Deoxyribonucleic Acid Synthesis in Mammalian Nuclei

INCORPORATION OF DEOXYRIBONUCLEOTIDES AND CHAIN-TERMINATING NUCLEOTIDE ANALOGUES

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The properties of a nuclear preparation from rat liver and thymus are described. (1) Nearest-neighbour analysis after incorporation of ^{32}P-labelled nucleotide residues from dATP, dCTP, dGTP, dTTP and arabinofuranosyl analogues of CTP and ATP shows template-dependent DNA synthesis. (2) Where primer termini are limiting, incorporation of arabinofuranosyl analogues of AMP and CMP residues proceeds to a limit indicating that both of these analogues are DNA chain terminators. (3) No large differences have been found between the priming potentialities or the intrinsic DNA polymerase activities of nuclei from resting or regenerating liver and the relationship of this DNA synthesis *in vitro* to DNA replication or repair *in vivo* is briefly discussed.

Though the intracellular distribution of DNA polymerases in animal tissues has been extensively studied (for a review see Keir, 1965), little is known of the factors that control DNA replication and repair in nuclei. When attempting to obtain rat liver and thymus nuclei suitable for studies of control of DNA synthesis, it was found that DNA synthesis *in vitro* was largely determined by the priming capacity of the nucleoprotein template and this was highly variable. However, this could be controlled by controlling a Ca^{2+}-dependent endonuclease activity that was producing primer termini (Burgoyne, Waqar & Atkinson, 1970a,b).

Accounts of the Ca^{2+} stimulation of DNA synthesis in liver (Burgoyne *et al.* 1970a) and thymus nuclei (Burgoyne *et al.* 1970b) have been published. This paper describes further properties of the deoxyribonucleotide-incorporation system of isolated nuclei and gives evidence that arabinosyl nucleotides, metabolites of the powerful growth inhibitors arabinosyl-adenine and arabinosyl-cytosine, are incorporated into nuclear DNA.

EXPERIMENTAL

Substrates and inhibitors. [α-^{32}P]dATP, [α-^{32}P]dCTP, [α-^{32}P]dGTP, [α-^{32}P]dTTP, [α-^{32}P]araATP† and [α-^{32}P]araCTP (1 Ci/mmol at date of synthesis) were

* Deceased 30 May 1970.
† Abbreviations: araCMP, araAMP, araCTP and araATP, arabinofuranosyl analogues of CMP, AMP, CTP and ATP; EGTA, ethanedioxybis(ethylamine)tetra-acetate.

prepared as described by Symons (1969). Unlabelled ATP, dATP, dCTP, dGTP, dTTP and phosphoenolpyruvate were from Sigma Chemical Company (St Louis, Mo., U.S.A.). Pronase was obtained from Calbiochem (Los Angeles, Calif., U.S.A.). Venom phosphodiesterase, micrococcal nuclease, calf spleen phosphodiesterase and deoxyribonuclease I were purchased from Worthington Biochemical Corporation (Freehold, N.J., U.S.A.). Arabinofuranosyl-cytosine was a gift from Dr W. J. Wechter and arabinofuranosyl-adenine was from the Cancer Chemotherapy National Service Centre. DNA was prepared from calf thymus (Kay, Simmons & Dounce, 1952).

Preparation of nuclei. Nuclei were isolated from livers of adult male rats and from thymus glands of young male rats (about 4 months old; 150–200g) by modification (Burgoyne *et al.* 1970a) of the Blobel & Potter (1966) method.

Isolation of nuclei in the presence of Ca^{2+} and Mg^{2+}. The highest priming capacities and rates of synthesis were obtained with nuclei isolated in the presence of bivalent cations. The preparation was as described above, but all solutions contained $1\,mM$-$CaCl_2$–$5\,mM$-$MgCl_2$ and no EDTA or EGTA.

