

AMINO ACID METABOLISM OF MYELOMA CELLS IN CULTURE

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SUMMARY

The growth of myeloma cells in Leibovitz medium supplemented with 20% serum was limited by the depletion of glutamine. A simple modification of the Leibovitz medium by increasing the concentrations of glutamine, lysine, isoleucine, leucine, sodium pyruvate, galactose, and vitamins resulted in over 100% increase in cell growth yield. The total myeloma protein produced by the cells was increased by approximately 90% in modified Leibovitz media. Analysis of spent culture media for 19 amino acids showed that the concentrations of 8 amino acids were reduced; those of 5 amino acids were increased and the other 6 did not change significantly.

INTRODUCTION

The importance of amino acids in synthetic media for *in vitro* growth of animal cells has long been recognized (Eagle, 1959). Studies on the rates of amino acid uptake have shown generally that L-glutamine was the most rapidly depleted, followed by L-lysine, L-leucine and L-isoleucine (McCarty, 1962; Kruse, Miedema & Carter, 1967; Griffiths & Pirt, 1967). L-glutamine in media is not only rapidly used by culture cells but also depleted by spontaneous conversion to pyrrolidone carboxylic acid and, if serum is present, L-glutamine is converted enzymically to L-glutamic acid (Wein & Goetz, 1973). For LS cells growing in Eagle's minimum essential medium (MEM), Griffiths & Pirt (1967) calculated that L-glutamine would limit growth. If excess L-glutamine were added, then L-leucine and isoleucine would limit growth (Griffiths & Pirt, 1967). Birch & Pirt (1970), using in part the above amino acid data, formulated a medium designed for LS cells. In this medium, LS cells attained an average maximum cell concentration of 3.3×10^6 cells/ml as compared to an average of 1×10^6 cells/ml for LS cells in Waymouth's MB752/I + Methocel (Birch & Pirt, 1970). Lambert & Pirt (1975) doubled the concentrations of L-cystine, L-glutamine, riboflavin, and glucose normal for MEM and added various other vitamins, coenzymes,

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hormones, organic and inorganic compounds to MEM to formulate a medium for MRC-5 cells. The new medium increased cell yield by 22%.

In this paper we report a simple modification of L-15 medium (Leibovitz, 1963) by increasing the concentrations of L-glutamine, L-lysine, L-isoleucine, L-leucine, vitamins, galactose, and sodium pyruvate. The growth yield of myeloma cells, MOPC-31C/R was increased by over 100% with the modified L-15 (or L-15/UT) medium as compared to regular L-15. Similarly, the myeloma protein, IgG, yield was increased by almost 90%. Also reported in this paper are the consumption-production rates of 19 amino acids for MOPC-31C/R cells.

MATERIALS AND METHODS

Abbreviations used

FCS	foetal calf serum	MEM	minimum essential medium
IgG	immunoglobulin G	MOPC-31 C/R	mouse plasmacytoma cells
L-15	Leibovitz medium	MRC-5	human diploid cell line
L-15/UT	modified Leibovitz medium		derived from foetal lung
LS	suspension culture cell derived		tissue
	from mouse fibroblast L strain	TCA	trichloroacetic acid

Cell lines

MOPC-31C mouse plasmacytoma cells were obtained from the American Type Culture Collection (Shannon & Macy, 1972). For over a year they have been maintained in stationary suspension in L-15 culture medium (Leibovitz, 1963) supplemented with 20%, v/v, foetal calf serum (FCS). MOPC-31C/R, a rabbit serum-medium adapted subline was obtained by gradually replacing the FCS with rabbit serum in the medium.

Culture media

L-15 was obtained from Grand Island Biological Co. (GIBCO) Grand Island, N.Y., in powder form and was freshly prepared and sterilized by filtration immediately prior to the experiments. A stock solution of supplements was prepared as shown in Table 1 for making

Table 1. *Composition of the stock solution used to prepare modified Leibovitz medium (L-15/UT)*

Component	Concentration, mg/l.
D-galactose	540
Na pyruvate	330
L-glutamine	996
L-leucine	415
DL-isoleucine	830
L-lysine	249
L-15 Vitamins	11 times *

* 11 times the normal concentration of the vitamins found in Leibovitz medium.

the modified L-15 and L-15/UT. The galactose was obtained from Sigma (St Louis, Mo.). The sodium pyruvate and the amino acids were obtained from Nutritional Biochemical (Cleveland, OH). The vitamins were obtained as a 100X solution from GIBCO. The stock solution was aliquoted and frozen until used. L-15/UT was prepared by mixing 0.9 part of

L-15 with 0.1 part of the stock solution. Penicillin and streptomycin were added to the medium to attain final concentrations of 100 units/ml and 100 µg/ml, respectively. Foetal calf serum and rabbit serum, also obtained from GIBCO, were heat-inactivated and added to L-15 and L-15/UT in a ratio of 0.2 to 0.8.

