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

of added histone. Further investigation with purified histone fractions (a gift from Dr A. J. MacGillivray, Beatson Institute for Cancer Research, Glasgow) indicated that, although the arginine-rich histone fractions were the most effective, the lysine-rich fractions would still suffice. On the other hand the only other basic protein that would fulfil the requirement was protamine. The incorporation reaction has optimum pH about 8, requires the presence of 2-mercaptoethanol and is not affected by large amounts of formate, by aminopterin or by puromycin. Addition to the system of native, denatured or deoxyribonuclease-digested DNA is inhibitory. On the other hand addition of trace amounts of deoxyribonuclease proved stimulatory whereas large amounts produced an inhibition. Although methyl-labelled *S*-adenosyl-L-methionine cannot be replaced as substrate by methyl-labelled L-methionine, some radioactivity is incorporated into protein from *S*-adenosyl-L-[Et-¹⁴C]ethionine.

Examination of the radioactive protein produced showed that, both with the crude chromatin system and the soluble system, not all the label was found in the histone fraction, up to one-third being associated with non-histone protein. Nevertheless acid hydrolysis of the total radioactive protein obtained with both systems yielded only one major labelled component as judged by paper chromatography and electrophoresis. Although this component is basic and would appear to be an L-amino acid possessing free α -amino and carboxylic groups from its reactivity towards L-amino acid oxidase, its chromatographic properties do not resemble those of ϵ -N-methyl-lysine, ϵ -NN-dimethyl-lysine, guanidino-methylated arginine or α -aminoguanidino-dimethylated arginine previously detected as products of protein methylase activity (Paik & Kim, 1967, 1968).

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Changes in Activity of Nuclear Deoxyribonucleic Acid Polymerase in Synchronized Mouse Cells

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Two reports (Littlefield, McGovern & Margeson, 1963; Gold & Helleiner, 1964) have appeared showing changes in the activity of DNA polymerase (DNA nucleotidyltransferase, EC 2.7.7.7) in cul-

tured mouse cells as they pass round the cell cycle. Both reported a slight fall in the activity of the enzyme present in supernatant fractions at the time the cells were in the DNA-synthetic (S) phase. In these estimations heat-denatured DNA was used as primer.

We have shown (Lindsay & Adams, 1968) that a substantial proportion of the DNA polymerase activity of exponentially growing L 929 mouse cells is associated with the nuclear fraction and that the preferred primer for this preparation is native DNA.

The present report is a reinvestigation of the changes in DNA polymerase distribution in synchronized cells, taking these later results into account.

On release from early-stationary-phase L 929 cells show a peak of DNA synthesis at about 18 hr. (Adams, 1969). Over this time the nuclear DNA polymerase preparation, measured with native DNA as primer, shows no more than a slight increase in activity. However, when denatured DNA is used as primer there is a peak of activity at 18 hr. representing a sevenfold increase in specific activity over a 3 hr. preparation. The native DNA/denatured DNA activity ratio shows a change from 4.2 at 3 hr. to 0.7 at 18 hr. The supernatant enzyme always prefers denatured DNA as primer and its activity increases 13-fold between 9 and 24 hr.

If cells are synchronized with aminopterin (Kishimoto & Lieberman, 1965) or excess of thymidine (Bootsma, Budke & Vos, 1964) then in the S phase and the G₂ phase there is little change in activity of the nuclear preparation with native DNA as primer. Over this period the nuclear fraction shows greater activity when denatured DNA is used as primer and there is a small increase in this activity during the S phase. The activity of the supernatant fraction falls rapidly with the onset of DNA synthesis to values 20–60% of those of cells harvested before reversal of inhibition of DNA synthesis. There is a rise in activity when DNA synthesis is complete. This last result is a confirmation of the two earlier reports.

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