AMINO ACID STARVATION IN AN *ESCHERICHIA COLI* AUXOTROPH

III. INCORPORATION OF ^{32}P INTO RIBONUCLEIC ACID AND OTHER CELL COMPONENTS

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SUMMARY

During amino acid starvation in leucine and proline auxotrophs of *E. coli*, although net protein and RNA synthesis have ceased, there is continuing incorporation of ^{32}P into RNA nucleotides. The turnover rate approximates 10% per hour initially and is nearly equal in the debris fraction, the ribosomes, and the soluble supernatant. Both early and late in starvation and in each centrifugal fraction the specific radioactivities of the four RNA nucleotides are approximately equal. These findings are discussed with reference to the interrelation of RNA and protein synthesis and the cessation of both during amino acid starvation.

INTRODUCTION

It was shown in the preceding papers[1,2] that transfer of an exponentially growing leucine auxotroph of *E. coli* into a medium deficient in the required amino acid, results in immediate cessation of protein and RNA synthesis, while DNA synthesis continues to about 30% net increase, and half the cells in the culture divide. The cells then remain viable for at least 90 min of starvation, and the machinery for protein and nucleic acid synthesis persists intact, for if leucine is again furnished, these macromolecular syntheses resume instantly at the former rate. The cells begin to divide again only after a lag of approximately half a generation time. During starvation the cells accumulate certain RNA precursors and related substances, notably ATP, UTP, UDPX compounds, CTP, DPN, uracil, and hypoxanthine.

It is by no means evident why normal RNA synthesis should be so completely dependent upon concurrent protein synthesis, although some speculative hypotheses have been advanced[3]. Since, as we have shown, the pathways leading to RNA precursors are plainly functional during amino acid starvation, and since the precursors themselves are abundant, the cause of the block must be sought further on,

Abbreviations: RNA, ribonucleic acid; DNA, deoxyribonucleic acid; ATP, adenosine triphosphate; UTP, uridine triphosphate; CTP, cytidine triphosphate; DPN, oxidized diphosphopyridine nucleotide; GMP, guanosine monophosphate; UMP, uridine monophosphate; CMP, cytidine monophosphate; AMP, adenosine monophosphate; BD, bacterial density; PCA, perchloric acid.

in the mechanisms concerned with the sequential arrangement and polymerization of the nucleotides. The studies reported here were therefore undertaken to see whether or not nucleotide precursors could be incorporated into RNA during amino acid starvation, despite the complete blockade of net RNA synthesis. Some of the findings have been reported in a preliminary communication[4].

METHODS

Growth and starvation

Leucine and proline auxotrophs of *E. coli* K12 were used as described previously[1]. Cells were grown at 37° in 1.5–3 l aerated mineral-glucose medium containing sufficient leucine (or proline), harvested at B.D. 0.12 g dry wt./l, washed twice with 250-ml portions of 0.15 % NaCl, and resuspended to the same B.D. in medium lacking the required amino acid. Samples were then withdrawn periodically during starvation. When originally harvested from the complete medium the cells were growing exponentially and had only attained one-quarter of the final B.D. that the medium will support. If the washed cells are resuspended in a complete medium instead of a deficient one, they resume growth immediately at the former rate. That the cells remain competent through at least 90 min starvation was shown in refeeding experiments[1].

3 mC of sodium radiophosphate (Abbott Laboratories, Oak Ridge) was used in each experiment. The contaminant non-orthophosphate ^{32}P which we describe elsewhere[5] did not significantly affect the data reported here because the nucleotides were adequately separated from non-nucleotide P, and because (in the later experiments) charcoal columns were used, as described below. In one type of experiment cells uniformly labelled by growth in $^{32}PO_4$ were resuspended in non-radioactive amino acid-deficient medium. A balance of ^{32}P exchanges among the various cellular fractions during starvation could thus be obtained, as well as a direct measure of ^{32}P excretion. In another type of experiment ordinary cells were starved in radioactive medium so that ^{32}P incorporation could be followed. For radioactivity determinations, appropriately diluted samples were dried on stainless steel planchets and counted with a thin-window G-M tube; counting rates in excess of 5 times background were usually achieved. Since the medium contained 63.5 mmoles P/l while total cell P was only 150 μmole/l, the uptake of ^{32}P by the cells could not appreciably lower the medium specific radioactivity. The specific activity of each cell component was compared with that of the whole culture (cells + medium). Incorporation was expressed as specific activity of sample × 100/specific activity of culture. Since there was little or no net synthesis of RNA during starvation, the incorporation data signify, in effect, % turnover.

