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MONOVALENT CATION EFFECTS ON A DNA-SYNTHESIZING SYSTEM

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ABSTRACT

The rate of incorporation of tritiated thymidine into DNA, in a multienzyme (predominantly cytoplasmic) system derived from rat thymus, is strongly affected by the nature and concentration of certain monovalent cations, tested one at a time, in the incubation mixture. The data indicate a narrow pH-activity range with a pH optimum at 7.3. K^+, at 50-60 mM concentrations, can increase the rate of thymidine incorporation into DNA by a factor of 3.4 over that attained in its absence; Na^+, causes a 2.5-fold increase. Under optimum conditions, maximum stimulation of DNA synthesis by selected cations increases in the order, Li^+, Na^+, C_s^+, Rb^+, NH_4^+, and K^+. These are specific ion effects.

Under standard conditions, the rate of DNA synthesis catalyzed by this multienzyme system reflects the degree of denaturation of the DNA primer. Undenatured DNA exhibits little, if any, primer activity. After prolonged water-dialysis at 2° C, DNA acquires primer properties in a degree proportional to the amount of dialysis. For primer purposes, thermal denaturation (100° C for 7 minutes) is superior to water-dialysis (80 hours at 2° C).

Preliminary experimental evidence suggests that the K^+-dependence, demonstrated for this thymus (cytoplasmic) multienzyme system, is a property of the polymerase system present therein.

SUMMARY

The Problem:

The importance of intracellular enzymatic DNA synthesis, a necessary premitotic event, leads one to seek answers to a number of questions concerning factors influencing such synthesis in a controlled system:

(a) Is the synthesis of DNA in a representative multienzyme system (principally of cytoplasmic origin) significantly affected by the common biological univalent cations sodium and potassium; if so, how is synthesis affected and at what ion concentrations?

(b) Are such effects, specific or non specific ion effects?

(c) How does the pH of the incubation media and the degree of denaturation of the DNA primer influence such cation-dependent effects?

(d) Are these cation-dependent effects a property of the kinase or of the polymerase systems involved.

The Findings:

A dialyzed multienzyme preparation (e.g. the supernatant fraction derived by high speed centrifugation of a homogenate of rat thymus tissue) was incubated at 38°C in the presence of Mg^{++}, an energy source (e.g. ATP), a suitable DNA primer, along with the 5'-deoxynucleotides of adenine, guanine and cytosine, plus tritium labeled thymidine. In this system isotope is incorporated into DNA during the course of new DNA synthesis. The rate of new DNA synthesis was estimated by measuring the resulting isotope incorporation by a liquid scintillation counting method. The pH-activity curves determined in the presence of three separate energy sources indicated a rather narrow pH-activity range and a pH optimum at 7.3. All activities between 50 and 100 per cent of optimum occurred at pH values within the range of 6.8 to 7.8.

When the potassium ion concentration in the incubation mixture was increased progressively from 1 to 160 mM at pH 7.3, an increase in rate of DNA synthesis occurred, which attained a maximum at 50-60 mM ion concentration, 3.4-fold over that attained at 1 mM. Sodium ions caused a 2.5-fold increase under similar conditions. As the concentration of either ion was increased, a point was reached (138 mM for K^+ and 106 mM for Na^+) above which inhibition of DNA synthesis occurred. Potassium ions were more effective than sodium ions in stimulating DNA synthesis, this difference being more marked at pH 6.9, although the optimum pH for both cations occurred at pH 7.3.

Under standard experimental conditions the magnitude of maximum stimulation of DNA synthesis at pH 7.3 by Li^+, Na^+, Cs^+, Rb^+, NH_4^+, and K^+, tested separately, increased in the order named. These were specific cation effects and were not attributable to ionic strength. The rate of DNA synthesis catalyzed by this multienzyme system was shown to reflect the degree of denaturation of the DNA primer.

Preliminary experimental evidence is presented which suggests that the K^+-dependence, demonstrated for this thymus (cytoplasmic) multienzyme system, is a property of the polymerase system present therein.

INTRODUCTION

In one of their classical papers concerning DNA*synthesis by enzymes derived from E. coli, Kornberg and coworkers[1] reported the inhibitory effect of sodium chloride (97% inhibition at 0.20 M) on the activity of purified polymerase. We were interested in determining this effect on a mammalian DNA-synthesizing system over the entire range of salt concentrations common to mammalian organisms. In a multienzyme system derived from rat thymus tissue, we observed (preliminary note, Walwick and Main[2])that sodium ions inhibit over-all DNA synthesis as a function of concentration (e.g., 80% inhibition at 0.100 M, pH 8.0).

When the above multienzyme preparation is incubated at 38° C in the presence of Mg^{++}, an energy source (e.g., ATP) and a suitable DNA primer, along with the 5'-deoxynucleotides of adenine, guanine and cytosine, plus ^{3}H-labeled thymidine, the isotope is incorporated into DNA during the course of new DNA synthesis. As we will show, the rate of (^{3}H-labeled) DNA synthesis depends upon the nature and concentration of monovalent cations in the incubation medium, upon certain pretreatments of the DNA primer and, within relatively narrow limits, upon the pH. The experiments demonstrate a stimulation of DNA synthesis in this system by certain univalent cations in concentrations up to 40-60 mM, followed by inhibition as cation concentrations are increased above these values. Stimulation is most evident at the pH optimum 7.3.

