2h and 4h the labelling of rRNA and its precursors is greatly increased.

By using 7.5% gels and isolating the 1.1A 2 and 4h after oestradiol treatment, large increases are observed in labelling of an RNA peak associated with the 4S RNA.

Actinomycin D affects synthesis of the various RNA species differentially. rRNA species are most affected and a dose of 30 µg/rat completely eliminates increased labelling of these fractions in response to oestradiol. The RNA that remains at the origin and tRNA of the gel are least affected and small extents of incorporation are observed even at actinomycin D doses of 125 µg/rat.

Cycloheximide at a dose causing 98% inhibition of protein synthesis, given before or simultaneously with oestradiol, seriously decreases the incorporation of precursors into all species of RNA. When administered 1h after the hormone its greatest effect is on the maturation of rRNA.

J. T. K. thanks the Science Research Council and Pfizer Ltd. for an Industrial Studentship.

Joel, P. B. & Haggerman, D. D. (1969). *Biochim. biophys. Acta*, **195**, 328.

Loening, U. E. (1969). *Biochem. J.* **113**, 131.

Weinberg, R. A., Loening, U., Willems, M. & Penman, S. (1967). *Proc. natn. Acad. Sci. U.S.A.* **58**, 1088.

Alkylated Components in Rat Liver Ribonucleic Acid: a Possible Index of True Biological Turnover

By A. W. CRAIG and P. J. O'CONNOR. (*Paterson Laboratories, Christie Hospital and Holt Radium Institute, Manchester M20 9BX, U.K.*)

In comparative studies of alkylating agents of different carcinogenic potency methyl methanesulphonate was injected into rats at a dose of 50mg/kg. Earlier observations had indicated that doses of this order did not produce toxic effects in rats other than a transient effect on late spermatogenesis (Jackson, Fox & Craig, 1961). Recently the time-course of methylation of rat liver RNA *in vivo* was followed for the first 24h after a similar dose (Whittle, 1969). Methylation was shown to be maximal by 2h and apparently remained constant until 24h, in contrast with the reported loss of 40% of methylated components by 24h when the carcinogen dimethylnitrosamine was used (Craddock & Magee, 1963). The study with methyl methanesulphonate was extended over a 14-day period, during which the apparent half-life for the biological turnover of rRNA was determined after a single injection of [5-³H]orotic acid and compared with the rate of disappearance of 7-alkylguanine

from RNA. To avoid any loss of RNA (Whittle & Potter, 1968) food intake was not restricted.

Two procedures were employed for the purification of RNA: the method of Ralph & Bellamy (1964), which preferentially extracts rRNA and tRNA, and Kirby's (1965) procedure, which extracts rRNA and some rapidly labelled RNA. The apparent half-life of rRNA determined by using [³H]orotic acid was approx. 5.0 days in both the control series and the methyl methanesulphonate-treated rats, a value in agreement with other reports (Blobel & Potter, 1968; Avdalović, 1970), indicating that the treatment had no detectable effect on the rate of breakdown of rRNA. The loss of 7-methylguanine was followed by analysis of acid hydrolysates of rat liver RNA preparations by using Dowex 50 (H⁺ form) column chromatography. The maximum extent of alkylation was 0.040–0.046% guanine and the loss of 7-methylguanine followed first-order kinetics with a half-life of approx. 3.5 days. The half-life of rRNA determined by orotic acid labelling must be an overestimate, owing to the reincorporation of labelled RNA degradation products. Since it is known that the glycosidic link of 7-methylguanosine is chemically stable at neutral pH (Kriek & Emmelot, 1964) the evidence strongly suggests that loss of 7-methylguanine under these conditions may give a better index of the true turnover of rat liver RNA.

Avdalović, N. (1970). *Biochem. J.* **119**, 331.

Blobel, G. & Potter, V. R. (1968). *Biochim. biophys. Acta*, **166**, 48.

Craddock, V. M. & Magee, P. N. (1963). *Biochem. J.* **89**, 32.

Jackson, H., Fox, B. W. & Craig, A. W. (1961). *J. Reprod. Fert.* **2**, 447.

Kirby, K. S. (1965). *Biochem. J.* **96**, 266.

Kriek, E. & Emmelot, P. (1964). *Biochim. biophys. Acta*, **91**, 59.

Ralph, R. K. & Bellamy, A. R. (1964). *Biochim. biophys. Acta*, **87**, 9.

Whittle, E. D. (1969). *Biochim. biophys. Acta*, **195**, 381.

Whittle, E. D. & Potter, V. R. (1968). *J. Nutr.* **95**, 238.