Centrifugal fractionation

The harvested cells were washed in cold 0.15 % NaCl and resuspended in 50 ml of 0.003 *M* $MgSO_4$. The B.D. of this suspension was measured turbidimetrically and an aliquot removed for chemical analyses (whole cell fraction). The remainder was treated in the cold for 2 min in a Raytheon 10 kc oscillator at maximum power, then centrifuged at 3,000 × *g* for 10 min to remove unruptured cells. The sediment was resuspended and assayed by turbidimetry or determination of total PCA in-

soluble P. These measures agreed well in estimating the fraction of cells that escaped rupture, from which could be calculated the dry wt. in the sonic lysate. The 3,000 × *g* supernatant was spun at 26,000 × *g* for 1 h, yielding a debris fraction; 105,000 × *g* for 1 h gave a sediment largely composed of 70 S particles (ribosome fraction) and a soluble fraction supernatant. (For the analytical ultracentrifuge runs we are indebted to Dr. F.-C. CHAO.)

Chemical fractionation

Aliquots of each centrifugal fraction (or of whole cells in some of the experiments) were made to 6 % with cold PCA, centrifuged after standing 1 h in ice, and the precipitates washed once with PCA. Phospholipids were removed with water–ethanol, ethanol–ether, and ether, and the residues were made up in 5 ml of 1 *N* KOH and incubated 16 h at 37°. A blank tube containing KOH was also incubated, carried through all subsequent steps, and used to correct the experimental data. The tubes were chilled, exactly neutralized with PCA, then made to 6 % with additional PCA and centrifuged cold to remove $KClO_4$, DNA, and protein. The supernatants, containing RNA nucleotides and non-nucleotide ("concomitant") P, were filtered through PCA-washed paper and each combined with a single cold PCA wash. DNA nucleotides (and "concomitant" P) were extracted from the residues with three 1.5-ml portions of 6 % PCA at 90°; the extracts were chilled, neutralized with KOH, centrifuged, filtered, and made up to 5 ml with water. The protein residues were dissolved in 1 *N* KOH. The cold acid-soluble, phospholipid, RNA, DNA, and protein fractions were assayed for absorbance at 260 mμ, total P (see ref. 6) and ^{32}P; however, as indicated in the data, not all chemical fractions were analyzed in every experiment.

We show elsewhere[5] that non-nucleotide P may seriously distort the RNA incorporation data, especially in the debris fraction. In most of the experiments, therefore, the RNA nucleotides were freed of such material on charcoal. Columns (1 × 11 cm) were prepared with a bottom layer of Whatman cellulose powder, then a 1:3 mixture of Norit A and cellulose powder, and finally a top layer of cellulose powder. The columns were operated under 0.2 atm. positive pressure. The cellulose powder prevented dispersion or leakage of charcoal and permitted adequate flow rates to the end of the run, but the columns could not be used again. The columns were washed well with 2 *M* NH_4OH in 50 % ethanol, then water, then 6 % PCA, and the RNA nucleotide solutions in PCA were poured on. The P and ^{32}P contents of the combined run-through and water washes were determined: these solutions absorbed negligibly at 260 mμ. The adsorbed mixture of nucleotides could be recovered quantitatively by eluting with sufficiently large volumes of ammonia–ethanol; in these experiments, however, the total eluate volumes were limited to 40 ml, giving recoveries of about 80 %. The eluates were taken to dryness in a flash-evaporator in the cold, then redissolved in water as required for the various determinations, or for separation of nucleotides.

Isolation of RNA nucleotides

The nucleotide mixture was applied to a 30 × 30 cm square of Whatman 3MM paper and run as an ascending chromatogram in a modified formulation of MARKHAM'S[7] solvent 5 (solvent 5f: 700 ml isopropanol, 63 ml conc. formic acid, water to 1 l). In some of the early experiments the charcoal column was omitted and the

