The Effect of Culture Age, Chloramphenicol and B₆ Inhibitors on Intra- and Extracellular Keto and Amino Acids of *Escherichia coli* B

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SUMMARY

Keto acids and free amino acids were assayed in the cells and the medium of *Escherichia coli* B growing in the presence of chloramphenicol, cycloserines, aminooxyacetate, and limiting nitrogen source.

Under these growth-limiting conditions the cells accumulated ketoglutarate and 'ketovaline' but no other keto acids. In all experiments only ketoglutarate, pyruvate, and 'ketovaline' were found in the medium. Amino acids are released into the medium in the early phases of growth and the composition of the extracellular amino acids is similar to that of the amino acid pool. The concentrations of free amino acids were 10^3 – 10^4 times higher in the cell than in the medium. The internal pool composition is fixed under all growth-limiting conditions. In the presence of the drugs the cells release amino acids into the medium.

INTRODUCTION

Every organism has a characteristic amino acid pool which is dependent on the genotype of the cell (Holden, 1962; Tempest, Meers & Brown, 1970). The amino acids released into the medium also depend on the genotype, as well as on the growth phase (Dagley & Johnson, 1956; Sorm & Cerna, 1960). The constancy of the pool is understandable in steady-state growth conditions, where the synthesis of macromolecules is regulated by small molecules in the cell.

The changes in the endogenously-synthesized amino acid pools of bacteria have been dealt with in several papers (Raunio & Rosenqvist, 1970; Lowry, Carter, Ward & Glaser, 1971; Clark, Peterson & Barnlohr, 1972). Mandelstam (1958) showed that growth rate, energy metabolism and nitrogen starvation had an influence on the amino acid pool, and amino acids were found in the medium only in the case of nitrogen starvation. Though few papers have dealt with the content of keto acids in the pool and in the medium of growing bacteria, pyruvate and 2-oxoglutarate are known to be the dominant keto acids in the medium (Suomalainen & Ronkainen, 1963; Raunio, 1966; Rosenqvist, Kallio & Nurmikko, 1972).

We here report the effect of certain growth inhibitors on keto and amino acid pools in growing *Escherichia coli* B cells. The powerful B₆ inhibitors, cycloserines and aminooxyacetate, were studied as well as the effect of nitrogen starvation and the blocking of protein synthesis by chloramphenicol.

METHODS

Cultivation of bacteria. *Escherichia coli* B was grown on stock slants containing micro inoculum broth (Difco) and 1.5 % agar and was kept in a freezer under glycerol between transfers. For the experiments *E. coli* B was transferred as a loop inoculum from the slant to an inoculation medium containing 1 % Bacto-tryptone (Difco), 1 % Bacto-yeast extract (Difco) and 0.5 % K_2HPO_4 , all of which had been autoclaved at 120 °C for 7 min. The cells in the inoculation medium were grown in a shaker and, after harvesting in the middle of the exponential growth phase, were washed twice with 0.9 % NaCl solution (room temperature). This procedure gave viable cells for the experiments.

A minimal mineral salt-glucose medium, containing 0.7 % Na_2HPO_4 , 0.3 % KH_2PO_4 , 0.01 % $MgSO_4 \cdot 7H_2O$, and 0.2 % D(+)-glucose plus 0.1 % $(NH_4)_2SO_4$ as nitrogen source, was inoculated with *E. coli* B cells to give an initial concentration of about 1.5×10^8 cells/ml growth medium. The cell suspension, either grown for inoculum or in the minimal medium, was shaken at 250 rev./min in a rotary G 10 gyratory shaker (New Brunswick Scientific Co., New Brunswick, New Jersey, U.S.A.) at 37 °C. The suspension was in a conical flask, with the volume of the medium less than one-fifth the total volume of the flask to ensure enough oxygen for growth. The cells were harvested by centrifugation at 5000 g for 10 min. For assays of keto acids in the minimal medium, 20 ml of the suspension was pipetted on to a Sartorius membrane filter, diam 5 cm, pore size 0.45 μm (Sartorius Membrane Filter, GmbH, Göttingen, Germany) and filtered with suction in less than two min.

Extraction of free amino acids. The extraction was carried out at pH 10 and at 100 °C for 10 min as described by Raunio & Rosenqvist (1970). For the extraction, cells in 70 ml of suspension were centrifuged at 5000 g for 10 min in a cold room, and they were not washed.

