tems utilizing exogenous inhibitors to attain the total inhibition of cytoplasmic protein synthesis for the study of control mechanisms involved in the regulation of mitochondrial DNA synthesis. It also provides a simple procedure for the preparation of large amounts of highly labeled yeast mitochondrial DNA.

Correction

Dr. Leland H. Hartwell informs us that mutant 314 used in this study has a lesion in cdc4, not in cdc8 as previously reported. Initiation of nuclear DNA synthesis is blocked in this mutant.

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Bone Marrow Cytoplasmic Deoxyribonucleic Acid Polymerase. Variation of pH and Ionic Environment as a Possible Control Mechanism†

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ABSTRACT: A cytoplasmic DNA polymerase has been purified 200fold from erythroid hyperplastic bone marrow. This enzyme shows an absolute requirement for a divalent cation, which may be either Mg^{2+} or Mn^{2+}, and is stimulated about fourfold by a monovalent cation, NH_4^+ or K^+. The enzyme preferentially uses activated calf thymus DNA and poly[d(A-T)] as a template, while native calf thymus DNA and synthetic DNA/RNA hybrids are rather poor templates. DNA synthesis with this enzyme is inhibited by low concentrations of heme or ethidium bromide. The rate of DNA synthesis with the cytoplasmic DNA polymerase is markedly dependent on pH as well as the concentrations of divalent and monovalent cations and the effects of these factors are interdependent and interrelated. Changes in pH have profound effects on the concentration of monovalent and divalent cations required for optimal activity and, conversely, changes in the concentrations of divalent and monovalent cations also influence the optimal pH of the reaction. The mechanism of salt activation has been shown to involve a dissociation of the DNA polymerase from an 11.6-S dimer to an 8-S monomer.

The presence of DNA polymerase in the cytoplasm of eukaryotic cells has been reported by several investigators (Lindsay *et al.*, 1970; Weissbach *et al.*, 1971; Chang and Bollum, 1972a; Wallace *et al.*, 1971; Baril *et al.*, 1971; Sedwick *et al.*, 1972). Although it was suggested that the presence of DNA polymerase in the cytoplasm could be artifactual and the result of leakage from nuclei during cell disruption (Keir *et al.*, 1962; Keir, 1965), it is becoming increasingly apparent that a DNA polymerase activity is, in fact, localized in the cytoplasm and that this enzyme is distinct from the polymerases present in nuclei or mitochondria (Lindsay *et al.*, 1970; Weissbach *et al.*, 1971; Chang and Bollum, 1972a; Wallace *et al.*, 1971; Baril *et al.*, 1971; Sedwick *et al.*, 1972).

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TABLE I: Purification of DNA Polymerase.[a]

Step	Total Activity (units)	Protein (mg)	Specific Activity (units/mg of protein)	Fold Purification	% Yield
30,000*g* supernatant	980,000	4200	234		100
Ribosomal homogenate	960,000	540	1,780	7.5	98
176,000*g* supernatant	960,000	340	2,840	12	98
60% ammonium sulfate precipitate	940,000	216	4,350	19	96
Phosphocellulose chromatography	340,000	43	7,800	33	35
DEAE-Sephadex-A25 chromatography	254,000	8.6	29,500	126	26
DNA-Cellulose chromatography	195,000	4.1	47,500	205	20

[a] Assay conditions were as described in Material and Methods. One unit of activity is defined as that amount of enzyme which incorporates 1 pmol of TMP in 15 min of incubation at 37°.

The function of the cytoplasmic DNA polymerase is not known. It was recently shown that the activity of this enzyme increases in response to cellular proliferation and it has been suggested that the cytoplasmic DNA polymerase is somehow involved in the process of replication (Chang and Bollum, 1972b; Chang *et al.*, 1973; Holmes and Johnston, 1973). Another possibility is that this enzyme is involved in cellular differentiation and gene amplification.

In order to understand how cellular proliferation and differentiation are regulated in mammalian cells, it is necessary to know how the synthesis of DNA is controlled. Accordingly, it is important to investigate the factors which may be significant in the regulation of cytoplasmic DNA synthesis. In this paper we will present the purification and partial characterization of DNA polymerase from the cytoplasm of rapidly proliferating bone marrow cells. In addition, we will present data showing that the rate of DNA synthesis with this enzyme can be regulated by varying the pH and the concentrations of monovalent and divalent cations and that these effects are interrelated and interdependent. Finally, the mechanism of salt activation will be discussed.