Studies on the Methylated Regions of HeLa-Cell Ribosomal and Nucleolar Ribonucleic Acids

By M. SALIM, R. WILLIAMSON and B. E. H. MADEN. (*Department of Biochemistry, University of Glasgow, Glasgow W.2, U.K., and Beatson Institute for Cancer Research, Hill Street, Glasgow C.3, U.K.*)

The methylation patterns of HeLa-cell rRNA and nucleolar RNA have been examined by the 'finger-printing' technique of Sanger, Brownlee & Barrell (1965) with RNA preparations methyl-labelled

in vivo to high specific radioactivity with [*Me*-¹⁴C]-methionine (Salim, Williamson & Maden, 1970). Results of this analysis were as follows. (i) Ribonuclease T₁ digests of 18S and 28S rRNA yielded characteristic 'methyl' 'fingerprints'. The 18S RNA yielded 15 well-resolved spots, and 28S RNA about 28 spots. Several spots are unique to 28S RNA, and four spots unique to 18S RNA. In addition, both 'fingerprints' showed some incompletely resolved material near the origin of the second dimension. (ii) The 'fingerprint' of 32S nucleolar RNA was similar to that of 28S rRNA. (iii) The 'fingerprint' of 45S nucleolar RNA resembled the 'fingerprints' of 28S and 18S RNA superimposed.

Further work has been aimed at structural characterization of some of the methylated oligonucleotides liberated from ribonuclease T₁ digestion of rRNA and nucleolar RNA. Alkaline hydrolysis of [¹⁴C]methyl-labelled material yields methyl-labelled mononucleotides or alkali-resistant (2'-*O*-methyl) dinucleotides. In most, but not all, cases examined a single spot yields a single methyl-labelled derivative on alkaline hydrolysis. When alkaline hydrolysis products from corresponding spots in 18S, 28S and 45S RNA are subjected to high-voltage electrophoresis it has been found, in all cases so far examined, that the material liberated from 18S or 28S RNA co-migrates with material from the corresponding spots in 45S RNA. These observations provide further chemical evidence on the nature of the relationship between rRNA and 45S nucleolar RNA in HeLa cells, previously deduced from several other lines of evidence (for reviews see Darnell, 1968; Maden, 1971).

These results from [¹⁴C]methyl-labelled material, together with analyses of rRNA double-labelled with [³²P]phosphate and [*Me*-³H]methionine, have permitted tentative sequence assignments for some of the smaller methylated oligonucleotides in HeLa-cell rRNA.

Darnell, J. E. (1968). *Bact. Rev.* **32**, 262.

Maden, B. E. H. (1971). *Prog. Biophys. molec. Biol.* **22**, 127.

Salim, M., Williamson, R. & Maden, B. E. H. (1970). *FEBS Lett.* **12**, 109.

Sanger, F., Brownlee, G. G. & Barrell, B. G. (1965). *J. molec. Biol.* **13**, 373.

'Fingerprinting' Studies on ³⁵S-Labelled Mammalian-Cell Ribosomal Proteins

By J. SHEPHERD and B. E. H. MADEN. (*Department of Biochemistry, University of Glasgow, Glasgow W.2, U.K.*)

Studies on the structure and assembly of animal-cell ribosomes have frequently relied on the use of one-dimensional protein fractionation techniques such as polyacrylamide-gel electrophoresis. In the present work a ³⁵S-labelled peptide 'mapping' technique previously applied to bacterial ribosomes (Traut, Moore, Delius, Noller & Tissières, 1967) has been adapted for use with mammalian-cell ribosomes. HeLa or other cultured cells were labelled to high specific radioactivity with [³⁵S]methionine or [³⁵S]cystine, ribosomal proteins were extracted and digested with trypsin, and the products were separated by two-dimensional electrophoresis and chromatography. Under standard experimental conditions reproducible 'fingerprints' were obtained. Results that relate primarily to ribosome structure are as follows. (i) HeLa-cell large (50S) and small (30S) subunits, labelled with [³⁵S]methionine and separated in EDTA-containing buffer, yield dissimilar and characteristic 'fingerprints'. [³⁵S]-Cystine-labelled ribosomal subunits also yield 'fingerprints' that are dissimilar both from each other and from the 'fingerprints' of [³⁵S]methionine-labelled subunits. (ii) [³⁵S]Methionine-labelled ribosomal subunits separated by dissociation at high salt concentration yield substantially similar 'fingerprints' to those yielded by subunits separated in EDTA. Together these findings imply the existence of major differences in the primary structures of HeLa-cell 50S and 30S ribosomal proteins, a result not fully evident from previous analyses using polyacrylamide-gel electrophoresis. (iii) Ribosomal subunits from methionine-labelled BHK (baby-hamster kidney) and HeLa (human) cells give very similar 'fingerprints'.

[³⁵S]Methionine-labelled peptide 'mapping' has also yielded information on ribosome assembly in HeLa cells. (i) The methionine-containing peptide 'map' of 55S nucleolar ribosomal precursor particles is similar to that of 50S ribosomes. The 55S nucleolar particles have previously been identified as direct precursors to cytoplasmic 50S ribosomes by various criteria (Warner & Soeiro, 1967). The methionine-containing peptide content of the kinetically more immature 80S nucleolar particle is presently being investigated. (ii) When formation of new ribosomes is inhibited by actinomycin D, a small number of distinctive methionine-containing peptides are found in 'fingerprints' of EDTA-separated 50S ribosomal subunits. This implies the