Assay of α -keto acids. α -Keto acids were determined as quinoxalines after condensation with *o*-phenylenediamine. For the preparation of the derivatives the method of Mowbray & Ottaway (1970) was used. The phenylenediamine keto acid ratio was about 100:1. No washings of the cells were performed and the quinoxalines were extracted with ethyl acetate and fractionated on silica gel G (Merck AG, Darmstadt, Germany), by using butyl acetate as solvent. Keto acid spots were illuminated under a 366 nm ultraviolet lamp and scraped into a centrifuge tube containing 0.5 N- NH_4OH . The intensity of the fluorescence was measured with a Perkin-Elmer MBF-2 fluorometer. The concentration of the keto acid in a certain spot was calculated from parallel experiments with known amounts of individual keto acids.

Assays of amino acids. Separation of free amino acids from the extract and from the medium was carried out on a Dowex 50 W (H^+ -form) column, as described by Raunio & Rosenqvist (1970). Amino acids were analysed as heptafluorobutyryl amino acid propyl esters by gas-liquid chromatography. Amino acid derivatives were prepared as described by Jöhnsson, Eyem & Sjöquist (1973), except that the esterification was performed only once, for 30 min, and benzene was used in the evaporation stage. The sample was dissolved in methylene dichloride before addition of heptafluorobutyryl anhydride. The fractionation conditions are set out in Table 2, below. The amount of total free amino acids was computed from the amounts of individual amino acids after g.l.c. fractionation and quantitation.

Table 1. *Accumulation of keto acids in the medium of E. coli B during the growth cycle*

Samples (20 ml) were withdrawn from *E. coli* B suspension growing in a minimal glucose-mineral salt medium and filtered immediately after harvesting on a 0.45 μ m filter. Keto acids were analysed from the cell-free medium as quinoxalines on a silica gel G plate and assayed fluorometrically, as described in Methods. No keto acids other than those listed were found in significant amounts in the medium.

Sampling time (h)	$10^{-8} \times$ No. of cells/ml	Ketoglutarate (μ M)	Pyruvate (μ M)	Ketovaline (μ M)	Total keto acids (μ M)
0	1.7	0	0	0	0
2	1.9	1.8	0.9	1.8	4.5
3	3.7	2.9	1.9	3.6	8.4
3.7	6.5	2.3	3.5	6.4	12.2
4.5	9.2	0.8	4.0	10.6	15.4
5.5	9.2	1.1	2.1	11.3	14.5

RESULTS

Accumulation of keto and amino acids during the growth cycle

Table 1 shows the concentrations of keto acids during growth as found on the thin-layer plate after extracting quinoxalines from the cell-free medium of *E. coli* B. Only three fluorescent spots were found: 2-oxoglutarate, pyruvate, and a spot which might have been ketovaline, ketoleucine or phenyl pyruvate. The separation of this last spot was not successful. The name ketovaline is used below because valine was one of the dominant amino acids in the pool (see Table 5, and Raunio & Rosenqvist, 1970). No ketobutyrate and ketoisoleucine spots were found. Keto acids began to accumulate during the early phases of growth (Table 1). The total concentration of keto acids per cell was surprisingly constant during the growth cycle. Also, the variations in amounts of pyruvate and ketovaline were small if keto acid content is calculated per cell. The concentration of ketoglutarate was highest in the first part of the growth cycle, again calculated per cell. The total amount of keto acids in the medium represented about 0.1 % of the glucose concentration in the medium.

Table 2 shows the peaks found in the gas-liquid chromatogram of the material from the pool and from the medium. Nineteen free amino acids were found, plus seven unidentified peaks belonging to non-protein amino acids or amino compounds. No characterization of the unidentified peaks was carried out at this stage.

The accumulation of different amino acids in the simple mineral salt-glucose medium in which *E. coli* B was grown is presented in Table 3. Amino acids begin to accumulate in the medium before cell division starts and their concentrations are maximal during the exponential phase of growth. This phenomenon was observed for all amino acids in the medium except isoleucine, which was found only in the first sample.

