The Assimilation of Amino-acids by Bacteria

3. Concentration of free Amino-acids in the Internal Environment of Various Bacteria and Yeasts

By E. SHIRLEY TAYLOR

*Medical Research Council Unit for Chemical Microbiology,
Biochemistry Department, Cambridge*

SUMMARY: Sixteen Gram-positive organisms (including three yeasts) were able to assimilate glutamic acid and lysine from the external medium and to concentrate these amino-acids in the internal environment, while eleven Gram-negative organisms were unable to do so.

It has been shown (Gale, 1947) that *Streptococcus faecalis* cells possess a high concentration of certain amino-acids existing in a free state within the cells, the internal concentration of lysine and glutamic acid being much greater than that in the external environment in equilibrium with the internal environment. Lysine is able to diffuse into the cell under certain conditions, while the migration of glutamic acid across the cell wall appears to be a process requiring energy, obtainable from fermentation processes. The gradient in concentration of lysine and glutamic acid across the cell wall appears to be maintained by properties of the cell wall itself, since rupture of the cell wall with tyrocidin, etc. results in the release of the internal amino-acids (Gale & Taylor, 1947). A biological consequence of this gradient across the cell wall is that the cell is able to select and concentrate certain amino-acids from a deficient medium. This mechanism should be of greater importance to organisms which are nutritionally exacting with regard to amino-acids than to organisms which can synthesize their amino-acid requirements (Gale, 1947). The present communication deals with the distribution amongst various bacterial genera and species of this cell wall gradient effect, as judged by the presence of free amino-acids in the internal environment of the cells.

EXPERIMENTAL

Organisms. The majority of the organisms listed in Table 1 were isolated by members of this Unit but we are indebted to Dr H. McIlwain for the strain of *Strep. haemolyticus* 'Richards', to Dr P. M. F. Shattock for strains of lactobacilli and the Cambridge Pathology Department for *Neisseria catarrhalis* and *Bacterium aerogenes*. The three yeasts were obtained from the Carlsberg Laboratory. Organisms obtained from or deposited in the National Collection of Type Cultures are indicated in Table 1 by their catalogue numbers.

Growth media. All organisms were grown on a basal medium consisting of tryptic digest of casein with the addition of glucose (2.0 % for coliform organisms and clostridia; 1.0 % for lactobacteriaceae); 0.1 % Marmite was added to

Table 1. *Free amino-acids in the internal environment of various organisms*

Organism	Gram-stain reaction	Time of growth (hr.)	Temp. of growth (° C.)	μl. amino-acid/100 mg.				
				Arginine	Glutamic	Histidine	Lysine	Tyrosine
Yeast foam	+	40	25	134	150	75	223	29
Dutch top yeast	+	40	25	187	378	64	543	30
<i>Saccharomyces carlsbergensis</i>	+	40	25	55	198	30	165	7
<i>Lactobacillus casei</i> , YCT 1	+	40	28	0	174	0	97	0
<i>L. delbrückii</i> , B	+	40	37	19	80	—	103	—
<i>L. helveticus</i> , B	+	40	37	—	74	8	70	—
<i>Strep. faecalis</i> , ST	+	16	37	0	230	10	309	—
<i>Strep. faecalis</i> , SF	+	16	37	6	107	—	110	—
<i>Strep. haemolyticus</i> 'Richards'	+	16	37	0	99	—	83	—
<i>Staph. aureus</i> , A	+	16	37	0	293	—	59	—
<i>Staph. aureus</i> , D	+	16	37	0	456	—	99	—
<i>Sarcina lutea</i>	+	56	25	6	225	16	61	0
<i>Micrococcus lysodeikticus</i>	+	24	37	0	203	—	108	—
<i>Cl. sporogenes</i>	+	14	37	0	81	—	31	—
<i>Cl. septicum</i> , P3 (547)	+	14	37	0	7	—	36	—
<i>B. mesentericus</i>	+	14	37	0	14	—	70	—
<i>B. subtilis</i>	+	14	37	0	19	—	29	—
<i>N. catarrhalis</i>	—	24	37	0	0	0	0	0
<i>B. brevis</i>	—	14	37	0	0	—	0	—
<i>Bact. coli</i> (86)	—	14	37	0	0	0	0	0
<i>Bact. coli</i> Taylor	—	14	37	0	0	—	0	—
<i>Bact. coli</i> (7020)	—	14	37	0	0	—	0	—
Organism N.C.T.C. No. 6578	—	14	37	0	0	0	0	0
<i>Bact. aerogenes</i> I	—	14	37	0	0	—	0	—
<i>Bact. aerogenes</i> II	—	14	37	0	0	—	0	—
<i>Proteus vulgaris</i>	—	14	37	0	0	—	0	—
<i>Pseudomonas pyocyanea</i> , G138	—	14	37	0	0	—	0	—

media used for lactobacteriaceae and 0.5 % glutamine for *Strep. haemolyticus* 'Richards'. The media were dispensed in flasks for coliforms, streptococci, lactobacilli and clostridia, conditions being made strictly anaerobic for lactobacilli and clostridia; for all other organisms the medium was dispensed in Roux bottles lying flat so as to expose a large surface to the air during growth. Yeasts were grown in Roux bottles containing Stephenson's inorganic medium (1939) with the addition of 0.2 % 'Difco' yeast extract and 4 % glucose, the pH being adjusted initially to 6.0.

Estimation of amino-acids. Amino-acids were estimated by the use of specific amino-acid decarboxylase preparations (Gale, 1945, 1946). The free amino-acids in the internal environment of the cells were estimated by the method of Gale (1947). To avoid respiratory gas changes with intact cells, manometers were filled with nitrogen during assay.