alkaline digest was neutralized, filtered, dried, redissolved, and directly chromatographed. Electrophoresis was then carried out in the second dimension in 0.1 *N* formic acid (pH 2.6, 16 V/cm) for 2.5 h. Each of the 3 rather large u.v. quenching spots was cut out, eluted with 0.1 *M* NH_4OH onto a strip of 3MM paper, and subjected to ascending chromatography in an ammonium sulfate–isopropanol solvent (300 g $(NH_4)_2SO_4$ in 500 ml water, 10 ml isopropanol, 6 g Tris, pH 7). This final chromatogram confirmed the homogeneity of 2 bands (GMP, UMP) and resolved the 3rd into well-separated spots of CMP and AMP. In later experiments it was found that satisfactory separations could be obtained in a single 2-dimensional run consisting of electrophoresis in formic acid as described above, followed by ascending chromatography in saturated ammonium carbonate. The electrophoretic separation gives (in order, from the anode) well separated spots of UMP, GMP and an AMP–CMP mixture. The chromatogram gives (in order of ascending R_F) AMP (2 spots), GMP, and a CMP–UMP mixture, all well separated. The 2 solvent systems not only complement each other from the standpoint of nucleotide separation, but offer the additional advantage of complete volatility. The spots were finally eluted and *u.v.* spectra recorded against appropriate paper blanks at acid and alkaline pH in the Beckman DK recording spectrophotometer. The spectra corresponded in all significant details with those of authentic 2′,3′-nucleoside monophosphates. The solutions were also assayed for P and ^{32}P. Further proof of purity was afforded by the ratio $O.D._{260}/P$ for each nucleotide.

RESULTS

Phosphate exchanges during amino acid starvation

Cells of the leucine auxotroph were labelled uniformly by growth in $^{32}PO_4$, then resuspended in non-radioactive medium lacking leucine, and harvested after 90 min. A typical experiment is shown in Table I. The characteristic chemical changes described previously are evident here. B.D. increased 17 % but protein N and RNA negligibly or not at all. (In other experiments the same changes often occurred without any increase of turbidity.) Acid-soluble and phospholipid P increased markedly, acid-soluble u.v.-absorbing substances somewhat less than P. Very considerable quantities of u.v.-absorbing substances were excreted from the cells. (Compare 154 A.U.*/l excreted during starvation with 144 A.U./l in the medium in which exponential growth had occurred.) There was net synthesis of DNA to about 30 % increase as measured by P or u.v. absorption. Protein residue P increased somewhat. The viable count increased during starvation to the same extent in ^{32}P as in ordinary medium.

The fate of P present in the cells at the onset of starvation can be followed in the 3rd column of the table. Except for DNA, all fractions lost ^{32}P and the aggregate loss was accounted for by ^{32}P appearing in the medium. The greatest amount of ^{32}P was lost from RNA, but the net loss of RNA was negligible (in most experiments zero). Thus about 12 % of the RNA finally present was derived from newly-formed precursors during the course of starvation. In contrast, DNA present before starvation was conserved. All estimates of the incorporation of P during starvation are neces-

* 1 A.U. = amount of material in 1 ml of solution with $O.D._{260} = 1.0$.

TABLE I

PHOSPHATE EXCHANGES DURING LEUCINE STARVATION

Cells were grown for 3 h with adequate leucine in medium containing $^{32}PO_4$, then harvested and washed twice. Half the cells were used for pre-starvation assays, the other half were resuspended for 90 min in non-radioactive medium without leucine. In the experiment shown here, B.D. increased during starvation from 0.124 to 0.146, protein N from 12.3 to 12.9 mg/l. All data given are per l culture. Data of columns A and B are based on counts and specific activity (53.5 counts/sec/μmole P) in the uniformly labelled cells; data of column C are from chemical determinations on the post-starvation cells. Upper part of table: P, as μmoles; lower part of table: absorbance units (1 A.U. = amount of material in 1 ml of solution with $O.D._{260}$ = 1.0).

	(A) *P before starvation*	*(B)* *Old P still present after starvation*	*(A)-(B)* *Loss of old P*	*(C)* *Total P after starvation*	*(C)-(B)* *Gain of new P*	*(C)-(A)* *Net gain of P*
Whole cells	116	103	13	136	33	20
Acid-soluble	8.40	4.37	4.03	15.8	11.43	7.40
Phospholipid	11.7	9.33	2.37	17.5	8.17	5.8
RNA	80.5	69.1	11.4	78.5	9.4	—2.0
DNA	19.6	19.3	0.3	25.6	6.3	6.0
Protein residue	1.84	1.38	0.46	2.04	0.66	0.20
Sum of fractions	122	104	19	139	36	17
Old P in culture filtrate after starvation			22			

	A.U. before starvation	*A.U. after starvation*	*Net gain of A.U.*
Acid-soluble	38.4	59.6	21.2
RNA	786	831	45
DNA	141	184	43
Culture filtrate	0	154	154

sarily minimum since incorporation of ^{32}P from the pre-starvation pool would not have been detected.