IgG concentration determination

IgG concentration was determined by the radial immunodiffusion method of Mancini (Mancini, Carbonara & Heremans, 1965) with the following modification. The antiserum-agar plates were stored in moist boxes rather than in paraffin oil. Rabbit anti-mouse IgG antisera and mouse IgG were obtained in lyophilized form from Cappel Laboratories (Downingtown, PA). Final concentrations of antisera in agar were 1 in 70. Three microlitres of each sample were added, using a Glenco micro syringe, into 2-mm diameter holes.

Cell counts

All cell counts were made using a haemocytometer. Cells excluding 0.5% trypan blue were considered to be viable.

Preparation of cell inoculum

Cells from stock stationary suspension culture were inoculated into two 500-ml Belco spinner flasks (Belco, Vineland, N.J.). A mixture of 8% oxygen, 4% carbon dioxide, and 88% nitrogen was sparged into the medium in each flask at a rate of approximately 5 ml per min.

Cell inoculum

Cells were taken from the 500-ml Belco spinner flasks, and after thorough mixing, placed in centrifuge bottles. The cells were spun down at 800 rev/min (180 g) for 6 min in an International PR-6 refrigerated centrifuge. The supernatant was decanted and the cells resuspended in either L-15 or L-15/UT supplemented with serum and the cell concentration adjusted to give a final viable cell concentration of 1.4×10^6 cells/ml.

Duplicate 250-ml Belco spinner flasks were prepared for each medium type. Each flask contained 100 ml of medium plus cells. The previously described gas mixture was sparged into each flask. Incubation temperature was 37 °C.

Amino acid analysis

Immediately after collection, particulate matter was removed from the samples by centrifugation and the supernatant frozen to prevent degradation. All samples were simultaneously thawed. Protein was precipitated from the samples with 10% TCA. The samples were left in a cold room overnight. They were centrifuged and the supernatant saved. The precipitates were washed once with 10% TCA, centrifuged, and the wash supernatant added back to the first wash. The TCA-soluble supernatant from 1 ml of the culture medium was diluted to 25 ml with pH 2.2, 0.2 N sodium citrate buffer and 0.8 ml of this diluted sample was applied to a JEOL 6-AH amino acid analyser (JEOL USA Inc., Cranford, N.J.) with the use of a semi-micro column system (0.8 × 30 cm). The first buffer consisted of 0.20 N sodium citrate, pH 3.25, containing 10% methanol which elutes aspartic acid, threonine, serine, asparagine, glutamine, glutamic acid, proline, glycine, cysteine, and valine. The second buffer consisted of 0.20 N sodium citrate at pH 4.25, which serves to elute methionine, isoleucine, leucine, tyrosine and phenylalanine. A temperature programmer changed the column temperature from 57° to 65 °C after elution of proline. The third buffer, made up of 0.35 N sodium citrate, 0.1 N sodium borate at pH 9.35 (Lin, unpublished, 1975), eluted histidine, ornithine, lysine, ammonia, and arginine. All 3 buffers contained thiodiglycol and caprylic acid. The column was re-equilibrated with the first buffer for 20 min before the next sample was run. The entire programme was controlled by a photoelectric tape unit; thus an unattended automated analysis of 12 samples could be achieved, using the above one column total amino acid analysis system.

The data were calculated by use of a set of standard amino acid mixtures. Asparagine and glutamine were eluted with threonine using the above programme. Quantitation of asparagine

and glutamine was made after 5-h acid hydrolysis of the sample with 6 N HCl at 110 °C. The difference in the amount of aspartic acid and glutamic acid with and without acid hydrolysis represented the asparagine and glutamine content in the original sample. One advantage of this analysis is that the pyrrolidone carboxylic acid was quantitatively converted back to glutamic acid after acid hydrolysis.

