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STUDIES OF DEOXYRIBONUCLEIC ACID SYNTHESIS AND CELL GROWTH IN THE DEOXYRIBOSIDE REQUIRING BACTERIA, *LACTOBACILLUS ACIDOPHILUS*

II. DEOXYRIBONUCLEIC ACID SYNTHESIS IN RELATION TO RIBONUCLEIC ACID AND PROTEIN SYNTHESIS*

TUNEKO OKAZAKI AND REIJI OKAZAKI
Biological Institute, Faculty of Science, Nagoya University, Nagoya (Japan)

(Received December 18th, 1958)

SUMMARY

1. Depletion of the deoxyriboside requirement of *Lactobacillus acidophilus* R-26 inhibits almost completely DNA synthesis and cell division, but little affects the synthesis of RNA and protein.

2. Omission of uracil from the medium causes a parallel inhibition of RNA and protein formation; but the synthesis of DNA is stimulated in the absence of uracil.

3. Addition of excess thymidine to the cells previously starved of both uracil and deoxyriboside causes a remarkable accumulation of acid-soluble deoxyribosidic compounds and active synthesis of DNA, with little or no concurrent increase of protein, RNA or cell number. Simultaneous addition of chloramphenicol and thymidine abolishes the small increase in protein observed under such conditions, with little effect on DNA synthesis.

4. Despite the fact that DNA is synthesized actively in the absence of RNA and protein increase, omission of amino acids from the uracil-deficient as well as complete medium strongly inhibits the increase of DNA.

INTRODUCTION

Syntheses of replicating materials such as DNA**, RNA and protein in the cell may be the most essential processes involved in cell growth. The question whether or not

* A preliminary account of some of the results reported in this paper has been published[1].
** The following abbreviations are used: deoxyribonucleic acid, DNA; ribonucleic acid, RNA.

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these synthetic processes are connected with each other has been the subject of a number of exptl. studies. It has been well established since the pioneer work of CASPERSSON *et al.*[2] and BRACHET[3] that protein synthesis depends on RNA or on its synthesis[4]. Some recent evidence has shown that RNA synthesis does not take place to a significant extent in systems lacking an essential amino acid[5–9]. The possible significance of protein synthesis for the synthesis of DNA has also been discussed[10–16]. On the other hand, it is believed that formation of RNA and protein does not depend directly on DNA synthesis[17–19].

Lactobacillus acidophilus R-26 requires, besides deoxyriboside, various nutrilites for its growth[20–22], so that the rates of RNA and protein syntheses as well as the rate of DNA synthesis are controlled in this bacterium by changing nutritional conditions. In the present study, an attempt is made to elucidate the interrelations between the synthetic processes which underly cell growth, by investigating the effects of depletion of the deoxyriboside, uracil or amino acid requirement of this bacterium.

METHODS

The routine handling of *L. acidophilus* R-26 was described previously[23]. Table I shows the composition of the complete culture medium, which is somewhat modified from that reported before[23].

TABLE I

COMPOSITION OF THE COMPLETE MEDIUM

Constituent	*Quantity/l*	
Enzyme-hydrolyzed casein	5	g
Acid-hydrolyzed casein	12.5	g
DL-tryptophane	100	mg
L-cysteine-hydrochloride	130	mg
KH_2PO_4	5	g
$FeSO_4(NH_4)_2SO_4 \cdot 6H_2O$	1.5	mg
NaCl	10	mg
$MnSO_4$	40	mg
$MgSO_4 \cdot 7H_2O$	200	mg
Potassium acetate	12.5	g
Cytidylic acid	25	mg
Adenine sulfate	20	mg*
Guanine hydrochloride	20	mg*
Uracil	10	mg
Thymine	10	mg**
p-Aminobenzoic acid	1.25	mg
Riboflavin	1.25	mg
Nicotinic acid	1.25	mg
Calcium pantothenate	1.25	mg
Vitamin B_6	1.25	mg
Folic acid	0.125	mg
Thioglycolic acid	50	mg
Glucose	15	g
Tween 80	0.5	g
Thymidine	6.05	mg***
Adjusted to pH 6.7 with KOH		

* In some cases half this amount was used.
** In recent expts. thymine was omitted without noticeable effect.
*** In some expts. more was used.