Methods and Materials

Tritium-labeled deoxyribonucleoside triphosphates were obtained from International Chemical and Nuclear Corp. Unlabeled deoxyribonucleoside triphosphates were purchased from Sigma Chemical Co. or Calbiochem. Calf thymus DNA was obtained from Worthington Biochemicals. Poly[d(A-T)] was purchased from General Biochemicals. Rifamycin AF/013 was a generous gift of Dr. Giancarlo Lancini and Renato Cricchio of Gruppo Lepetit, Milan, Italy. Rifampicin, ethidium bromide, actinomycin D, heme, nalidixic acid, and PCMB were obtained from Calbiochem. Poly(rA)·poly(dT) and poly(rA)·oligo(dT) were obtained from Collaborative Research. Activated calf thymus DNA was prepared as described by Aposhian and Kornberg (1962).

Purification of DNA Polymerase

Erythroid hyperplastic bone marrows were induced in rabbits by the procedure of Borsook *et al.* (1952). New Zealand rabbits weighing 4–6 lb were given daily injections of 1.0 ml of a 2.5% neutralized phenylhydrazine solution for 4 days. The rabbits received no injections on the 5th and 6th days and were sacrificed on the 7th day. The humerus, femur, and tibia were removed and immediately placed in ice-cold washing buffer (10 mM Tris-HCl (pH 7.8)–0.13 M NaCl–5 mM KCl–7 mM $MgCl_2$). The bones were cracked open and the marrow was removed into washing buffer. Wright stain of the marrow cells revealed an intense erythroid hyperplasia with greater than 70% erythroid precursors. The marrow cells were sedimented at 4000*g* for 10 min and lysed by the addition of 10 volumes of lysing buffer (5 mM Tris-HCl (pH 7.4)–7.5 mM KCl–10% glycerol–0.25 mM 2-mercaptoethanol). The suspension was gently dispersed with a loose-fitting glass–Teflon homogenizer and stirred for 20 min. Nuclei, mitochondria, and stroma were removed by centrifugation at 30,000*g* for 15 min. The purification of the enzyme from the 30,000*g* supernatant is summarized in Table I.

The 30,000*g* fraction was brought to 5 mM in $MgCl_2$ and centrifuged at 78,000*g* for 2.5 hr. The microsomal pellets were suspended in buffer A (50 mM Tris-HCl (pH 7.8)–1.0 mM dithiothreitol–0.1 mM EDTA–25% glycerol) containing 1 M KCl by gentle homogenization in a loose-fitting glass homogenizer and the suspension was left overnight at 0°. The next day the suspension was centrifuged at 152,000*g* for 75 min and the supernatant was separated from the microsomal fraction. The 152,000*g* supernatant was adjusted to 60% saturation with ammonium sulfate and the precipitate was collected by centrifugation at 30,000*g* for 15 min. The precipitate was dissolved in buffer A and dialyzed against 1 l. of buffer A for 4 hr with one change of buffer.

Phosphocellulose Chromatography. The dialyzed ammonium sulfate fraction (80 ml) was applied to a phosphocellulose column (2.3 × 9 cm), equilibrated with buffer A containing 0.05 M KCl and washed with the same buffer. A linear gradient of 0.05–1.0 M KCl in buffer A with a total volume of 300 ml was applied and 4-ml fractions were collected. The fractions containing DNA polymerase activity (Figure 1) were pooled and dialyzed against 1 l. of buffer A for 4 hr with one change of buffer.

DEAE-Sephadex A-25 Chromatography. Approximately 25 ml of the dialyzed phosphocellulose eluate was applied to a column of DEAE-Sephadex A-25 (1.5 × 10 cm) previously equilibrated with buffer A. A linear gradient of 0.05–0.5 M KCl in buffer A with a total volume of 100 ml was applied and 3-ml fractions were collected. DNA polymerase activity was eluted as a single peak at 0.07 M KCl (Figure 2) and the fractions containing DNA polymerase activity were pooled and dialyzed against buffer A for 4 hr.