Activation of nuclei and assay of nucleotide incorporation. Procedures were described by Burgoyne *et al.* (1970a). Assay solutions normally contained $20\,\mu M$-[α-^{32}P]-dTTP and $0.4\,mM$-ATP–$0.4\,mM$-dATP–$0.4\,mM$-dCTP–$0.4\,mM$-dGTP–$2\,mM$-phosphoenolpyruvate–$1\,mM$-EDTA–$0.2\,mM$-EGTA–$10\,mM$-$MgCl_2$. Where other labelled nucleotides were used as substrates the three appropriate non-radioactive deoxyribonucleotides were present at $0.4\,mM$. Specific activities and concentrations of nuclei in assays are shown in figure legends.

Isolation of labelled DNA. Nuclear suspensions, after labelling as described above, were shaken with 0.5 vol. of redistilled 78% (v/v) phenol to stop the reaction and the precipitate formed on addition of 5 vol. of ethanol to the

emulsion was collected by centrifuging at 10000g for 10min, then washed three times with 75% ethanol containing 10mM-EDTA (sodium salt), pH7.0. The precipitate was incubated for 2.5h at 37°C in 10mM-EDTA (sodium salt)–pronase (50μg/ml) that had been pre-incubated for 2h at 37°C to inactivate nucleases. Sodium chloride was added to a final concentration of 0.25M and the solution was extracted with an equal volume of 78% phenol; 2.5vol. of ethanol was added to the aqueous layer to precipitate DNA. The precipitate was dissolved in 0.1M-EDTA (sodium salt), pH7.0, and the nucleic acid fraction was transferred into 0.01M-tris chloride–0.1mM-EDTA, pH7.4, by passage through a column of Sephadex G-50. After incubation in 0.3M-KOH at 37°C for 18h to hydrolyse RNA, the DNA was precipitated by adjustment to pH7.0 with dilute acetic acid and addition of 3vol. of ethanol, and the DNA was washed with 75% ethanol.

Hydrolysis and nearest-neighbour analysis. Nearest-neighbour analysis was carried out after hydrolysis with micrococcal nuclease and calf spleen phosphodiesterase (Josse, Kaiser & Kornberg, 1961; Wu & Kaiser, 1968). The identity of incorporated dAMP, dCMP, dGMP, dTMP, araAMP and araCMP was confirmed by electrophoresis in 0.05M-tris citrate, pH3.4, and by chromatography in isobutyric acid–aq. 1M-NH₃ soln.–0.1M-EDTA (sodium salt) (100:60:1.6, by vol.) after hydrolysis with pancreatic deoxyribonuclease and venom phosphodiesterase (Lehman, Bessman, Simms & Kornberg, 1958).

RESULTS

DNA synthesis in liver nuclei when DNA was extensively nicked by exposure to Ca^{2+} during isolation. Liver nuclei incorporated deoxyribonucleotide into endogenous DNA at a rate that depended on the conditions of isolation and pretreatment of the nuclei. Incorporation of $[^{32}P]dAMP$, $[^{32}P]dCMP$, $[^{32}P]dGMP$, $[^{32}P]dTMP$, $[^{32}P]araCMP$ or $[^{32}P]$-araAMP from the corresponding $[\alpha\text{-}^{32}P]$deoxyribonucleoside triphosphates, or $[\alpha\text{-}^{32}P]$arabinoside triphosphates in the presence of the three other non-radioactive deoxyribonucleoside triphosphates, was shown by isolation of the labelled DNA and liberation of 5'-nucleotides with deoxyribonuclease I and venom diesterase. With the exception of $[^{32}P]$-araCMP, 95% of the label was in the nucleotide corresponding to the radioactive triphosphate and with $[^{32}P]araCMP$ more than 86% radioactivity was released as $[^{32}P]araCMP$ when $[\alpha\text{-}^{32}P]araCTP$ was used as the radioactive substrate. Nearest-neighbour analysis (Tables 1–4) showed incorporation adjacent to each of the four possible primer nucleotides.

With nuclei prepared in the presence of Ca^{2+} and Mg^{2+}, when single radioactive triphosphates were incubated in the absence of other added non-radioactive deoxyribonucleoside triphosphates there was still extensive incorporation continuing for at least 80min at about 50% of the rate in the complete system (Fig. 1). Under these conditions there were

Table 1. *Nearest-neighbour analysis*

Nuclei were labelled and nearest-neighbour analysis was carried out as described in the Experimental section.