Phosphate incorporation into RNA during starvation

In order to ascertain whether the observed turnover of P in the RNA fraction during starvation actually represented a turnover of RNA nucleotides, cells were grown in nonradioactive medium and starved in the presence of $^{32}PO_4$. RNA nucleotides were then isolated as described and their specific activity compared with that of P in the culture medium. Typical experiments with leucine and proline auxotrophs are shown in Table II. Absence of concomitant P in the isolated mononucleotides is indicated by the good agreement of data in the first 2 columns. The final column shows that the extent of incorporation of all 4 nucleotides was the same, within experimental error. On the other hand, RNA turnover was consistently less in proline than in leucine starvation. We do not know whether this has anything specific to do with proline lack as against leucine lack, or whether (as seems more likely) it arises from a non-specific difference between the 2 auxotrophic strains.

Cultures starved in the presence of $^{32}PO_4$ were sampled periodically in order to obtain information on the rate of RNA turnover. Typical of such experiments with

TABLE II

PHOSPHATE INCORPORATION INTO RNA DURING LEUCINE AND PROLINE STARVATION

Cells were grown for 3 h with adequate amino acid; in the first experiment a leucine auxotroph, in the 2nd a proline auxotroph was used. The cells were washed twice and resuspended for 90 min in amino acid-deficient medium containing $^{32}PO_4$. Culture was sampled initially and finally for B.D., protein N, ribose, and viable count. RNA fraction and individual RNA nucleotides were obtained as described under METHODS. Initial and final data in the leucine experiment were: B.D. 0.157, 0.157; protein N 16.0, 16.2 mg/l; ribose 74, 78 μmoles/l; viable 261, 470·10^9/l. In the proline experiment the corresponding data were: B.D. 0.131, 0.142; protein N 13.5, 14.4; ribose 65, 68; viable 273, 414. Published data on ε_{260}(P) are from BEAVEN *et al.*[8]. Experimental data are from O.D.$_{260}$ and P determinations. For ^{32}P counting 1-ml samples were plated. Nucleotide solutions did not require dilution; RNA samples were diluted 1:10, whole culture samples 1:1331. Data are corrected for background (0.34 counts/sec), decay, and dilution. In the last column incorporation is expressed as 100 × specific activity of sample/specific activity of whole culture; in the absence of net change this represents turnover of RNA as % of that present.

Sample	ε_{260}(P) *published*	ε_{260}(P) *found*	*Counts/sec/ml*	*μmole P/ml*	*Specific activity Counts/sec/μmole P*	*Incorporation %*
Leucine starvation						
Whole culture	—	—	4672	53.1	88.0	—
RNA fraction	—	—	117	8.86	13.2	15.0
CMP 1.	6.8	6.4	5.23	0.383	13.7	15.6
2.		6.4	5.74	0.430	13.3	15.1
AMP 1.	14.2	14.6	4.82	0.377	12.8	14.6
2.		14.1	5.08	0.411	12.4	14.1
GMP 1.	11.8	11.9	4.81	0.360	13.4	15.2
2.		11.6	4.58	0.365	12.6	14.3
UMP 1.	10.0	9.9	4.84	0.359	13.5	15.3
2.		10.0	3.92	0.303	13.0	14.8
Proline starvation						
Whole culture	—	—	5192	54.0	96.1	—
RNA fraction	—	—	87.3	8.41	10.4	10.8
CMP	6.8	6.6	3.87	0.363	10.7	11.1
AMP	14.2	14.2	4.16	0.377	11.0	11.4
GMP	11.8	12.1	3.45	0.306	11.3	11.7
UMP	10.0	9.9	3.93	0.358	11.0	11.4

leucine and proline auxotrophs is that shown in Fig. 1. Turnover began at the onset of starvation and continued at a decreasing rate, for as long as 3 h. The initial rate of turnover in leucine starvation approximated 10% per hour. The decline in the slope of the curve with time, while so little of the RNA has yet turned over, suggests the possibility that the asymptote is not 100% but some smaller fraction.