DNA-Cellulose Chromatography. DNA-cellulose was prepared according to the procedure of Alberts and Herrick (1971). A 1.5 × 5 cm column was equilibrated with buffer A and 10 ml of the dialyzed DEAE eluate was loaded at a rate

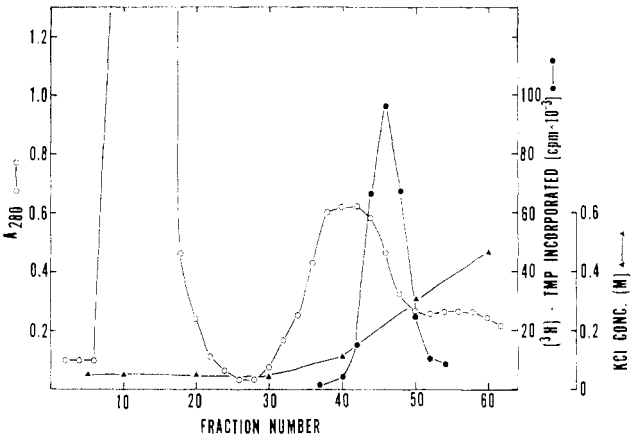

FIGURE 1: Phosphocellulose chromatography of cytoplasmic DNA polymerase. The experimental details were as described in Methods and Materials except that the concentration of TTP was 0.1 μM, 75 mCi/μmol.

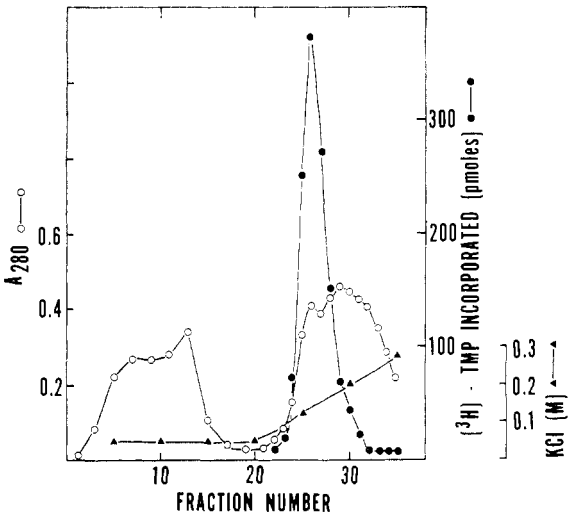

FIGURE 2: DEAE-Sephadex A-25 chromatography of cytoplasmic DNA polymerase. See Methods and Materials for experimental details.

of 24 ml/hr. The enzyme was eluted stepwise with buffer A containing 0.2 M KCl.

DNA polymerase at this stage was purified about 200-fold as compared to the 30,000*g* supernatant and the yield varied from 20 to 25%. The enzyme was relatively stable at 0° for 1–2 weeks.

We have not been able to determine the purity of the DNA polymerase by disc gel electrophoresis since the enzyme does not enter 5% gels either at low or high pH. However, sedimentation of the enzyme in a 5–20% sucrose gradient resulted in two symmetrical peaks of activity with sedimentation coefficients of 8 and 11.6 S.

Assay for DNA Polymerase. The standard assay mixture contained in a final volume of 0.25 ml: 40 mM Hepes[1] buffer (pH 7.0)–0.4 mM $MnCl_2$–60 mM $(NH_4)_2SO_4$–0.16 mM each dATP, dGTP, and dCTP, 0.004 mM [^{3}H]TTP, 2000 Ci/mol, 20 μg of activated calf thymus DNA, and 5–10 μg of enzyme. The reaction mixture was incubated for 15 min at 37° and stopped by the addition of 2 ml of cold 5% trichloroacetic acid. The precipitate was collected on a glass fiber filter (Whatman GF/C) and washed with trichloroacetic acid and ethanol. The filter was dried and counted in a liquid scintillation counter.

[1] Abbreviation used is: Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid.

TABLE II: Requirements for DNA Synthesis.[a]

Conditions	[^{3}H]-TMP Incorporated (pmol)	% Activity
Complete	188	100
−dATP	29	15
−dATP, dCTP	18	10
−DNA	0	0
−Divalent cation	1.2	0.6
−Monovalent cation	49	26
+Native calf thymus DNA	4.5	2.4
+rA/dT	4.8	2.5
+rA/oligo dT	3.6	1.9
+Poly[d(A-T)]	193	102

[a] Incubation conditions were the same as described in Methods and Materials except that (1) the individual components of the reaction mixture were omitted as indicated and (2) other templates were substituted for activated calf thymus DNA as indicated.