Isolated 3'-deoxyribonucleotide	Reaction no. 1 [α-32P]dATP			Reaction no. 2 [α-32P]dTTP			Reaction no. 3 [α-32P]dGTP			Reaction no. 4 [α-32P]dCTP		
	Sequence	c.p.m.	Fraction	Sequence	c.p.m.	Fraction	Sequence	c.p.m.	Fraction	Sequence	c.p.m.	Fraction
(a) Complete assay mixture												
3'-dTMP	T-A	2064	0.234	T-T	524	0.142	T-G	7095	0.443	T-C	8588	0.294
3'-dAMP	A-A	1305	0.148	A-T	748	0.203	A-G	5074	0.317	A-C	6722	0.230
3'-dCMP	C-A	3957	0.449	C-T	1482	0.402	C-G	1049	0.065	C-C	6915	0.236
3'-dGMP	G-A	1485	0.169	G-T	934	0.253	G-G	2808	0.175	G-C	7032	0.240
Total		8811	1.000		3688	1.000		16026	1.000		29257	1.000
(b) Omission of the three unlabelled deoxyribonucleoside 5'-triphosphates from the assay mixture												
3'-dTMP	T-A	2126	0.281	T-T	454	0.233	T-G	5085	0.354	T-C	11744	0.295
3'-dAMP	A-A	2218	0.294	A-T	523	0.263	A-G	4661	0.324	A-C	9396	0.236
3'-dCMP	C-A	2150	0.285	C-T	517	0.265	C-G	813	0.057	C-C	13234	0.333
3'-dGMP	G-A	1055	0.140	G-T	464	0.239	G-G	3815	0.265	G-C	5414	0.136
Total		7549	1.000		1948	1.000		14374	1.000		39788	1.000

Table 2. *Nearest-neighbour frequencies of rat liver nuclear DNA*

Primary data for this table are presented in Table 1 but here dinucleotide frequencies are expressed relative to all possible dinucleotide frequencies (cf. Josse *et al.* 1961). Percentage values in parentheses are those found in the absence of the three unlabelled triphosphates.

α-^{32}P-labelled triphosphate	Net percentage dinucleotide sequence			
dATP	T-A	A-A	C-A	G-A
	5.2 (7.8)	3.3 (8.1)	10.0 (7.9)	3.8 (3.9)
dTTP	T-T	A-T	C-T	G-T
	3.8 (6.6)	5.5 (7.5)	10.9 (7.5)	6.9 (6.8)
dGTP	T-G	A-G	C-G	G-G
	9.5 (6.8)	6.8 (6.2)	1.4 (1.1)	3.7 (5.1)
dCTP	T-C	A-C	C-C	G-C
	8.6 (7.3)	6.7 (5.9)	6.9 (8.3)	7.0 (3.4)

Table 3. *Nearest-neighbour analysis ([α-^{32}P]araCTP as substrate)*

Nuclei were labelled and nearest-neighbour analysis was carried out as described in the Experimental section.

Isolated 3'-deoxyribonucleotide	Labelled triphosphate [α-^{32}P]araCTP		
	Sequence	c.p.m.	Fraction
(a) Complete assay mixture			
3'-dTMP	T-araC	15830	0.327
3'-dAMP	A-araC	14069	0.291
3'-dCMP	C-araC	5726	0.118
3'-dGMP	G-araC	12779	0.264
Total		48404	1.000
(b) Omission of the three unlabelled deoxyribonucleotides (dATP, dGTP, dTTP)			
3'-dTMP	T-araC	9458	0.311
3'-dAMP	A-araC	9369	0.308
3'-dCMP	C-araC	6421	0.212
3'-dGMP	G-araC	5140	0.169
Total		30388	1.000

Table 4. *Nearest-neighbour analysis ([α-^{32}P]araATP as substrate)*

Nuclei were labelled and nearest-neighbour analysis was carried out as described in the Experimental section.

