THE RELATION OF POLYNUCLEOTIDE PHOSPHORYLASE AND GROWTH OF ESCHERICHIA COLI

by

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B. S., National Taiwan University, 1962

A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

In Genetics

KANSAS STATE UNIVERSITY
Manhattan, Kansas
1963

Approved by:

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INTRODUCTION

Polynucleotide phosphorylase was originally discovered in extracts of Azotobacter vinlandii by Grunberg-Manago and Ochoa in 1955. The availability of highly purified polynucleotide phosphorylase has permitted extensive investigations into its mechanism of action in vitro, but the in vivo role of this enzyme is not yet clear.

Lark et al., (1963) observed that the restoration of amino acids following amino acid deprivation reinitiated chromosome replication at the origin and suggested that some structural component was involved. Further, Lark and Lark (1964) demonstrated that this structural component was synthesized during thymine starvation, and that the synthesis of the component was resistant to 25 μg/ml of chloramphenicol. Since the synthesis of polynucleotide phosphorylase was also resistant to the presence of low concentration of chloramphenicol (Levin et al, 1963; Thang et al., 1963), polynucleotide phosphorylase may be related to this structural component.

This investigation was attampt to study the synthesis of polynucleotide phosphorylase in different conditions of growth. The physiologicol role of this enzyme may thus be made clearer through the information gained from this investigation.

LITERATURE REVIEW

Polynucleotide phosphorylase has been found in a wide variety of microorganisms and may also be present in certain mammalian cell nuclei (Hilmore and Heppel, 1957). This enzyme catalyzes the synthesis of polyribonucleotides from nucleoside 5'-diphosphates with elimination of in-

organic orthophosphate in the presence of Mg^{2+}. The reverse reaction, phosphorolytic cleavage of polynucleotides, is also catalyzed. Polyribonucleotides formed by the action of polynucleotide phosphorylase do not require the presence of DNA as a templete.

Mg^{2+} is absolutely required in the reactions which are catalyzed by polynucleotide phosphorylase. The optimal levels of Mg^{2+} depend on the ADP concentration and are generally attained at an ADP/Mg^{2+} ratio of 1.5 (Littauer and Kornberg, 1957) with purified E. coli enzyme.

In vitro, polynucleotide phosphorylase catalyzes the reactions of polyribonucleotide synthesis, phosphorolysis of polyribonucleotides, ^{32}P-exchange and arsenolysis of nucleoside diphosphates (Grunberg-Manago, 1963). Specificity is conferred by the nature of the sugar moiety of the nucleotide and the number of phosphates terminally esterified to the nucleoside. No reaction occurs when the ribonucleoside diphosphates are replaced by their corresponding deoxyribose derivatives or nucleoside mono- and tri-phosphates.

Highly purified preparations of polynucleotide phosphorylase obtained from Azotobacter agilis (Ochoa and Mii, 1961; Mii and Ochoa, 1957) and from Escherichia coli (Williams and Grunberg-Manago, 1964) catalyze the polymerization reaction only after an initial lag period. This lag phase may be eliminate by long-chain polynucleotides or oligonucleotides. Singer and his co-workers observed that long chains of polynucleotides were formed by the addition of monomer units to the 3'-hydroxyl-oligonucleotides, and that oligonucleotides with an esterified 3'-hydroxyl group overcame the lag period in the polymerization but were not incorporated into the polymer (Singer et al., 1957; 1960). Grunberg-Manago (1960) observed that ^{14}C-sRNA eliminated the lag phase in the polymerization of poly A but was not incorporated into

the poly A and could be recovered intact from the incubation mixture. It was subsequently observed that priming with polynucleotides showed a certain degree of specificity, i.e., stimulation of homopolymer synthesis by the addition of the corresponding, and not the complementary, homopolymer. The polymerization of ADP and UDP is inhibited by poly U and poly A respectively (Mii and Ochoa, 1957). This inhibition can be overcome by adding the corresponding homopolymer.

The rates of phosphorolysis of several different species of polyribonucleotides by polynucleotide phosphorylase were studied by Grunberg-Manago (1959). It was observed that biosynthetic polynucleotides containing only one base unit were phosphorolyzed very rapidly in the presence of Mg^{2+} and inorganic phosphate. The rate of poly G phosphorolysis was low. Polynucleotides containing four different bases, A, G, U and C, were phosphorolyzed at about one third of the homopolymer rate.

Several compounds have been found to be inhibitory. Littauer and Kornberg (1957) found Mn^{2+} and streptomycin to be inhibitory. The exchange rate of ^{32}Pi with ADP was increased by low concentrations of protamine whereas higher concentrations of protamine were inhibitory. Beers et al., (1958) found that acridine orange inhibited the synthesis and phosphorolysis of poly A. An inhibitory action of tetracyclines on polynucleotide phosphorylase has been observed by Fuwa and Okuda (1966). Several diphosphate analogs are inhibitory. Cardeilhac and Cohen (1964) reported complete inertness of arabinosyl cytosine diphosphate as a substrate for polynucleotide phosphorylase with marked inhibition of the polymerization of ADP and CDP into poly A and poly C respectively. Subsequently, Lucas-Lenard and Cohen (1966) reported induction of a lag by deoxycytidine 5'-diphosphate, deoxyadenosine

5'-diphosphate, and 9-β-D-arabinosyladenine 5'-diphosphate during the early part of the ADP and CDP polymerization which could not be eliminated by the addition of Mg^{2+}, poly A or free 3'-OH oligonucleotides. If these analogues were added after the onset of polymerization reaction, at which time some oligonucleotides had presumably been synthesized, the rate of release of inorganic phosphate suddenly dropped and a lag period ensued. This result suggested that the lag period did not stem from the slow formation of initial oligonucleotides. It was also observed that the ratio of inhibitor to substrate determined the extent of inhibition by the substrate analogues. These substrate analogues may compete with the substrate for active sites on the enzyme.

That the addition of RNA inhibits the phosphorolysis of poly A or poly U was first reported by Grunberg-Manago et al., (1956). Recently, Futai and Mizuno (1966) found sRNA and rRNA (ribosomal RNA) to be strikingly inhibitory. When added to an equal amount of poly A, sRNA caused 60% inhibition and rRNA 40% inhibition. This inhibition reached 80% when these RNAs were added in excess. Although the effect of DNA was negligible, it was observed that adenosine monophosphate, uridine monophosphate, guanosine monophosphate, deoxyadenosine monophosphate, thymine monophosphate, deoxyquanosine monophosphate and deoxycytosine monophosphate brought about 20% inhibition.

In crude bacterial extracts, the assay of enzyme activity is linear only during the first few minutes of incubation and linearity can be extended by the addition of polymer or by heating at 60°. Grunberg-Manago (1963) suggested that this phenomenon was due to the presence of nucleases which were inactivated at 60°. Subsequently, Lucas and Grunberg-Manago (1964) observed that purified enzyme from E. coli was heat stable below 55°. At

higher temperature there was a precipitous fall in enzymatic activity with nearly 100% inhibition at 65°. The rate of temperature inactivation was shown to decrease in the presence of either poly A or ADP.

The localization of the enzyme is uncertain, but it is usually found in the soluble portion of the cell. In Escherichia coli and Pseudomonas aeruginosa (Wade and Lovett, 1961; Strasdine et al., 1962), it was found that up to 40 % of the total polynucleotide phosphorylase was contained in the ribosomal fraction. Abrams and McNamara (1962) found most of the enzyme in Streptococcus faecalis located in cell membranes.

That the activity of the enzyme varied with the stage of growth was reported by Grunberg-Manago (1963). In E. coli B grown on peptone medium, polynucleotide phosphorylase activity was highest at the beginning of the logarithmic phase. In a tryptophanless E. coli strain, Levin et al., (1963) found that the polynucleotide phosphorylase activity and RNA content of bacteria treated with chloramphenicol both were increased two fold relative amounts of the enzyme synthesized in its absence, although protein synthesis was inhibited. This effect was tryptophan dependent and 5-methytryptophan could replace tryptophan, even though this compound did not support growth.

Subsequently, Thang et al. (1963) have shown that the effect of chloramphenicol in increasing polynucleotide phosphorylase activity was not due to change in substrate concentration, the presence of chloramphenicol, tryptophan, RNA or other similar factor. If 5-methyltryptophan replaced tryptophan in a tryptophanless E. coli culture, the increment of polynucleotide phosphorylase was a function of concentration of chloramphenicol up to 4 to 5 μg/ml. With synchronous cultures of A. agilis, a parallel has also been found between the level of the enzyme and RNA synthesis during various con-

dition of growth (Grunberg-Manago, 1963).

Since it is known that messenger RNA is very unstable and in cell-free extracts of E. coli it is degraded to 5'-ribonucleotides, Grunberg-Manago (1963) has postulated that in vivo the enzyme might be primarily responsible for the degradation of RNA to yield nucleoside diphosphates and further to control the level of inorganic phosphate of the cell. This enzyme might thus be the most efficient system for generating DNA precursors from RNA and for eliminating the information contained in mRNA.

MATERIAL AND METHODS

Bacterial Strain and Growth Conditions

The quadruple auxotroph of Escherichia coli $15T^-$ requiring thymine, methionine, tryptophan and arginine was used in all experiments except when otherwise indicated. It was grown at 37° in M9 minimal salts medium (Na_2HPO_4, 0.7%; KH_2PO_4, 0.3%; NaCl, 0.5%; NH_4Cl, 0.1%; glucose, 0.4%; $CaCl_2$ 0.002%; $MgSO_4$, 0.02%, adjusted to pH 7 to 7.2) supplemented with 20 µg/ml thymine, 140 µg/ml L-tryptophan, 340 µg/ml L-arginine and 300 µg/ml L-methionine. The following compounds were used to replace glucose as an energy source: sodium succinate (succinate medium), final concentration 0.09%; aspartic acid (aspartate medium) neutralized to pH 7.0 with NH_4OH, final concentration 0.1%. The L-broth was used as a complete medium (yeast extract 1.0%; L-tryptophan, 0.5%; NaCl, 0.5%; glucose, 0.4%, adjusted to pH 7) supplemented with thymine and required amino acids. When DL-5-methyltryptophan was used to replace L-tryptophan, the final concentration of DL-5-methyltryptophan was 40 µg/ml.