Sucrose Density Gradient Analysis of Cytoplasmic DNA Polymerase. DNA polymerase was dialyzed for 4 hr against 5% sucrose either in high salt buffer containing 4 mM Hepes buffer (pH 7)–0.4 mM $MnCl_2$–1.0 mM dithiothreitol–100 mM $(NH_4)_2SO_4$ or in low salt buffer containing 20 mM $(NH_4)_2SO_4$. The enzyme solution (0.2 ml) containing 320 μg of protein was layered on a 5–20% sucrose gradient containing either high or low salt buffer. Sedimentation was carried out in a Spinco SW 39 rotor for 15 hr at 37,000 rpm at 2–4°. 4 S tRNA was simultaneously sedimented as a marker. Thirty-eight equal fractions were collected from the bottom of the tubes and assayed for DNA polymerase activity.

Results

Requirements for DNA Synthesis. The cytoplasmic DNA polymerase from bone marrow shows an absolute requirement for a template and a divalent cation which may be either Mn^{2+} or Mg^{2+} (Table II). The reaction is stimulated about fourfold by a monovalent cation, either NH_4^+ or K^+. With this enzyme the synthesis of DNA requires all four deoxyribonucleoside triphosphates for maximal activity. Omission of one deoxyribonucleoside triphosphate in the reaction mixture reduced the activity to 15% of the control value and omission of two deoxyribonucleoside triphosphates resulted in less than 10% of the control activity.

This enzyme has little activity with native DNA, but preferentially uses "activated" DNA as template. As is the case with the DNA polymerase I from *E. coli* (Richardson *et al.*, 1964), presumably the cytoplasmic DNA polymerase is unable to initiate DNA synthesis on an intact template and, therefore, requires single-strand nicks in the template to act as primers. Synthetic DNA/RNA hybrids are also poor templates for this enzyme, but the synthetic copolymer poly-[d(A-T)] is a very good template, being as active as activated calf thymus DNA.

Inhibitors of Cytoplasmic DNA Synthesis. The effects of various inhibitors of DNA synthesis are shown in Table III.

TABLE III: Inhibitors of DNA Synthesis.[a]

Inhibitor	Concn	[^{3}H]TMP Incorporated (pmol)	% Inhibition
Control		210.0	0
Nalidixic acid	40 μg/ml	210.0	0
Rifampicin	20 μg/ml	210.0	0
Rifamycin AF/013	8 μg/ml	112.0	42
Rifamycin AF/013	40 μg/ml	8.2	96
Ethidium bromide	8 μg/ml	44.0	79
Ethidium bromide	20 μg/ml	7.5	96.5
Actinomycin D	20 μg/ml	37.0	82
Heme	4×10^{-5} M	0	100
p-Chloromercuribenzoate	1×10^{-5} M	0	100

[a] Reaction conditions were as described in Materials and Methods except (1) the concentration of activated calf thymus DNA was 20 μg/ml and (2) inhibitors were added as indicated.

The cytoplasmic DNA polymerase is extremely sensitive to PCMB, although a requirement for added dithiothreitol cannot be demonstrated. However, the enzyme has been purified in the presence of dithiothreitol. The synthesis of DNA with this enzyme is also very sensitive to the intercalating dye, ethidium bromide, as is mitochondrial DNA synthesis (Meyer and Simpson, 1968). At a concentration of 8 μg/ml the synthesis of DNA is inhibited about 80% and at 20 μg/ml of ethidium bromide causes 97% inhibition. Cytoplasmic DNA synthesis is also sensitive to rifamycin AF/013 and high concentrations of actinomycin D. The former at a concentration of 8 μg/ml results in 42% inhibition and the latter at a concentration of 20 μg/ml inhibits about 82%. Rifamycin AF/013 has been shown to inhibit eukaryotic DNA-dependent RNA polymerases, both nucleoplasmic and nucleolar (Meilhac *et al.*, 1972; Juhasz *et al.*, 1972; Adman *et al.*, 1972). This enzyme, however, is completely insensitive to nalidixic acid and rifampicin. Nalidixic acid has been shown to be a potent inhibitor of DNA replication in microbial systems (Gross *et al.*, 1964; Moses *et al.*, 1971; Pedrini *et al.*, 1972) while rifampicin inhibits DNA-dependent RNA polymerase of bacterial origin (Wehrli and Staehelin, 1971; Reid and Parsons, 1971).

The synthesis of DNA with the cytoplasmic DNA polymerase from erythroid hyperplastic bone marrow, similar to RNA synthesis with the reticulocyte RNA-dependent RNA polymerase (Downey *et al.*, 1973), is markedly inhibited by heme at a concentration of 10^{-5} M. It is of interest that this heme concentration is optimal in stimulating hemoglobin synthesis in reticulocyte lysates (Bruns and London, 1965).