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*Abbreviations: DNA, deoxyribonucleic acid; ^{3}HTdR, tritiated thymidine; ^{3}HTTP, tritiated thymidine-5'-triphosphate; DPN, diphosphopyridine nucleotide; Tris, tris (hydroxymethyl) aminomethane; Tris-OH, free base form of Tris; ATP, adenosine-5'-triphosphate; PEP, phosphoenolpyruvate; CPM, counts per minute, a measure of DNA synthesized under specific experimental conditions.

MATERIALS AND METHODS

Calf thymus DNA was obtained from Worthington Biochemical Corporation; ^{3}H-TdR (specific activity 0.36 C/mmole) from Schwarz Bio Research, Inc; tricyclohexylammonium PEP, carrier thymidine and the 5'-deoxynucleotides of cytosine, adenine and guanine from California Corporation for Biochemical Research; DPN, Tris, and disodium ATP from Sigma Chemical Co. ATP was converted to its Tris salt by passage through a column of Dowex-50 (X8, Tris form). Female Sprague-Dawley rats, 5-1/2 weeks old, were obtained from the Specific Pathogen-Free Colony of this Laboratory. All other chemicals were commercial products of analytical grade.

The multienzyme preparation from rat thymus tissue was prepared as described by Bollum and Potter[4], with the following exceptions: (1) KCl was omitted from the Tris-sucrose extraction fluid, and (2) the enzyme preparation was dialyzed. For example, 45 ml of this preparation in Visking tubing was dialyzed at 2°C in a rocking dialyzer against 2.5 l of Tris-sucrose solution (0.23 M sucrose, 11 mM Tris Cl buffer pH 8.0). The latter was changed hourly for three hours. DNA primer solution (66.6 mg DNA/100 ml cold H_2O) was dialyzed against four 2-liter portions of deionized H_2O at 1-2°C for periods stated in the legends to Figures 1-4. The sum of the Na^+ and K^+ concentrations introduced into the final incubation mixture via the combined dialyzed enzyme preparation and dialyzed DNA primer solution was less than 1 mM*. The protein concentration of the enzyme preparation, as determined by the method of Lowry, et al[5] was 9 mg/ml. Without further purification, multienzyme preparations were stored at -196°C until used.** DNA solutions were stored at -20°C.

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*By dialysis of the enzyme preparation, potassium ions were reduced from 20 mM to 2.0 mM; sodium ions from 2.0 mM to 0.2 mM (flame photometry). After a 24-hour dialysis period, Na^+ in the DNA primer solution were reduced from 3 mM to 0.3 mM. K^+ concentration was negligible. Further dialysis of the primer solution (e.g., 80 hours) reduced these ion concentrations to slightly lower values.

** As measured in terms of its ability to incorporate ^{3}HTdR into DNA under standardized conditions, this enzyme preparation was found labile. When stored at -20°C, it lost 50% of its initial activity by the 12th day; 65% by the 26th day; 70% by the 55th day. When stored at -196°C it lost none of its initial activity by the 55th day and only 23% by the 98th day.

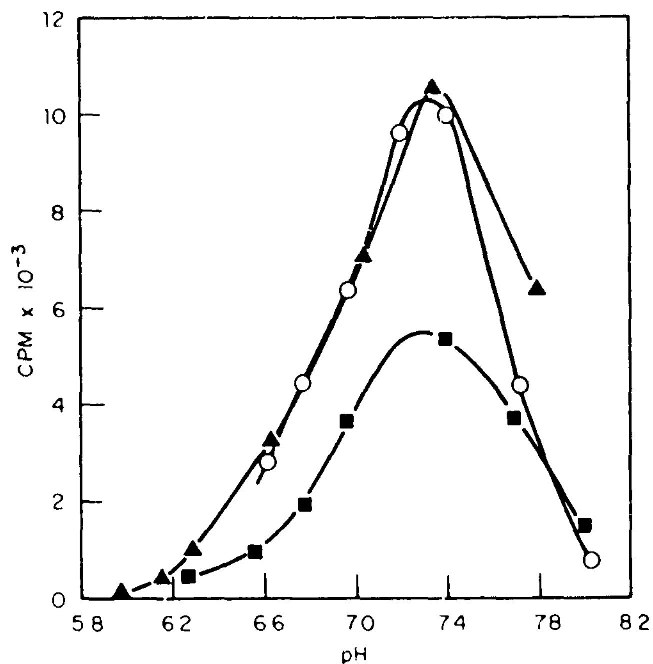

Fig. 1. Effect of varying the pH on the rate of ^{3}HTdR incorporation into DNA. The radioactivity of the total DNA isolated after 40 minutes incubation at 38°C is plotted against the pH of the incubation mixture. Energy substrates employed were: ——▲——▲—— , glucose 50 mM, potassium ATP 3 mM, and DPN 2.5 mM; ——○——○—— , tricyclohexylammonium PEP 10 mM, potassium ATP 0.5 mM; ——■——■—— , potassium ATP 3 mM. Phosphate buffer (Tris form, adjusted to desired pH with Tris-OH) was 20 mM; $MgCl_2$ 6 mM; each of the deoxynucleoside-5'--monophosphates of adenine, guanine and cytosine 50 µM; ^{3}HTdR 66 µM (37.5 mC/mmole). Concentrations indicated are those in complete incubation mixtures (0.50 ml). The complete incubation mixture also included 0.20 ml of multienzyme preparation (1.8 mg protein) and 0.15 ml DNA primer solution (100 µg DNA). In preparation as primer for this experiment alone, DNA solution was dialyzed (see text for details) against deionized H_2O for 56 hours at 2°C.