E. coli 15T⁻U⁻ was grown in M9 glucose medium plus 20 μg/ml of uracil, 20 μg/ml thymine and the required amino acids.

Fresh cultures were prepared by diluting the over-night culture with fresh medium of the same composition as the original medium. All experiments were started when the culture titer reached 2×10^8 cells/ml. Changes of media were accomplished by collecting and washing cells on a membrane filter and resuspending them in pre-warmed fresh medium.

Preparation of Bacterial Extracts

Samples were taken from the culture and immediately frozen in an ethanol-dry ice bath and preserved in dry ice. The preserved sample was thawed and put in an ice bath. All procedures were carried out at 0-4°. The cells were collected by centrifugation, washed once with 0.01 M Tris buffer (pH 7.4) and resuspended in 0.01 M Tris buffer. The cell titer was measured in a Coulter Counter. Toluene, 5 μl, was added to one ml of bacterial suspension contained in a stoppered test tube and mixed for 20 seconds on a vibrator. The tube was then incubated for 10 minutes at 37° and cooled quickly in an ice bath. In order to eliminate inhibitory reactions and to obtain a linear reaction curve, 0.5 ml of the toluene-treated bacterial suspension was placed in a test tube and heated for 5 min. at 60°. The heated and unheated bacterial suspensions were used directly for enzyme assay.

Assay of Polynucleotide Phosphorylase

The activity of polynucleotide phosphorylase was measured by the incorporation of (^{3}H)ADP into the acid-insoluble precipitate. Each assay tube contained 20 μl of 0.01 M Tris buffer (pH 8.1), 40 μl of 0.01 M Mg^{2+}, and

20 µl of 0.01 M EDTA (Ethylenediaminetetraacetate). A 0.2 ml aliquot of heated or unheated toluene-treated bacterial suspension was added directly to the assay tube. Sixty µl of 32 mM (^{3}H)ADP (specific activity 3.2×10^4 counts/min/umole) were added to the tube which was incubated immediately at 30°. Fifty µl sample aliquots were removed at 10 min. intervals and quickly placed into an ice cold test tube containing 1.0 ml of 5% TCA (Trichloroacetic acid) and one drop of dupanol. The reaction was allowed to continue for 40 min. The precipitate was collected by membrane filtration and washed with 2% cold TCA, followed by water. The radioactivity was counted in a Packard Tricarb scintillation spectrometer, using an organic scintillation fluid (3 g of 2,5 diphenyl-oxazole and 300 mg 1,4-bis-2-4-methyl-oxazolebenzene dissoved in one liter toluene). The specific activity of enzyme was calculated as µmoles of (^{3}H) ADP incorporated per 30 min. per mg of protein or per 10^8 cells.

For every sample, the kinetics of the enzyme activity was studied. Linearity was obtained when the bacterial extract was pre-heated at 60° for 5 min. The enzyme activity without pre-heating leveled within 30 min. Fig. 1 showed the effect of pre-heating on enzymatic activity. The sample was taken from a glucose culture during exponential growth.

It was assumed that the enzyme activity of an unheated sample was linear for the first ten min. and reached a plateau within 40 min. The initial specific activity of unheated samples was calculated from the enzyme activity at 10 min. after (^{3}H) ADP had been added to reaction mixture. The level of enzyme activity of such unheated samples was calculated from the highest value of (^{3}H) ADP incorporation within 40 min.

The inhibitory effectiveness in each sample is expressed by the heated activity divided by the initial activity and the heated activity

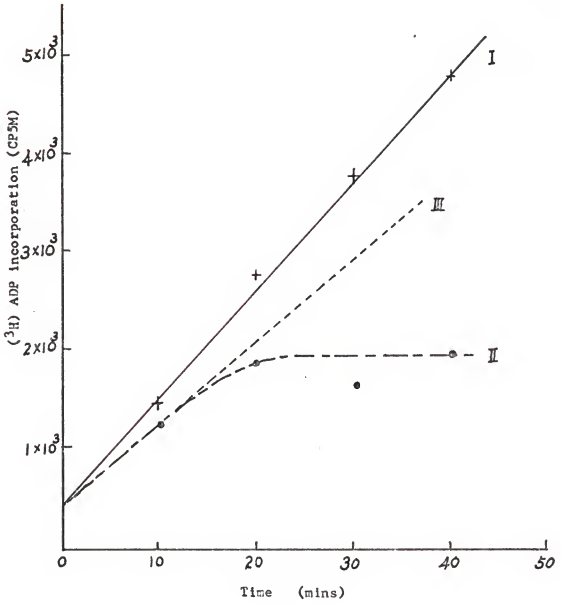

Fig. 1. The effect of heat on the activity of polynucleotide phosphorylase of E. coli. Samples were taken from a glucose culture ($2x10^3$ cells/ml). Curve I. toluene treated bacterial extract pre-heated 60° for 5 min. Curve II. without pre-heating. Curve III. initial unheated activity.

divided by the plateau activity.

Assay of Protein

Total protein was measured by the method of Lowry et al. (1951), using bovine serum albumine as a standard. To 20 µl of a toluene-treated bacterial suspension, 0.1 ml of 1 N NaOH was added and incubated for one hour at 37°. One ml of alkaline sodium carbonate (1.0 ml of a solution of 0.5% $CuSO_4 \cdot 5H_2O$ in 1.0% Na-K tartrate mixed with 50 ml of 2% Na_2CO_3) was then added, mixed well, and allowed to stand for 10 min. at room temperature. To this, 0.1 ml of 1 N phenol reagent was added with immediate mixing. The color development was allowed to proceed for 30 min. at room temperature, and samples were read with a Zeiss spectrophotometer at 750 mµ.

Assay of β-Galactosidase

Thiomethyl-β-D-galactoside was added as an inducer to the bacterial culture used for induced β-galactosidase assay. The final concentration of inducer was 2×10^{-3} M.

One ml of the frozen bacterial culture was thawed and placed in an ice cold tube. To this, 5 µl of β-mercaptoethanol was added, followed by the addition of one drop of toluene, and the tube was mixed on a vibrator for ten sec.. It was then incubated in a 37° water bath for 30 min.

Two hundred µl of the toluene treated sample was removed and placed in a tube containing 100 µl of H_2O. Fifty µl of a 5 mg/ml solution of o-nitrophyenyl-β-D-galactoside was added to the tube, then, the tube was immediately incubated at 30°. The reaction was stopped by adding 150 µl of 1.0 M Na_2CO_3. Color was measured at 420 mµ with a Zeiss spectrophoto-

meter. Enzymatic activity was calculated as millimicromole of nitrophenol produced/min/ml (one millimicromole/ml of o-nitrophenol has an optical density of 0.0075) (Pardee et al., 1959).

EXPERIMENTAL PROCEDURES AND RESULTS

Effect of Chloramphenicol

The ability of cells to synthesize enzyme in the presence of chloramphenicol was investigated. A fresh culture (titer = $2x10^8$ cells/ml) was divided into 5 equal aliquots. Chloramphenicol (CAP) (concentration 2 mg/ml) was added to each. The final concentration of CAP in each culture was 2 μg/ml, 5 μg/ml, 8 μg/ml, 10 μg/ml, and 25 μg/ml respectively. Cultures were incubated at 37° in a shaking water bath and samples taken at intervals. These experiments were performed with cultures in broth, glucose and succinate medium. Results are shown in Tables 1, 2, and 3.

In a glucose culture containing chloramphenicol, polynucleotide phosphorylase activity per mg of protein increased as the incubation time increased. As CAP concentration approached 25 μg/ml, the increase in enzyme activity was less. It has been observed that CAP at low concentration permitted growth at a reduced rate, whereas concentrations of CAP of 10 μg/ml or higher, stopped cell division within 40 min. in glucose culture.

In broth cultures (Table 2), with 2 μg/ml CAP the activity per mg of protein and per 10^8 cells of heated preparations decreased as the incubation time increased. At a chloramphenicol concentration of 5 μg/ml, the enzyme activity per mg of protein of heated preparation remained almost constant, but the enzyme activity of unheated preparation increased. It is also

Table 1. Effect of chloramphenicol on glucose culture.
When a fresh glucose culture titer reached 2×10^8 cells/ml, sixty ml of the culture was transferred to each of five pre-warmed flask containing chloramphenicol. The final concentration of CAP in each flask was 2 μg/ml, 5 μg/ml, 8 μg/ml, 10 μg/ml, and 25 μg/ml respectively. Cultures were continously incubated. Ten ml samples were taken at intervals as indicated. Polynucleotide phosphorylase activity and protein were measured.

