their sedimentation coefficient, the size of the constituent rRNA species and the presence of '5S' RNA, and are not of the 'bacterial' type.

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The Distinction between Various Functional States of Hepatic Ribosomes, and Different Distributions of Ribosomes between these States in Fed and in Starved Mice

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Active ribosomes are found as 'polyribosomes' or as 'native subunits'. A third group of ribosomes, the unprogrammed 'single ribosomes', usually co-sediment with monoribosomes. In appropriate ionic conditions, however, single ribosomes are split into derived subunits that apparently co-sediment with native subunits. Since single ribosomes could be artifacts produced by association of subunits, we have investigated possible differences between the behaviour of derived and native subunits.

Single ribosomes obtained from mouse liver were split into derived subunits by lowering the Mg^{2+} concentration in the buffer from 5 to 1 mM and increasing the K^+ concentration from 25 to 150 mM, and a preparation of native subunits was subjected to similar changes. About 40% of derived subunits reassociated when the buffer was adjusted to its original composition, although this did not result in any obvious association of native subunits. Clear differences between the two types of particle were also evident in their sedimentation coefficients. Although both native and derived large subunits sedimented at 61 S, the native and derived small subunits appeared to sediment at 50 S and 40 S respectively.

To investigate mechanisms that might control the distribution of ribosomes between their different functional states, hepatic polyribosomes of mice that had been starved for 18 h were analysed. A double-labelling technique (with [3H], [^{14}C] orotate) was used for comparing the ribosomes of starved and of fed mice, so that artifacts were minimized. Zonal centrifugation showed that starvation for 18 h produces a specific increase in the monomer fraction (i.e. single ribosomes and monoribosomes) without noticeably affecting polyribosomes or native subunits. When the isolated monomer fraction was treated in a way that dissociates single ribosomes into subunits but does not affect monoribosomes (Lawford, 1969), the proportion of ribosomes in the monomer fraction that dissociated was shown to be increased by starva-

tion. These results suggest that the increased monomer fraction produced by starvation is the result of an accumulation of unprogrammed single ribosomes.

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The Effects of Trimethoprim on Ribonucleic Acid Synthesis in *Escherichia coli*

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Trimethoprim [2,4-diamino-5-(3,4,5-trimethoxybenzyl)pyrimidine] inhibits the bacterial enzyme dihydrofolate reductase (Burchall & Hitchings, 1966), leading to a decrease in the intracellular tetrahydrofolate pool. Thus the lack of tetrahydrofolate prevents the effective production of *N*-formylmethionyl-tRNA in protein synthesis (Eisenstadt & Lengyel, 1966), the formation of the purine and pyrimidine moieties of nucleic acids (Magasanik, 1962) and the 5'-methyl group of dTMP (Bertino & Stacey, 1966). I have studied the effect of trimethoprim on RNA synthesis in RC mutants of *Escherichia coli*, using cultures supplemented with exogenous nucleotide bases.

As shown by Shih *et al.* (1966), the state of the RC gene determined whether or not RNA accumulation could continue after an inhibition of protein synthesis was brought about by trimethoprim. In cultures of RC^{str} strains RNA accumulation was much diminished 3 min after the addition of trimethoprim, whereas it continued normally in RC^{rel} strains for 30–60 min afterwards. In both RC^{str} and RC^{rel} strains the rate of polymerization of nascent RNA chains was unaffected by trimethoprim. Any blockade of RNA synthesis is thus located at the process of chain initiation. In the absence of base supplementation, chain polymerization of RNA was decreased about tenfold, and this resulted in a crowding of DNA-dependent RNA polymerases on the cistrons. The inhibitory effect on RNA polymerase binding of a blockade of RNA chain initiation can therefore be ameliorated by a rate-limiting step imposed at the level of polymerization.

Most of the RNA produced during trimethoprim inhibition of RC^{str} strains was readily hybridized with DNA at low DNA/RNA ratios (w/w), i.e. it behaved like mRNA. On the other hand much of the RNA produced in RC^{rel} strains was stable RNA (rRNA + tRNA). Experiments in which the technique of 'coincident hybridization' (Midgley & Gray, 1971) was used showed that in RC^{str} strains the amount of mRNA declined quickly after the addition of trimethoprim, but later rose slowly to values almost identical with those found during normal growth.