The experimental conditions for individual experiments as well as the composition of incubation mixtures are indicated in the legends to respective Figures. The components of each incubation mixture were combined in the cold, vortex mixed, and incubated in annealed pyrex glass tubes (Corning No. 9820, 10 x 75 mm) at 38° C. Incubations were stopped by chilling in an ice water bath followed by acidification. The method of isolation of the DNA and determination by liquid scintillation counting of radioactivity incorporated therein has been described[6]. Each incubation and determination was performed in triplicate. All experiments were performed at least twice, except that illustrated by Figure 4. A point for point duplication of results was obtained in repetitive experiments.

RESULTS

Determination of pH-Activity Curves for Rat Thymus Multienzyme System

Figure 1 illustrates the results obtained from experiments concerning pH-activity curves in the presence of three energy sources. The pH values (glass electrode) obtained for each point at 0 time, as compared with those at the end of 40 minutes' incubation, differed by no more than 0.05 pH unit. For most points these values were identical. The average of these two values (abscissa) was plotted against CPM, in thousands (ordinate). These three independent curves indicate a rather narrow pH-activity range and a pH optimum at approximately 7.3. All activities between 50 and 100 per cent of optimum occur at pH values within the range 6.8 to 7.8.

Effect of Dialysis of Primer (Unheated DNA) on DNA Synthesis at pH 7.4

Dialysis of the DNA primer solution at 1-2° C against deionized water caused both a lowering of the Na^+ concentration and a decrease in pH within the dialysis bag with time. Thus, at 0 time the pH was 7.0; after 24 hours dialysis, pH 5.1, after 48 hours, pH 4.5; after 80 hours, pH 3.9. Conditions of low ionic strength and/or low pH cause denaturation of DNA in aqueous solution[7-11], physically indistinguishable from that produced by heat denaturation[10]. Highly polymerized DNA preparations, found inactive as primers for purified calf thymus polymerase, become primers after denaturation by heating at 99° C for 10 minutes[12]. Fig. 2 illustrates the effect on our DNA synthesizing system of primers denatured to various degrees by water dialysis (for 0 hours, 24 hours, 48 hours and 80 hours). DNA synthesis, as affected by varying amounts of added K^+, was tested in the presence of these four primers. These data clearly show: (a) undialyzed (undenatured) calf thymus DNA exhibits little, if any primer

