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Growth and metabolism of marine fish Chinook salmon embryo cells: response to lack of glucose and glutamine

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Abstract

A peculiar phenomenon, differing from the response of mammalian cells, occurred when Chinook salmon embryo (CHSE) cells were passaged in the medium lacking of both glucose and glutamine. To elucidate metabolic mechanism of CHSE cells, the metabolism parameters, key metabolic enzymes, and ATP levels were measured at different glucose and glutamine concentrations. In the glutamine-free culture, hexokinase activity kept constant, and lactate dehydrogenase (LDH) activity decreased. This indicated that lack of glutamine did not expedite glucose consumption but made it shift to lower lactate production and more efficient energy metabolism. The results coincided with the experimental results of unaltered specific glucose consumption rate and decreased yield coefficients of lactate to glucose. In the glucose-free culture, simultaneous increase of glutaminase activity and of specific ammonia production rate suggested an increased flux into the glutaminolysis pathway, and increases of both glutamate dehydrogenase activity and yield coefficient of ammonia to glutamine showed an increased flux into deamination pathway. However, when glucose and glutamine were both lacking, the specific consumption rates of most of amino acids increased markedly, together with decrease of LDH activity, indicating that pyruvate derived from amino acids, away from lactate production, remedied energy deficiency. When both glucose and glutamine were absent, intracellular ATP contents and the energy charge remained virtually unaltered.

Introduction

Although marine fish cell lines are increasingly used in virology, toxicology, biotechnology and basic fish researches, investigations on their growth and metabolism have seldom been reported. Bols *et al.* (1994) found that glutamine requirement for the *in vitro* proliferation of fish cells was conditional and dependent on the basal medium and serum supplementation. Ganassin & Bols (1992) reported that purines could promote growth of salmon cell line in the medium with different mammalian sera. Unfortunately, from then on, little has been reported about growth and metabolism, especially metabolic pathways, of marine fish cell lines.

Glucose and glutamine are main nutrients of animal cells in culture. Glucose is utilized either through the pentose phosphate pathway to provide nucleotides for biosynthesis or through the glycolysis pathway to provide metabolic intermediates and energy. A large portion of glucose may end up as lactate and, normally, only a small portion of glucose enters the tricarboxylic acid (TCA) cycle. Although glutamine can be either directly used as protein and peptide constituent or converted to precursors of purines and pyrimidines of nucleic acids, a large portion of glutamine may undergo deamination and transamination reactions. Most of mammalian cell lines die immediately once glucose is depleted (Mercille & Massie 1994). However, if glutamine is depleted, some cell

lines cannot survive but others can adapt to the condition lacking of glutamine (McDermolt & Butler 1993, Sun & Zhang 2004). In the work we have found that Chinook salmon embryo (CHSE) cells can be passaged into the medium lacking of both glucose and glutamine, which is completely different from mammalian cell culture. To elucidate the metabolic mechanism, the metabolism parameters, key glycolytic and glutaminolysis enzymes, intracellular ATP and energy charge of CHSE cell were investigated.

Materials and methods

Cell line and cell culture

Chinook salmon embryo cell line (CHSE-214), established by Fryer *et al.* (1965), was kindly provided by Institute of Aquatic Biology, Chinese Academy of Science. Cells were grown at 20 °C and pH 7 in tissue culture flasks with DMEM without glucose and glutamine (Sigma) supplemented with 10% (v/v) newborn calf serum (Sijiqing Biotechnology Co., Hangzhou, China) and, when necessary, 1 g glucose l^{-1} and 0.1 g glutamine l^{-1}. To elucidate the metabolic mechanism of CHSE cell, after washing with phosphate buffer solution, cells were inoculated into four media with different glucose and glutamine concentrations to exhaust endogenous glucose and glutamine and exclude their residual effects.

Analytical methods

Cell density was measured by microscopic counting with hemacytometer. Cell viability was estimated by the method of trypan blue dye exclusion. Glucose and ammonia were analyzed enzymatically by special analytical kits (Shanghai Institute of Biologicals, China). Lactate was quantified by lactate dehydrogenase method (Gutmann & Wahlefeld 1974). Amino acids were assayed by reversed-phase HPLC after pre-column (Agilent Hypersil AA-ODS) derivation with *o*-phthaldialdehyde (Fiorino *et al.* 1989).

Enzyme activities were determined in quadruplicate on different samples. Enzymes were extracted according to the method of Yallop *et al.* (2003) and kept on ice during the experiments. In assays involving NAD^+/NADH, measurements were taken at 340 nm and the extinction coefficient $E_{340\ nm}$ of 6.2×10^{-3} mol^{-1} l cm^{-1} was used. Hexokinase (HK, E.C. 2.7.1.1) was assayed according to the methods of Du & Chen (1987). Glutaminase (GA, E.C. 3.5.1.2) and NAD-dependent glutamate dehydrogenase (GDH, E.C. 1.4.1.2) were assayed using the methods of Meister (1985) and Bergmeyer (1983). Lactate dehydrogenase (LDH, E.C. 1.1.1.27) was assayed using an analytical kit (Jiancheng Biotechnology Co., Nanjing, China). Glutamate/pyruvate aminotransferase (GPT, E.C. 2.6.1.2) and glutamate/oxaloacetic aminotransferase (GOT, E.C. 2.6.1.1) were also assayed using special analytical kits (Shanghai Institute of Biologicals).

Protein concentration of cell-free extract was determined in triplicate by Coomassie Brilliant Blue method using a special analytical kit (Jiancheng Biotechnology Co.).