Effect of chloramphenicol on free amino acids and keto acids

In cells treated with inhibitors, the growth rate always decreased. Therefore the effect of chloramphenicol on the keto acid and amino acid pools was investigated. Tables 4 and 5 show the effect of this drug. The effect of chloramphenicol on the accumulation of keto acids in the medium is shown in Table 4. The lower the growth rate the higher the concentrations of ketoglutarate and ketovaline, while the concentration of pyruvate in the

Table 2. *Fractionation of heptafluorobutyric amino acid propyl esters by gas-liquid chromatography*

Twenty L-amino acids were acetylated and esterified as described in Methods. Instrumental details are: Perkin-Elmer F-11 fitted with FID, glass column $\frac{1}{8}$ ins $\times$ 6 ft, packed with 3 % OV-1 on Gas Chrom Q (80 to 100 mesh), nitrogen gas flow 35 ml/min, hydrogen pressure 17 lbf/in², and air pressure 22 lbf/in². Sample size was 1 μ l in ethyl acetate, and 1 μ l acetic acid anhydride was injected at the same time. Programming rate was 4 min isothermally at 100 °C, and from 100 °C to 250 °C, 4 °C/min. Norleucine (nleu) was used as internal standard. Arginine, histidine, and cysteine gave low yields in the separation of free amino acids in the derivation process. Peaks represented by numbers were not identified.

Peaks	Relative retention time*		Peaks	Relative retention time*	
	Cells	Medium		Cells	Medium
Ala	28	27	I		101w
Gly	31	30	Lys	114	115
Val	45	44	Tyr	116	117
Thr	50	—	2	123m	124w
Ser	52	51	Arg	127	128
Leu	54	—	His	128	—
Ile	56	56	3	138w	138w
Nleu	61	60	Trp	141	141
Pro	66	—	4	143s	145w
Hyp	80	—	Cys	161	163
Met	83	—	5	162m	—
Asp	85	85	6	167m	167m
Phe	95	95	7	180m	179w
Glu	100	100			

* Relative to glu taken as 100. w, Weak; m, medium; s, strong.

Table 3. *Accumulation of amino acids in the medium of E. coli B during the growth cycle*

Samples were taken from the cell suspension growing in a simple mineral salt-glucose medium at the beginning of the acceleration phase (A), and at the beginning (B), in the middle (C), and at the end (D) of the exponential phase of growth. The separation of free amino acids and the preparation of their derivatives for g.l.c. is described in Methods and in Table 2.

Amino acid	Concn (μ M)			
	A	B	C	D
Ala	0.4	1.1	5.8	2.5
Gly	0.4	1.0	3.2	2.4
Val	0.1	0.3	1.1	0.1
Ser	1.2	1.4	2.8	2.6
Leu	—	0.2	0.2	0.2
Ile	2.4	—	—	—
Pro	—	—	—	0.3
Met	—	—	—	0.2
Asp	—	0.2	0.4	0.4
Phe	—	—	—	0.1
Glu	0.7	2.3	2.0	0.8
Lys	0.9	1.2	1.8	2.0
Tyr	0.3	0.1	0.2	0.3
Cys	0.2	0.2	0.2	0.2
Total	6.6	8.1	17.6	12.2
$10^{-8} \times \text{No. of cells/ml}$ (equivalent to $10^7 \times \text{concentration}$)				
	2.0	3.2	7.0	10.3

Table 4. *Effect of chloramphenicol on the accumulation of keto acids in the medium during the growth of E. coli B*

Experimental details are given in Table 1. Chloramphenicol was added 3 h after inoculation when the cells were in the acceleration phase.

Sampling time (h)	Chloramphenicol concn ($\mu\text{g/ml}$)	$10^{-8} \times$ No. of cells/ml	Keto acids in the medium (μM)			
			Ketoglutarate	Pyruvate	Ketovaline	Total keto acids
0	0	1.4	0	0	0	0
5.5	0	9.8	1.0	4.8	9.8	15.2
5.5	0.5	8.7	1.3	8.2	12.8	22.3
6	2	5.4	3.8	3.7	9.5	17.0
6.5	5	2.8	6.4	3.3	11.8	21.5
6.5	20	1.6	8.5	1.9	8.1	18.5

Table 5. *Effect of chloramphenicol on the intracellular and extracellular amino acids in E. coli B*

Escherichia coli B was grown in a simple mineral salt-glucose medium supplemented with different concentrations of chloramphenicol. Separation and preparation of derivatives of free amino acids from the medium and from the cell pool was as described in Methods and in Table 2.