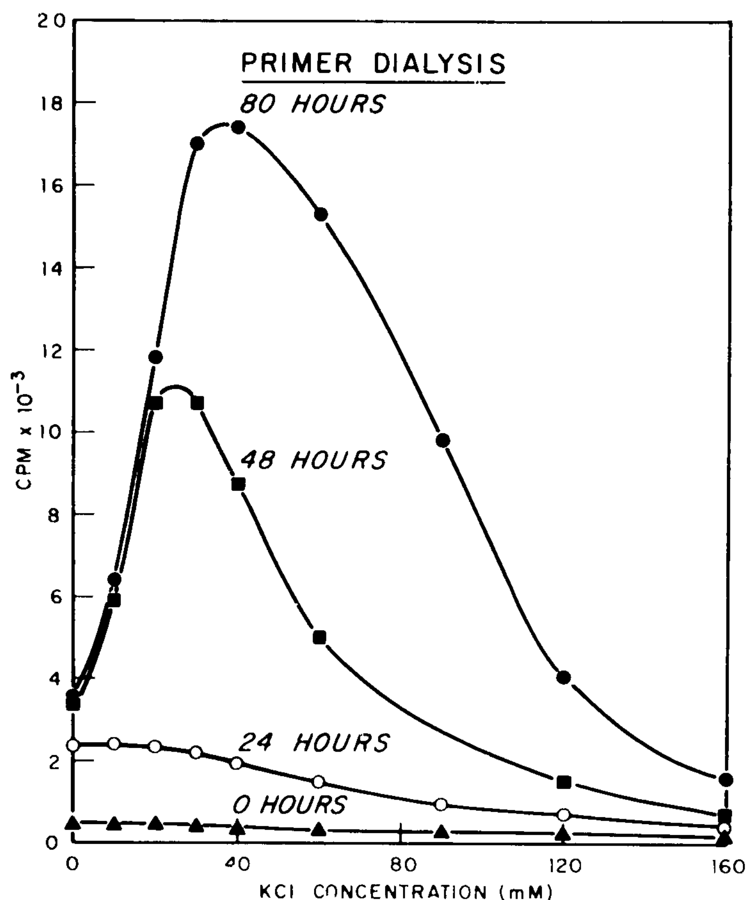

Fig. 2. The comparative effects of four primers (DNA, dialyzed against H_2O for differing periods of time) on the rates of ^{3}HTdR incorporation into DNA in the presence of various concentrations of K^+. The radioactivity (CPM) of the total DNA isolated after 70 minutes incubation at 38°C is plotted against the K^+ concentration in the incubation mixture. The incubation mixture (0.50 ml) contained: phosphate buffer (Tris form, pH 7.40), 5.0 mM; ATP (Tris form, pH 7.4), 6.0 mM; $MgCl_2$, 6.0 mM; each of the deoxynucleoside-5'-monophosphates of adenine, guanine and cytosine, 50 µM; and ^{3}HTdR (specific activity 75 mC/mmole), 66µM. The mixture also included 0.20 ml of multienzyme preparation (1.8 mg protein) and 0.15 ml DNA primer solution (100 µg DNA). The four primers were prepared by dialyzing solutions of DNA against deionized H_2O for 0 hours, 24 hours, 48 hours, and for 80 hours at 2°C (see text for details). Buffer capacity was sufficient to maintain the pH (glass electrode determinations) of incubation mixtures between the limits 7.40 (0 time) and 7.35 (70 minutes) of the incubation period.

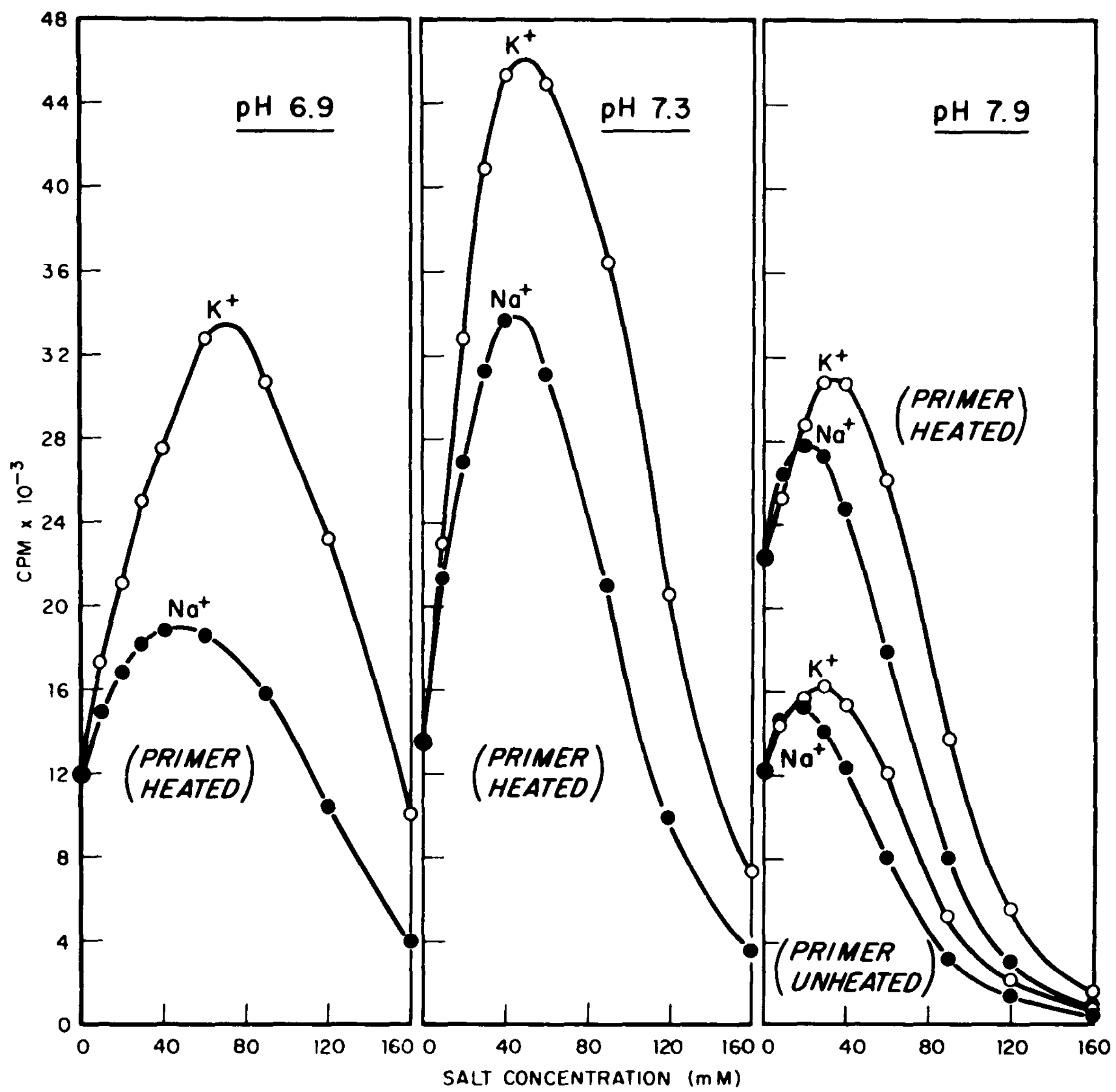
pH 6.9
pH 7.3
pH 7.9
K+
Na+
(PRIMER HEATED)
(PRIMER HEATED)
(PRIMER HEATED)
(PRIMER UNHEATED)
CPM x 10-3
SALT CONCENTRATION (mM)
0
4
8
12
16
20
24
28
32
36
40
44
48
0
40
80
120
160