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To test the effects of various deficient media, bacteria previously grown up to the late logarithmic phase in the complete medium were resuspended, after washing in saline, in a larger vol. of one of those media at 0.2 to 0.1 the original cell concn., and incubated at 35°. During incubation, growth was followed by determining the O.D. at 650 mμ in a Beckman DU spectrophotometer. The number of cells/unit vol. of culture was determined at intervals by counting the cells fixed with formalin in an appropriately diluted suspension in a Thoma haemocytometer.

At desired times during incubation, an aliquot of culture was taken and chilled, and cells were sedimented by centrifugation and washed twice in cold saline. In many expts., a part of the cell suspension in saline was used for the determination of total nitrogen. A large aliquot of the cell suspension was centrifuged, and sedimented cells were frozen with dry ice-ethanol. Usually the samples were stored for one to three days at —20° before being used for the analyses of DNA, RNA, protein and acid-soluble compounds.

The acid-soluble, nucleic acid and protein fractions were prepared as described in the previous paper[23]. DNA was estimated by the *p*-nitrophenylhydrazine reaction[24], RNA by the orcinol reaction[25], and total and protein nitrogen by the method of LEVY AND PALMER[26]. Assay of the deoxyribosidic compound in the acid soluble fraction was made microbiologically, by using *L. acidophilus* as the test organism, after digesting the sample with snake venom[23, 27]. U.V. absorption of the acid-soluble extract was determined at 260 mμ in the Beckman DU spectrophotometer.

RESULTS AND DISCUSSION

Effect of deoxyriboside depletion

The bacteria previously grown in the complete medium were transferred into the fresh media containing the following amounts of thymidine/ml: (a) 0.00 mμmole, (b) 0.50 mμmole, (c) 1.75 mμmoles, (d) 25.00, mμmoles.

Changes in the contents of various cellular constituents during incubation in

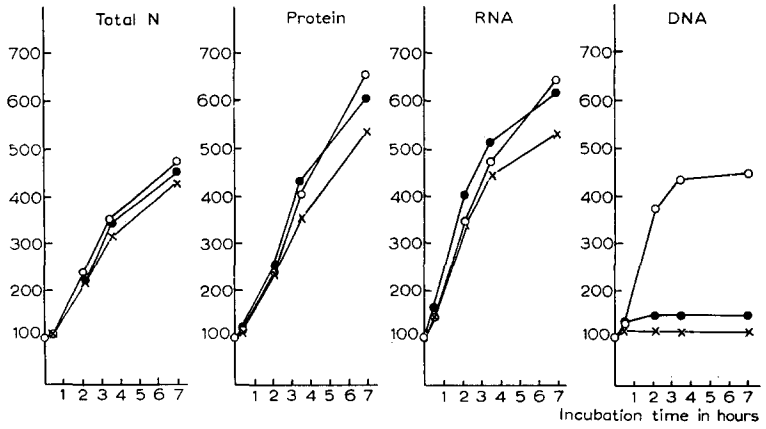

Fig. 1. Effects of thymidine concn. on the increases in total nitrogen, protein, RNA and DNA. Values at zero time are taken as 100. × Medium (b): 0.50 mμmole thymidine/ml. ● Medium (c): 1.75 mμmoles thymidine/ml. ○ Medium (d): 25.00 mμmoles thymidine/ml.

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media (b), (c) and (d) are shown in Figs. 1, 2 and 3. In Fig. 4 are illustrated the increases in total nitrogen, protein, RNA, DNA and cell number during 7-h incubation in the media (a), (b), (c) and (d), and that of acid-soluble u.v. absorbing substances found during 3.5 h.

