INVOLVEMENT OF NEWLY-FORMED PROTEIN IN THE SYNTHESIS OF DEOXYRIBONUCLEIC ACID

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SUMMARY

By the combined use of thymine starvation and chloramphenicol, it was shown that DNA synthesis requires the formation of protein which may precede or accompany it.

INTRODUCTION

Escherichia coli 15 t^-h^- (thymine- and histidine-less strain) is unable to synthesize both thymine and histidine. As described by BARNER AND COHEN[1], this strain shows a characteristic death as a consequence of unbalanced growth during thymine starvation in a medium lacking thymine but otherwise complete. During thymine starvation there is little, if any, synthesis of DNA but a vigorous synthesis of RNA and protein takes place. If after thymine starvation, the bacteria are suspended in medium now provided with thymine but lacking histidine, there is a remarkable synthesis of DNA without any concomitant net increase of either RNA or protein. Experiments were therefore carried out to examine the role of prior protein synthesis in this synthesis of DNA.

METHODS

Cells of *E. coli* 15 t^-h^- grown on a glucose-salts medium (GRAY AND TATUM's medium[2] with 0.1 % glucose) supplemented with thymine (5 μg/ml) and L-histidine (10 μg/ml) were harvested during the exponential phase of growth, washed twice and suspended in two lots of thymine starvation medium (glucose-salts with histidine but no thymine) at a concentration of approx. $4 \cdot 10^8$/ml. Chloramphenicol (20 μg/ml) was added to one of these lots. Both were incubated at 37° with shaking and aliquots withdrawn at 0, 30, 60 and 120 min, washed twice, and resuspended in thymine medium (glucose-salts with thymine but no histidine). These timed aliquots from the two original lots were further divided into two identical series. To one of these chloramphenicol was added, and all cultures were incubated for an additional 120 min.

Throughout the experiment 20 ml aliquots of the bacterial suspensions were sampled from each series of flasks and treated with 5 % TCA in the cold. After sedimentation the pellets were washed in cold 5 % TCA and then extracted by 5 %

Abbreviations: DNA, deoxyribonucleic acid; RNA, ribonucleic acid; TCA, trichloroacetic acid.

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TCA at 90° for 15 min. Aliquots of this extract were used for the estimation of both DNA and RNA by the diphenylamine method[3] and orcinol reaction[4], respectively. The indole reaction[5] was also used for DNA estimation in parallel with the diphenylamine method to confirm the measurements. Protein was estimated by the use of Folin reagent[6] taking aliquots from the TCA precipitated fraction.

All experiments reported in this paper were repeated at least once with similar results.

RESULTS AND DISCUSSION

E. coli 15 t^-h^- was found to synthesize a significant amount of DNA in the absence of exogenous histidine when exponentially growing cells were transferred to a thymine medium without previous starvation. Thymine starvation prior to incubation in thymine stimulated an even faster DNA synthesis. This is in good agreement with the report of BARNER AND COHEN[1] describing DNA synthesis in the absence of phenylalanine in *E. coli* 15 t^-pa^- (thymine- and phenylalanine-less).

During thymine starvation, while the synthesis of DNA was negligible, large amounts of RNA and protein were synthesized. As is clear from Table I, the longer the period of thymine starvation, the more DNA was synthesized during subsequent incubation in thymine medium. After 60 and 120 min of thymine starvation, the number of viable cells had already decreased to 36.4% and 0.4% respectively. Nonetheless, the cells were still metabolically active and there was an approximately three-fold increase in DNA after 2 h subsequent incubation in thymine medium. During this period, however, no significant net increase of either RNA or protein was observed and viable cell numbers remained constant. This is evidence for the separation of DNA synthesis from the synthesis of other macromolocules, although an active

TABLE I

EFFECT OF THYMINE STARVATION ON SUBSEQUENT SYNTHESIS OF DNA, RNA AND PROTEIN IN *E. coli* 15 t^-h^-

	Time of thymine starvation (min)	*Percent over initial value during thymine starvation*	*Percent over 0 min value during subsequent incubation in thymine medium*					
			No chloramphenicol			*With chloramphenicol*		
			0 min	*60 min*	*120 min*	*0 min*	*60 min*	*120 min*
Surviving cells per ml	0	100	100	90	107	100	90	105
	30	90.5	100	105	109	100	103	108
	60	36.4	100	91	100	100	96	93
	120	0.4	100	104	107	100	106	107
Amount of DNA per ml	0	100	100	150	151	100	163	180
	30	100	100	196	216	100	191	219
	60	102	100	224	289	100	220	286
	120	105	100	203	295	100	176	225
Amount of RNA per ml	0	100	100	127	133	100	190	209
	30	151	100	126	126	100	136	188
	60	228	100	103	102	100	150	147
	120	317	100	100	96	100	110	120
Amount of protein per ml	0	100	100	103	107	100	101	103
	30	120	100	100	99	100	100	98
	60	155	100	97	95	100	102	100
	120	222	100	95	93	100	100	100

turnover of some fractions of protein and/or RNA may not be denied. This critical point is now under investigation.