CAP concentration (μg/ml)	Time after adding CAP (mins)	(^{3}H)ADP incorporation/30' per 10^8 cells	per mg protein			Heated Initial	Heated Level	μg protein 10^8 cells
		Heated	Heated	Unheated (Initial)	(Level)			
2	0'	2.82	0.58	0.35	0.14	1.66	4.15	47.5
	40'	3.86	0.86	0.38	0.19	2.26	4.53	45.6
	80'	4.23	0.86	0.54	0.19	1.58	4.53	52.0
	120'	5.50	1.06	0.64	0.33	1.66	3.20	52.6
	180'	4.86	1.06	0.64	0.20	1.66	5.30	42.5
5	0'	3.89	0.78	0.61	0.21	1.28	3.72	51.2
	40'	5.15	1.21	1.14	0.70	1.06	1.73	40.6
	80'	7.20	1.99	1.60	1.86	1.24	1.07	38.0
	120'	8.65	2.28	1.63	1.60	1.40	1.42	38.9
	180'	7.15	2.15	1.50	1.61	1.43	1.42	34.2
8	0'	2.90	0.64	0.43	0.16	1.52	4.00	51.6
	40'	4.65	0.89	0.54	0.26	1.65	3.42	51.2
	80'	5.38	1.18	0.93	0.83	1.28	1.41	46.6
	120'	5.43	1.41	1.51	0.93	1.23	1.52	38.7
	180'	6.58	1.28	1.60	0.86	0.80	1.48	52.0
10	0'	2.37	0.48	0.24	0.11	2.00	4.36	51.0
	40'	2.94	0.77	0.45	0.36	1.71	2.14	38.0
	80'	4.15	1.02	0.67	0.57	1.52	1.79	40.2
	120'	3.90	0.99	1.12	no level	0.88	no level	41.2
	180'	4.85	1.28	0.99	no level	1.29	no level	38.8
25	0'	2.08	0.42	0.26	0.10	1.62	4.20	49.6
	40'	2.56	0.61	0.26	0.26	2.35	2.35	49.0
	80'	2.17	0.61	0.42	0.29	1.45	2.10	35.8
	120'	2.27	0.58	0.19	0.19	3.65	3.65	33.4
	180'	2.46	0.74	0.42	0.32	1.76	2.30	34.0

Table 2. Effect of chloramphenicol on the ability of L-broth culture to synthesize polynucleotide phosphorylase. Experimental conditions were as described in Table 1.

CAP concentration (µg/ml)	Time after adding CAP (mins.)	(^{3}H) ADP incorporation µmoles/30' per 10^8 cells	per mg protein			Heated Initial	Heated Level	µg protein 10^8 cells
		Heated	Heated	Unheated (Initial)	Unheated (Level)			
2	0'	9.2	1.12	1.09	0.62	1.03	1.81	75.0
	20'	6.4	1.04	0.93	0.97	1.12	1.07	63.0
	40'	6.4	1.14	0.90	0.97	1.27	1.17	59.0
	80'	4.95	0.83	0.80	no level	1.00	-	62.0
	120'	5.28	0.80	0.80	:	1.00	-	66.0
	180'	3.81	0.67	0.67	:	1.00	-	57
5	0'	11.30	1.10	0.70	0.63	1.57	1.78	70.0
	20'	8.3	1.19	1.21	0.95	0.98	1.25	55.0
	40'	6.6	1.07	1.23	no level	0.87	-	53
	80'	6.55	1.05	1.80	:	0.58	-	53
	120'	6.30	1.00	1.75	:	0.57	-	52
	180'	5.5	0.91	1.75	:	0.52	-	45
10	0'	9.3	1.09	0.99	0.61	1.10	1.79	82
	20'	7.6	1.05	0.99	0.80	1.06	1.31	71
	40'	4.8	0.88	1.09	no level	0.81	-	54
	80'	4.6	0.91	1.28	:	0.71	-	50
	120'	5.9	0.92	0.93	:	0.99	-	63
	180'	4.4	0.69	0.99	:	0.69	-	62
25	0'	10.9	1.12	0.93	0.60	1.21	1.87	96
	20'	6.95	1.05	0.80	0.70	1.32	1.50	67
	40'	6.36	0.99	1.38	0.86	0.76	1.15	66
	80'	4.2	0.70	0.90	0.90	0.78	0.78	59
	120'	4.7	0.82	0.77	no level	1.07	-	54
	180'	3.3	0.85	0.86	:	1.99	-	39

Table 3. Effect of chloramphenicol on the ability of succinate culture to synthesize polynucleotide phosphorylase. Experimental conditions were as described in Table 1.

CAP concentration (μg/ml)	Time after adding CAP (mins.)	(^{3}H) ADP incorporation μmoles/30' per 10^8 cells	per mg protein			Heated Initial	Heated Level	μg protein 10^8 cells
			Heated	Unheated (Initial)	Unheated (Level)			
2	0'	2.7	0.74	0.29	0.13	2.56	5.68	37.4
	40'	2.9	0.83	0.42	0.38	1.98	2.18	34.4
	80'	3.1	0.93	0.90	0.87	1.03	1.07	34.6
	120'	3.1	1.02	0.64	0.89	1.59	1.28	31.2
	180'	3.0	0.90	0.90	no level	1.00	-	33.2
5	0'	2.4	0.73	0.70	0.15	1.00	4.86	33.4
	40'	3.0	1.05	0.38	0.42	2.76	2.45	28.7
	80'	3.7	1.14	0.54	0.48	2.11	2.37	31.8
	120'	3.1	1.05	0.80	0.51	1.31	2.06	29.0
	180'	2.5	1.12	0.70	0.53	1.60	1.93	24.4
8	0'	3.0	0.92	0.48	0.29	1.91	3.17	32.8
	40'	2.6	0.93	0.61	0.58	1.52	1.6	28.0
	80'	2.7	0.83	0.61	0.43	1.36	1.93	31.8
	120'	2.9	0.83	0.61	0.58	1.36	1.43	35.2
	180'	4.3	1.21	0.64	0.48	1.89	2.52	34.2
10	0'	2.43	0.77	0.42	0.20	1.83	3.85	32.2
	40'	2.33	0.80	0.29	0.27	2.76	2.96	29.0
	80'	2.80	1.02	0.70	0.38	1.46	2.69	27.4
	120'	3.7	1.15	0.55	0.51	2.10	2.26	31.4
	180'	4.2	1.24	0.90	0.48	1.38	2.59	37.2
25	0'	3.6	0.96	0.61	0.45	1.57	2.13	37.2
	40'	2.3	0.80	0.45	0.26	1.78	3.08	28.2
	80'	2.0	0.80	0.54	0.35	1.48	2.29	25.0
	120'	2.2	0.93	0.96	0.48	0.97	1.49	23.4
	180'	2.0	0.77	0.54	0.35	1.43	2.20	25.6

shown that the activity of unheated enzyme did not level out in column 3 of Table 2.

Table 3 shows that chloramphenicol also induced a small but consistant preferential synthesis of polynucleotide phosphorylase in succinate culture.

In order to examine the possibility that changes of polynucleotide phosphorylase activity and total protein/cell were simply the result of an effect of residual CAP in the incubation mixture on (^{3}H) ADP incorporation, samples were taken before and immediately after CAP addition. The enzyme activity and protein/cell in the presence and absence of chloramphenicol are shown in Table 4. Although in the presence of chloramphenicol, protein/cell tends to be higher and the level of activity of the unheated perparations tends to be slightly decreased, these changes do not account for the observed effects of incubation in chloramphenicol seen in Table 1, 2, and to a lesser degree in Table 3.

The Relation of Polynucleotide Phosphorylase Activity to Growth Rate

Different growth rates were observed for the organisms when grown in different culture media. In broth medium, the generation time of E. coli was about 20 min., in glucose medium 40 min., in succinate 70 min., and in aspartate 120 min. The levels of polynucleotide phosphorylase of cells which were grown in different culture media were investigated. E. coli was grown in L-broth, glucose, succinate and aspartate medium. When the culture titer reached 2×10^8 cell/ml, samples were taken for the enzyme and protein assays. The results are shown in Table 5.

Cells grown in L-broth medium had the highest polynucleotide phosphorylase activity per mg of protein and per 10^8 cells. There was no dif-

Table 4. Effect of chloramphenicol on the incorporation of (^{3}H) ADP into acid insoluble material. Fresh cultures were grown to $2x10^9$ cells. Samples were taken immediately before and after adding different concentration CAP. Polynucleotide phosphorylase and protein were measured.

CAP concentration (µg/ml)	(^{3}H) ADP incorporation µmoles/mg protein/30' Heated	Unheated (Initial)	Unheated (Level)	Heated / Initial	Heated / Level	µg protein / 10^8 cells
Glucose culture						
0	0.78	0.42	0.26	1.85	3.00	42.0
2	0.58	0.35	0.14	1.66	4.15	47.5
5	0.78	0.61	0.21	1.28	3.72	51.2
8	0.64	0.42	0.16	1.52	4.00	51.6
10	0.48	0.24	0.11	2.00	4.36	51.0
25	0.42	0.26	0.10	1.62	4.2	49.6
Succinate culture						
0	0.88	0.53	0.24	1.66	3.66	33.6
2	0.74	0.29	0.13	2.56	5.68	37.4
5	0.73	0.70	0.15	1.00	4.86	33.4
8	0.92	0.48	0.29	1.90	3.17	32.8
10	0.77	0.42	0.20	1.83	3.85	32.2
25	0.96	0.61	0.45	1.57	2.13	37.2
L-broth culture						
0	1.11	0.77	0.64	1.44	1.74	67.0
2	1.12	1.09	0.62	1.03	1.81	64.0
5	1.10	0.7	0.62	1.57	1.78	70.0
10	1.09	0.99	0.61	1.10	1.79	81.5
25	1.12	0.93	0.60	1.21	1.87	95.5

Table 5. Polynucleotide phosphorylase activity of E. coli grown on different media.