Influence of pH on the Optimal Divalent Cation Concentration. In vitro studies have shown that the rate of DNA synthesis is affected by pH and the concentrations of divalent and monovalent cations when each factor is investigated individually (Green and Korn, 1970; Baril *et al.*, 1971; Schlabach *et al.*, 1971; Chang and Bollum, 1972a). Since the effects of all these factors are interdependent, it is important to investigate the interrelationships between these effects in order to understand how DNA synthesis may be regulated. The effect of varying the Mn^{2+} concentration on the rate of DNA synthesis at three different pH values is shown in Figure 3. It is clear that the Mn^{2+} concentration resulting in maximal

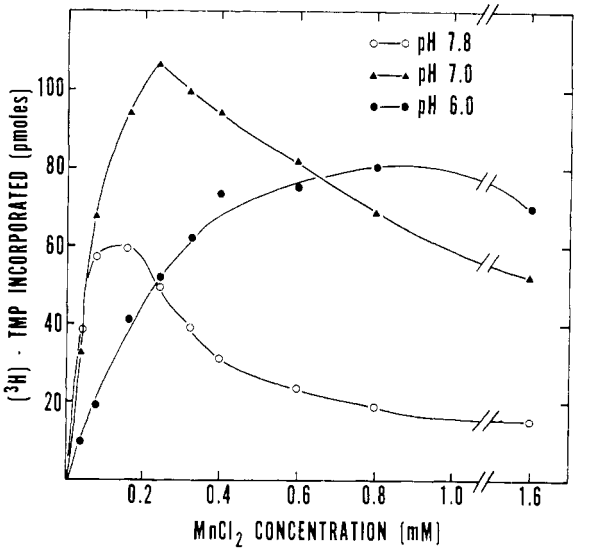

FIGURE 3: Effect of pH on $MnCl_2$ optimum. Assay conditions were as in Methods and Materials except for the concentration of $MnCl_2$ and pH as indicated. For pH 7.8, Tris-HCl buffer (40 mM) was used instead of Hepes buffer.

activity increases with increasing H^+ concentration. At pH 7.8 the optimal Mn^{2+} concentration is 0.12–0.16 mM while higher Mn^{2+} concentrations at this pH markedly inhibit DNA synthesis. Decreasing the pH to 7.0 increases the optimal Mn^{2+} concentration to 0.24 mM and at pH 6.0 a very broad Mn^{2+} optimum of 0.4–1.6 mM is obtained.

A similar pattern is seen with Mg^{2+} (Figure 4). At pH 7.8 the Mg^{2+} optimum is 2 mM and at this Mg^{2+} concentration there is little activity at pH 7.0 or 6.5. At pH 7 the optimal Mg^{2+} concentration increases to 8–10 mM and at pH 6.5, it ranges from 10 to 20 mM. Thus, similar to Mn^{2+}, the concentration of Mg^{2+} required for maximal activity increases with increasing H^+ concentration. Conversely, as can be seen in Figures 3 and 4, the pH optimum increases as the divalent cation concentration decreases, indicating that the divalent cation concentration also has a marked influence on the hydrogen ion concentration required for optimal activity.

Influence of pH on the Optimal Monovalent Cation Concentration. The activity of DNA polymerases prepared either from mammalian or microbial sources has been shown to be influenced by the concentration of monovalent cations. With

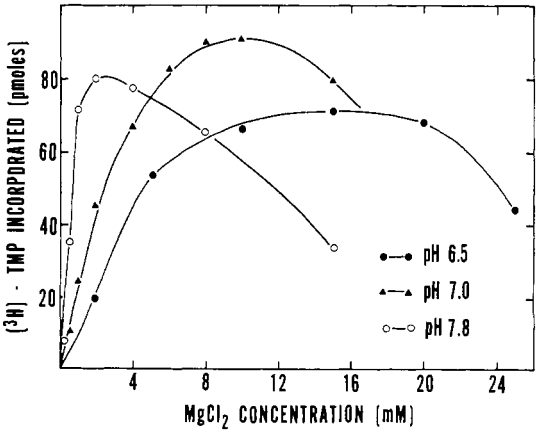

FIGURE 4: Effect of pH on $MgCl_2$ optimum. Assay conditions were as described in Methods and Materials except for pH and $MgCl_2$ concentration as indicated. For pH 7.8, Tris-HCl buffer (40 mM) was used instead of Hepes buffer.

