# TURNOVER OF PROTEIN AND NUCLEIC ACID IN SOLUBLE AND RIBOSOME FRACTIONS OF NON-GROWING *ESCHERICHIA COLI*

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SUMMARY

The stability of protein and of RNA has been examined in the ribosome and soluble fractions of non-growing *E. coli*. In both fractions there is balanced degradation and re-synthesis of protein at about 5% per h. The RNA of the ribosomes is also degraded at this rate but re-synthesized at only 1.5% per h. In the soluble fraction the rate of nucleic acid synthesis exceeds the rate of degradation, so that there appears to be net transfer of material from the ribosomes to the soluble fraction. Degradation of ribosomes supplies much of the free amino acids and almost all of the ribonucleotides passing through the free pool during starvation.

## INTRODUCTION

It has been shown in a number of independent studies that, in actively growing cultures of *Escherichia coli*, there is little, if any, breakdown of protein[1-4]. The stability of RNA has not been examined so thoroughly, but here too the evidence suggests a high degree of stability[4,5].

By contrast it has been found that in populations of *E. coli*, the growth of which has been prevented by nitrogen or carbon limitation, there is balanced degradation and resynthesis of protein which proceeds for some hours at about 5%/h[6-8]. This increased lability in the protein of stationary populations appears to be general among micro-organisms. In *Bacillus cereus* the rates of protein turnover in growing and non-growing cells are 1 % and 7 %/h respectively[9]. In yeast essentially similar results were obtained for breakdown of protein[10,11] and there were similar changes in the stability of the RNA. In these studies in bacteria and yeast the turnover was too great to be accounted for by death and lysis of the cells and it must therefore be presumed to be true intracellular turnover.

When individual enzymes have been measured, they have usually appeared to be stable under conditions where general protein turnover occurs. This appears to be true for nitratase and tetrathionase[12], lysine apo-decarboxylase[13] and β-galactosidase[7], and it raises the question whether turnover affects only non-enzymic proteins. It now appears that the active enzymes of the bacterial cell are distributed between the

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

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soluble and cell membrane fractions and that the ribonucleoprotein particles (ribosomes) seem to be associated with very little enzymic activity[14]. It therefore seemed possible that the material for turnover is provided by the ribosome particles, which are drawn upon under conditions of carbon or nitrogen starvation to provide amino acids.

The present study had two objects: first to determine to what extent the soluble and ribosome fractions were involved in turnover, and second, whether turnover of RNA was co-ordinated with that of protein. The degradation of protein was measured by the loss of radioactive leucine from the soluble and ribosome portions of nitrogen-starved cells; resynthesis was measured by incorporation of labelled leucine into these fractions. The corresponding values for nucleic acid turnover were obtained by measuring incorporation or loss of labelled guanine.

## METHODS

*Organism*: A strain of *E. coli* K12 requiring leucine and threonine was used. The cells were grown with aeration at 35° in minimal medium with 1% succinate as the carbon source and with the required amino acids[7].

### *Measurement of leucine incorporation*

Bacteria were harvested in the exponential phase of growth (0.7–0.9 mg dry wt./ml) and starved by incubation for 20 min in the same medium with threonine omitted. The cells were then washed in phosphate buffer (0.05 *M*; pH 7.0), and suspended in the same buffer containing 1% succinate and incubated at a density of about 1 mg dry wt. cells/ml with DL-[1-$^{14}$C]leucine (500 $\mu$g/ml; specific activity about 10 counts/min/$\mu$g). Samples containing about 170 mg dry wt. of cells were taken at intervals and chloramphenicol added to give a final concentration of 25 $\mu$g/ml to stop further incorporation. The cells were centrifuged and washed in 40 ml tris-(hydroxymethyl) aminomethane (0.001 *M*; pH 7.4) containing magnesium acetate (0.01 *M*)[14]. They were suspended in 11 ml of the same solution and disrupted for 15 min in a Raytheon oscillator (10 kc). This treatment gave very reproducible yields of both ribosomal and soluble fractions. The suspension was centrifuged for 10 min at 10,000 rev./min to remove unbroken cells and debris and the supernatant was centrifuged at 40,000 rev./min (100,000 × *g*) for 1 h. The sedimented ribosomes were washed with 11 ml of Tris–Mg solution and the washings added to the supernatant fraction. The ribosomes were suspended in 5 ml of Tris–Mg solution and both fractions treated in the cold with an equal volume of 10% trichloroacetic acid (TCA). The precipitate was extracted with hot TCA (5%) and organic solvents and radioactivity measured as previously described[7]. The relative specific activity has been expressed as a percentage of the activity of completely labelled protein obtained from cells which had been grown from a small inoculum in the presence of labelled leucine.