It will be seen that in medium (a), (b) or (c) which contained no or only a small amount of thymidine, DNA synthesis was largely inhibited (by 87 to 98%), but increases in total nitrogen, protein and RNA were affected only slightly (inhibited by 4 to 28%). Since increase in cell number was also inhibited under these conditions, the amounts of total nitrogen, protein and RNA per cell as well as per DNA became several times higher than in the control incubated in the complete medium (d). The situation would be characterized as "unbalanced growth" similar to that found in *Escherichia coli* 15T⁻ in the absence of thymine[17,18]. The result lends additional support to the assumption that the synthesis of the bulk of cellular protein and RNA does not depend directly on the formation of new DNA molecules[17–19].

On microscopic observation, the cells subjected to deoxyriboside starvation proved to have an elongated form as found in many cases where DNA synthesis and cell division were inhibited relative to growth in mass[17,18].

As described in the previous report[23], the major part of the deoxyribosidic compounds in the acid-soluble extract of this bacterium are those deoxynucleotide-like compounds which show little deoxyribosidic growth effect unless digested enzymically. It is obvious from Fig. 3 that the amount of these intracellular deoxyribosidic compounds increased actively when cells were incubated in the complete medium, but it showed a decrease soon after the beginning of incubation in the media of low thymidine contents. This behavior, together with some other facts, suggested that these acid-soluble deoxyribosidic compounds represent the intracellular precursor pool of DNA[23,33].

Another noteworthy effect was found in the acid-soluble fraction. As shown in Figs. 3 and 4, upon deoxyriboside starvation a remarkable accumulation of u.v. absorbing material occurred in this fraction. In the complete medium the concn. of acid-soluble u.v. absorbing substances increased during the first 30 min and then dropped sharply (Fig. 3). The drop was prevented when cells were cultured in the media of low thymidine contents. In the case of medium (a) or (b), the increase in the concn. of acid-soluble u.v. absorbing material continued for 3.5 h. The result of a preliminary chromatographic study indicated that some derivatives of uracil ribonucleotide are mainly responsible for the observed accumulation of acid-soluble material.

Effects of uracil depletion

Cells previously grown in the complete medium were transferred, after washing, to a fresh medium deficient in uracil. In Fig. 5, the changes in the amount of various cellular constituents during subsequent incubation in this medium are shown together with those observed in the complete and amino acid-deficient media. Another expt. on the effect of uracil depletion is shown in Fig. 6.

It can be seen that the omission of uracil caused parallel inhibition of increases in total nitrogen, protein and RNA. These constituents showed some increase until about 3 h after the beginning of incubation, but thereafter their increase was blocked almost completely. However, the increase in DNA was rather stimulated by uracil

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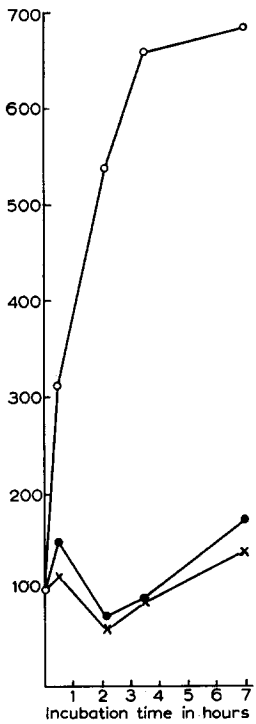

Fig. 2. Effects of thymidine concn. on intracellular acid-soluble deoxyribosidic compounds. The amount at zero time is taken as 100. × Medium (b): 0.50 mμmole thymidine/ml. ● Medium (c): 1.75 mμmoles thymidine/ml. ○ Medium (d): 25.00 mμmoles thymidine/ml.

Fig. 2.

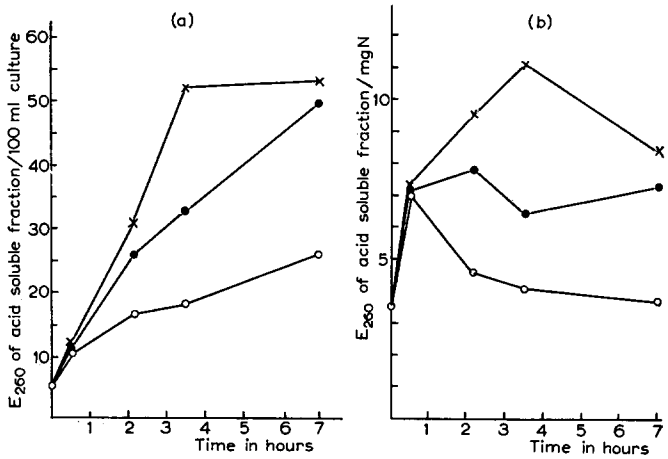

Fig. 3.