RNA turnover in various centrifugal fractions

The RNA of different particle sizes is known to differ functionally. Ribosomes are believed to be sites of protein synthesis[9]. The soluble fraction contains "transfer RNA" which accepts activated amino acids and presumably participates in their sequential arrangement on ribosomal templates[10]. Debris RNA is less well understood but may represent ribosomes bound to membrane fragments[9]. In our experience[5] the RNA of *E. coli* is distributed between debris, ribosome and soluble fractions in the

approximate ratio 1:4:1. The ribosomal RNA is unchanged during amino acid starvation (Table III), and there was also no change in the amounts of 30 S and 50 S material (from dissociated 70 S particles[11]) in analytical ultracentrifuge runs at 0.001 *M* Mg^{++} and below. Which of the fractions are involved in RNA turnover and at what relative rates? Some typical experiments are summarized in Table IV. Both early and late in starvation RNA turnover occurred in all the centrifugal fractions.

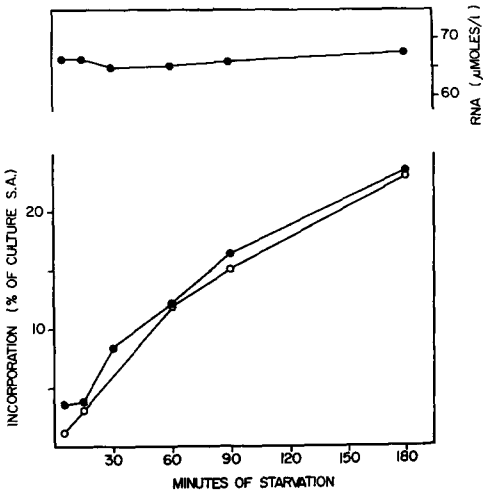

Fig. 1. Kinetics of ^{32}P incorporation into RNA during amino acid starvation (leucine auxotroph). Cells grown 3 h in adequate leucine, washed and resuspended in leucine-deficient medium containing $^{32}PO_4$. Samples taken as shown and worked up for RNA and combined CMP + AMP derived from RNA, as described under METHODS. ●—●, RNA, based on $O.D._{260}$ and $\varepsilon_{260} = 12.0$; upper curve, right ordinate, total cell RNA, μmole/l; lower curve, left ordinate, incorporation, % of RNA turned over. O—O, CMP + AMP, based on P. All counts except some at 5 and 15 min were greater than 5 times background.

TABLE III

STABILITY OF RIBOSOME RNA DURING LEUCINE STARVATION

A culture was sampled during exponential growth at the time of harvesting, then periodically after resuspension of the washed cells in leucine-deficient medium. The procedures described under METHODS were followed for sonic rupture, centrifugal fractionation and chemical isolation of RNA in each sample. Ribose (by the orcinol reaction) and $O.D._{260}$ were then determined. Data are μmoles ribose and absorbance units per g original cells.

	Ribosome fraction	
	Ribose	*A.U.*
Exponential growth	155	3180
15 min starved	157	3080
90 min starved	155	2950

In all experiments and at all times during starvation, although the differences were small, turnover was greatest in the soluble and least in the ribosome fraction. The minor differences between nucleotides within a single fraction were not consistent and are not thought to be real.

TABLE IV

PHOSPHATE INCORPORATION INTO RNA OF VARIOUS CENTRIFUGAL FRACTIONS DURING LEUCINE STARVATION

Procedures as described under METHODS. In this experiment 80% of the cells were lysed by 2 min sonic oscillation. Data are % turnover, calculated as 100 × specific activity of sample/specific activity of whole culture; since there was no net change in RNA this is equivalent to % turnover The data are from 3 different experiments. In Expt. a the UMP was not isolated; in Expt. b the CMP and AMP were isolated together.

Expt.		*a*	*b*	*c*
Duration of starvation (min)		10	10	90
Centrifugal fraction	*Nucleotide*		*% turnover*	
Debris (26,000 × *g* sediment)	Whole RNA	2.0	—	10.5
	CMP	1.7	1.7	
	AMP	1.9		
	GMP	1.7	1.9	
	UMP	—	2.0	
Ribosomes (105,000 × *g* sediment)	Whole RNA	1.6	—	10.0
	CMP	1.2	1.1	
	AMP	1.4		
	GMP	1.2	1.1	
	UMP	—	0.7	
Soluble (105,000 × *g* supernatant)	Whole RNA	2.6	—	13.7
	CMP	2.1	1.5	
	AMP	2.2		
	GMP	2.0	1.4	
	UMP	—	1.7	

DISCUSSION

The experiments reported here show that during amino acid starvation in *E. coli* auxotrophs, after net synthesis of RNA and protein have ceased, there is continued incorporation of ^{32}P from the medium into the nucleotides of RNA. About 10% of the RNA is thus renewed during the first hour. The conservation of DNA phosphate speaks against a simultaneous lysis and re-synthesis of whole cells, and MANDELSTAM[12] has shown in another way that lysis is negligible under these conditions. Nor is there any general degradation of RNA or of ribosomes. In this respect amino acid starvation in an otherwise complete medium differs from incubation in buffer alone[13]. Finally, the magnitude of the turnover makes it evident that we are not dealing primarily with an exchange of terminal nucleotides. Our data on the rate of RNA turnover are not unlike those of GROS AND GROS[14], although they considered the observed incorporation of ^{32}P and [^{14}C]adenine to be negligible.