### *Loss of labelled leucine from protein*

Cells were grown and harvested as described above, but with [$^{14}$C]leucine in the growth medium. They were then starved, washed and incubated as before in the presence of DL-[$^{12}$C]leucine (500 $\mu$g/ml) to trap any labelled leucine that might be released by the degradation of protein. Samples were taken at the beginning and end of the incubation period and protein counted after isolation, as above, from the

ribosomal and soluble fractions. Because of the difficulty of measuring accurately a small percentage loss in radioactivity, a sample was taken only after 4 h, when the expected loss would be about 20 %.

*Incorporation of guanine*

After growth and the same preliminary starvation and washing as before, cells were incubated in 1 % succinate-buffer with [8-$^{14}$C]guanine (50 $\mu$g/ml; specific activity 60–100 counts/min/$\mu$g). Ribosomal and soluble fractions were prepared and treated in the cold with TCA (final concentration 5 %). The precipitate was washed twice with ice-cold 5 % TCA, and then twice with acetone, ethanol, ethanol–ether and ether[7]. The residue consisting of nucleic acid and protein was weighed, and then extracted twice at 90° for 20 min with 7 ml TCA (5 %)[15]. The residue of protein was dried by washing with acetone and ether. Purines were isolated by the following modification of the method described by KERR *et al.*[16]. The combined TCA extracts were acidified with *N* $H_2SO_4$ (1 ml) and extracted three times by shaking with a double volume of ether to remove TCA. The volume of the aqueous layer was reduced to about 8 ml and the solution heated at 100° for 30 min to convert the purines to free bases which were then precipitated by the addition of 100 mg $AgNO_3$ in a volume of 0.5 ml. Tubes were left overnight in the cold and the precipitate washed twice with 0.1 *N* $H_2SO_4$ (5 ml). It was then extracted twice for 5 min at 100° with 0.1 *N* HCl (5 ml) and the combined extract evaporated to dryness *in vacuo*. The purines thus obtained were dissolved in 2 ml $H_2O$ and a suitable amount (about 0.2 ml) placed on Whatman No. 3MM paper together with 100-$\mu$g amounts of the original labelled guanine used in the incubation medium. Chromatograms were developed for 36 h with isopropanol–HCl–$H_2O$ (75:18:17)[17] and dried at room temperature. The guanine spots which were easily visible under a u.v. lamp, were cut out and extracted three times with $H_2O$ (10 ml). "Blanks" of similar size were cut from the sheet and treated in the same way. The combined extracts were evaporated to a small volume and then made up to 5 ml in graduated tubes. The concentration of guanine was determined by the absorption at 249 and 290 m$\mu$, and a suitable volume (usually 2 ml) plated in 3.5 cm$^2$ planchets for measurement of radioactivity. Correction for selfabsorption was unnecessary.

*Loss of labelled guanine from nucleic acids*

When cells growing in the usual synthetic medium had reached 0.3 mg dry wt./ml, [$^{14}$C]guanine (final concentration 2.5 $\mu$g/ml; specific activity about 100 counts/min/$\mu$g) was added, and the culture grown further to about 0.8 mg/ml. The cells were harvested and after the same preliminary treatment as in previous experiments, they were incubated in succinate-buffer with [$^{12}$C]guanine (50 $\mu$g/ml). At the beginning and end of the incubation period samples were taken for preparation of the ribosomal and soluble fractions. The procedure outlined above for the isolation of purines was then followed, except that guanine was not separated from adenine. The purines were extracted by HCl from the silver precipitate, taken to dryness, and counted.

## RESULTS

*Rates of degradation in soluble and microsomal protein*

K12 (leu$^-$thr$^-$) was grown in 1 % succinate synthetic medium (400 ml) in the

presence of DL-[1-$^{14}$C]leucine (300 $\mu$g/ml; specific activity 5 counts/min/$\mu$g). The cells were harvested at 0.8 mg dry wt./ml, starved and washed and incubated with shaking in 1 % succinate-buffer (350 ml) with unlabelled leucine for 4 h (see METHODS). Samples (175 ml) were taken at the beginning and end of this period. During the incubation the O.D. of the suspension increased 20 %. This is commonly found in coliform bacteria during nitrogen starvation and is probably due to formation of polysaccharides from succinate. The specific activity of the protein isolated from the soluble and microsomal cell fractions was measured.