Isolated 3'-deoxyribonucleotide	Labelled triphosphate [α-^{32}P]araATP		
	Sequence	c.p.m.	Fraction
(a) Complete assay mixture			
3'-dTMP	T-araA	2775	0.263
3'-dAMP	A-araA	538	0.051
3'-dCMP	C-araA	5689	0.540
3'-dGMP	G-araA	1538	0.146
Total		10540	1.000
(b) Omission of the three unlabelled deoxyribonucleotides (dGTP, dCTP, dTTP)			
3'-dTMP	T-araA	870	0.315
3'-dAMP	A-araA	476	0.172
3'-dCMP	C-araA	1109	0.402
3'-dGMP	G-araA	307	0.111
Total		2762	1.000

Table 5. *Inhibition of DNA synthesis by araCTP*

Nuclei (1.4×10^6/assay), previously activated to a limited extent by incubation for 10min at 0–2°C, were assayed for polymerase in the presence of 1mM-EDTA, 0.2mM-EGTA, 10mM-MgCl$_2$, 2mM-phosphoenol-pyruvate, 0.34M-sucrose, dATP, dCTP, dGTP and ATP (each 0.4mM) and 9.5μM-[α-^{32}P]dTTP (1.01×10^9c.p.m./μmol). The incorporation was carried out for 30min.

Experimental conditions	Total [^{32}P]TMP incorporated into DNA (pmol)	Inhibition (%)
(1) Complete	1.115	0
(2) dCTP omitted	0.458	59
(3) dCTP, dGTP and dATP omitted	0.341	70
(4) dCTP replaced by araCTP (5μM)	0.469	58
(5) Standard reaction mixture plus araCTP (5μM) and dCTP decreased to 200μM	0.688	39
(6) Standard reaction mixture plus araCTP (10μM)	0.632	44
(7) Complete reaction mixture plus pancreatic deoxyribonuclease (25μg)	0.131	89
(8) Complete reaction mixture plus pancreatic ribonuclease A (20μg)	1.041	7
(9) Complete reaction mixture plus CaCl$_2$ (1mM)	6.700	0
(10) dCTP replaced by araCTP (5μM) plus CaCl$_2$ (1mM)	3.991	41
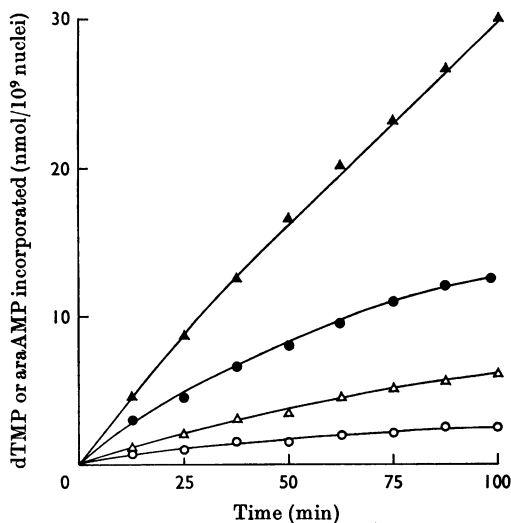
Fig. 1. Incorporation of [α-^{32}P]araATP and [α-^{32}P]dTTP into extensively activated nuclear DNA. Nuclei (1.9×10^6/assay) were exposed to Ca^{2+} and Mg^{2+} during isolation in assay solution with 1mM-CaCl$_2$, 9mM-MgCl$_2$, 2mM-phosphoenolpyruvate, 0.4mM-ATP and 0.34M-sucrose. Assays were carried out in the presence of: O, 21.5μM-[α-^{32}P]araATP (4.9×10^8c.p.m./μmol); $\triangle$, [α-^{32}P]araATP and dCTP, dGTP and dTTP (each 0.4mM); $\bullet$, 50μm-[α-^{32}P]dTTP (3.2×10^8c.p.m./μmol); $\blacktriangle$, [α-^{32}P]dTTP and dCTP, dGTP and dATP (each 0.4mM).
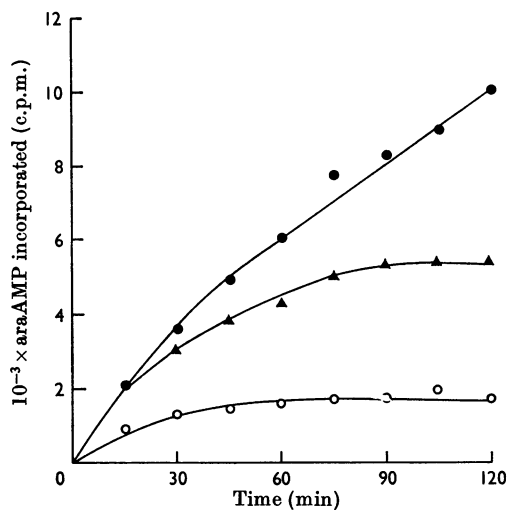