Fig. 3. Comparative effects of K^+ and Na^+ on ^{3}HTdr incorporation into DNA at three pH values (6.90, 7.30, and 7.90) of the incubation mixture. The radioactivity (CPM) of the total DNA, isolated after 70 minutes incubation at 38°C, is plotted as a function of the salt concentration (mM). The final incubation mixture (0.500 ml) contained: phosphate buffer (Tris form) 5.0 mM; ATP (Tris form), 6.0 mM; $MgCl_2$, 6.0 mM; each of the deoxynucleoside-5'-monophosphates of adenine, guanine, and cytosine, 50 μM; ^{3}HTdR (specific activity 75 mC/m mule), 66 μM; and the chlorides of sodium and of potassium to produce the final concentrations shown in the figure. The mixture also included 0.200 ml of multienzyme preparation (1.8 mg protein) and 0.150 ml DNA primer solution (100 μg DNA). Additional incubation conditions specific for experiments illustrated by the left-hand curves (pH 6.9), by the middle curves (pH 7.3) and by the right-hand curves (pH 7.9) are as follows:

At pH 6.9. Solution (1), a mixture of ^{3}HTdR, ATP, $MgCl_2$, and the deoxynucleotides of adenine, guanine, and cytosine, was adjusted to pH 5.65 with 1 M Tris-OH and to millimolarities 5-fold their respective concentrations indicated in the final incubation mixture. Solution (2), of the chlorides of either sodium or potassium were prepared (pH 7.0) at 10-fold the final concentrations indicated in the figure. Solution (3), the multienzyme preparation was at pH 7.60. Solution (4), the DNA primer, was prepared from DNA solution which had been dialyzed against deionized H_2O for 80 hours at 1-2°C. To 10 ml of this solution (pH 3.9) were added 0.020 ml of 1 M Tris-OH and 0.165 ml of 1 M phosphate buffer (Tris form, pH 6.3). The resulting solution (pH 6.3) was heated at 100°C for 7 minutes and then rapidly cooled. The pH of the final incubation mixture comprising (Solution (1), 0.100 ml; Solution (2), 0.050 ml; Solution (3), 0.200 ml; and Solution (4), 0.150 ml)) was 6.90.

At pH 7.3. Solution (1) (as described in preceeding paragraph) was adjusted to pH 7.30 with 1 M Tris-OH. Solutions (2) and (3) remained unchanged. Solution (4) was adjusted to pH 7.30 with 1 M Tris-OH before heating as DNA primer. The pH of the final incubation mixture was 7.30.

At pH 7.9. Solutions (1) and (4) (see paragraph At pH 6.9) were adjusted to pH 7.90 with 1 M Tris-OH. The pH of the final incubation mixture was 7.90. For experiments illustrated by the upper curves, (Primer heated), Solution (4) after adjustment to pH 7.9 was heated as described previously. For experiments illustrated by the lower curves, (Primer unheated), Solution (4) was unheated.

activity in this system, (b) after water dialysis, DNA becomes a primer in degree depending on the amount of dialysis, (c) at certain concentrations K^+ stimulate the rate of DNA synthesis, and (d) the K^+ concentration at which peak stimulation occurs is affected (increased) by the degree of denaturation of the primer. The latter relationship (d) extends to DNA primer, obtained by 80-hour water dialysis, further denatured by thermal means. This is shown in the following section.

<u>Potassium and Sodium Ion Effects on Tritiated Thymidine Incorporation into DNA</u>

^{3}HTdR is utilized for DNA synthesis by multienzyme systems of cytoplasmic origin derived from mammalian cells[4,13]. In studying this incorporation, we compared the levels of ^{3}HTdR incorporation in K^+- and Na^+-containing incubation mixtures at different pH values. The results of this comparison are shown in Fig. 3, in which the radioactivity of the DNA isolated, after 70 minutes incubation, is plotted against the salt (KCl or NaCl) present in the incubation mixture. In the two lower curves (Primer Unheated) pH 7.9, the DNA primer had been denatured solely by water dialysis for 80 hours as described previously (Fig 2). In all other cases (vis., Primer Heated; upper curves at pH 7.9 and all those at pH 7.3 and 6.9), water-dialyzed DNA was further denatured by heating for 7 minutes at 100° C in preparation as primer. This additional (thermal) denaturation of the primer enhanced the maximum rates of DNA synthesis at pH 7.9 by a factor of 1.84 for either K^+ or Na^+ *.