The results of such an experiment appear in Table I. It will be seen that, while the total amount of protein in each fraction is virtually unchanged, there is a fall in

TABLE I

RATE OF PROTEIN DEGRADATION IN SOLUBLE(S) AND RIBOSOMAL (R) FRACTIONS OF NITROGEN-STARVED BACTERIA AS MEASURED BY THE LOSS OF LABELLED LEUCINE

*E. coli* previously grown in the presence of [$^{14}$C] leucine was incubated 4 h in succinate-buffer containing [$^{12}$C] leucine (500 $\mu$g/ml) (See text.) Radioactivity of protein isolated from the two fractions was measured.

| *Time (h)* | *Fraction* | *Protein isolated (mg)* | *Counts/min/mg protein* | *% Counts lost* |
|---|---|---|---|---|
| 0 | S | 32.0 | 286 | |
| | R | 28.6 | 268 | |
| 4 | S | 34.0 | 214 | 25 |
| | R | 29.4 | 210 | 21.5 |

the specific activity of both fractions: 25 % in 4 h in the soluble fraction and 21.5 % in the particulate. This difference between the two fractions is not significant, and in some experiments the rate of degradation of the particulate fraction was equal to, or slightly higher than, that of the soluble fraction. The values found are in good agreement with an overall degradation rate of about 5 %/h for coliform protein.

*Rates of synthesis of soluble and ribosomal proteins*

Cells were grown in 600 ml medium supplemented with unlabelled leucine and threonine. They were harvested at a density of 0.75 mg/ml, starved, washed and incubated in 1 % succinate-buffer (525 ml) containing DL-[$^{14}$C]leucine (final concentration 500 $\mu$g/ml; specific activity 10 counts/min/$\mu$g). Samples were taken at 0, 120 and 240 min and treated with 4.5 ml chloramphenicol (1 mg/ml) to stop further incorporation. Protein was then isolated from the soluble and ribosome fractions and its radioactivity measured.

It was again found that the amount of protein in each fraction remained the same during the 4 h of nitrogen-starvation (Table II). Both fractions appeared to become labelled at a more or less linear rate. The specific activity of the protein has been calculated as a percentage of that of fully labelled protein, obtained by growing the cells in labelled leucine. On this basis the soluble proteins are 8 % labelled after 2 h and 16.5 after 4 h. The corresponding values for the particulate proteins were 10 and 21.5 %. The proteins of both fractions are thus being re-synthesized at a rate (4–5 %/h) corresponding fairly closely to their rate of degradation.

TABLE II

RATE OF PROTEIN SYNTHESIS IN SOLUBLE(S) AND RIBOSOMAL (R) FRACTIONS OF NITROGEN-STARVED BACTERIA

*E. coli* was incubated with [$^{14}$C]leucine (500 $\mu$g/ml) in succinate-buffer for 4 h (See text). The specific activity of protein isolated from the two cell fractions has been calculated as a percentage of the activity of fully labelled protein (608 counts/min/mg) isolated from cells grown in the presence of [$^{14}$C]leucine.

| *Time (h)* | *Fraction* | *Protein isolated (mg)* | *Counts/min/mg protein* | *% labelling* |
|---|---|---|---|---|
| 0 | S | 38.0 | 1 | 0 |
| | R | 24.7 | 1 | 0 |
| 2 | S | 39.2 | 48 | 8 |
| | R | 26.3 | 61 | 10 |
| 4 | S | 38.3 | 101 | 16.5 |
| | R | 24.2 | 129 | 21.5 |

*Rates of degradation of soluble and ribosomal nucleic acids*

Cells were grown as before in synthetic medium (400 ml). When the culture had reached a density of 0.34 mg/ml, the medium was supplemented with [8-$^{14}$C]guanine (final concentration 2.5 $\mu$g/ml; specific activity 100 counts/min/$\mu$g). The culture was harvested at 0.8 mg/ml and, after the usual preliminary treatment, the cells were incubated in 1 % succinate-buffer (350 ml) containing [$^{12}$C]guanine (50 $\mu$g/ml) as a "trap". Samples (175 ml) were taken at the beginning of the incubation and after 4 h, and the soluble and ribosomal fractions were separated and precipitated in the cold with TCA. The dried precipitates were weighed and extracted with hot 5 % TCA (See METHODS.) The nucleic acid in each fraction is taken to be the amount of material extracted in this step. The total radioactivity of the purine fraction was then measured.

Table III indicates that the weights of nucleic acid, like those of the protein remained fairly constant during the starvation period.

The particulate fraction lost 24 % of its radioactivity in 4 h. This rate compares closely with the loss of radioactivity from the protein of the same fraction and is of the same order as that previously reported for RNA turnover in non-growing *E. coli*[18].

