a 'chase' of this labelled material, in the presence of actinomycin D and excess of unlabelled uridine, almost all of this 'pre-tRNA' moved to the 4S position. Incubation of this 'pre-tRNA' *in vitro* with extracts of infected or uninfected cells also caused this material to move to the 4S position. As with the RNA from uninfected cells, we obtained two peaks in the pre-tRNA position, and movement of the RNA appeared to be from peak I to peak II to 4S RNA. Incubation of infected cells with radioactive label for periods ranging from 10 to 75 min indicated that label first appeared in the pre-tRNA I position.

Unlike the larger rRNA species, 5S RNA production is apparently not appreciably inhibited by pseudorabies infection. At least part of this 5S RNA produced after infection appears not to be associated with ribosomes.

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Specificity of Transfer Ribonucleic Acid Methylases from Normal Mouse Colon and 1,2-Dimethylhydrazine-Induced Colon Tumours

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Numerous reports of enhanced activity of tRNA methylases in neoplastic tissues have been published (summarized by Borek, 1971). Such increases could be due either to the stimulation of methylases present in the normal tissue or to the presence of additional methylases recognizing sites in the tRNA added as substrate not recognized by the enzymes present in normal tissue. This question can best be answered by direct studies of the nucleotide sequences containing the methylated bases formed *in vitro*, but the pattern of methylated products produced by crude mammalian tissue extracts acting on unfractionated tRNA is too complex for such analysis. The methylation of a number of highly purified tRNA species by extracts prepared from normal mouse colon and colon tumours has therefore been studied. Tumours were induced by 1,2-dimethylhydrazine, and extracts containing tRNA methylase activity were prepared and assayed as previously described (Pegg, 1971; Pegg & Hawks, 1971).

The following tRNA preparations from *Escherichia*

coli were found to be substrates for the formation of the methylated bases shown (in parentheses): glutamic acid tRNA (5-methylcytosine and 1-methyladenine), serine tRNA₃ (5-methylcytosine, 1-methyladenine, *N*²-methylguanine and *N*²*N*²-dimethylguanine) and formylmethionine tRNA (1-methyladenine, *N*²-methylguanine, 1-methylguanine and 5-methylcytosine). Purified yeast tRNA species (Gillam *et al.*, 1968) were also tested. Phenylalanine tRNA and arginine tRNA were not methylated, but aspartic acid tRNA (*N*²-methylguanine), alanine tRNA+threonine tRNA₁ (5-methylcytosine), threonine tRNA₂ (*N*²-methylguanine and 1-methyladenine), threonine tRNA₃ (*N*²-methylguanine and 1-methyladenine) and methionine tRNA₁ (5-methylcytosine) were substrates. Methylation was activated by Mg²⁺, NH₄⁺, putrescine or spermidine, and the cation giving maximal activity depended on the tRNA present as substrate. In general, NH₄⁺ or polyamine was required for the formation of methylated adenine and cytosine whereas methylated guanines were produced when only Mg²⁺ was present. Enzymes prepared from tumours produced products with each of the substrates tested similar to those found with extracts from normal colon, although the rate and extent of methylation were greater with the tumour extracts.

The sequences containing the methylated bases produced with formylmethionine tRNA and aspartic acid tRNA were determined by standard methods of enzymic degradation and fractionation of the oligonucleotides (Tener, 1967; Sanger & Brownlee, 1967). *N*²-Methylguanosine was present at residue 26 of aspartic acid tRNA in the sequence G-A-A-U-2MeG-G-G-C-. In formylmethionine tRNA *N*²-methylguanosine was present at residue 27 in the sequence -C-U-C-2MeG-U-C-, 1-methyladenosine was present at residue 59 in the sequence -T-ψ-C-A-1Me-A-U-, 1-methylguanosine was present at residue 9 in the sequence -G-U-1MeG-G-A- and 5-methylcytidine was tentatively identified as present in position 49 in the sequence -G-7MeG-U-5MeC-G-. All of these sites in the tRNA structure are known to be occupied by methylated bases in some known tRNA sequences (Staehelin, 1971), and similar sites were methylated by both the tumour and the normal colon extracts.

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