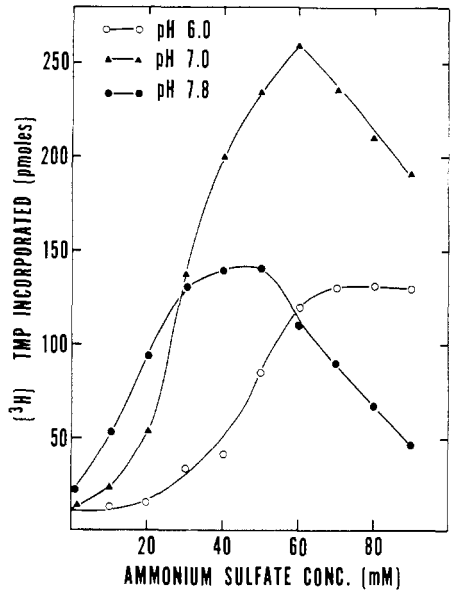

FIGURE 5: Effect of pH on ammonium sulfate optimum. Assay conditions were as described in Methods and Materials except for pH and the concentration of ammonium sulfate as shown. For pH 7.8, Tris-HCl buffer (40 mM) was used instead of Hepes buffer.

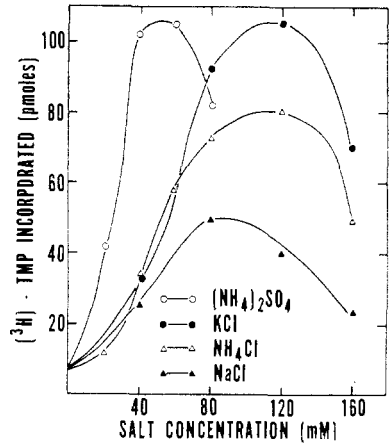

FIGURE 6: Effect of monovalent cations. Assay conditions were as described in Methods and Materials except for the concentraion of monovalent cations.

the cytoplasmic DNA polymerase the optimal concentration of ammonium sulfate is dependent on the H^+ concentration in the assay medium (Figure 5). The optimal concentration of ammonium sulfate decreases with decreasing H^+ concentration. At pH 6, the optimal $(NH_4)_2SO_4$ concentration ranges from 70 to 90 mM. Increasing the pH to 7 results in a lower $(NH_4)_2SO_4$ optimum (60 mM) and it is further reduced to 30–50 mM when the pH is increased to 7.8. Thus, the requirement for ammonium sulfate increases with decreasing pH.

The effects of salt are not merely due to ionic strength, since not all salts show the same degree of stimulation (Figure 6). At their optimal concentrations $(NH_4)_2SO_4$ and KCl are equally active but NH_4Cl is less active and NaCl is the least active in stimulating the reaction.

Effect of Monovalent Cation Concentration on the pH Optimum. Figure 7 shows the effects on the pH optimum that result from varying the ammonium sulfate concentration. With 20 mM $(NH_4)_2SO_4$ the pH optimum is 7.5. Increasing the $(NH_4)_2SO_4$ concentration to 40 mM shifts the pH optimum to 7.25 and at 80 mM $(NH_4)_2SO_4$ the pH optimum is 7.0. Furthermore, at pH values above 7.0, 80 mM $(NH_4)_2SO_4$ inhibits the synthesis of DNA, while at pH values below 7.0 the same concentration of $(NH_4)_2SO_4$ markedly stimulates the rate of DNA synthesis. In the range of 20–40 mM $(NH_4)_2SO_4$ DNA synthesis is stimulated at all pH values examined.

Effect of Salt on the Sedimentation Behavior of the Enzyme. The sigmoidal shapes of the curves obtained when rate is plotted as a function of salt concentration (Figure 6) suggest the possibility that this enzyme is an allosteric protein and that monovalent cations are positive effectors modifying the conformation or subunit interactions of the enzyme. Thus it is important to investigate the sedimentation characteristics of this enzyme at different salt concentrations.

When the cytoplasmic DNA polymerase is sedimented in a sucrose density gradient under optimal conditions of pH and divalent cation (*i.e.* pH 7.0, 0.4 mM $MnCl_2$) but at suboptimal $(NH_4)_2SO_4$ concentration (20 mM), two peaks of enzyme activity are detected (Figure 8), a large peak sedimenting at 11.6 S and a smaller peak sedimenting at 8 S. Under identical conditions of pH and divalent cation concentration but at high salt concentration (100 mM $(NH_4)_2SO_4$) only one large peak of activity is detected sedimenting at 8 S.