TABLE III

RATE OF NUCLEIC ACID DEGRADATION IN SOLUBLE(S) AND RIBOSOMAL (R) FRACTIONS OF NITROGEN-STARVED BACTERIA AS MEASURED BY THE LOSS OF LABELLED GUANINE

*E. coli* previously grown in the presence of [$^{14}$C]guanine was incubated 4 h in succinate buffer containing [$^{12}$C]guanine (50 $\mu$g/ml). The total radioactivity present in the purines isolated from the two cell fractions was measured (see METHODS).

| *Time (h)* | *Fraction* | *Total nucleic acid extracted (mg)* | *Total purine radioactivity counts/min* | *% counts lost* |
|---|---|---|---|---|
| 0 | S | 9.4 | 13,960 | — |
| | R | 16.9 | 14,220 | — |
| 4 | S | 10.0 | 13,250 | 5 |
| | R | 16.3 | 10,780 | 24 |

By contrast the soluble fraction lost only 15 % of its labelled guanine in the same period. It should be emphasized, at this point that, while the ribosomal fraction contains only RNA, the soluble fraction contains both RNA and DNA. If the latter is stable and also the major component of this fraction, the actual rate of breakdown of the soluble RNA may be considerably higher than appears from the values in Table III.

*Rates of synthesis of soluble and ribosomal nucleic acids*

Cells were grown as before to a density of 0.9 mg/ml. After starvation and washing they were incubated in 1 % succinate-buffer (525 ml) containing [8-$^{14}$C]guanine (50 $\mu$g/ml; specific activity 66 counts/min/$\mu$g).

Samples (175 ml) were taken at 0, and 240 min, and the specific activity of the guanine was measured after its isolation from the soluble and ribosomal fractions.

TABLE IV

RATE OF NUCLEIC ACID SYNTHESIS IN SOLUBLE(S) AND RIBOSOMAL (R) FRACTION OF NITROGEN-STARVED BACTERIA

*E. coli* was incubated with [$^{14}$C]guanine (50 $\mu$g/ml) in succinate-buffer for 4 h. The specific activity of guanine isolated from the nucleic acids of the soluble and ribosomal fractions has been calculated as a percentage of the activity (66 counts/min/$\mu$g) of the guanine in the incubation medium.

| *Time (h)* | *Fraction* | *Total nucleic acid extracted (mg)* | *Guanine isolated ($\mu$g)* | *Counts/min per $\mu$g guanine* | *% labelling* |
|---|---|---|---|---|---|
| 0 | S | 10.8 | 30 | 0.7 | 1 |
| | R | 18.7 | 35 | 0.8 | 1 |
| 4 | S | 11.1 | 34 | 9.5 | 14.5 |
| | R | 17.6 | 40 | 4.0 | 6.0 |

In Table IV, the specific activity of the isolated guanine is expressed as a percentage of that of the guanine added to the incubation medium. The rates of nucleic acid synthesis, as measured by the degree of guanine labelling in the two fractions, differed considerably. The soluble fraction was 14.5 % labelled at the end of 4 h, while the ribosomal fraction was only 6.0 % labelled. This is in contrast to the rate of degradation in this fraction which was almost four times as high (compare Table III).

## DISCUSSION

In *E. coli* in a non-growing state the proteins of the ribosomes and those of the soluble fraction appear to become equally labile and are both degraded at a rate of about 5 %/h. This process is balanced by a more or less equal rate of re-synthesis, so that the amount of protein in each fraction remains constant during a 4-h period in which the total turnover may exceed 20 %.

The nucleic acids present an entirely different picture. The nucleic acid of the ribosomes, which is virtually all RNA is degraded at the same rate as the protein. This suggests that if one of the components of the ribosome is attacked the other also becomes unstable and that the ribosome as a whole is broken down to amino acids and nucleotides. These results are consistent with the observation of TISSIÈRES AND

WATSON[14] that the ribosomes are far more resistant to the action of ribonuclease than purified ribonucleic acid. The rate of re-synthesis of the particulate RNA appears to be at a much lower rate viz. about 1.5 %/h, assuming that guanine incorporation is an accurate measure of the rate of synthesis. If this assumption is made, it follows that the ribosomes synthesized under conditions of nitrogen-deprivation have an abnormally high ratio of protein to RNA. The soluble nucleic acids behave in the opposite way during nitrogen-starvation. They are characterized by a negligible rate of degradation and a fairly high rate of synthesis. The net result would be a transfer of material from the particulate to the soluble state through the nucleotide pool.

It thus appears that when cells enter a state of starvation a balanced flow of amino acids and ribonucleotides to the free pool is established. Such states of starvation are likely to arise temporarily whenever bacteria growing in a complex medium reach a point where one substrate has been exhausted and some alternative is available which requires an induced enzyme. As a result of the turnover process a supply of amino acids becomes available for enzyme synthesis under these conditions. The kinetics of synthesis of β-galactosidase during starvation has been examined and it was found that there was no specific diversion of amino acids to this enzyme. Instead, the β-galactosidase constituted the same fraction of the total protein synthesized as it did in the growing cells[6].

It is currently believed that the ribonucleoprotein particles in mammalian cells are essential sites of protein synthesis, and it has been assumed that this is also their function in bacteria. The present findings indicate that they can, in addition, act as a reservoir which supplies almost half of the amino acids and virtually all of the ribonucleotides passing through the free pools during starvation.

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