Fractionation of the Lysine-Rich Histones

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Histone F1 has been extracted from rat thymus nuclei with 5% (w/v) perchloric acid (Johns, 1964, method 1) and fractionated by preparative polyacrylamide-gel electrophoresis (Buckingham & Stocken, 1965). Four subfractions were obtained. These were very similar in amino acid composition, although some differences, notably in threonine content, were significant. Separation of the peptides obtained by digestion with trypsin, although incomplete, showed that the subfractions shared a large common complement of peptides and suggested that they possessed closely related primary structures.

Histone F1 has been shown to contain phosphorus in the form of phosphoserine (Ord & Stocken, 1966a,b), and evidence has been found of a relation between the phosphorus content of this histone and the rate of DNA synthesis in the tissue of origin. When derived from rat thymus nuclei labelled *in vitro* with ^{32}P , the histone F1 subfractions showed marked differences in radioactivity. The subfraction with lowest electrophoretic mobility, F1(3), was unlabelled, and the next slowest-moving subfraction, F1(2), contained both labelled and unlabelled components. The two other subfractions were both labelled. The differences in amino acid composition between the subfractions showed that the extent of phosphorylation was not the sole factor that led to their resolution by polyacrylamide-gel electrophoresis.

A comparison of histone F1 preparations from rat thymus, rat brain (M. Ellis, unpublished work), rat liver and rat ascites hepatoma 223 revealed significant differences. Histone subfraction F1(3) was absent from rat liver and the hepatoma. The subfraction in rat brain corresponding in electrophoretic mobility to histone F1(2) in thymus was present in a significantly greater proportion.

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Lack of Correlation between the Presence of 7-Methylguanine in Deoxyribonucleic Acid and Ribonucleic Acid of Organs and the Localization of Tumours after a Single Carcinogenic dose of *N*-Methyl-*N*-nitrosourethane

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Alkylation of nucleic acids, DNA in particular, has been suggested to be relevant for the initiation of the carcinogenic process by alkylating agents (Brookes & Lawley, 1960, 1964). This hypothesis appeared to receive support from the finding of 7-methylguanine in rat liver nucleic acids after a single dose of the hepatocarcinogen dimethylnitrosamine (Magee & Farber, 1962). Dimethylnitrosamine requires enzymic activation before it can release a methylating entity, and the presence of the appropriate enzyme mainly in rat liver made the interpretation of these results difficult.

N-Methyl-*N*-nitrosourethane, although related to dimethylnitrosamine, is free from this disadvantage. It readily releases its methylating moiety on chemical interaction with cellular free thiol groups, without the need of enzymes (Schoental, 1961). Moreover, although *N*-methyl-*N*-nitrosourethane is a very effective carcinogen, and induces tumours with a single dose in the gastrointestinal tract (and in the lung), it does not induce tumours in the liver, whatever the dosage schedule or route of administration. *N*-Methyl-*N*-nitrosourethane allows one therefore to test whether alkylation of nucleic acids is significant in relation to the development of tumours in the respective organs.

$7\text{-}^{14}\text{C}$ -Methylguanine has been found to be present in the total nucleic acids of rat liver after intragastric administration of $N[^{14}\text{C}]$ -methyl-*N*-nitrosourethane (Schoental, 1967). When a single dose of *N*-methyl-*N*-nitrosourethane is administered intraperitoneally, it readily induces gastrointestinal tumours, but again no liver tumours (Schoental & Bensted, 1968). By using this procedure it became possible for DNA and RNA to be isolated separately by the method of Kirby (1962) from the livers and from the intestines of rats after intraperitoneal injection of a single carcinogenic dose of $N[^{14}\text{C}]$ -methyl-*N*-nitrosourethane (5 mg./100 g. body wt.; about 1×10^6 c.p.m.). Acid hydrolysates of the respective nucleic acids were subjected to column chromatography on Dowex 50; a radioactive peak corresponding to $7\text{-}^{14}\text{C}$ -methylguanine was found in both the DNA and the RNA. Its proportion was larger in the DNA from the liver than in that from the intestines.

The finding, that *N*-methyl-*N*-nitrosourethane, a

carcinogen that does not induce liver tumours, yet methylates guanine at the 7-position in the DNA and in the RNA of rat liver, indicates that this reaction cannot be of significance for the initiation of carcinogenesis.

Similar conclusions were reached by Krüger, Ballweg & Maier-Borst (1968), who used *N*-methyl-*N*-nitrosourea, and by Lijinsky & Ross (1969) and Lijinsky, Tomatis & Wenyon (1969), who used various nitrosamines.

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undersimilar conditions, gave levels of incorporation corresponding to about 1% methyl group content.

The extent of methylation of the viral DNA was calculated by using the incorporation obtained above and taking into account the diluting effect of methionine present in the cells. This dilution factor had been obtained both by direct chemical and by isotopic methods. The calculated extent of methylation was found to represent less than one methylated nucleotide/herpes-simplex-virus particle. This suggests that herpes-simplex virus DNA is not a substrate for methylation *in vivo*, and therefore normally contains no methyl groups other than that of thymine. The possible implications of this finding will be discussed.

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Methylation of Herpes-Simplex-Virus Deoxy-ribonucleic Acid

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Methylated bases have been reported to be present in the DNA of several organisms, e.g. bacteria (Gold, Hurwitz & Anders, 1963; Doskočil, 1965), mammalian cells (Burdon, 1966) and sea-urchin embryos (Grippo, Iaccarino, Parisi & Scarano, 1968). Several DNA-containing bacteriophages also contain methylated bases (Gold, Hausmann, Maitra & Hurwitz, 1966), but bacteriophages T3 and T5 are not methylated *in vivo* (Geffer, Hausmann, Gold & Hurwitz, 1966). Recently it was reported that polyoma-virus DNA was not a substrate for methylation *in vivo* (Kaye & Winocour, 1967), and we now present evidence for the absence of methylation in the DNA of a large animal virus, herpes simplex.

Herpes-simplex virus, strain α of HFEM, was grown in BHK 21/C13 cells in the presence of L-[Me-³H]methionine. Virus was purified and the DNA extracted from it was analysed and further purified by equilibrium centrifugation in CsCl. DNA of buoyant density corresponding to that of herpes-simplex virus was found to contain a very low level of radioactivity, whereas BHK 21 DNA,

Enzymic Methylation of Chromatin Protein: a New Reaction

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A particulate chromatin preparation, isolated from nuclei of Krebs 2 tumour cells by the method of Burdon, Martin & Lal (1967) and suspended in 0.02 M-tris-HCl (pH 7.8)-0.01 M-2-mercaptoethanol (TM buffer), will catalyse the incorporation of only small amounts of radioactivity from *S*-adenosyl-L-[Me-¹⁴C]methionine or *S*-adenosyl-L-[Me-³H]methionine into protein. Surprisingly, however, treatment of the chromatin preparation with pancreatic deoxyribonuclease caused a marked increase in the radioactivity incorporated into protein.

To investigate this phenomenon more closely, the crude chromatin preparation was suspended gently in TM buffer containing NaCl (1.5 M) and the resulting viscous solution dialysed against TM buffer alone. After dialysis, the aggregate of DNA and histone that forms was removed by centrifugation to yield a clear preparation free of histone. Such a soluble preparation of non-histone chromatin protein will catalyse the incorporation of radioactivity from the methyl group of *S*-adenosyl-L-methionine into protein, but only in the presence