Medium	(^{3}H) ADP incorporation μmoles/30' per 10^9 cells	per mg protein			Heated Initial	Heated Level	μg protein 10^9 cells
	Heated	Heated	Unheated	(Initial) (Level)			
L-broth	9.3	1.35	0.75	0.69	1.82	1.96	65.1
Glucose	3.3	0.76	0.4	0.19	1.9	4.00	45.3
Succinate	3.3	0.88	0.53	0.24	1.66	3.66	37.7
Aspartate	1.8	0.70	0.52	0.32	1.34	2.18	28.3

Table 6. Polynucleotide phosphorylase activity of E. coli grown in broth medium. Cell counts were made at the time samples were taken.

Time (mins)	cell count (10^8/ml)	(^{3}H) ADP incorporation μmoles/30' per 10^9 cells	per mg protein			Heated Initial	Heated Level	μg protein 10^9 cells
		Heated	Heated	Unheated	(Initial) (Level)			
0'	2.2	7.55	1.11	0.77	0.64	1.44	1.74	67.0
20'	4.4	6.90	1.09	0.61	0.64	1.78	1.70	64.0
40'	6.9	6.30	0.99	0.64	0.74	1.54	1.33	63.4
80'	8.5	4.40	0.74	0.45	0.45	1.64	1.64	61.6
120'	10.0	2.85	0.43	0.22	0.29	1.85	1.65	64.4
180'	10.8	1.70	0.29	0.29	0.13	1.00	2.23	59.0

ference in the enzymatic activity per mg of protein of cells grown on glucose, succinate and aspartate cultures, though cells grown in aspartate medium had a lower enzyme activity per 10^8 cells.

Table 6 shows, in L-broth culture, polynucleotide phosphorylase activity was highest during the early stage of exponential growth, but decreased very rapidly as the culture became older.

Shift-up and Shift-down Experiments

Maaløe and his co-workers (1958) investigated cell size and chemical composition as a function of medium composition during balanced growth. Subsequently, Kjeldaard et al. (1958) reported that after a change of medium from one which supported a slow growth rate to one which permitted a faster growth rate (shift-up), RNA synthesis increased instantaneously, whereas the rate of DNA and protein synthesis increased slowly. The pre-shift cell division rate was maintained for a period and then shifted abruptly to the new rate. After a change of medium from one which supported a faster growth rate to one which supported a slow growth rate (shift-down), RNA and protein synthesis both stopped almost completely but cell division and DNA synthesis were maintained at the pre-shift rate for a short period. The ability of cells to synthesize enzyme during the transition period of shift-up and shift-down was investigated.

Shift-up experiments were performed on cultures shifted from glucose or succinate or aspartate media to broth. After the shift, polynucleotide phosphorylase activity, protein per cell and cell number were measured at intervals. The results are shown in Fig.2, 3, 4 and Tables 6, 7, 8.

After a shift-up, the total protein of the cell maintained the pre-

shift value for a while and then increased rapidly to reach a new level. Polynucleotide phosphorylase activity per mg protein and per 10^9 cells was increased immediately. Cell division rates were maintained for 60 min. and increased at the time when polynucleotide phosphorylase activity reached a new level.

Shift-down experiments were performed on a broth culture shifted to glucose medium, a glucose culture shifted to succinate medium, a glucose culture shifted to aspartate medium and a succinate culture shifted to aspartate medium. The results are shown in Fig. 5, 6, 7, 8 and Tables 10, 11, 12 and 13.

When balanced growing cells were transferred from broth medium to glucose medium, polynucleotide phosphorylase activity and protein content of cells dropped in 30 min, whereas cell division rate maintained the pre-shift rate for a short period and then slowed down. Similar results were also found when a glucose culture was shifted to succinate medium, when a glucose culture was shifted to aspartate medium, and when a succinate culture was shifted to aspartate medium. However, the polynucleotide phosphorylase activity per mg protein did not change signifigantly over the period measured.

In order to investigate the effect of medium transfer on polynucleotide phosphorylase activity and protein content of cells, samples were taken before and immediately after medium change by filtration. Table 14 shows that medium changes do not affect the value of polynucleotide phosphorylase activity and protein content of samples which were pre-heated. However, the unheated polynucleotide phosphorylase level activity was affected.

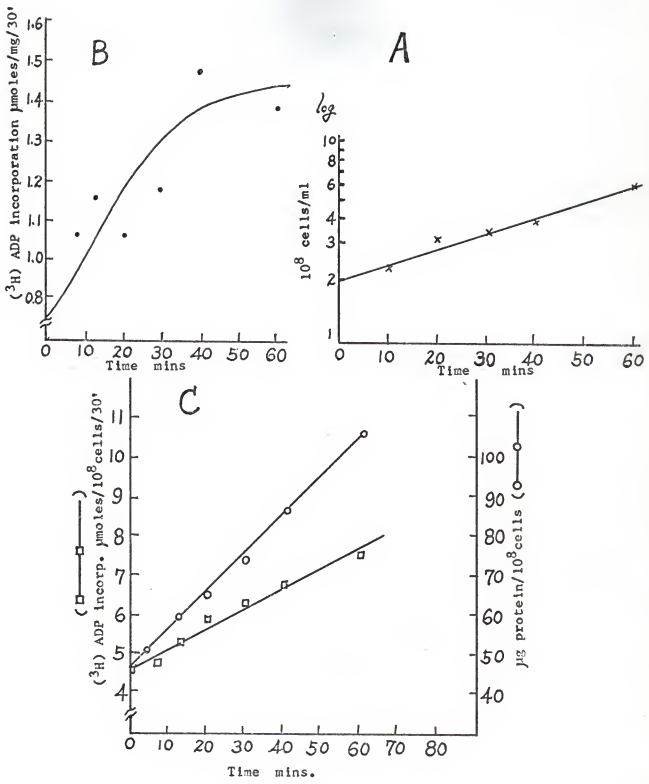

Fig. 2. Synthesis of protein, enzyme and increase of cell number when the E. coli culture shifted from glucose medium to L-broth medium. A.cell number; B. enzyme per protein; C. enzyme/10^8cells and protein/10^8cells

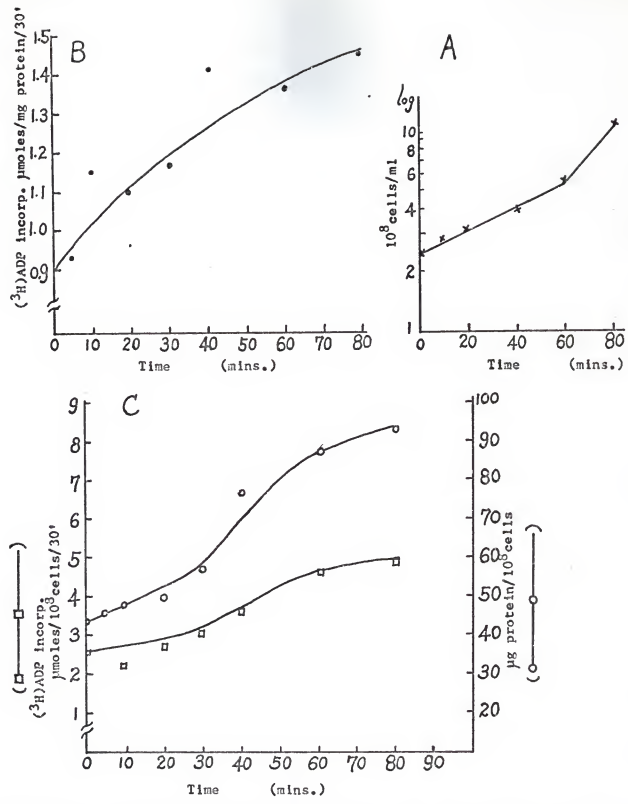

Fig. 3. Synthesis of protein, polynucleotide phosphorylase and increase of cell number when the E. coli culture shifted from succinate medium to L-broth medium at time zero. A. cell number; B. enzyme activity/mg protein; C. enzyme activity/10^8cells and µg protein/10^8cells.

Table 7. *E. coli* culture shifted from glucose medium to L-broth at time zero. Enzyme activity and protein determined at intervals.

Time after shift (mins)	(^{3}H) ADP incorporation μmoles/mg protein/30' Heated	Unheated (Initial)	Unheated (Level)	Heated Initial	Heated Level	μg protein 10^8cells
0'	0.99	0.51	0.58	1.94	1.70	46.3
5'	0.99	0.51	0.42	1.94	2.36	46.3
8'	1.06	0.70	0.59	1.52	1.83	48.5
12'	1.15	0.70	0.54	1.64	2.13	52.5
20'	1.06	0.58	0.48	1.83	2.21	59.3
30'	1.17	0.74	0.58	1.58	2.02	63.0
40'	1.47	1.09	0.93	1.35	1.58	66.6
60'	1.38	0.07	0.58	1.97	2.38	75.4

Table 8. E. coli culture shifted from succinate medium to L-broth at time zero. Enzyme activity and protein determined at intervals.

