinomycin D was added to the incubation. With all four nucleoside triphosphates present 900pmol of UMP was incorporated in 5min. If the nucleoside triphosphate-dependent methylation (17pmol of methyl group) represented incorporation into newly synthesized RNA, then 17×30 pmol, or 57% of the UMP incorporation, should represent synthesis of ribosomal precursor RNA.

Starvation leads to an inhibition of ribosome synthesis in rat liver (Hirsch & Hiatt, 1966). When the total methylase activity of the nuclei was measured in the presence of *S*-adenosyl-L-[Me-¹⁴C]-methionine and added yeast RNA, no change was found after 24h starvation. However, the nucleoside triphosphate-dependent methylation by these nuclei was decreased by 47% to 9pmol of methyl group, and it was calculated that of the total UMP incorporation (610pmol) only 45% represented the synthesis of ribosomal precursor RNA.

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Control of Histone Modification within Chromatin by Adenine Nucleotides and Steroid Hormones

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Chromatin from Krebs 2 ascites-tumour cells can catalyse the transfer of methyl groups from *S*-adenosyl-L-methionine to its histone and DNA. Certain lysine residues of the histone are modified resulting in the formation of *N*^ε-mono-, -di-, and -trimethyl-L-lysine residues, and kinetics suggest that DNA methylation actually occurs as a result of transfer of methyl groups from these residues (Burdon & Garven, 1969; Burdon, 1971). The histone-methylating enzyme(s) can be solubilized from the intact chromatin and have been shown to

have a unique requirement for histone (Burdon & Garven, 1969, 1971). Studies with the soluble preparation, as well as with the intact chromatin, now reveal AMP, even at low concentrations (50nM), to be a specific stimulator of this modification reaction.

Examination of other histone-modifying enzymes, both in the intact chromatin of Krebs 2 cells and in soluble preparations from chromatin, have indicated that phosphorylation of histone by a histone kinase-like enzyme (Langan, 1968) is inhibited specifically by low concentrations of AMP. Cyclic AMP (adenosine cyclic 3':5'-monophosphate), contrary to expectation, proved to have no convincing stimulatory effect. Nevertheless cyclic AMP stimulated the histone kinase in soluble preparations obtained by high-speed centrifugation of homogenates of Krebs 2 cells, as has been found to be the case for soluble preparations from acetone-dried powders of rat liver (Langan, 1968). Although histone acetylase activity (Galliwitz, 1970) was readily detectable in both the chromatin of Krebs 2 cells and in the soluble preparation from it, the activities were not influenced by either cyclic AMP or AMP.

Attempts were made to determine whether chromatin from Krebs 2 cells itself had any special potential to regulate *in situ* the concentrations of AMP (or cyclic AMP) and hence the methylation and phosphorylation of histone moieties. These revealed the presence of both adenylate cyclase and cyclic AMP phosphodiesterase activities. Moreover, of these chromatin enzymes the latter was found to be inhibited by low concentrations of hormones such as testosterone, oestradiol-17 β and progesterone.

Preliminary studies, in which intact chromatin is either 'methylated' or 'phosphorylated' as a result of prior incubation with *S*-adenosyl-L-methionine or ATP, have indicated that these respective treatments can influence, either negatively or positively, the amount of RNA made subsequently by the endogenous RNA polymerase of that chromatin.

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