Estimation of the dry weight of cells. The dry weight per ml. of cell suspension was estimated turbidimetrically by the use of the Hilger photoelectric absorptiometer, calibrated for each of the organisms used by drying a known volume of thick suspension of known turbidity to constant weight.

Method. The cultures were incubated until it was judged that active cell division had just ceased. The organisms were then centrifuged out of the medium, washed once with distilled water and made up into thick suspension of which the dry weight was determined. The internal amino-acid contained in 1 ml. of the cell suspension was then estimated by comparison of the amino-acid content of the suspension before and after boiling the cells (Gale, 1947). For comparative work, the results are expressed as $\mu\text{l.}$ amino-acid in the internal environment of 100 mg. dry weight of cells.

RESULTS

Table 1 shows the amounts of arginine, glutamic acid, histidine, lysine and tyrosine found in the internal environment of the cells of various species of micro-organisms. The tyrosine and histidine values were generally small and were carried out in a few cases only. No arginine was found in the majority of bacterial species tested, many of which are known to possess enzymes which attack the arginine molecule (Hills, 1940). The three yeasts tested all had a high internal concentration of arginine. An interesting correlation can be observed between the presence of lysine and glutamic acid in a free state in the internal environment of the cells, and a positive reaction to the Gram-stain. None of the eleven Gram-negative organisms tested show any internal free amino-acids, whereas all sixteen Gram-positive organisms show internal concentrations of lysine and glutamic acid.

The external medium in all cases consisted essentially of a tryptic digest of casein in which the amounts of free lysine and glutamic acid varied from 100–200 $\mu\text{l.}/\text{ml.}$; the histidine content was *c.* 20–30 $\mu\text{l.}/\text{ml.}$ As found by Gale (1947) 100 mg. *Strep. faecalis* cells possessed an internal environment of volume 0.27–0.34 ml. Consequently, if the concentration of free amino-acid within the cell is the same as that in the external environment, then the amounts of lysine and glutamic acid within the cell would be of the order 30–70 $\mu\text{l.}/100\text{ mg.}$

or of histidine 5–10 $\mu\text{l.}/100\text{ mg.}$ Gale (1947) showed in the case of *Strep. faecalis* that the internal concentrations of lysine and glutamic acid within the cell were considerably greater than those outside, whereas histidine appeared inside the cell in approximately the same concentration as outside. Assuming that these approximate calculations hold true for other bacteria, it is possible to decide whether the amounts of free amino-acid estimated in the internal environment represent an increase of concentration over those existing in the external medium during growth. Inspection of the values in Table 1 shows that the glutamic acid content of the Gram-positive organisms with the exception of *Clostridium septicum*, *B. subtilis* and *B. mesentericus*, showed an internal concentration higher than that in the external medium. In the case of lysine, exceptions may occur with *Staph. aureus* A., *Sarcina lutea*, *Cl. septicum*, *Cl. sporogenes* and *B. subtilis*.

The results suggest that the Gram-positive organisms possess a cell wall which is permeable to amino-acids only under certain conditions as described by Gale (1947) for *Strep. faecalis*. The Gram-negative species do not show this effect.

The question arises whether the Gram-negative cells are completely permeable to amino-acids, in which case they would have an internal concentration of amino-acid equal to that of the medium at harvesting. On washing prior to assay, this amino-acid would be lost in the washing water. To test this a thick suspension of *Bact. coli* cells was prepared as usual and suspended in a solution of lysine containing 200 $\mu\text{l.}$ lysine/ml. After 30 min. the cells were centrifuged down and an attempt made to estimate the lysine in the supernatant fluid and in the packed cell mass. The cell mass was then re-suspended in water, centrifuged down, the supernatant liquid collected, evaporated to small bulk *in vacuo* and assayed for lysine. No evidence could be obtained that the cells had carried down any lysine from the solution other than that mechanically entangled with the cell mass and the washings from the cells were devoid of lysine. These results appear to suggest that the Gram-negative cells are unable to take up amino-acids in a free state into their internal environment.

DISCUSSION

The results above show that there is a clear-cut distinction between Gram-negative and Gram-positive bacterial species in that the latter possess an internal environment in which free amino-acids exist sometimes in a concentration considerably greater than that in the external environment at equilibrium, while the former do not show this property. The effect in *Strep. faecalis* depends partly upon the properties of the cell wall and partly on the existence of a negative charge inside the cell (Gale, 1947). Henry & Stacey (1943) have demonstrated that the reaction to the Gram stain depends upon the presence in the Gram-positive species of a magnesium ribonucleotide in the surface layers of the cell which combines with protein, and confers upon the nucleoprotein so formed the property of combining with the violet dye used in the staining technique. The present studies show that a further difference lies in the

properties of the cell walls of the two groups such that the Gram-positive group can assimilate free amino-acids into the cell and concentrate them in the internal environment. The Gram-positive organisms are, in general, more exacting in their amino-acid requirements than Gram-negative species and the concentration gradient existing across the cell wall must be of biological importance in facilitating the assimilation of the essential amino-acids.

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REFERENCES

- GALE, E. F. (1945). *Biochem. J.* **39**, 46.
GALE, E. F. (1946). *Nature, Lond.*, **157**, 265.
GALE, E. F. (1947). *J. gen. Microbiol.* **1**, 53.
GALE, E. F. & TAYLOR, E. S. (1947). *J. gen. Microbiol.* **1**, 77.
HENRY, H. & STACEY, M. (1943). *Nature, Lond.*, **151**, 671.
HILLS, G. M. (1940). *Biochem. J.* **34**, 1057.
STEPHENSON, M. (1939). *Bacterial Metabolism*, p. 318, 2nd ed. London: Longmans, Green and Co.

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