Figs. 3 (a) and (b). Changes in u.v. absorbing material in the culturegrown in media of different thymidine contents. Fig. 3 (a): Extinction at 260 mμ/100 ml culture. Fig. 3 (b): Extinction at 260 mμ/mg nitrogen. × Medium (b): 0.50 mμmole thymidine/ml. ● Medium (c): 1.75 mμmoles thymidine/ml. ○ Medium (d): 25.00 mμmoles thymidine/ml.

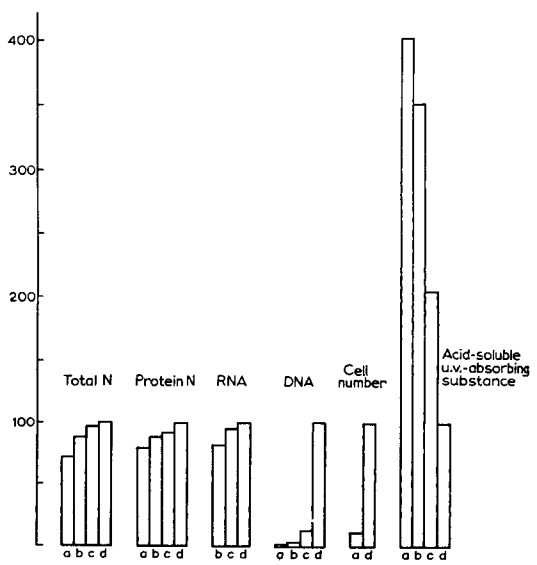

Fig. 4. Effects of deoxyriboside concn. on the increases of various cellular constituents and cell number. The increases in total nitrogen, protein, RNA, DNA and cell number during 7-h incubation and the increase in acid-soluble u.v. absorbing material during 3.5 h are shown. a: No thymidine. b: 0.50 mμmole thymidine/ml. c: 1.75 mμmoles thymidine/ml. d: 25.00 mμmoles thymidine/ml.

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deficiency. Thus depletion of uracil, which would primarily interfere with RNA synthesis, produced a concomitant inhibition of protein formation but tended to accelerate DNA synthesis*. This fact suggests that RNA and protein syntheses are coupled with each other, whereas the system for DNA synthesis is competitively related to that of RNA synthesis. Under the conditions of this expt., the increase in cell number was also inhibited to 38 % of the control.

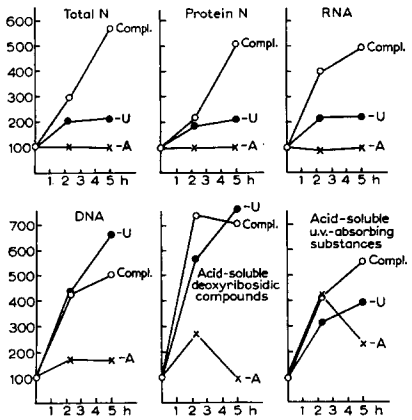

Fig. 5. Changes in the amounts of various cellular constituents during incubation in complete, uracil-deficient and amino acid-deficient media. Values at zero time are taken as 100. Concn. of adenine sulfate and of guanine hydrochloride: 10 mg/l. Compl: Complete medium. –U: Uracil-deficient medium. –A: Amino acid-deficient medium.