Amino acid	Amino acid contents									
	Medium (μM)					Cells (10^{-8} mol/cell)				
	Chloramphenicol concn ($\mu\text{g/ml}$)									
	0	0.5	2	5	20	0	0.5	2	5	20
Ala	9.0	47.8	13.2	9.8	39.8	5	16	15	24	19
Gly	8.8	14.0	13.2	12.6	23.4	14	27	22	39	26
Val	1.6	30.8	2.4	—	2.6	3	12	8	7	5
Thr	1.0	0.8	0.8	—	—	1	2	2	5	4
Ser	4.8	3.6	2.6	12.4	22.2	1	3	1	8	5
Leu	1.6	1.2	1.2	—	—	1	3	1	10	5
Ile	1.2	1.2	0.6	—	—	2	2	1	7	5
Pro	2.0	1.4	2.6	16.4	30.2	1	3	1	9	5
Hyp	0.4	0.2	0.2	—	—	3	4	4	9	7
Met	0.4	0.2	0.2	0.4	0.6	1	1	—	1	1
Asp	4.0	2.2	2.2	1.6	1.6	3	6	2	13	7
Phe	1.0	0.6	0.6	2.6	2.8	1	1	—	4	3
Glu	4.4	9.2	32.0	480.0	600.0	15	22	15	61	45
Lys	3.0	1.6	1.8	2.8	7.6	4	4	3	11	7
Tyr	0.8	0.4	0.6	0.6	1.2	1	2	1	5	3
Trp	0.8	0.4	0.6	1.6	1.6	1	2	1	24	7
Cys	2.2	1.2	1.6	1.0	0.8	—	1	—	10	7
Total	47.0	116.8	76.2	541.8	734.4	57	111	76	246	159
	$10^{-8} \times$ No. of cells/ml									
	9.8	8.7	5.4	2.8	1.6					

medium is unaffected. Compared with the normal growth rate the increase of ketoglutarate in the medium, per cell, was 20- to 50-fold; ketovaline increased 4- to 5-fold. These values were obtained, however, when growth was almost completely inhibited. The samples were harvested in the exponential phase of growth.

The contents of the amino acids in the pool and the concentrations of free amino acids in the medium in the presence of chloramphenicol for the same samples as in Table 4 are

Table 6. *Effect of L- and D-cycloserines, amino-oxyacetate, and nitrogen limitation on the accumulation of keto acids in the medium by E. coli B*

Experimental details are given in Table 1. Inhibitors were added to the growing *E. coli* B suspension 2 h after inoculation. D-CS, D-cycloserine; L-CS, L-cycloserine; AOA, amino-oxyacetate; AS, ammonium sulphate.

Sampling time (h)	$10^{-8} \times$ No. of cells/ml	Inhibitor	Keto acids in the medium (μ M)			
			Keto-glutarate	Pyruvate	Ketovaline	Total keto acids
Expt 1						
0	1.6	None	0	0	0	0
5.0	9.7	None	0.3	4.9	8.6	13.8
4.8	9.8	None	1.2	5.3	8.0	14.5
5.3	9.5	D-CS (0.03 mM)	6.8	2.7	9.1	18.6
6.0	4.7	D-CS (0.035 mM)	8.8	8.5	6.4	23.7
6.8	6.8	L-CS (0.02 mM)	7.2	6.2	10.5	23.9
7.0	5.3	L-CS (0.03 mM)	1.2	3.3	9.4	13.9
Expt 2						
4.5	9.2	None	1.7	9.6	9.8	21.1
5.5	5.9	AOA (0.16 mM)	2.5	4.8	13.8	21.1
6.0	4.8	AOA (0.18 mM)	2.0	3.3	10.6	16.1
4.8	5.9	AS (0.02 %)	70.0	5.0	32.0	107.0
5.0	5.2	AS (0.015 %)	75.0	4.3	60.0	139.0

given in Table 5. The release of amino acids is increased in the presence of the protein synthesis inhibitor. The increase is caused mainly by the accumulation of glutamate, alanine, glycine, serine and proline. The increase of glutamate is several hundred-fold if the accumulated amounts are calculated per cell. The effect of chloramphenicol on the intracellular free amino acids is negligible if the content per cell is calculated.