Several things are evident. K^+ is more effective than Na^+ in stimulating the rate of ^{3}HTdR incorporation into DNA. This difference is most marked at pH 6.9, although the optimum pH for both cations occurs at 7.3. The range of concentration over which these cations are effective in stimulating the rate, becomes narrower as the incubation mixture is made more alkaline. Potasium ions at 50-60 mM

* As indicated in the legend to Figure 3, DNA (previously water-dialyzed for 80 hours) was heated <u>after</u> pH adjustment from 3.9 to the more alkine values indicated, in preparation as primer. In contrast, water-dialyzed DNA completely lost its primer properties with heating at 100°C for 7 minutes at pH 3.9. When the latter preparation was substituted for primer in the experiments described, no significant net radioactivity was found incorporated into DNA (CPM ranged between 0 and 29). This loss may be ascribed to depurinization[8, 14, 15] of the primer DNA during this latter treatment.

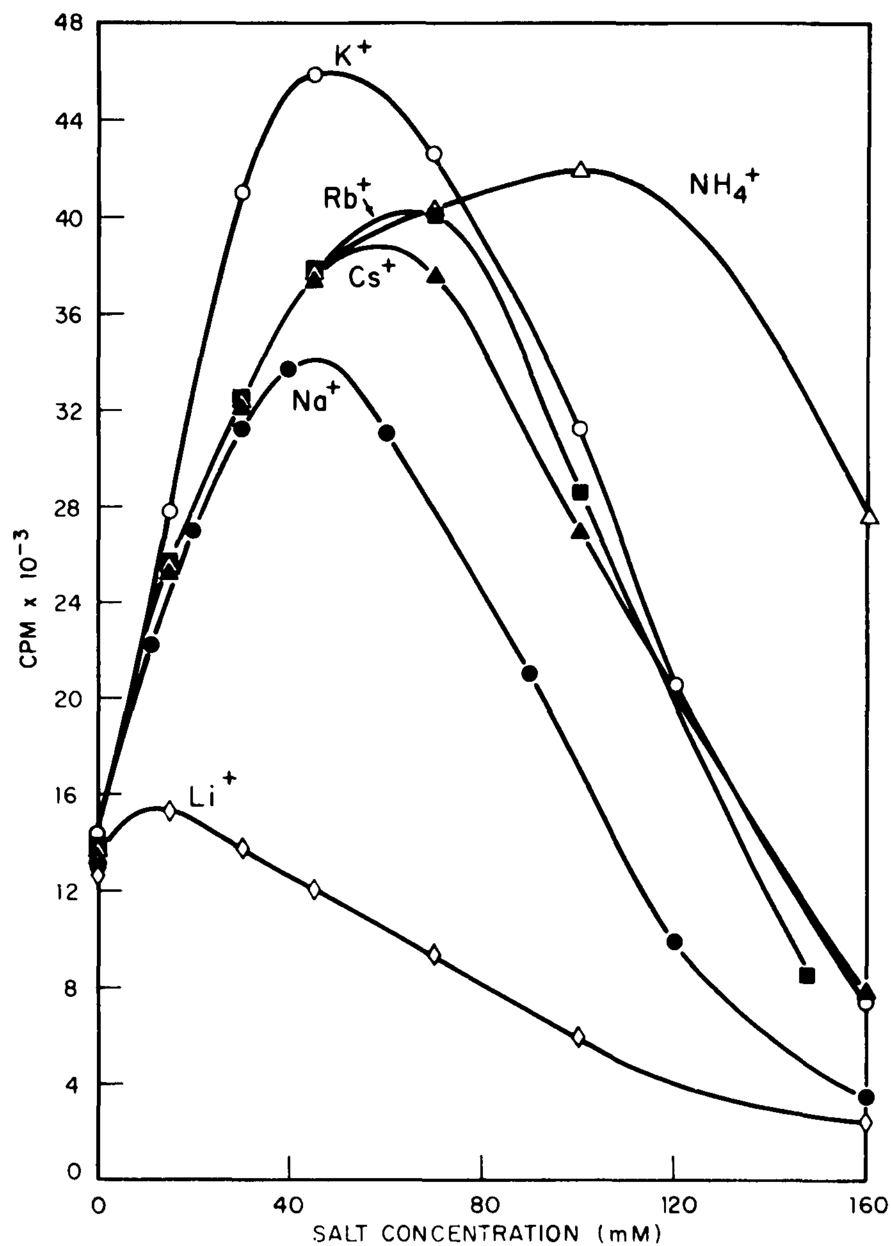

Fig. 4. Comparative effects of different monovalent cations on the incorporation of ^{3}HTdR into DNA at pH 7.30 of the incubation mixture. The specific ^{3}H-activity (CPM) of the total DNA, isolated after 70 minutes incubation, is plotted as a function of the salt concentration (mM). Incubation conditions as in legend to Fig. 3, At pH 7.3.

concentrations and at pH 7.3, can increase the rate by 3.4-fold over that attained in its absence; Na^+ causes a 2.5-fold increase under similar conditions. As the concentration of either ion is increased a point is reached (138 mM for K^+ and 106 mM for Na^+) above which inhibition of DNA synthesis now occurs. From a biological viewpoint, of most significance is the predominance of K^+ stimulation over that effected by Na^+.