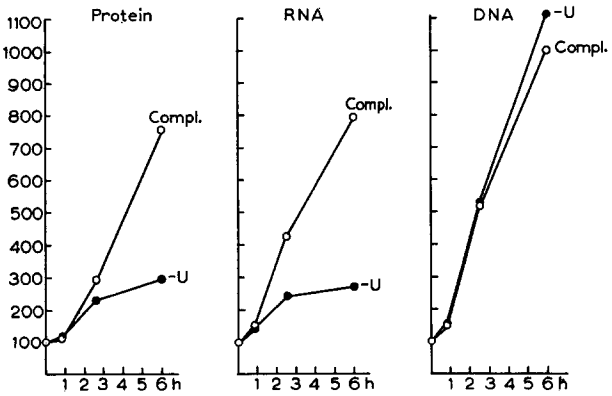

Fig. 6. Effects of uracil-depletion in syntheses of protein, RNA and DNA. Concn. of adenine sulfate and of guanine hydrochloride: 20 mg/l. Compl: Complete medium. –U: Uracil deficient medium.

As seen from Fig. 5, the increase in the quantity of acid-soluble deoxyribosidic compounds was little affected by uracil depletion. At 2 h 15 min after the beginning of incubation, the amount of these compounds was somewhat lower in the uracil deficient culture than in the control, but slightly higher after 5-h incubation. The

* As a result, DNA concn. relative to the amount of RNA or protein reached a value about 3 times the normal. Under certain conditions, DNA concn. higher than this was produced in the cells starved of uracil. A similar observation was made by PARDEE AND PRESTIDGE[38] in *E. coli*; they succeeded in producing surplus DNA twice the normal by treatment with β-2-thienylalanine.

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concn. of these deoxyribosidic compounds within the cell, however, was higher in the uracil depleted cells than in the control at both stages examined.

In connection with the above results, the very recent report of BARNER AND COHEN[29] attracts special attention. These authors studied nucleic acid and protein metabolism in a newly isolated auxotrophic mutant of *Escherichia coli* strain 15, possessing thymine and uracil requirements. They observed that the medium containing thymine but lacking uracil abolished almost completely the increase in RNA but permitted significant increase of DNA and protein, though the rates of both, especially the rate of DNA synthesis, were considerably reduced. The present expt. shows that the depletion of uracil in the presence of thymidine stimulated DNA synthesis in a system which requires both deoxyriboside and uracil. One plausible explanation for this difference would be that in the *E. coli* 15T⁻U⁻ studied by BARNER AND COHEN, uracil may be required, more or less, as precursor of cytosine of RNA and DNA as well as for RNA uracil as observed in other uracil requiring strains of *E. coli*[30–32], whereas in *L. acidophilus* R-26 uracil seems to be used more specifically for RNA uracil. The latter point is suggested by the fact that uracil is essential for the growth of *L. acidophilus* even in the presence of added cytidylic acid. There is another difference between observations made in *L. acidophilus* R-26 and *E. coli* 15T⁻U⁻; RNA and protein increases were inhibited in parallel by uracil depletion in *L. acidophilus*, while in *E. coli* protein synthesis was only slightly affected, under conditions where the RNA increase was completely blocked in a uracil deficient medium. An explanation of this difference may be found in the observation of BARNER AND COHEN that a fraction of RNA showed a marked turnover without net increase of RNA.

Effect of amino acid depletion

The amino acid deficient medium was prepared by omitting casein hydrolysates and L-cysteine and DL-tryptophane from the complete medium. As shown in Fig. 5, the omission of amino acids completely abolished the increase of total nitrogen, protein and RNA. This may indicate, in agreement with recent observations in other microorganisms[5–9], that the presence of amino acids is required directly or indirectly for RNA synthesis as well as for protein formation. DNA synthesis was also inhibited, although some increase was observed in the early phase. Deoxyribosidic compounds and u.v. absorbing substances in the acid-soluble fraction increased during the early phase of incubation and decreased later. The accumulation of these compounds may be a result of the inhibition of DNA, RNA and/or protein syntheses.

Further studies in the uracil-depleted system

It was shown above that in the cells incubated in the uracil deficient medium DNA synthesis was accelerated, although RNA and protein formation was inhibited. Under the above conditions, however, DNA synthesis was not separated completely from the increase of RNA and protein. But, it should be possible to cause DNA synthesis in the absence of RNA and protein synthesis, if the system for DNA synthesis is in fact independent from RNA and protein formation.