Effect of B₆ inhibitors on the free amino acids and keto acids

B₆ inhibitors block many vital reactions in the biosynthetic and catabolic pathways of amino acids. Therefore one would expect to find the accumulation of keto acids in the cell or in the medium of *E. coli* affected B₆ inhibitors. The B₆ inhibitors used were L- and D-cycloserines and amino-oxyacetate. Table 6 gives the results of the experiments for which keto acid concentrations in the medium of *E. coli* B were assayed. D-Cycloserine increased the amount of ketoglutarate and ketovaline in the medium, although the increase was very small if the growth rate is taken into account. The effect of L-cycloserine and amino-oxyacetate on the accumulation of keto acids was negligible.

When the samples mentioned in Table 6 were analysed for their free amino acid content the values shown in Tables 7 and 8 were obtained. D-Cycloserine increased not only the accumulation of keto acids as shown in Table 6 but also the accumulation of most amino acids. The release of free amino acids from cells was about ten-fold higher compared with the uninhibited growth condition. The other B₆ inhibitors, L-cycloserine and amino-oxyacetate, increased the accumulation of extracellular amino acids in *E. coli* B, though the effects were smaller. The increased accumulation of amino acids was characteristic of the drug, and not due to the lowered growth rate as in the chloramphenicol experiments. The effect of B₆ inhibitors on the intracellular amino acid pool was negligible. In the experiments where D-cycloserine was used as inhibitor, some cells clumped together during growth.

Table 7. *Effect of B₆ inhibitors and nitrogen limitation on the free amino acid pool of E. coli B*

Experimental details are given in Table 6. Separation and preparation of derivatives of amino acids is presented in Methods and in Table 2. Cycloserines gave peaks with the same retention times as serine.

Amino acids in the cells	$10^{18} \times$ Amino acid concn (mol/cell), with the following inhibitors:									
	None		AS (%)		$10^{-4} \times$ AOA (M)		$10^{-5} \times$ L-CS (M)		$10^{-5} \times$ D-CS (M)	
	Expt. 1	Expt. 2	0.02	0.015	1.6	1.8	2.0	3.0	3.0	3.5
Ala	20	19	26	26	24	26	38	4	22	37
Gly	35	42	44	45	44	44	57	14	49	58
Val	8	3	8	7	7	15	16	4	9	11
Thr	4	4	4	5	6	6	8	2	5	7
Ser	7	9	10	4	11	10	—	—	—	—
Leu	8	9	10	13	12	12	13	2	10	16
Ile	7	8	7	10	10	12	11	11	8	12
Pro	8	8	9	11	11	10	11	4	9	13
Hyp	2	3	3	3	2	2	3	4	3	4
Met	2	3	3	2	2	1	3	2	3	3
Asp	11	14	12	16	14	14	16	4	13	20
Phe	3	4	4	5	4	4	5	1	4	6
Glu	19	26	31	42	24	45	60	48	27	36
Lys	6	6	9	8	7	7	6	2	8	10
Tyr	4	4	5	7	6	6	5	1	4	8
Trp	4	2	4	8	6	1	4	1	4	16
Cys	0	0	0	3	3	0	4	1	0	6
Total	149	168	188	215	201	213	270	102	189	280
$10^{-8} \times$ No. of cells/ml										
	9.2	9.8	6.0	5.2	6.0	4.8	6.8	5.2	9.4	4.7

Table 8. *Effect of amino-oxyacetate, L- and D-cycloserines, and the limiting nitrogen source on the release of free amino acids in E. coli B*

Experimental details are given in Table 7.