The mode of this DNA synthesis, occurring separately from an accompanying synthesis of protein, was investigated by the density gradient centrifugation method of MESELSON AND STAHL[7]. From an experiment reported elsewhere[8] evidence was obtained that DNA synthesis involves replication in non-dividing as well as in dividing cells. There may be a relation between this replication and the mutations claimed to be induced by thymine starvation[9–11].

No significant inhibitory effect of chloramphenicol on the synthesis of DNA was observed when chloramphenicol was added to the thymine medium after thymine starvation for different times as shown also in Table I, except for a little inhibition in the 120 min thymine starvation series. Chloramphenicol has been shown to inhibit protein synthesis in *E. coli* whereas nucleic acid synthesis continues in its presence[12]. The necessity for a concomitant net synthesis of protein during this period of active DNA synthesis is therefore ruled out. There is particular interest in the fact that the amount of protein synthesized during thymine starvation is proportional to the amount of DNA synthesized upon subsequent incubation with thymine. This relationship is much more obvious when chloramphenicol is added to the thymine starvation medium to prevent the synthesis of protein.

Although chloramphenicol failed to inhibit DNA synthesis in the thymine medium, it had a tremendous effect when added to the thymine starvation medium. The inhibition of the synthesis of protein by chloramphenicol during thymine starvation subsequently eliminated the stimulated synthesis of DNA which otherwise would have taken place in thymine medium. As shown in Table II, almost the same amounts of DNA were synthesized in each of the thymine media regardless of the length of previous thymine starvation. Chloramphenicol allowed RNA synthesis to a decreased extent in the thymine starvation medium, but this increase of RNA did not influence the amount of DNA synthesized afterwards. And also there appeared to be almost no breakdown of this RNA such as was previously observed by NEIDHARDT AND GROS[12] and HOROWITZ, LOMBARD AND CHARGAFF[13] in other amino acid auxotrophs of *E. coli* upon transferring the chloramphenicol treated cells to amino acid starvation medium without chloramphenicol. This is possibly due to the active turnover of RNA in thymine medium.

Another interesting finding is that chloramphenicol prevented thymineless death to a remarkable extent, *i.e.*, while in the control only 0.4 % of the cells were colony-formers after 2 h, 73.2 % remained so when chloramphenicol was present. BILLEN[14] reported a similar effect of chloramphenicol in preventing thymine-less death of *E. coli* 15 t^- (thymine-less).

The experiments reported here verify the hypothesis that prior protein synthesis is a prerequisite for subsequent DNA synthesis. They support similar findings of TOMIZAWA AND SUNAKAWA[15] on DNA synthesis in a system of multiplying bacteriophage, and of HAROLD AND ZIPORIN[16] and DRAKULIC AND ERRERA[17] on DNA synthesis of *E. coli* treated with ultra-violet light. Recently DOUDNEY[18] found a similar relationship between the synthesis of protein and DNA in a synchronous culture of *E. coli* by the use of chloramphenicol. From these results, it is suggested that the amount of protein previously synthesized may determine the amount of DNA formed subsequently. This protein seems to constitute, at least in part, a DNA-forming system.

The small amount of DNA formed in thymine medium, independent of the length of previous thymine starvation in chloramphenicol, is probably due to protein that was formed before the starvation.

TABLE II

EFFECT OF THYMINE STARVATION IN THE PRESENCE OF CHLORAMPHENICOL ON SUBSEQUENT SYNTHESIS OF DNA, RNA AND PROTEIN IN *E. coli* 15 t⁻h⁻

	Time of thymine starvation with chloramphenicol	*Percent over initial value during thymine starvation with chloramphenicol*	*Percent over 0 min value during subsequent incubation in thymine medium*					
			No chloramphenicol			*With chloramphenicol*		
			0 min	*60 min*	*120 min*	*0 min*	*60 min*	*120 min*
Surviving cells per ml	0	100	100	97	103	100	103	103
	30	100	100	—	100	100	—	100
	60	93.0	100	—	98	100	—	100
	120	73.2	100	—	100	100	—	102
Amount of DNA per ml	0	100	100	150	151	100	163	180
	30	102	100	129	145	100	131	144
	60	100	100	128	146	100	133	147
	120	104	100	121	145	100	122	134
Amount of RNA per ml	0	100	100	127	133	100	190	209
	30	154	100	93	105	100	114	123
	60	167	100	95	102	100	113	114
	120	180	100	99	103	100	111	110
Amount of protein per ml	0	100	100	103	107	100	101	103
	30	100	100	93	96	100	94	95
	60	100	100	90	105	100	96	100
	120	102	100	92	98	100	100	102

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