When cells previously grown in the complete medium were transferred to the uracil deficient medium, the increase in RNA and protein continued up to 3 to 5 h, though at an inhibited rate, and thereafter leveled off. On the other hand, DNA was

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synthesized actively also during first 3 h. Hence it was considered that separation of DNA synthesis from the increase of RNA and protein might be achieved if the cells were first incubated in the medium deficient in both uracil and deoxyriboside, and, after the increase in RNA and protein had been suppressed, a sufficient amount of deoxyriboside were added. (In some later expts., increase of RNA and protein or turbidity was found to continue even after 6–7-h incubation in the medium lacking uracil and deoxyriboside. However, a closer examination of exptl. conditions showed that this was observed when the depth of culture medium in the Erlenmeyer flask exceeded 4–5 cm, though the reason is not known. Therefore it was necessary to perform the expts. described below with medium depth below 4 cm.)

The results of such expts. are given in Table II and Fig. 7. In one of these (expt. 1), cells were incubated for 4 h 50 min in the medium deficient in uracil but with a small amount (1.75 mμmoles/ml) of thymidine before the addition of excess thymidine. DNA increased 438 % during the 6-h period following the addition of excess thymidine, whereas the increment of protein was as small as 25 % and no significant increase of RNA was observed. Similar results were obtained in expt. 2, in which excess thymidine was added to the cells previously incubated for 6 h in the medium lacking both uracil and deoxyriboside.

TABLE II

CHANGES AFTER THE ADDITION OF EXCESS THYMIDINE TO THE CELLS PREVIOUSLY STARVED OF URACIL AND DEOXYRIBOSIDE

Bacteria grown overnight in the complete medium were resuspended, at 0.1 the original cell concn., in the medium lacking uracil and containing 1.75 mμmoles thymidine/ml. After 3-h 50-min incubation in this medium at 35°, cells were supplied with excess (about 40 mμmoles/ml) thymidine, and further incubated at the same temp.

Time of incubation after addition of excess thymidine	*DNA*	*RNA*	*Protein N*	*Acid-soluble*		*Cell number*
				Deoxy ribosidic compounds	*u.v. absorption*	
	Actual values/l culture					
h min	*mg*	*mg*	*mg*	*mμmoles*		
0.00	0.74	18.6	10.9	44.8	94.2	$904 \cdot 10^8$
0.30	1.13	18.6	11.2	731	95.5	
1.30	2.00	18.6	12.5	881	114.3	
3.00	3.62	19.7	13.4	1151	130.7	
6.00	4.00	18.3	13.6	676	106.4	$928 \cdot 10^8$
	Relative values					
0.00	100	100	100	100	100	100
0.30	152	100	103	1627	101	
1.30	269	100	115	1962	121	
3.00	439	106	124	2572	139	
6.00	538	98	125	1509	113	103

Thus it appears that DNA synthesis can proceed without concurrent synthesis of the bulk of cellular RNA and protein. Under these conditions, the increase in cell number was also inhibited completely, indicating that RNA and/or protein syntheses as well as DNA synthesis are prerequisites for cell division.

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This DNA synthesis in the "resting" cells follows a remarkable change in the acid-soluble deoxyribosidic compounds; these increased sharply during the first 30 to 60 min and later tended to decline. This behavior is also in agreement with the assumption that these deoxyribosidic compounds are the precursors of DNA*[23,33]. On the other hand, the total ultraviolet absorbing material in the acid-soluble fraction showed only a small change.