Effects by Different Ions On DNA Synthesis

The effects of different monovalent cations on the rate of incorporation of ^{3}H-TdR into DNA at pH 7.3 are illustrated in Fig. 4. Under these experimental conditions the maximum magnitude of stimulation increases in the order Li^+, Na^+, Cs^+, Rb^+, NH_4^+, and K^+. It is clear that these are specific ion effects. *

DISCUSSION

The experiments described here are unique in several respects; (a) they are concerned with cation effects in the lower as well as in the upper range of K^+ and Na^+ concentrations commonly found in biological systems, (b) while buffer concentrations in incubation mixtures were kept to a minimum consistent with adequate pH control, and (c) rates of incorporation were determined at one pH value slightly above and one slightly below that (pH 7.3) which permits maximum stimulation of DNA synthesis by K^+ or Na^+.

Bollum[4] did not observe stimulation of DNA synthesis by monovalent cations in systems employing a partially purified calf thymus polymerase at pH 7.0. Although calf thymus polymerase conceivably could respond differently to monovalent cations than does rat thymus polymerase,

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* Most recently we have obtained evidence which suggests that the stimulation effect of K^+ demonstrated above is characteristic, in part, of the polymerase system in our dialyzed multienzyme preparation. In preliminary experiments to be reported in detail elsewhere, ^{3}HTTP was substituted for ^{3}HTdR and the deoxynucleoside 5'-triphosphates of adenine, guanine and cytosine were substituted for the deoxynucleoside 5'-monophosphates in equivalent amounts under experimental conditions as described in the legend to Fig. 3. For example, at pH 7.3 and with no K^+ in the incubation mixture, the count rate was 42,900 CPM. This increased to 80,800 in the presence of 45 mM K^+. Whether the kinases in our multienzyme preparation exhibit a similar sensitivity to monovalent cations remains to be determined.

Bollum's results also could be attributed to his choice of experimental design. In this work[4], he tested the effect of various salts ($CaCl_2$, NaCl, KCl, NH_4Cl, Tris-Cl buffer at pH 7, and KH_2PO_4 buffer at pH 7) which were added to 40 mM KH_2PO_4-K_2HPO_4 buffer (60 mM in K^+) pH 7.0. In our experiments, care was taken to achieve low levels of Na^+ and K^+ by dialysis of the enzyme preparation and DNA primer. Initial salt concentrations in general and Na^+ and K^+ concentrations in particular were kept low by using Tris-phosphate as a buffer at the lowest levels consistent with adequate pH control. Consequently, we were able to detect a stimulation of the DNA synthesizing system by K^+ at levels below 60 mM concentration. This K^+ level is approximately the concentration yielding maximum stimulation in our system. To the extent to which a comparison can be made, there appears to be no conflict between the experimental results of these two papers.

In an elegant study, Allfrey, Meudt, Hopkins and Mirsky[16] have shown that protein and nucleic acid synthesis in isolated calf thymus nuclei are Na^+-dependent processes and that this dependence reflects the operation of specific transport mechanisms, energy dependent and enzymatic in nature. Although they were able to detect and measure accurately a small amount of DNA synthesis in nuclei (by thymidine incorporation), it should be noted that they did not report Na^+ dependence to be a property characteristic of the DNA polymerase (or kinase) activity of isolated nuclei.

In a more speculative vein, discovery of such a property for nuclear polymerase would lead to the provocative conclusion that the DNA polymerase in nuclei possesses different properties from that found in cytoplasm. Smellie and Eason[19] recently have presented evidence of another type which tends to suggest that the small amount of DNA polymerase found in isolated nuclei, derived from Ehrlich and Landschurtz ascites cells, is not of nuclear origin.

The Na^+ requirement for amino acid and thymidine transport into a calf thymus intranuclear pool[16], contrasts with the usual observation that K^+ is the specific cofactor needed for the incorporation of amino acids into protein by isolated microsomal (cytoplasmic) systems[17], and for amino acid transport into intact (Ehrlich ascites) cells[18]. In like manner, the polymerase activity, described herein as predominantly K^+-dependent, was exhibited by an enzyme in the soluble cytoplasmic fraction. It is worthy of mention that the K^+ concentration range (50-60 mM) for maximum stimulation of thymidine incorporation in this cytoplasmic fraction compares favorably with that found for other K^+-dependent cytoplasmic processes[17] (maximum at

60-75 mM salt concentration) and for the Na^+-dependent nuclear processes[16] (maximum at 50-60 mM salt concentration) cited above. One is encouraged to investigate the possible influence of these monovalent cation concentrations, and of their regulation within the cell on intracellular DNA and protein synthesis.