Time after shift (mins)	(^{3}H) ADP incorporation μmoles/mg protein/30' Heated	Unheated (Initial)	Unheated (Level)	Heated Initial	Heated Level	μg protein 10^8cells
0'	0.89	0.45	no level	1.98	-	37.7
5'	0.93	0.51	"	1.82	-	37.7
10'	1.15	0.77	"	1.49	-	32.5
20'	1.09	1.03	"	1.06	-	36.7
30'	1.15	1.09	"	1.06	-	40.0
40'	1.41	0.96	"	1.47	-	45.0
60'	1.35	1.12	"	1.20	-	55.0
80'	1.44	1.12	"	1.29	-	57.0

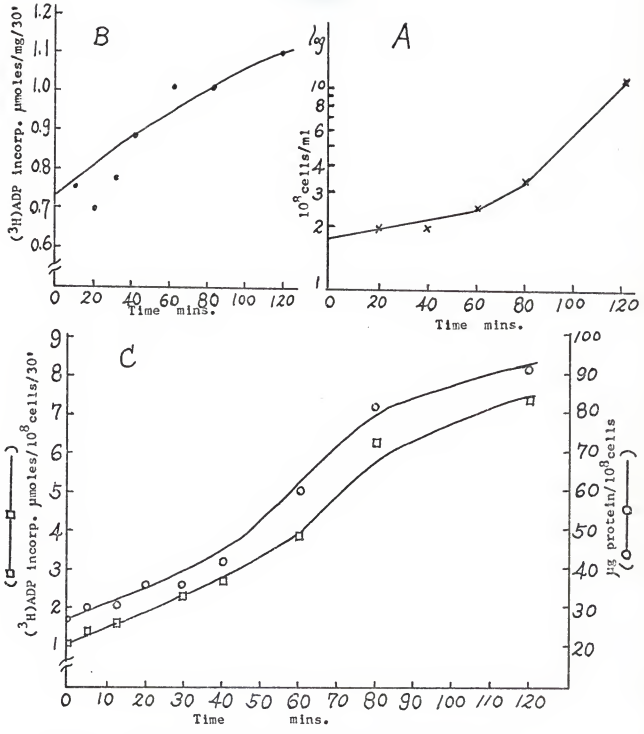

Fig. 4. Synthesis of protein, enzyme and increase of cell number when the E. coli culture shifted from aspartate medium to L-broth medium. A. cell number; B. enzyme/protein; C. enzyme/10^8cells and protein/10^8cells

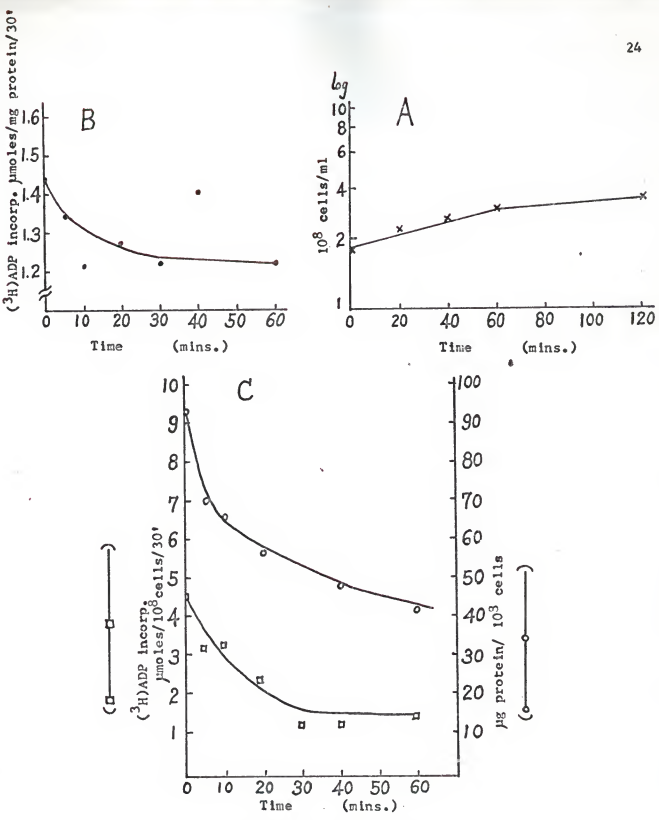

Fig. 5. Synthesis of protein, polynucleotide phosphorylase and increase of cell number when the E. coli culture shifted from L-broth medium to glucose medium at time zero. A. cell number; B. enzyme activity/mg protein; C. enzyme activity/10^8cells and μg protein/10^8cells.

Table 9. E. coli culture shifted from aspartate to L-broth medium at time zero. Enzyme activity and protein determined at intervals.

Time after shift (mins)	(^{3}H) ADP incorporation μmoles/mg protein/30' Heated	Unheated (Initial)	Unheated (Level)	Heated Initial	Heated Level	μg protein 10^8 cells
0'	0.89	0.80	no level	1.11	-	21.8
5'	0.83	0.83	:	1.00	-	25.0
12'	0.74	0.87	0.86	0.85	0.86	26.0
20'	0.70	0.54	0.61	1.30	1.15	28.3
30'	0.77	0.22	0.12	3.50	6.40	33.3
40'	0.89	0.89	0.93	1.00	0.95	36.0
60'	1.06	0.67	no level	1.58	-	48.0
80'	0.99	0.93	:	1.06	-	73.0
120'	1.15	1.03	:	1.12	-	82.5

Table 10. E. coli culture shifted from L-broth medium to glucose medium at time zero. Polynucleotide phosphorylase activity and protein of cells determined at intervals.

Time after shift (mins.)	(^{3}H) ADP incorporation μmoles/mg protein/30' Heated	Unheated (Initial)	Unheated (Level)	Heated Initial	Heated Level	μg protein 10^8 cells
0'	1.41	0.83	0.83	1.70	1.70	62.5
5'	1.34	0.54	0.38	2.54	3.53	53.0
10'	1.22	0.80	0.45	1.53	2.71	54.3
20'	1.28	0.99	0.51	1.28	2.51	45.0
30'	1.22	1.22	0.48	1.00	2.55	33.6
40'	1.41	0.61	0.64	2.31	2.20	34.8
60'	1.22	0.58	0.32	2.11	3.82	36.0

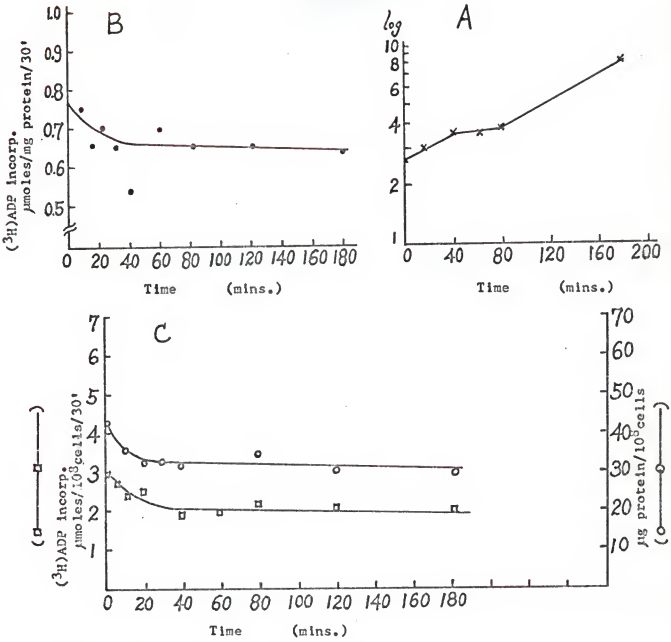

Fig. 6. Synthesis of protein, polynucleotide phosphorylase and increase of cell number when the E. coli culture shifted from glucose medium to succinate medium at time zero. A. cell number; B. enzyme activity/mg protein; C. enzyme activity per 10^8cells and μg protein/10^3cells.

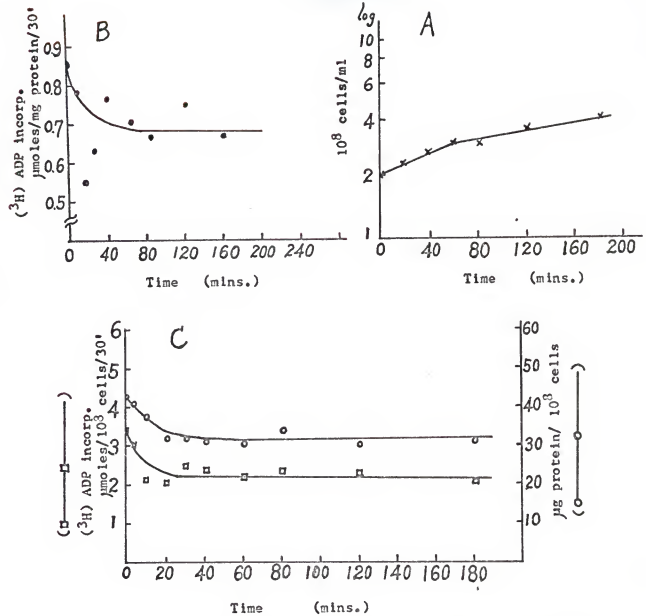

Fig. 7. Synthesis of protein, polynucleotide phosphorylase and increase of cell number when the E. coli culture shifted from glucose medium to aspartate medium at time zero. A. cell number; B. enzyme activity/mg protein; C. enzyme activity/ 10^8 cells and μg protein/10^3 cells.

Table 11. *E. coli* culture shifted from glucose medium to succinate medium at time zero. Polynucleotide phosphorylase and protein determined at intervals.

Time after shift (mins)	(^{3}HO ADP incorporation μmoles/mg protein/30' Heated	Unheated (Initial)	Unheated (Level)	Heated Initial	Heated Level	μg protein 10^8cells
0'	0.76	0.39	0.24	1.95	3.17	41.0
7'	0.76	0.42	0.15	1.81	5.06	36.0
12'	0.65	0.27	0.16	2.40	4.06	37.0
20'	0.70	0.41	0.13	1.70	3.89	34.6
30'	0.65	0.22	0.14	2.95	4.64	33.3
40'	0.57	0.27	0.09	2.11	5.70	32.7
60'	0.72	0.24	0.44	3.00	1.60	28.4
80'	0.66	0.13	0.09	3.66	6.69	34.4
120'	0.67	0.30	no level	2.23	-	32.3
180'	0.65	0.44	:	1.48	-	30.8

Table 12. *E. coli* culture shifted from glucose medium to aspartate medium at time zero. Polynucleotide phosphorylase and protein determined at intervals.