Amino acids in the medium	Amino acid concn (μ M), with the following inhibitors:									
	None		AS (%)		$10^{-4} \times$ AOA (M)		$10^{-5} \times$ L-CS (M)		$10^{-5} \times$ D-CS (M)	
	Expt 1	Expt 2	0.02	0.015	1.6	1.8	2.0	3.0	3.0	3.5
Ala	5.4	3.8	2.4	1.8	2.8	2.8	0.8	1.2	6.6	21.0
Gly	2.6	3.4	4.2	2.8	21.6	13.2	4.0	7.6	7.6	31.8
Val	1.2	2.6	0.2	0.2	0.8	0.6	0.2	0.2	5.0	4.8
Thr	—	0.2	0.2	—	0.8	1.2	—	0.2	1.4	4.4
Ser	1.6	3.2	1.6	1.6	3.4	5.6	—	—	—	—
Leu	—	0.4	0.4	—	—	—	—	—	3.2	9.6
Ile	—	0.4	0.2	—	0.6	2.2	—	—	2.0	6.6
Pro	—	0.6	0.4	—	1.2	1.2	0.2	0.4	3.2	7.6
Hyp	—	0.2	0.2	—	—	—	—	—	4.4	2.4
Met	—	0.4	0.2	—	0.8	0.4	1.6	2.4	2.2	3.8
Asp	2.0	1.2	0.8	0.4	1.2	1.4	0.6	0.8	5.2	12.4
Phe	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	1.2	3.8
Glu	2.8	3.0	5.6	2.8	21.8	25.6	11.2	9.0	9.4	28.0
Lys	0.6	0.6	0.6	0.8	1.6	1.8	1.6	1.6	3.4	6.2
Tyr	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	1.6	4.2
Trp	1.0	0.8	0.8	—	—	—	—	—	1.8	5.8
Cys	0.2	0.4	0.2	—	—	—	0.2	—	3.0	8.4
Total	17.8	21.6	18.6	10.8	57.0	56.4	27.0	32.8	71.6	173.4
$10^{-8} \times$ No. of cells/ml										
	9.2	9.8	6.0	5.2	6.0	4.8	6.8	5.2	9.4	4.7

Effect of low nitrogen concentration on keto acids and amino acids

Nitrogen limitation led to an increased accumulation of keto acids in the medium (Table 6). This increase was found in the case of ketoglutarate and ketovaline but not in the case of pyruvate; the increase was 60- to 70-fold for ketoglutarate and 5- to 10-fold for ketovaline, compared with normal growth conditions. The effect of lowered nitrogen concentration on intra- and extracellular amino acids was negligible, as shown in Tables 7 and 8.

Concentrations of intra- and extracellular amino acids

Qualitatively, intra- and extracellular amino acids were very similar except for isoleucine and serine (Table 5). The calculated ratios for the concentrations of glutamate and alanine, respectively, in the intracellular space and in the medium were about 10^3 and 10^4 . The concentration of glutamate was 10 mM inside the cell and $0.3 \mu\text{M}$ in the medium; for alanine the corresponding values in one experiment were 8 mM and $0.4 \mu\text{M}$. In these calculations we assumed that an extinction of 1.0 corresponds to 5.4×10^8 cells/ml suspension and the volume of the cell is 2.5×10^{-15} l. An electronic particle counter (Celloscope 302, L. L. Ljungberg & Co., Stockholm, Sweden) was used to assay the number of cells. In one experiment the number of alanine molecules per cell was 1.1×10^7 .

DISCUSSION

Earlier results from our laboratory have shown that the intracellular amino acid pool in *E. coli* is fairly constant during growth. Only the amounts of glutamate, valine, and alanine changed significantly (Raunio & Rosenqvist, 1970). This constancy indicated that the regulatory system for metabolites in the bacterial cell is very efficient, and our aim in this study was to test this efficiency using powerful metabolic inhibitors. Because all these inhibitors affected growth rate we tested this rate effect using chloramphenicol. The effect of the growth rate on the intracellular free amino acids was negligible (Table 5), but the concentrations of extracellular amino acids were altered, indicating that the cells are able to regulate the intracellular pool of amino acids and have an active system or systems to keep the free amino acid content inside the cell at a fixed level. One way in which the amino acid pool in the cell might be regulated is through degradation, e.g. to keto acids. Tables 4 and 6 show that ketoglutarate and ketovaline were formed in greater amounts under abnormal growth conditions. However, this may have been due to reduced rates of amino acid biosynthesis and not to increased degradation. Cycloserines and amino-oxyacetate are powerful inhibitors affecting the anabolic and catabolic pathways of almost all amino acids. One would thus expect these inhibitors to bring about changes in the free amino acid and keto acid contents. The effect of these drugs on the amino acid pool was negligible (Table 5) and the total concentrations of free amino acids inside the cell did not change markedly. Another way to keep the pool at fixed level is to transport the amino acids out of the cells to the medium. This is a probable method of regulation, because the concentrations of amino acids in the medium increased even under normal growth conditions without any inhibitors (Table 3), under chloramphenicol inhibition (Table 5), and especially under abnormal growth conditions caused by B_6 inhibitors (Table 8). It is also possible that the cells degrade proteins and this degradation is reflected in the pool or in the medium. The results show that the intracellular and extracellular amino acids do not correspond to protein amino acid composition in *E. coli* (Roberts, Abelson, Cowie, Bolton & Britten, 1957).

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