Fig. 2. DNA-chain termination by [α-^{32}P]araATP in nuclei activated at 37°C for 10min. Nuclei (1.7×10^6/assay) were treated in assay solution as described for Table 5 and the incubation was in the presence of: O, 17.2μM-[α-^{32}P]araATP (9.2×10^8c.p.m./μmol); $\blacktriangle$, [α-^{32}P]araATP and dCTP, dGTP and dTTP (each 0.4mM); $\bullet$, [α-^{32}P]araATP, 0.4mM each of dCTP, dGTP and dTTP, and 1mM-CaCl$_2$.

small but repeatable changes in the pattern of nearest-neighbour frequencies (Table 2).

The radioactivity in labelled DNA purified from nuclei as described in the Experimental section was

completely converted into an acid-soluble form by pancreatic deoxyribonuclease.

As expected, pancreatic ribonuclease A had no effect on the DNA synthesis but pancreatic deoxyribonuclease, added during incubation with labelled [α-^{32}P]dTTP, abolished incorporation of ^{32}P into acid-precipitable material (Table 5).

DNA synthesis in nuclei when DNA was only partially nicked by limited exposure to Ca^{2+}. $[\alpha\text{-}^{32}P]$-araATP and $[\alpha\text{-}^{32}P]$araCTP were not incorporated into non-activated nuclei. When the nuclear DNA underwent a limited amount of nicking, by a controlled exposure to Ca^{2+} and Mg^{2+}, incorporation of $[\alpha\text{-}^{32}P]$araATP and $[\alpha\text{-}^{32}P]$araCTP commenced but in time reached a limit, indicating that incorporation of both araAMP and araCMP depended on the availability of priming sites (cf. Fig. 2 and 3). On re-exposure of the nuclear DNA to Ca^{2+}, incorporation resumed (Fig. 3) and at a rate comparable with that obtained with nuclei incubated in the presence of Ca^{2+}. Addition of activated calf thymus DNA could also fulfil the need for additional priming sites (Fig. 4).

The chain-terminating arabinosyl analogues were also incorporated into DNA of isolated rat thymus nuclei but in this case, as with $[\alpha\text{-}^{32}P]$dTTP incorporation (Burgoyne *et al.* 1970*b*), the net incorporation reached a maximum and then decreased during the assay period owing to the presence of degradative enzymes in the nuclei (Fig. 5).
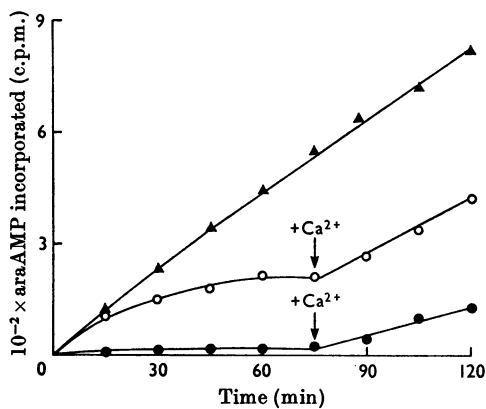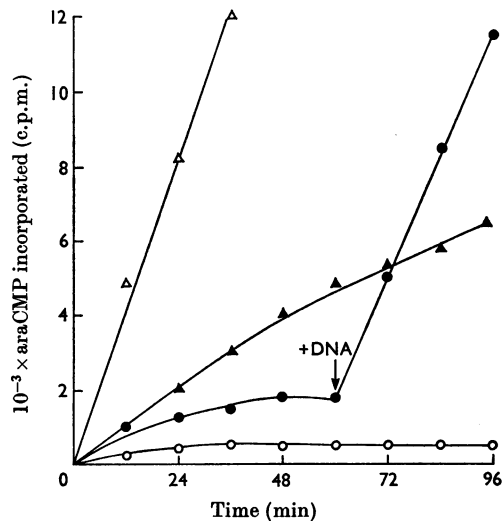