Time after shift (mins)	(^{3}H) ADP incorporation μmoles/mg protein/30' Heated	Unheated (Initial)	Unheated (Level)	Heated Initial	Heated Level	μg protein 10^8cells
0'	0.85	0.27	0.27	3.14	3.14	41.5
5'	0.79	0.29	0.09	2.72	8.75	38.6
10'	0.55	0.34	0.12	1.62	4.56	33.0
20'	0.63	0.41	0.14	1.54	4.51	34.0
30'	0.77	0.20	0.07	3.85	11.0	32.7
40'	0.76	0.29	0.11	2.62	6.09	30.8
60'	0.72	0.17	0.04	4.24	18.0	35.3
80'	0.67	0.20	0.09	3.34	7.45	30.8
120'	0.75	0.33	0.09	2.27	8.30	27.7
180'	0.69	0.27	0.12	2.55	5.76	-

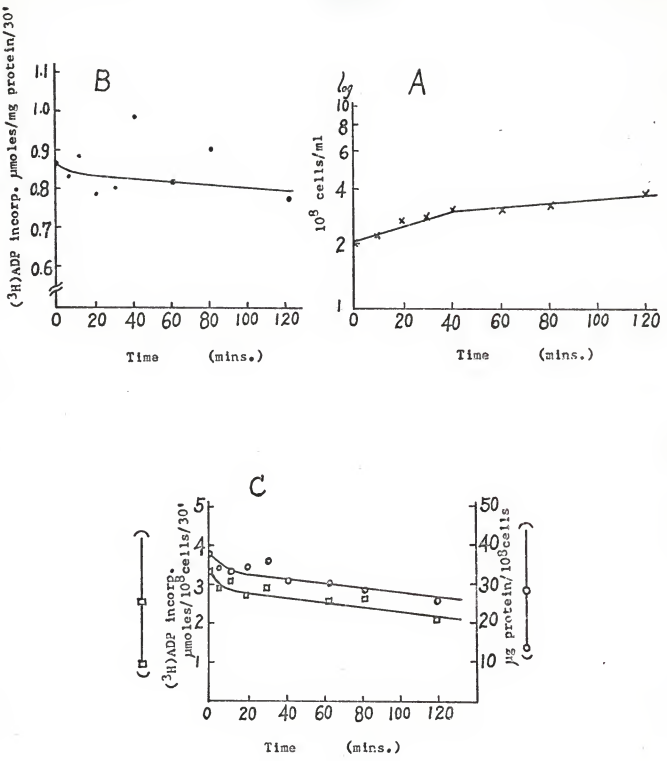

Fig. 8. Synthesis of protein, polynucleotide phosphorylase and incerase of cell number when the E. coli culture shifted from succinate medium to aspartate medium at time zero. A. cell number; B. enzyme activity/mg protein; C. enzyme activity/10^8 cells and μg protein/10^8 cells.

Table 13. *E. coli* culture shifted from succinate medium to aspartate medium at time zero. Polynucleotide phosphorylase and protein determined at interval.

Time after shift (mins.)	(^{3}H) ADP incorporation μmoles/mg protein/30'			Heated Initial	Heated Level	μg protein 10^8cells
	Heated	Unheated (Initial)	(Level)			
0'	0.86	0.60	no level	1.43	-	35.0
5'	0.83	0.25	0.14	3.30	5.90	34.5
10'	0.89	0.20	0.20	4.45	4.45	35.4
20'	0.79	0.33	0.35	2.40	2.26	34.2
30'	0.80	0.74	0.27	1.08	2.96	36.7
40'	0.97	0.74	0.27	1.31	3.58	31.0
60'	0.83	0.35	no level	2.37	-	31.0
80'	0.93	0.75	:	1.24	-	28.3
120'	0.77	0.48	:	1.60	-	26.5

Table 14. Effect of medium transfer on the incorporation of (^{3}H) ADP into acid insoluble material. Samples were taken before and immediately after medium change by filtration.

Sample	(^{3}H) ADP incorporation μmoles/mg protein/30'			Heated Initial	Heated Level	μg protein / 10^8 cells
	Heated	Unheated (Initial)	Unheated (Level)			
Glucose to L-broth						
before	0.86	0.39	0.19	2.20	4.50	48.0
after	0.99	0.51	0.51	1.94	1.94	46.3
Glucose to succinate						
before	0.67	0.32	0.22	2.09	3.04	43.0
after	0.76	0.39	0.24	2.24	3.16	41.0
Glucose to aspartate						
before	0.85	0.39	0.15	2.18	5.65	43.0
after	0.85	0.27	0.27	3.14	3.14	42.0
Glucose to -A.A.*						
before	0.74	0.43	0.17	1.64	4.35	46.1
after	0.74	0.42	0.14	1.76	5.25	45.0
Succinate to L-broth						
before	0.87	0.37	0.37	2.35	2.35	34.6
after	0.89	0.45	0.45	1.98	1.98	34.6
Succinate to aspartate						
before	0.83	0.69	0.69	1.20	1.20	36.0
after	0.86	0.60	0.60	1.43	1.43	35.0
Aspartate to L-broth						
before	0.70	0.68	0.29	1.03	2.40	30.0
after	0.89	0.80	0.80	1.11	1.11	21.8
L-broth to Glucose						
before	1.44	0.70	0.67	2.06	2.15	67.0
after	1.19	0.51	0.42	2.33	2.84	53.0

*-A. A. :glucose medium without required amino acids.

Effect of Thymine Starvation

It has been reported (Hanawalt and Maaløe, 1961; Maaløe and Hanawalt, 1961) that during thymine starvation, cell division and DNA synthesis stop while net synthesis of protein and RNA are somewhat less sensitive. The effect of thymine on polynucleotide phosphorylase synthesis was investigated. After a glucose culture of E. coli reached a titer of 2×10^8 cells/ml, it was shifted to pre-warmed glucose medium without thymine. The culture was immediately divided into two aliquots, A and B; to culture A, thiomethyl-β-D-galactoside was added (final concentration 10^{-3} M). Samples were taken at intervals from cultures A and B for β-galactosidase assay and from culture B for polynucleotide phosphorylase assay. The results are shown in Table 15.

Table 15. Effect of thymine starvation on the ability of enzyme synthesis of cells. E. coli cultures deprived of thymine at time zero. Experimental conditions were as described in the text.

Time (mins)	Polynucleotide phosphorylase specific activity per 10^8 cells	per mg protein	β-galactosidase specific activity +TMG*	-TMG	μg protein per 10^8 cells
0'	3.2	0.76	1.85	2.35	42.0
10'	3.0	0.76	12.95	2.35	45.8
20'	3.8	0.87	44.00	2.35	44.6
30'	4.3	0.96	68.4	2.85	44.0
40'	3.88	0.74	80.9	2.55	44.0
60'	3.71	0.58	106.3	2.35	64.8

TMG: thiomethyl-β-D-galactoside.

Table 15 shows that during thymine starvation, the total protein of the cell was increased. The constitutive β-galactosidase activity did not change and the capacity to form the inducible enzyme remained. Polynucleotide

phosphorylase activity per cell and per mg of protein increased. When 25 μg/ml of chloramphenicol was added to a thymine deprived culture, the capacity to form the inducible enzyme, β-galactosidase, was repressed completely whereas polynucleotide phosphorylase activity per mg protein was retained unchanged. The results are shown in Table 16.

Table 16. E. coli culture deprived of thymine and to which was added 25 μg/ml of CAP at time zero. Polynucleotide phosphorylase and β-galactosidase were determined at intervals.

Time (mins)	Polynucleotide phosphorylase specific activity/mg protein	β-galactosidase specific activity	
		+TMG	-TMG
0'	0.62	2.5	2.35
10'	0.56	2.6	2.65
20'	0.62	2.5	2.35
30'	0.58	2.04	2.75
40'	0.61	2.04	2.25
60'	0.59	2.5	2.55

Table 17 shows the specific activity of polynucleotide phosphorylase when a culture was returned to thymine containing medium after one hour of thymine starvation. Restoration of thymine caused polynucleotide phosphorylase activity to increase slightly. The increment of the enzyme activity per mg of protein was also seen when samples were not pre-heated.

Effect of Amino Acids

The effect of amino acid starvation on the ability of polynucleotide phosphorylase synthesis of cells was investigated. An E. coli culture (titer 2×10^8 cells/ml) was filtered and resuspended in pre-warmed glucose medium without amino acids. After 90 min. incubation, required amino acids were added to the culture. Samples were taken at the indicated time for

Table 17. E. coli culture deprived of thymine for 60 min, then thymine was added at time zero. Polynucleotide phosphorylase and protein were determined at various intervals.

Time (mins.)	(^{3}H) ADP incorporation μmoles/mg protein/30' Heated	Unheated (Initial)	Unheated (Level)	Heated Initial	Heated Level
0'	0.62	0.25	0.11	2.48	5.64
5'	0.61	0.37	0.15	1.65	4.06
10'	0.83	0.50	0.19	1.66	4.36
20'	0.86	0.70	0.22	1.23	3.92
30'	1.02	0.83	0.29	1.23	3.52
40'	0.8	0.61	0.23	1.31	3.48
60'	0.73	0.38	0.13	1.92	5.62

Table 18. Effect of amino acids on the activity of polynucleotide phosphorylase of E. coli. The E. coli culture was deprived of amino acids at time zero. After 90 min. incubation, required amino acids were added and incubation continued. Polynucleotide phosphorylase and protein were determined at various intervals.