These data suggest that a single DNA polymerase can exist in two forms (8 and 11.6 S), since they can be interconverted, and that the predominant form of the enzyme is determined by the salt concentration. Such a large change in sedimentation constant is unlikely to be the result of a conformational change in the protein and is consistent with a monomer–dimer interconversion.

Discussion

Cytoplasmic DNA polymerases have been demonstrated in several cell lines in culture as well as in regenerating liver and bone marrow, and several hypotheses have been put forward to account for the presence of these enzymes in the cytoplasm. It has been suggested that the enzyme originates in the cytoplasm and is transported to the nucleus during the S-phase of the cell cycle (Littlefield *et al.*, 1963; Gold and Helleiner, 1964; Fansler and Loeb, 1970; Lindsay *et al.*, 1970), or that the enzyme is transported into the cytoplasm as a complex with DNA following DNA replication (Chang *et al.*, 1973). Another suggestion is that the enzyme might have leaked from the nucleus during purification (Keir *et al.*, 1962; Keir, 1965). Our purification procedure, using hypotonic shock to lyse the cells, is quite gentle and it is unlikely that the cytoplasmic location of the DNA polymerase in bone marrow is the result of leakage from nuclei or mitochondria, since these organelles usually remain intact following hypotonic lysis of cells, and can be eliminated by differential centrifugation.

The erythroid cytoplasmic DNA polymerase preferentially utilizes activated DNA as a template, although the synthetic copolymer poly[d(A-T)] is also an efficient template. Native DNA and synthetic DNA/RNA hybrids are rather poor templates for the enzyme. Presumably, this template specificity reflects the inability of the cytoplasmic DNA polymerase to initiate DNA synthesis on an intact template unless a primer is present, as is the case with the *E. coli* DNA polymerase I.

With activated DNA as a template, the enzyme requires all four deoxyribonucleoside triphosphates for maximal activity. Omission of one deoxyribonucleoside triphosphate in the reaction mixture reduces the activity to approximately 15% of the control. This is similar to other mammalian DNA poly-

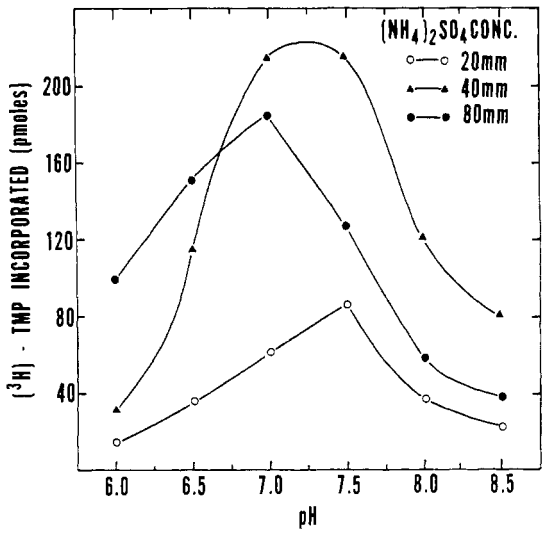

FIGURE 7: Effect of ammonium sulfate concentration on the pH optimum. Assay conditions were as in Methods and Materials except for pH and the concentration of ammonium sulfate as indicated.

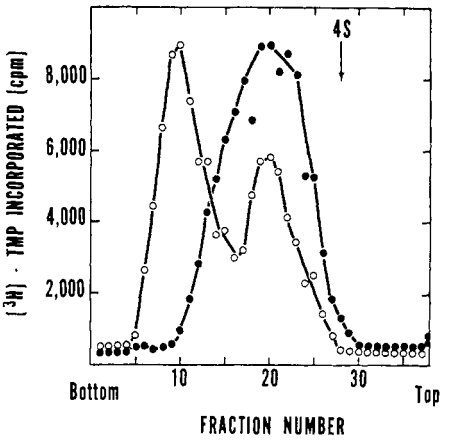

FIGURE 8: Sucrose gradient analysis of cytoplasmic DNA polymerase. The experimental details were as described in the text and Methods and Materials: (●) 100 mM $(NH_4)_2SO_4$; (○) 20 mM $(NH_4)_2SO_4$. The assay was as described in Methods and Materials except that the concentration of TTP was 0.1 μM, 75 mCi/μmol.

merases which have been reported to synthesize considerable amounts of DNA in the absence of one or more deoxyribonucleoside triphosphates (Green and Korn, 1970; Chang and Bollum, 1972a).