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82	CO, Radiological Health Laboratory Division
83	Commander, Air Force Systems Command
84	Director, USAF Project RAND
85-86	Commandant, School of Aerospace Medicine, Brooks AFB
87	CO, School of Aviation Medicine, Gunter AFB
88	CO, 6571st Aeromedical Research Lab., Holloman AFB
89	Radiobiological Laboratory
90	Office of the Surgeon (SUP3.1), Strategic Air Command
91	Office of the Surgeon General
92	Commander, Special Weapons Center, Kirtland AFB
93	Director, Air University Library, Maxwell AFB
94-95	Commander, Technical Training Wing, 3415th TTG
96	Hq., Second Air Force, Barksdale AFB
97	Commander, Electronic Systems Division (CRZT)

OTHER DOD ACTIVITIES

98-100	Chief, Defense Atomic Support Agency (Library)
101	Commander, FC/DASA, Sandia Base (FCDV)
102	Commander, FC/DASA, Sandia Base (FCTG5, Library)
103	Commander, FC/DASA, Sandia Base (FCWT)
104	Armed Forces Institute of Pathology
105-114	Armed Services Technical Information Agency
115	Director, Armed Forces Radiobiology Research Institute

OCD

116	Office of Civil Defense, Battle Creek
117	Office of Civil Defense, Washington

118	Research Analysis Corporation
119	Life Science Officer, AEC, Washington
120	Division of Biology and Medicine (Benson)
121	NASA, Ames Research Center, Moffett Field
122	Naval Attache, Stockholm (for Commodore Troell)
123	Aerojet General, Azusa
124	Alco Products, Inc.
125-129	Argonne Cancer Research Hospital
130-139	Argonne National Laboratory
140-141	Atomic Bomb Casualty Commission
142	AEC Scientific Representative, France
143	AEC Scientific Representative, Japan
144-146	Atomic Energy Commission, Washington
147-150	Atomic Energy of Canada, Limited
151-153	Atomics International
154-155	Battelle Memorial Institute
156-159	Brookhaven National Laboratory
160	Chicago Patent Group
161	Columbia University (Rossi)
162	Committee on the Effects of Atomic Radiation
163-164	Convair Division, Fort Worth
165-167	Defence Research Member
168-169	duPont Company, Aiken
170	duPont Company, Wilmington
171	Edgerton, Germeshausen and Grier, Inc., Goleta
172	Edgerton, Germeshausen and Grier, Inc., Las Vegas
173-174	General Electric Company (ANPD)
175-182	General Electric Company, Richland
183	General Electric Company, St. Petersburg
184	Glasstone, Samuel
185	Hawaii Marine Laboratory
186	Hughes Aircraft Company, Culver City
187	Iowa State University
188	Journal of Nuclear Medicine
189	Knolls Atomic Power Laboratory
190	Lockheed Aircraft Corporation
191-192	Los Alamos Scientific Laboratory (Library)
193	Lovelace Foundation
194	Martin Company
195	Massachusetts Institute of Technology (Hardy)
196	Mound Laboratory
197	National Academy of Sciences
198	National Bureau of Standards (Taylor)
199	National Cancer Institute
200	National Lead Company of Ohio
201	National Library of Medicine
202	New York Operations Office
203	New York University (Eisenbud)
204	Oak Ridge Institute of Nuclear Studies

205	Patent Branch, Washington
206-207	Phillips Petroleum Company
208-211	Pratt and Whitney Aircraft Division
212-213	Public Health Service, Washington
214	Public Health Service, Las Vegas
215	Public Health Service, Montgomery
216	Sandia Corporation, Livermore
217	Union Carbide Nuclear Company (ORGDP)
218-222	Union Carbide Nuclear Company (ORNL)
223	Union Carbide Nuclear Company (Paducah Plant)
224	United Nuclear Corporation (NDA)
225	U.S. Geological Survey, Denver
226	U.S. Weather Bureau, Washington
227-229	University of California Lawrence Radiation Lab., Berkeley
230-231	University of California Lawrence Radiation Lab., Livermore
232	University of California, Davis
233	University of California, Los Angeles
234	University of California, San Francisco
235	University of Chicago Radiation Laboratory
236	University of Puerto Rico
237	University of Rochester (Atomic Energy Project)
238	University of Tennessee (UTA)
239	University of Utah
240	University of Washington (Donaldson)
241-244	Western Reserve University
245	Westinghouse Electric Corporation
246-270	Technical Information Service, Oak Ridge

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271-300	USNRDL, Technical Information Division

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MONOVALENT CATION EFFECTS ON A DNA-SYNTHESIZING SYSTEM by R.K. Main and E.R. Walwick 2 April 1962 20 p. illus. 19 refs.
UNCLASSIFIED

The rate of incorporation of tritiated thymidine into DNA, in a multienzyme (predominantly cytoplasmic) system derived from rat thymus, is strongly affected by the nature and concentration of certain monovalent cations, tested one at a time, in the incubation mixture. The data

(over)

1. Nucleic acids - Enzymatic synthesis.
2. Cations.
3. Ions.
I. Main, R.K.
II. Walwick, E.R.
III. Title.
IV. MR005-08-1200.

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Under standard conditions, the rate of DNA synthesis catalyzed by this multienzyme system reflects the degree of denaturation of the DNA primer. Undenatured DNA exhibits little, if any, primer activity. After prolonged water-dialysis at 2° C, DNA acquires primer properties in a degree proportional to the amount of dialysis. For primer purposes, thermal denaturation (100° C for 7 mins) is superior to water-dialysis (80 hours at 2° C).

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