Time	(^{3}H) ADP incorporation μmoles/30' per 10^8 cells Heated	per mg protein Heated	Unheated (Initial)	Unheated (Level)	Heated Initial	Heated Level	μg protein 10^8 cells
0'	3.2	0.74	0.42	0.14	1.76	5.25	45.0
10'	2.6	0.74	0.15	0.12	4.93	6.16	36.2
20'	2.2	0.64	0.51	0.10	1.25	6.40	34.2
30'	2.0	0.67	0.32	0.16	2.09	4.2	27.7
40'	2.0	0.64	0.19	0.12	3.37	5.35	30.3
60'	2.0	0.64	0.25	0.16	2.56	4.0	30.7
90'	1.9	0.64	0.22	0.10	2.90	6.40	30.0
+A. A.							
95'	2.5	0.77	0.42	0.19	1.83	4.05	33.3
100'	2.4	0.83	0.51	0.32	1.63	2.59	43.0
120'	2.9	0.90	0.60	0.42	1.50	2.14	38.4
150'	3.5	0.83	-	-	-	-	-

polynucleotide phosphorylase assay. The results are shown in Table 18.

Table 18 shows that during deprivation of required amino acids, the polynucleotide phosphorylase activity per 10^8 cells dropped as well as protein per 10^8 cells and its level was restored by restoring required amino acids.

RNA Synthesis and Polynucleotide Phosphorylase Synthesis

In order to investigate the relation of RNA and polynucleotide phosphorylase in cells, two experiments were carried out. One was to determine the effect of uracil starvation on E. coli $15T^-U^-$ which requires uracil to support its growth. When this organism is deprived of uracil, RNA synthesis is retarded preferentially. Table 19 shows that polynucleotide phosphorylase activity per 10^8 cells and per mg of protein declined during uracil starvation but was restored by adding uracil.

The effect of 200 μg/ml of chloramphenicol on an amino acid starved culture was investigated. The results are shown in Table 20. The activity of polynucleotide phosphorylase per 10^8 cells declined with time in amino acid starved cells in the presence of chloramphenicol, but the enzyme activity per mg of protein decreased slightly. If chloramphenicol was removed and amino acids were added to the culture after 80 min, the enzyme activity per 10^8 cells and protein per 10^8 cells increased.

Table 19. Effect of uracil starvation on the activity of polynucleotide phosphorylase of E. coli. A culture was deprived of uracil at time zero and incubation continued. At 80 min, uracil was added to the culture. Enzyme activity and protein were determined at the indication time.

Time (mins.)	(^{3}H) ADP incorporation μmoles/30' per 10^8 cells	per mg protein			Heated / Initial	Heated / Level	μg protein / 10^8 cells
	Heated	Heated	Unheated (Initial)	(Level)			
-U 0'	3.0	0.64	0.29	0.1	2.2	6.4	46.6
20'	2.3	0.59	0.18	0.13	3.28	4.55	40.4
40'	2.0	0.53	0.30	0.11	1.78	4.83	38.1
60'	2.0	0.46	0.24	0.09	1.96	5.12	42.1
80'	2.0	0.47	0.24	0.09	1.96	5.21	42.6
+ U							
85'	1.8	0.47	0.31	0.12	1.52	3.92	37.6
90'	2.1	0.56	0.34	0.12	1.65	4.66	38.4
100'	2.7	0.60	0.39	0.14	1.54	4.30	45.6
120'	4.1	0.77	0.33	0.12	2.33	6.40	57.6
140'	3.6	0.85	0.48	0.16	1.77	5.30	42.6
160'	2.8	0.65	0.34	0.15	1.92	4.32	42.6

Table 20. Effect of 200 μg/ml of chloramphenicol on the polynucleotide phosphorylase of an amino acid starved culture. An E. coli culture was shifted to a glucose medium(without amino acidd) containing 200 μg/ml of CAP at time zero. After 80 min. of incubation at 37°, the cells were filtered and resuspended in an equal volume of prewarmed glucose medium. Polynucleotide phosphorylase and protein were determined at intervals.

Time (mins.)	(^{3}H) ADP incorporation μmoles/30' per 10^8 cells	per mg protein			Heated Initial	Heated Level	μg protein 10^8 cells
	Heated	Heated	Unheated (Initial)	Unheated (Level)			
CAP - AA							
0'	3.3	0.74	0.26	0.11	2.8	6.70	44.8
10'	3.0	0.70	0.36	0.14	1.94	5.00	42.2
20'	2.5	0.68	0.30	0.16	2.26	4.26	36.0
30'	2.4	0.68	0.15	0.09	4.54	7.56	30.6
40'	2.6	0.72	0.21	0.17	3.43	4.24	33.4
50'	2.2	0.72	0.28	0.16	2.57	4.50	30.6
60'	2.2	0.69	0.31	0.20	2.22	3.45	31.8
80'	1.9	0.66	0.29	0.20	2.28	3.30	28.0
- CAP + AA							
0'	1.9	0.68	0.41	0.16	1.61	4.12	27.9
5'	1.7	0.62	0.20	0.22	3.1	3.06	28.6
10'	2.2	0.72	0.22	0.23	3.28	3.13	31.0
15'	2.1	0.72	0.34	0.18	2.12	4.0	29.2
20'	2.5	0.99	0.60	0.30	1.65	3.30	24.4
30'	2.5	0.90	0.64	0.37	1.40	2.43	29.4
40'	2.7	0.92	0.92	0.61	1.00	1.50	26.5
50'	-	1.11	0.81	0.63	1.37	1.76	42.5
60'	3.4	0.97	1.24	0.85	0.78	1.14	-
80'	3.8	0.85	1.15	0.54	0.74	1.57	45.3
120'	-	1.03	0.64	0.33	1.61	3.12	44.1

Effect of 5-methyltryptophan

Sharon and Lipmann (1957) reported that 5-methyltryptophan, an analogue of tryptophan, inhibited growth and was not incorporated into protein. When this amino acid analogue replaces tryptophan in a culture of E. coli, a tryptophan auxotroph, polynucleotide phosphorylase activity increases in the presence of chloramphenicol (Levin et al., 1963; Thang et al., 1963). When 5-methyltryptophan replaced tryptophan in a glucose culture, the polynucleotide phosphorylase activity was investigated. Table 21 shows that the enzyme activity per mg of protein did not changed, but enzyme activity per 10^8 cells decreased. The cell division rate of the culture declined.

Table 21. Effect of 5-methyltryptophan on polynucleotide phosphorylase. 5-methyltryptophan (final concentration 40 µg/ml) replaced tryptophan at time zero.

Time (mins)	(^{3}H)ADP incorporation umole/30' per 10^8 cells		per mg protein		Initial divid	Level divid	µg protein per 10^8 cells
		Heated	Unheated				
			Initial	Level	Heated	Heated	
0'	2.95	1.01	0.38	0.14	2.64	7.20	40.6
20'	2.05	1.40	0.22	0.11	6.36	12.70	40.5
40'	1.76	0.98	0.31	0.15	3.16	6.55	31.4
60'	1.49	0.84	0.36	0.19	2.30	4.38	27.7
90'	1.76	1.06	0.37	0.26	2.86	4.06	25.8

DISCUSSION

Kinetic studies of the enzyme activity showed that linearity was obtained when bacterial extracts were pre-heated at 60° for 5 min. The results in Fig. 1 indicate that there are two factors involved in the inhibition of polynucleotide phosphorylase polymerization. One reduces the rate of the enzyme reaction and the other may degrade the end products of polymerization. Such a degradation may be due to nucleases which are inactivation at 60°. Deoxynucleoside diphosphates may act as allosteric inhibitors of polynucleotide phosphorylase polymerization since such deoxynucleoside diphosphates induce an initial lag period in polymerization (Lucas-Lenard, and Cohen 1966). Bacterial cultures grown under different conditions do not exhibit significantly different variations between the initial rates, or levels of unheated enzyme and heated enzyme activities. However, it seems that the best results are obtained with pre-heated samples.

Levin et al. (1963) observed that the presence of chloramphenicol induced an increment in polynucleotide phosphorylase synthesis. We found that the increment of polynucleotide phosphorylase content of cells is a function of chloramphenicol concentration, up to 10 μg/ml. This increment is significant in cells grown in glucose medium, but is less so for cells grown in succinate medium. No effect was observed for cells grown in L-broth medium.

Schaechter et al. (1958) observed that a fast-growing culture contains cells with a larger volume, a greater mass, more DNA, more RNA and more proteins than cells from a culture of the same organisms growing slowly. We have observed that the polynucleotide phosphorylase content of cells

and its rate of synthesis varies with the growth rate. In exponentially growing cultures, the cells from L-broth cultures contained higher enzyme activity than the cells from glucose cultures, succinate cultures, or aspartate cultures. The high enzyme activity observed in cells grown in L-broth medium was not entirely attributable to the increased amounts of total protein per cell that was found. The cells grown in glucose medium, succinate medium and aspartate medium had the same level of enzyme activity per mg of protein, but the cells grown in aspartate medium had a lower enzyme activity per 10^8 of cells.

In an L-broth culture, cells had the highest enzyme activity at the early stage of exponential growth, but enzyme synthesis decreased as growth rate declined. Thus, once again, polynucleotide phosphorylase activity appears to be closely related to cell growth.

The observation that the relationship to changes in cell division rates are accompanied by changes in enzyme activity also indicates that polynucleotide phosphorylase is related to cell growth and cell division. When cells were transferred from a medium permitting slow growth to a medium permitting fast growth the rates of cell division remained at the pre-shift rate until enzyme activities had increased and reached a new level. Pre-shift rates of cell division were maintained for approximately 60 min. in transfers from succinate to broth medium, and 90 min. in transfers from aspartate to broth medium. When cells were transferred from medium permitting fast growth to medium permitting slow growth, the enzyme activities per cell and per mg of protein dropped slightly while cell division proceeded at the pre-shift rates for a short period and then shifted to a new lower rate. It is obvious that polynucleotide phosphorylase synthesis is repressed immediately after a

shift-down, whereas its synthesis is preferentially accelerated by a shift-up.