RESULTS AND DISCUSSION

We found that the growth of MOPC-31C cells in L-15 supplemented with 20% FCS (the medium recommended by the American Type Culture Collection) was limited by depletion of L-glutamine in the medium and that L-leucine, L-isoleucine and L-lysine were severely depleted (Roberts, 1975). The high rate of depletion of these amino acids is in agreement with the findings of others using various cell lines and media (McCarty, 1962; Kruse *et al.* 1967; Griffiths & Pirt, 1967). Unfortunately, L-15 is a high molarity medium (approximately 188 mM). Adding significant quantities of compounds to increase yields would result in a medium of over 200 mM which would be physiologically undesirable. In order not to change the molarity of the medium significantly, the concentrations of the less essential components of L-15 were reduced.

The modified L-15 medium, L-15/UT, was formulated to have: (1) 4.3 times the normal L-15 concentrations of L-glutamine, L-leucine, DL-isoleucine, and L-lysine; (2) 1.5 times the normal concentrations of galactose and sodium pyruvate; and (3) twice the normal concentration of vitamins. All other compounds in L-15 were reduced by 10% in L-15/UT. L-15/UT has an approximate molarity of 191 mM as compared to 188 mM for L-15.

The above modification significantly increased both the growth yield of myeloma cells and the myeloma protein, IgG, produced by the cells. As is shown in Fig. 1, the growth yield of MOPC-31C/R cells (adapted from FCS to rabbit serum) in L-15/UT supplemented with 20% rabbit serum is over 100% higher than that in L-15 supplemented with 20% rabbit serum. The yield of IgG was increased by almost 90% in L-15/UT supplemented with 20% rabbit serum as compared to that of L-15 supplemented with 20% rabbit serum which is shown in Fig. 2. It is interesting to note in Figs. 1 and 2 that the initial rates of cell growth and IgG production were almost identical. Similar results to the above were also obtained using myeloma cells growing in FCS medium.

Attempts to increase yields further by the addition of carbohydrates, amino acids and vitamins were unsuccessful. The reason(s) for the abrupt halt of cellular growth is unclear. The L-15/UT culture media taken during the declining phase of growth had pH values of 7.0 and 7.1 for the 2 duplicate flasks used in the experiment, indicating that pH change was not the cause. The above media did not exhibit any inhibitory effects on myeloma cell growth when added to fresh media in ratios as high as 1 to 1. The reason(s) for the abrupt halt of cellular growth thus appear to be depletion of an unknown factor(s) in the serum, rather than production of an inhibitory factor in the aged culture.

Samples of the L-15/UT media, which were taken at the start of the experiment

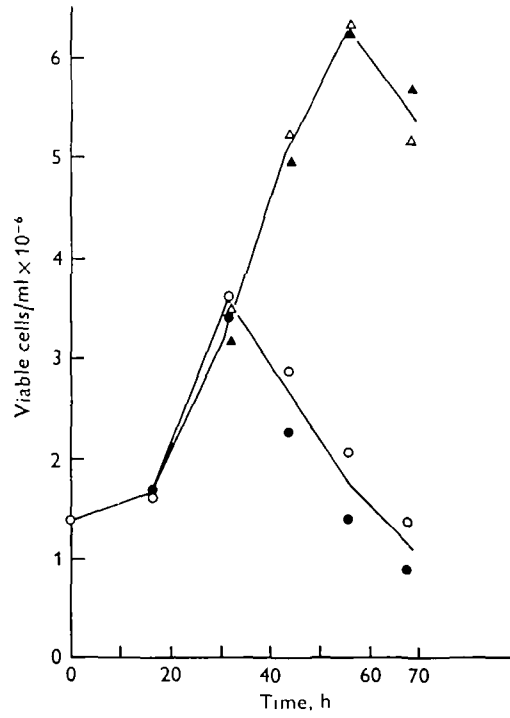


Fig. 1. Myeloma cell growth in Leibovitz medium (L-15) and modified Leibovitz medium (L-15/UT). Batch culture in duplicate spinner flasks of MOPC-31C/R cells. ○, ●, in L-15; △, ▲, in L-15/UT.