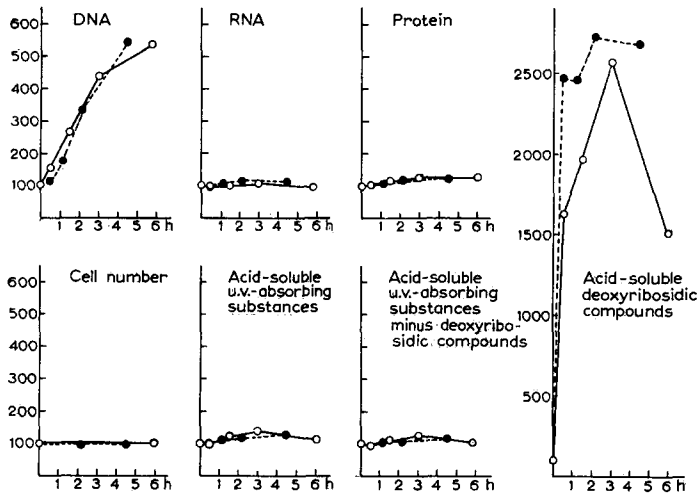

Fig. 7. Changes after addition of excess thymidine to the cells previously starved of uracil and deoxyriboside. Values at zero time (time of thymidine addition) are taken as 100. —○—○— Expt. 1: The same expt. as shown in Table II. ---●---●--- Expt. 2: Bacteria grown overnight in the complete medium were resuspended in the medium lacking uracil and deoxyriboside at 0.1 the original cell concn. After incubation for 6 h, excess thymidine was added, and the culture was incubated further at 35°.

Although under the above exptl. conditions DNA synthesis proceeded almost exclusively in the absence of synthesis of the bulk of cellular RNA and protein, the fact should not be overlooked that the DNA synthesis was still accompanied by the formation of a limited amount of protein (20 to 25 % of the preexisting amount). Most of this increase in protein seems to be in some way coupled with DNA synthesis induced by addition of thymidine, since an increase of less than 10 % was observed if thymidine was not added under otherwise identical conditions (*cf.* lines 5 and 8 in Table III).

In an attempt to clarify the nature of this coupling between DNA synthesis and protein increase, the effect of chloramphenicol, which is known as a relatively specific inhibitor of protein synthesis[34], was tested. It can be seen from Table III that the addition of chloramphenicol abolished almost completely the increase in protein, but showed little inhibitory effect on the DNA synthesis. Thus the protein increase observed in this uracil-depleted system may depend on the DNA synthesis, but the reverse seems not to be the case. If this assumption is valid, the nature of

* The major deoxyribosidic compound that accumulated under these conditions was a derivative of thymidine diphosphate (the deoxyribosidic compound in fraction Kc reported in the previous paper[23]).

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TABLE III

EFFECT OF CHLORAMPHENICOL ON SYNTHESES IN THE URACIL-DEPLETED SYSTEM

The uracil-depleted system was prepared as in Expt. 2 in Fig. 7. Chloramphenicol was added to the medium together with thymidine.

Addition to the uracil and deoxyriboside-depleted cells	*Incubation time h min*	*DNA*	*RNA*	*Protein*	*Acid-soluble*	
					Deoxy-ribosidic compounds	*u.v. absorption*
	0.00	100	100	100	100	100
Thymidine	0.30	102	98	108	3330	120
Thymidine	1.30	156	99	115	2985	127
Thymidine	3.00	289	98	120	1462	90
Thymidine	5.00	355	95	122	918	98
Thymidine and chloramphenicol (25 μg/ml)	5.00	301	102	104	1531	108
Thymidine and chloramphenicol (100 μg/ml)	5.00	324	118	104	1406	108
None	5.00	84	97	109	424	113

protein whose synthesis is directly controlled by DNA synthesis attracts special attention.

Further, the addition of chloramphenicol before the initiation of DNA synthesis on thymidine addition also failed to interfere strongly with DNA synthesis (Table IV). It has been reported that the initiation of DNA synthesis after phage infection, mustard treatment or u.v. irradiation is blocked by chloramphenicol[12–15]. In such cases reconstruction or repair of DNA synthesizing machinery would have to take place before the initiation of actual DNA synthesis, and this would involve the synthesis of new proteins. However, such an alternation of the DNA synthesizing machinery would not be involved in the present case, because DNA synthesis was induced by addition of thymidine to the system which had previously ceased synthesizing DNA because of deficiency of the precursor material.

TABLE IV

EFFECT OF THE ADDITION OF CHLORAMPHENICOL BEFORE THE INITIATION OF DNA SYNTHESIS IN THE URACIL-DEPLETED SYSTEM

The uracil-depleted system was prepared as in Expt. 2 in Fig. 7. Chloramphenicol was added at the concn. of 50 μg/ml.