This short period of maintaining pre-shift rates of cell division after a shift-down may be explained as follow: after a shift, those chromosomes which have started replication may continue their cycle and end in cell division, but the subsequent cell division rate could be limited by the rate of polynucleotide phosphorylase formation directly or indirectly, or by the total growth rate.

Since chromosomal replication has a polarity and is sequential at a constant rate (Cairns, 1963; Lark et al., 1963; Nagata, 1963; Meselson and Stahl, 1958; Yoshikawa and Sueoka, 1963), the ratio of genes located toward the origin of replication to genes located toward the terminus is 2:1 in an exponentially growing culture. Also, in an early exponentially growing population, the occurrence of chromosomes whose replication has just started is much more frequent than that of chromosomes which are about to complete their replication (Yoshikawa and Sueoka, 1963). The possible average stage of a replicating chromosome in cells from exponentially growing cultures is shown in Fig. 9.

Furthermore, each gene is replicated at a definite time in the cell duplication cycle (Yoshikawa and Sueoka, 1963). Doubling the gene is supposed to double the potential for synthesis of the corresponding enzyme (Donachie, 1964). It has also been observed that the order of enzyme syntheses appears to be the same as that of gene replication (Masters et al., 1964; Kuemple et al., 1965; Tauro and Halvorson, 1966), though the exact timing of enzyme syntheses may be varied by changing feed-back inhibition, de-repression etc. (Masters and Donachie, 1966; Kuempel et al., 1965; Masters

and Pardee, 1965).

According to the above hypotheses, it seems reasonable to expect that enzymes whose controlling gene is located near the origin of replication will appear to be synthesized preferentially in those cultures in which the occurrence of chromosomes undergoing early replication is higher, or in the cells in which chromosomes have more initial replicationg points.

The highest polynucleotide phosphorylase activity per mg of protein was observed in the cells of an early exponentially growing culture of broth. It is possible to account for this fact by stating that the controlling gene population is high in those cells, since it has been observed that the chromosome replication units of cells grown in broth medium are more numerous than those of cells grown in glucose, succinate and aspartate medium (Lark et al., 1966). Possible models of replicating chromosome are shown in Fig.9.

When a broth culture becomes older, the replication points of chromosomes may decrease, and eventually the population of chromosomes which are about to complete their replication increase. Thus, the occurrence of the gene which is located near the origin is decreased. Therefore, the data which shows that polynucleotide phosphorylase activity per mg of protein declined in an outgrown broth culture may indicate that the gene controlling synthesis of polynucleotide phosphorylase is possibly located very near the beginning of the replicating chromosome.

The observation of the preferential synthesis of polynucleotide phosphorylase after amino acid starvation and thymine starvation is consistent with the possibility of a polynucleotide phosphorylase controlling gene located near the origin of chromosome. After amino acid starvation chromosome replication is re-initiated from the origin (Lark, 1966; Lark et al.,

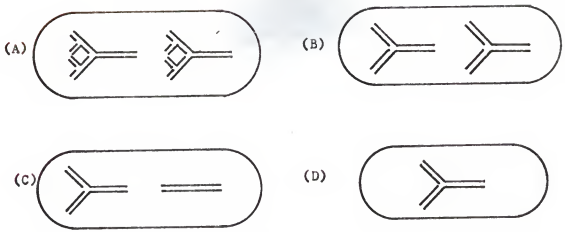

Fig. 9. The possible models of average replicating chromosome in cells from an exponentially growing culture. (A) broth culture. (B) glucose culture. (C) succinate culture. (D) aspartate culture.

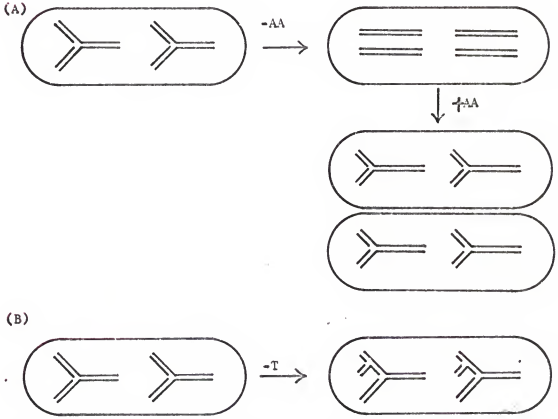

Fig. 10. Effects of amino acid starvation (A), and thymine starvation (B) on chromosome replication in cells growing in glucose medium.

1963), but cells do not appear to commence chromosome replication simultaneously (Hanawalt and Maaloe, 1961). Thymine starvation also will induce an extra replication fork on one of the two partially completed daughter chromosomes (Pritchard and Lark, 1964). Thus, we found that polynucleotide phosphorylase was preferentially synthesized after amino acid starvation and thymine starvation. Fig. 10 shows the possible models of the effects on chromosome replication of amino acid starvation or thymine starvation.

During thymine starvation, we observed that polynucleotide phosphorylase was preferentially synthesized, whereas DNA replication had ceased. This may suggest that transcription is not necessarily linked to genome replication. Masters and Donachie (1966) also observed that enzyme was periodically synthesized in a thymineless strain of B. cereus in which DNA synthesis was inhibited by 5-fluoro-deoxyuridine.

SUMMARY

The results presented show that the characteristics of polynucleotide phosphorylase are:

(1) Its synthesis is not inhibited by the presence of chloramphenicol in the culture. The increment of polynucleotide phosphorylase in the cells is a function of chloramphenicol concentration, up to 10 μg/ml.

(2) The enzyme activity of cells and enzyme synthesis rates vary with the growth rate and the age of cells. In the early stage of exponential growth, cells had the highest enzyme activity, but activity declined as the culture became older.

(3) There is a lower limit of enzyme activity per cell. The lower limit is about 1.8 units of enzyme per 10^3 cells found in exponentially grow-

ing cells from aspartate medium and in cells from an outgrown broth culture.

(4) The enzyme activity per mg of protein increased in a thymine starved culture.

(5) The enzyme activity per mg of protein decreased in amino acid starved cultures, and its synthesis is preferentially restored by adding the required amino acids to the culture.

(6) 5-methyltryptophan inhibits the synthesis of the enzyme.

(7) Deprivation of uracil also blocks the enzyme synthesis.

(8) Enzyme synthesis as well as total protein is decreased in an amino acid starved culture simultaneously treated with 200 μg per ml of chloramphenicol. The enzyme was found to be synthesized preferentially by removing chloramphenicol and restoring required amino acids to the above culture after 80 min. of incubation.

These results indicate that polynucleotide phosphorylase is closely related to cell growth. The possibility that the gene which controls the synthesis of polynucleotide phosphorylase is located very near the origin of the replicating chromosome is dicussed.

ACKNOWLEDGMENT

Sincere appreciation is expressed to Dr. Karl G. Lark, the major advisor, and Dr. C. Lark for their encouragment and helpful suggestions during this study. The author also wishes to thank Dr. T. H. Pittenger and Dr. C. Hedgcoth for their helpful suggestions in the preparation of this manuscript.

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THE RELATION OF POLYNUCLEOTIDE PHOSPHORYLASE AND GROWTH OF ESCHERICHIA COLI

by

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AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

In Genetics

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1968

ABSTRACT

Polynucleotide phosphorylase was described by Grunberg-Manago and Ochoa in 1955. This enzyme catalyzes the synthesis of polyribonucleotides from nucleoside 5'-diphosphates in the presence of Mg^{2+}. The reverse reaction also is catalyzed. The physiological role of this enzyme is not clear.

The purpose of this investigation was to attempt to assess some effects culture medium changes had on the enzyme activity in the cell.

The quadruple auxotroph of Escherichia coli 15T⁻ requiring thymine, methionine, tryptophan and arginine was used in all experiments except when otherwise indicated. Several different media which permitted different growth rates were used. Changes of media were accomplished by collecting and washing cells on a membrane filter and resuspending them in pre-warmed fresh medium. The activity of polynucleotide phosphorylase was measured by a modification of the method of Levin et al. (1963), which permits the measurement of (^{3}H) ADP incorporation into the acid insoluble precipitate.

The results indicate that: (1) Chloramphenicol, when present in the culture permits an increase in polynucleotide phosphorylase activity. (2) The total polynucleotide phosphorylase activity of cells varies with the growth rate and the age of cells. The lower limit is about 1.8 units of enzyme per 10^8 cells found in exponentially growing cells from aspartate medium and in cells from an outgrown broth culture. (3) Thymine starvation induces an increase of the enzyme activity of the cells. (4) Polynucleotide phosphorylase synthesis requires the presence of amino acids and uracil in the culture. (5) 5-methyltryptophan inhibits polynucleotide phosphorylase synthesis as well as protein synthesis. (6) In shift-up cultures, cell division rate changes after the enzyme activity has reached a new level. Polynucleotide

phosphorylase activity decreases in shift-down cultures whereas the pre-shift cell division rate is maintained for a longer period. (7) Polynucleotide phosphorylase activity decreases in amino acid starved cultures simultaneously treated with 200 μg per ml of chloramphenicol, and was found to be synthesized preferentially by removing chloramphenicol and restoring required amino acids after 30 min. of incubation.

In summary, these results indicate that polynucleotide phosphorylase is related to the growth of E. coli. The possibility that the gene which controls the synthesis of polynucleotide phosphorylase is located very near the origin of the replicating chromosome is discussed.