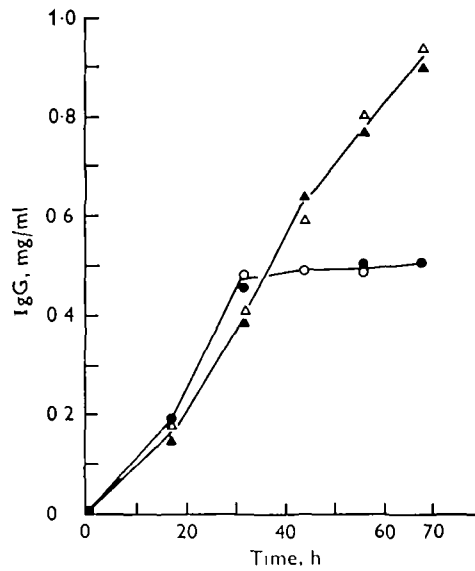


Fig. 2. IgG production by myeloma cells cultured in Leibovitz medium (L-15) and in modified Leibovitz medium (L-15/UT). Batch culture in duplicate spinner flasks of MOPC-31C/R cells. ○, ●, in L-15; △, ▲, in L-15/UT.

and at the point of maximum viable cell concentration (56 h after the start of the experiment), were analysed for 19 amino acids and the percent consumed was calculated for each. The percent consumption of each amino acid is shown in Table 2; L-methionine, L-lysine, and L-isoleucine were the most heavily depleted, 98, 89, and 85%, respectively. Increasing the concentration of these amino acids did not

Table 2. *Amino acid metabolism of myeloma cells MOPC-31C/R in batch spinner flask culture*

Amino acid	Medium			Percent* consumed	Rate of † consumption or pro- duction, mmol × 10 ⁻¹¹ /cell/h	Cell yield/mmol amino acid consumed cells × 10 ⁹ /mmol
	Rabbit serum, mM	L-15/UT, mM	80% L-15/UT + 20% serum, mM			
DL-α-Alanine	0.510	4.55	3.74	N	—	—
L-Arginine	0.092	2.58	2.08	N	—	—
L-Asparagine	0.017	1.70	1.36	N	—	—
L-Aspartic acid	0.131	0.00	0.03	P	+0.08	—
L-Cysteine	0.000	0.89	0.71	N	—	—
L-Glutamine	0.399	8.66	7.01	68	-2.62	1.02
L-Glutamic acid	0.244	0.00	0.05	P	+0.31	—
L-Glycine	1.030	2.40	2.13	P	+0.19	—
L-Histidine	0.124	1.45	1.18	N	—	—
DL-Isoleucine	0.068	8.04	6.45	85	-1.51	1.78
L-Leucine	0.102	4.02	3.24	57	-1.01	2.64
L-Lysine	0.241	2.17	1.78	89	-0.87	3.22
DL-Methionine	0.000	0.90	0.72	98	-0.19	13.9
L-Proline	0.195	0.00	0.04	P	+0.15	—
DL-Phenylalanine	0.079	1.36	1.10	23	-0.13	38.5
L-Serine	0.172	1.71	1.40	P	+0.26	—
DL-Threonine	0.145	4.53	3.65	N	—	—
L-Tyrosine	0.050	1.49	1.20	11	-0.07	58.9
DL-Valine	0.295	1.54	1.29	57	-0.16	13.3

* Analysed at peak of growth. N = negligible concentration change (less than 10%). P = produced. All DL mixtures were assumed to be in 50-50 ratio of D to L and the % consumption was based only on L form. Medium was not analysed for tryptophan. Concentration of tryptophan in L-15/UT is 0.09 mM.

† Calculated by equation:

$$\int_{C_i}^{C_f} dc = kN_o \int_0^T e^{\alpha t} dt \quad \text{or} \quad k = \frac{C_f - C_i}{T} \left[\frac{\ln N - \ln N_o}{N - N_o} \right],$$

k = rate of consumption or production, mmol/cell/h. N_o = initial number of cells, N = final number of cells, C_i = initial concentration, C_f = final concentration, T = time interval, $\alpha = 0.693/G$, G = mean generation time.

significantly increase maximum cell concentration. The rate of consumption or production and, in the case of consumption, the cellular yield of the nineteen amino acids are also shown in Table 2. The rates of consumption or production of the different amino acids are in the ranges previously reported for 5 different cell lines (McCarthy, 1962).

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