Thus it appears that phosphodiester bonds in RNA may be labilized to permit nucleotide exchange, or that entire RNA molecules are depolymerized and resynthesized. Either interpretation bespeaks the presence and continued functioning of templates for RNA synthesis during amino-acid starvation. The near-equality of

specific radioactivities in the debris, ribosome, and soluble fractions suggests parallel and independent turnover of the different types of RNA. About 4 nucleotides exchange in the ribosomes for each one in the soluble or debris fraction. Since this is precisely the synthetic ratio during balanced growth, it may be that the turnover process represents the continued operation during starvation of those mechanisms that would ordinarily result in net RNA synthesis.

We showed previously[2] that there is an ample supply of nucleoside di- and triphosphates, and it now appears that their arrangement and polymerization into RNA can and do occur during amino-acid starvation; yet the total amount of RNA does not increase. The cessation of RNA synthesis on withholding a single required amino acid (and of protein synthesis when a single pyrimidine is absent) have been attributed to the need for amino-acid-nucleotide compounds as common intermediates in both synthetic pathways[14]. It is evident, however, from MANDELSTAM's[12] experiments, that during starvation the deficient amino acid should be available within the cell as a result of protein turnover. The hypothetical common intermediates should then also be present, and one should see a continued net synthesis of RNA and ribonucleoprotein at the expense of soluble protein. Likewise, when chloramphenicol blocks protein synthesis in an amino-acid auxotroph, RNA synthesis will not proceed unless the deficient amino-acid is supplied. That which is derived from protein turnover does not suffice, but traces furnished in the medium are quite adequate[15]. These findings suggest that protein turnover may not actually yield amino acids in the form required for RNA synthesis. Alternatively, the turnover of RNA and protein may be tightly coupled so that all protein degradation within the cell is accompanied by an equivalent depolymerization of RNA, making net synthesis impossible.

In MANDELSTAM's[12] experiments amino acid residues of protein were replaced at about 5 %/h, representing 2 % of cell dry wt. In our experiments RNA nucleotides were replaced at 10 %/h, which also amounts to 2 % of cell dry wt. Assuming an average nucleotide to be 3 times heavier than an average amino acid, it follows that 3 amino acids are replaced in protein for each nucleotide replaced in RNA. It has been proposed that much of a cell's RNA may be the inert end-product of "co-synthesis", oligonucleotide "coding units" being polymerized after carrying amino acids to the sites of protein synthesis. For the continued cycling of such a process during starvation we should expect a turnover ratio (nucleotides: amino acids) of at least 3:1, a trinucleotide being the smallest possible "coding unit". The observed ratio (1:3) departs very widely from this value. On the other hand, a coupled turnover during starvation might be expected to reflect the normal synthetic ratio of nucleotides in RNA to amino acids in protein during balanced growth (about 1:8 in this strain), but here the observed ratio falls conspicuously short.

The equal specific radioactivity of all four nucleotides in RNA, even early in starvation when only 1–2 % have exchanged, requires explanation. We have shown[2] that the nucleotide pools of adenine and uracil are much larger than those of guanine and cytosine. It might be expected, therefore, that newly-formed RNA precursors would be diluted unequally prior to their incorporation. The equal specific activities observed in our experiments may therefore indicate (as also proposed recently for mammalian cells[16]) that each of the four pools turns over at a rate proportional to its own size; *i.e.*, the pool size is determined by the cell's total need for a particular nucleotide. In support of this generalization is the fact that the larger pools are just

those which are called upon to supply nucleotides for the more abundant coenzymes as well as for the nucleic acids.

MANDELSTAM[17] has pointed out the probable survival value of protein turnover in providing amino acids for the initial expanded synthesis of repressed enzymes in sudden shifts from rich to deficient media. Since enzyme biosynthesis requires a supply of nucleotides as well as amino acids, RNA turnover could serve an analogous purpose in sudden "shifts down" to purine and pyrimidine deficiency. Related problems have been investigated extensively by MAALOE[18].

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