Addition	*Duration of incubation after the time of thymidine addition; h*	*DNA*	*Turbidity*
	0.00	100	100
Thymidine	4.00	639	126
Thymidine and chloramphenicol (2 h 45 min before thymidine addition)	4.00	455*	110*
Thymidine and chloramphenicol (at the time of thymidine addition)	4.00	471	104
None	4.00	115	106

* Expressed taking the amounts in the same culture at the time of thymidine addition as 100.

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Thus the synthesis of cellular DNA in this deoxyriboside-requiring bacterium would not depend on both protein and RNA synthesis, although the possibility is not necessarily ruled out that DNA synthesis depends on synthesis or on turnover of a small quantity of protein or RNA which cannot be detected by measuring the net amount.

In this connection, the above-mentioned fact that depletion of amino acid inhibited DNA synthesis is of special interest. On the basis of the above assumption, the inhibition of DNA synthesis in cells starved of amino acids cannot be the secondary effect of the inhibition of protein or RNA formation. In the expts. shown in Table V, the effect of amino acid depletion was tested in the uracil deficient system. It is seen that here also amino acid starvation largely inhibited DNA synthesis and that amino acids could not be replaced by an inorganic nitrogen source. It appears very probable that the presence of amino acids might be required in some way for DNA synthesis in the cell. A similar suggestion has been made by SPIEGELMAN[35] about DNA synthesis in osmotically shocked protoplasts of *Bacillus megaterium*. The situation might be explained, if, for example, amino acid-conjugated deoxynucleotides are involved in DNA synthesis.

TABLE V

EFFECT OF AMINO ACID DEFICIENCY ON THE SYNTHESIS OF DNA AND OTHER CONSTITUENTS IN URACIL-DEPLETED SYSTEM

The cells preincubated in the medium lacking uracil and deoxyriboside were transferred into the medium as indicated and incubated at 35°.

Medium	*Time of incubation after transfer to the medium indicated; h*	*DNA*	*RNA*	*Protein*	*Acid-soluble*	
					Deoxyribosidic compounds	*u.v. absorption*
Experiment 1						
	0.00	100	100	100	100	100
Uracil deficient but otherwise complete	4.00	400	140*	137*	825	130
Lacking uracil and amino acids	4.00	156	62	95	380	76
Lacking uracil and amino acids but containing 0.1 % NH_4Cl	4.00	118	72	97	415	79
Experiment 2						
	0.00	100	100	100	—	—
Uracil deficient but otherwise complete	4.00	637	130*	139*	—	—
Lacking uracil and amino acids	4.00	105	73	94	—	—

* Increase of RNA was observed when cells previously starved of uracil and deoxyriboside were transferred to a fresh uracil-deficient medium with excess thymidine, instead of adding excess thymidine to the original medium. Under these conditions increase in protein was also larger than in ordinary expts.

However, an alternative interpretation of the inhibitory effect of amino acid depletion on DNA synthesis is not impossible, although it appears less probable at present: DNA synthesis might depend on the synthesis or turnover of a small fraction of protein or RNA, and this could not be blocked completely by depleting uracil and adding chloramphenicol but virtually abolished under conditions of amino acid depletion.

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The possibility that the protein synthesis is necessary for DNA synthesis in normal cell growth was stressed by BARNER AND COHEN[16] based on the finding in *E. coli* 15T⁻PA⁻ requiring thymine and phenylalanine that the preincubation of cells with phenylalanine alone greatly stimulated the DNA synthesis occurring immediately after transfer to the complete medium. They stated that the preincubation with amino acid may stimulate the formation of a protein possibly essential to the subsequent synthesis of DNA. However, their observation may also be explained by the assumption that small molecular cofactors or precursors containing amino acid are indispensable for DNA synthesis, and that these are accumulated during the preincubation with phenylalanine.

ACKNOWLEDGEMENTS

We are grateful to Professor TUNEO YAMADA for his interest and advice on this work and to Mr. YOSHITAKA KURIKI for kind assistance in some of the experiments.

This work is supported in part by a grant from the Rockefeller Foundation and a subsidy from the Tokai Gakujutsushoreikai.

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