DNA synthesis with the cytoplasmic DNA polymerase from bone marrow is inhibited by high concentrations of actinomycin D and by low concentrations of ethidium bromide. The inhibition by ethidium bromide is quite interesting in that this intercalating dye is a potent cytoplasmic mutagenic agent and an efficient inhibitor of mitochondrial DNA synthesis (Meyer and Simpson, 1968). However, the efficiency of ethidium bromide as an inhibitor of DNA synthesis with this enzyme is most likely due to its ability to interact with the activated DNA template.

It is also of interest that the cytoplasmic DNA polymerase, like the reticulocyte RNA-dependent RNA polymerase (Downey *et al.*, 1973), is inhibited by low concentrations of heme. Similar concentrations of heme have been shown to be required for optimal and continued synthesis of hemoglobin at the translation step.

These studies clearly show that the activity of this cytoplasmic DNA polymerase is dependent on pH as well as the concentrations of divalent and monovalent cations, and that the effects of these factors are interrelated. Slight changes in pH have profound effects on the concentrations of monovalent and divalent cations required for maximal activity and, conversely, changes in the concentrations of monovalent or divalent cations influence the optimal pH of the reaction.

Although the effects of these variables could be on the enzyme, the template, the substrate, or the interaction of any combination of these, we believe that the major effects we have observed are on the enzyme since the sedimentation pattern of the enzyme is markedly influenced by the salt concentration. Depending on the relative concentration of salt, the enzyme either exists predominantly in a dimer form having a sedimentation coefficient of 11.6 S or in a monomeric form at 8 S. At high salt concentrations the enzyme sediments primarily in the monomeric form; thus, salt activation involves the dissociation of the enzyme from a dimer to a monomer.

Two forms of a DNA polymerase sedimenting at 10 and 7 S have also been demonstrated in human lymphocytes, in addition to the 3.3-S nuclear enzyme (Smith and Gallo, 1972). More recently Hecht and Davidson (1973) have reported that most of the 6–8-S DNA polymerase isolated from the cytoplasm of mouse testes sedimented at 3.5 S in the presence of high salt, and suggested that a subunit of the cytoplasmic DNA polymerases was identical with the nuclear enzyme. Chang and Bollum (1972c) have also suggested a common subunit or polypeptide sequence between the nuclear and cytoplasmic enzymes based on their observation that antibody prepared against the 6–8-S cytoplasmic DNA polymerase from calf thymus also inhibits the 3.3-S nuclear enzyme. However, with the cytoplasmic DNA polymerase prepared from bone marrow, we have not observed activity sedimenting at 3.3 S either at high or low salt.

The observation that the cytoplasmic DNA polymerase can undergo activation corresponding to a dimer → monomer dissociation under relatively mild conditions suggests the possibility that the control of enzyme activity by dimer–monomer interconversion may be an important regulatory mechanism in the control of DNA synthesis in the cytoplasm. However, it is not known whether these effects of pH, monovalent cations, and divalent cations represent physiological control mechanisms. The variation of pH within physiological range as well as changes in the concentration of divalent and monovalent cations have also been suggested as an important control mechanism in the regulation of enzymes involved in glycolysis and gluconeogenesis (Bygrave, 1967; Scrutton and Utter, 1968; Sols and Marco, 1970; Seubert and Schoner 1971). Other physiologically important factors such as the concentrations of energy metabolites and the role they play in the control of cytoplasmic DNA synthesis are currently under investigation in our laboratory.

The function of cytoplasmic DNA polymerase is still not known. However, the recent demonstration of cytoplasmic DNA (Lerner *et al.*, 1971) is consistent with our suggestion that this enzyme may be important in cellular differentiation. Furthermore, we have recently observed that DNA synthesis in crude cytoplasmic extracts is sensitive to RNase and relatively resistant to actinomycin D, suggesting that the cytoplasmic synthesis of double-stranded DNA involves an RNA/DNA intermediate (McDonnell *et al.*, 1970; Verma *et al.*, 1972; J. J. Byrnes *et al.*, manuscript in preparation). Thus it is possible that this enzyme is involved in the cytoplasmic processing of genetic information. This is consistent

with reports that cytoplasmic DNA polymerase activity increases in response to cellular proliferation (Chang and Bollum, 1972b; Chang *et al.*, 1973).

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