Fig. 4. Re-initiation of $[\alpha\text{-}^{32}P]$araCTP incorporation by deoxyribonuclease-I-activated calf thymus DNA. Nuclei (1.05×10^6/assay) were in assay solution as described for Table 5 with dATP, dGTP and dTTP (each 0.4mM). The incubation was carried out in the presence of: $\bigcirc$, 6μM-$[\alpha\text{-}^{32}P]$araCTP (1.87×10^9c.p.m./μmol); $\bullet$, $[\alpha\text{-}^{32}P]$araCTP (the nuclei were activated at 37°C for 4min and deoxyribonuclease-I-activated calf thymus DNA was added after 60min incubation); $\blacktriangle$, $[\alpha\text{-}^{32}P]$araCTP and 1mM-CaCl$_2$; $\triangle$, $[\alpha\text{-}^{32}P]$araCTP, deoxyribonuclease-I-activated calf thymus DNA was added at zero time.

Fig. 3. Dependence of $[\alpha\text{-}^{32}P]$araATP incorporation on the presence of priming ends. Activated nuclei (1.8×10^6/assay) were treated in assay solution as described for Table 5 and the incubation was in the presence of: $\bullet$, 17.2μM-$[\alpha\text{-}^{32}P]$araATP (6.0×10^7c.p.m./μmol), 0.4mM each of dCTP, dGTP and dTTP (1mM-CaCl$_2$ was added after 75min incubation; activation of the nuclei was at 0°C for 25min); $\bigcirc$, $[\alpha\text{-}^{32}P]$araATP, dCTP, dGTP and dTTP (each 0.4mM) (1mM-CaCl$_2$ was added after 75min incubation; activation of the nuclei was at 37°C for 6min); $\blacktriangle$, $[\alpha\text{-}^{32}P]$araATP, 0.4mM each of dCTP, dGTP and dTTP (1mM-CaCl$_2$ was added immediately when the incubation started; activation of the nuclei was at 37°C for 6min). Almost identical results were obtained when $[\alpha\text{-}^{32}P]$-araCTP (6.9×10^8c.p.m./μmol) was used as the labelled substrate.
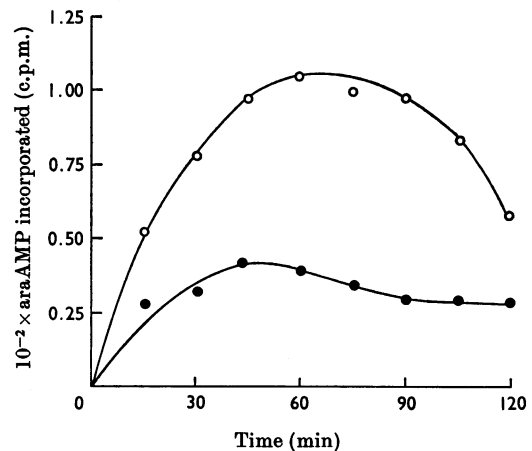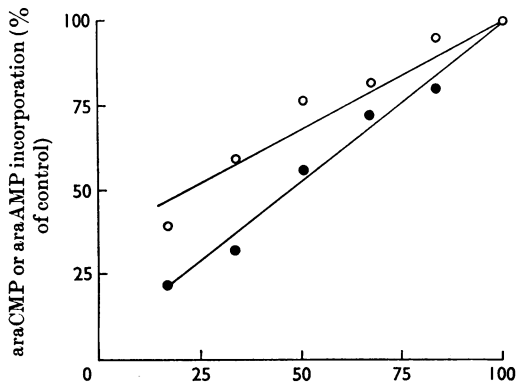

