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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