In the RC^{el} mutant the amounts of mRNA increased in the presence of trimethoprim.

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The Formation of MS Nucleotides in *Escherichia coli* Inhibited by Trimethoprim

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The unusual nucleotide MS I, guanosine tetraphosphate (Travers *et al.*, 1970), accumulates in RC^{tr} mutants of bacteria when required amino acids are removed (Cashel & Gallant, 1969), when levallophan is added (Boquet *et al.*, 1971) and when a mutant containing a temperature-sensitive aminoacyl-tRNA synthetase is incubated at a non-permissive temperature (Ehrlich *et al.*, 1971). It also accumulates in both RC^{tr} and RC^{el} strains when the bacteria are subjected to a 'stepdown' or diauxic lag in the growth medium (Harshman & Yamazaki, 1971; Winslow, 1971).

I have studied the formation of MS I nucleotide in RC^{tr} and RC^{el} strains of *Escherichia coli* inhibited with trimethoprim. In the presence of added exogenous purines and pyrimidines, RC^{tr} strains of *E. coli* accumulated MS I nucleotide to a concentration similar to that found in organisms deprived of required amino acids. In cultures not supplemented with purines or pyrimidines, the rate of accumulation of MS I nucleotide was lower, and reached lower values than when purines were supplied exogenously. In RC^{el} strains, MS I nucleotide concentrations did not increase, even when RNA accumulation was inhibited by purine deprivation.

If RC^{tr} strains were inhibited with both trimethoprim and rifampicin, MS I nucleotide concentrations rose rapidly and did not fall detectably over a further period of 20 min. In this, the cultures behaved like the temperature-sensitive valyl-tRNA synthetase RC^{tr} mutants at non-permissive temperatures (Ehrlich *et al.*, 1971), and not like amino acid-starved RC^{tr} strains (Wong & Nazar, 1970). Purine deprivation of RC^{tr} cultures did not affect the stability of the MS I fraction. The role of MS I nucleotide in the control of RNA synthesis was discussed.

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Effect of Pseudorabies Virus on the Metabolism of Low-Molecular-Weight Ribonucleic Acid in BHK-C13 Cells

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We have shown that shortly after infection of exponentially growing BHK-C13 cells with pseudorabies virus there is a gradual decrease in total RNA synthesis, and that this decline is mainly due to a large decrease in rRNA synthesis; similar results have been reported by Rakusanova *et al.* (1971). However, we have found that the synthesis of low-molecular-weight RNA species is less inhibited by pseudorabies infection than is rRNA synthesis.

Excellent separation of 4S and 5S RNA from the larger RNA species, and from each other, was obtained by electrophoresis on 10% polyacrylamide gels (Loening, 1967).

[5-³H]Uridine was used to label RNA in experiments, and RNA from cells labelled to equilibrium with [¹⁴C]uridine was used as internal control in all gels. After electrophoresis, the gels were sliced and solubilized (by a modification of the method of Tishler & Epstein, 1968) and their radioactivities counted by liquid-scintillation spectrometry.

When uninfected cells were labelled for 10 min the resulting cytoplasmic RNA showed a small 5S peak and a very small 4S peak. However, the bulk of radioactive material appeared between the 5S and 4S positions; this material appears to be precursor to tRNA (pre-tRNA) (Burdon, 1967; Bernhardt & Darnell, 1969; Shepherd, 1969). We have consistently found two peaks moving in the pre-tRNA position, and have designated the electrophoretic peak closer to 5S RNA as pre-tRNA I and the other as pre-tRNA II.

A longer label of uninfected cells (30 min or more) produced RNA that corresponded almost exactly to that of the internal 4S marker. However, after infection with pseudorabies virus the bulk of the material labelled for 30 min or longer remained in the pre-tRNA position on electrophoresis. During