Fig. 5. Incorporation of $[\alpha\text{-}^{32}P]$araATP by rat thymus nuclei. Incubation conditions were as described in Table 5 with the assay solution containing 25.8μM-$[\alpha\text{-}^{32}P]$araATP (6.0×10^7c.p.m./μmol), dCTP, dGTP and dTTP (0.4mM each). Each assay (50μl) contained nuclei corresponding to 0.89μmol of DNA P. Incorporation was measured: $\bullet$, in the absence of Ca^{2+}; $\bigcirc$, in the presence of 1mM-CaCl$_2$.

Fig. 6. Competition between labelled arabinoside triphosphate incorporation and the corresponding deoxyriboside triphosphate. Nuclei were exposed to Ca^{2+} and Mg^{2+} during isolation. Assays were for 15 min and assay conditions were the same as those described for Fig. 1 with modifications as stated below. The ratio of arabinoside triphosphate to its corresponding deoxyribonucleotide was varied as shown in the figure. In both cases the control incorporation corresponds to the incorporation when the test mixture contains $30\,\mu$M-arabinoside triphosphate without corresponding deoxyribonucleotide. With cytosine arabinoside this was $0.57\,$nmol/10^9 nuclei and with adenine arabinoside this was $0.94\,$nmol/10^9 nuclei. $\bigcirc$, $30\,\mu$M (sum of dCTP and [α-^{32}P]araCTP 2.18×10^9 c.p.m./μmol), 1.2×10^6 nuclei/assay with dATP, dGTP and dTTP (each 0.4mM). $\bullet$, $30\,\mu$M (sum of dATP and [α-^{32}P]araATP 2.39×10^9 c.p.m./μmol), 1.02×10^6 nuclei/assay with dCTP, dGTP and dTTP (each 0.4mM).

Both the compounds araATP and araCTP were found to inhibit DNA synthesis in isolated rat liver and thymus nuclei. The results indicated that incorporation of [α-^{32}P]araCTP and [α-^{32}P]araATP competed successfully with dCTP and dATP respectively (Fig. 6).

Intrinsic DNA polymerase activities of nuclei from resting and regenerating liver. Nuclei prepared in the absence of Ca^{2+} and Mg^{2+} from normal or 24 h regenerating rat liver had incorporation rates close to the lowest limit of detection in our system. The rate was 1–2% of that observed in the presence of 1 mM-calcium chloride. Moreover, even in the presence of calcium this nuclear preparation did not show significant differences between nuclei from normal and regenerating livers.

DISCUSSION

Nearest-neighbour frequencies observed in the presence of all four deoxyribonucleoside triphosphates did not differ significantly from the values expected for rat liver DNA, and in the absence of the other three deoxyribonucleoside triphosphates there was no extensive homopolymer formation (Table 1) of the type that would result from template-independent terminal transferase (Krakow, Coutsageorgopoulos & Canellakis, 1962; Bollum, Groeniger & Yoneda, 1964). The small increase in homodinucleotide frequencies (4.8% for A-A, 1.4% for C-C, 1.4% for G-G and 2.8% for T-T; Table 2) was consistent with template-dependent limit attachment at endogenous primer termini. So attachment to endogenous primer termini was probably a major component of incorporation *in vitro*. When chain-terminating nucleotide analogues, i.e. [α-^{32}P]araATP or [α-^{32}P]araCTP, were used as the labelled substrate (Tables 3 and 4) nearest-neighbour frequencies similar to those with the deoxyribonucleotides were obtained.

To reconcile the apparent template-dependence of incorporation from single triphosphates with their relatively rapid and continued incorporation in nuclei prepared in the presence of Ca^{2+} and Mg^{2+}, the following two possibilities were considered. (a) The nuclei might contain endogenous deoxyribonucleoside triphosphates sufficient to maintain the observed rates of incorporation. These triphosphates might have been present *in vivo* or have been formed from endogenous DNA by nuclease and kinase action. (b) The nuclear DNA might be so extensively nicked that observed incorporation might involve attachment to one or a few nucleotides at each primer terminus.

The results reported here further support the conclusion (Burgoyne *et al.* 1970a) that the availability of primer ends (alternative b) was the major factor controlling the incorporation in the absence of the complete complement of deoxyribonucleotides.

The prime objective of the nearest-neighbour frequency studies was to demonstrate that both the ordinary deoxyribonucleotides and the arabinosyl nucleotides were being incorporated into a heteropolymer with a composition similar to that expected for rat DNA. We think that these results have demonstrated this; nevertheless there were deviations from the ratios expected for a complementary double-stranded DNA (e.g. G-G is not equal to C-C, Table 2). A complete evaluation of the significance of these deviations was not possible with these results. However, as incorporations in the complete system were carried out under conditions that gave a large excess of primer ends it might be expected that some base sequences would be preferentially copied and thus cause deviations in the gross nearest-neighbour ratios.

In the cases of omission of the three unlabelled deoxyribonucleoside triphosphates additional deviations would arise from the preferential copying of the limited number of homonucleotide sequences available.

The limit attachment of [^{32}P]araCMP or [^{32}P]-araAMP to nicked DNA in activated nuclei, and the further incorporation achieved when new primer sites were made available by the addition of either Ca^{2+} or activated DNA, indicated that DNA synthesis was limited by the availability of priming sites and that the arabinosyl nucleotides appeared to function as chain terminators. The chain-termination mechanism observed *in vitro* would be expected to be operable *in vivo*. Both these chain-terminating arabinoside triphosphates acted as a substrate for mammalian DNA polymerase and appeared to terminate chains by terminal addition of arabinosyl nucleotide to the growing polydeoxy-ribonucleotide chains, which then prevented further addition of other nucleotides. This agrees with studies on partially purified DNA polymerase from animal (Momparler, 1969) and bacterial (Atkinson, Deutscher, Kornberg, Russel & Moffatt, 1969) sources.

If the synthesis we were observing were true replication we would have expected to find more intrinsic priming ability in nuclei from regenerating liver than in nuclei from normal liver. Although nuclei from regenerating liver have been found to have slightly higher intrinsic (i.e. Ca^{2+}-free) priming activity, we consider these differences too small to be convincing. Thus we consider that the processes we are observing *in vitro* are still a very imperfect reflection of the process of DNA synthesis, or repair, *in vivo*.

REFERENCES

Atkinson, M. R., Deutscher, M. P., Kornberg, A. Russel, A. F. & Moffatt, J. G. (1969). *Biochemistry, Easton*, **8**, 4897.

Blobel, G. & Potter, V. R. (1966). *Science, N.Y.*, **154**, 1662.

Bollum, F. J., Groeniger, E. & Yoneda, M. (1964). *Proc. natn. Acad. Sci. U.S.A.* **51**, 853.

Burgoyne, L. A., Waqar, M. A. & Atkinson, M. R. (1970a). *Biochem. biophys. Res. Commun.* **39**, 254.

Burgoyne, L. A., Waqar, M. A. & Atkinson, M. R. (1970b). *Biochem. biophys. Res. Commun.* **39**, 918.

Josse, J., Kaiser, A. D. & Kornberg, A. (1961). *J. biol. Chem.* **236**, 864.

Kay, E. R. M., Simmons, N. S. & Dounce, A. L. (1952). *J. Am. chem. Soc.* **74**, 1724.

Keir, H. M. (1965). *Prog. nucleic Acid Res. molec. Biol.* **4**, 81.

Krakow, J. S., Coutsageorgopoulos, C. & Canellakis, E. S. (1962). *Biochim. biophys. Acta*, **55**, 639.

Lehman, I. R., Bessman, M. J., Simms, E. S. & Kornberg, A. (1958) . *J. biol. Chem.* **233**, 163.

Momparler, R. L. (1969). *Biochem. biophys. Res. Commun.* **34**, 465.

Symons, R. H. (1969). *Biochim. biophys. Acta*, **190**, 548.

Wu, R. & Kaiser, A. D. (1968). *J. molec. Biol.* **35**, 523.