Metabolism parameter calculations

Specific rates of substrate consumption and metabolite production were calculated using the following equations:

$$Q_{Glc} = 2 \times 10^{-3}(C_{Glc,t_0} - C_{Glc,t_1})/[(X_{t_1} + X_{t_0}) \times t],$$

$$Q_{Gln} = 2 \times 10^{-3}(C_{Gln,t_0} - C_{Gln,t_1})/[(X_{t_1} + X_{t_0}) \times t],$$

$$Q_{Lac} = 2 \times 10^{-3}(C_{Lac,t_1} - C_{Lac,t_0})/[(X_{t_1} + X_{t_0}) \times t],$$

$$Q_{Amm} = 2 \times 10^{-3}(C_{Amm,t_1} - C_{Amm,t_0})/[(X_{t_1} + X_{t_0}) \times t],$$

$$Q_{AA} = 2 \times 10^{-3}(C_{AA,t_0} - C_{AA,t_1})/[(X_{t_1} + X_{t_0}) \times t],$$

where Q is specific rate, mmol $cell^{-1}$ d^{-1}; C is the metabolite concentration, mmol l^{-1}; t_0 and t_1 are the seeding and harvest times, d; X_{t_0} and X_{t_1} are the viable cell densities at t_0 and t_1, cells ml^{-1}. Subscripts Glc, Gln, Lac, Amm, Ala and AA indicate glucose, glutamine, lactate, ammonia, alanine and amino acid, respectively.

The yield coefficients of lactate to glucose, ammonia and alanine to glutamine (mmol $mmol^{-1}$) were calculated from the equations:

$$Y_{Lac/Glc} = Q_{Lac}/Q_{Glc},$$
$$Y_{Amm/Gln} = Q_{Amm}/Q_{Gln},$$
$$Y_{Ala/Gln} = Q_{Ala}/Q_{Gln}.$$

Results and discussion

Cell growth

The growth of CHSE cells is shown in Figure 1. The maximum cell densities at 12 d in the glutamine-free culture (with 1 g glucose l^{-1}) and glucose-free culture (with 0.1 g glutamine l^{-1}) were 11.2×10^5 cells ml^{-1} and 9.3×10^5 cells ml^{-1}, respectively, lower than that in the regular culture with 1 g glucose l^{-1} and 0.1 g glutamine l^{-1}. In the culture lacking of both glucose and glutamine, cells grew much slowly to a maximum cell density of 7.1×10^5 cells ml^{-1} but could be passaged steadily. In hybridoma and myeloma cell cultures, cells die immediately once glucose is depleted (Mercille & Massie 1994). HeLa cells could grow normally in the glucose-free medium but needed extra addition of nucleotides (Reitzer *et al.* 1979). Up to now, no cell line has been reported that can grow normally in the medium lacking of both glucose and glutamine.

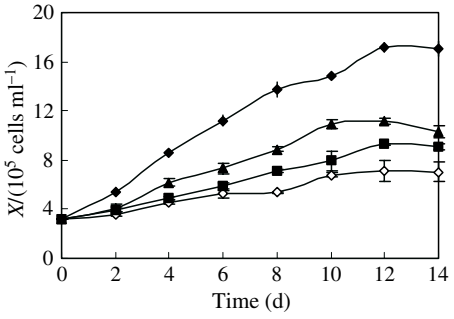

Fig. 1. Growth of CHSE cells in batch cultures with different concentrations of glucose and glutamine. CHSE cells were inoculated at 3.2×10^5 cells ml^{-1}. ◇ Glc (0 g l^{-1}), Gln (0 g l^{-1}); ■ Glc (0 g l^{-1}), Gln (0.1 g l^{-1}); ▲ Glc (1 g l^{-1}), Gln (0 g l^{-1}); ◆ Glc (1 g l^{-1}), Gln (0.1 g l^{-1}).

Metabolism parameters

The metabolism parameters of glucose and glutamine and specific consumption rates of amino acids at 6 d in the batch culture with different glucose and glutamine concentrations are shown in Tables 1 and 2. In the glutamine-free culture, Q_{Glc} kept constant, Q_{Lac} and $Y_{Lac/Glc}$ decreased by 17% and 20% respectively, showing that lack of glutamine did not expedite glucose consumption but make it shift to lower lactate production and more efficient energy metabolism. This result differed from pulse culture of baby hamster kidney (BHK) cells, whose Q_{Glc} reduced with the decrease of glutamine concentration (Martinelle *et al.* 1998).

In the glucose-free culture, the specific consumption rates of glutamine and other amino acids increased greatly, suggesting that lack of glucose forced the cells to utilize amino acids as energy sources. Combined increase of Q_{Gln} and $Y_{Amm/Gln}$ by 19% and 18%, respectively, did not offset the high increase of Q_{Amm} by 41%, meaning the vigorous catabolism of cells to some amino acids other than glutamine. Increased $Y_{Amm/Gln}$ and decreased $Y_{Ala/Gln}$ gave an evidence of the shift of glutamine consumption towards energy efficient deamination metabolism. In murine hybridoma and Ehrlich ascites tumor cell cultures, depletion of glucose also propelled the cells to shift to more efficient pathway of glutaminolysis (Barnabé & Butler 2000, Skog & Tribukait 1985). In hybridoma cell culture, glucose limitation increased glutamine consumption and led to most of the glutamine entering TCA cycle through the glutamate dehydrogenase pathway (Ljunggren & Häggström 1994).

Amino acids take part in both anabolism and catabolism when both glucose and glutamine were lacking. The specific consumption rates of amino

Table 1. Metabolism parameters of glucose and glutamine in Chinook salmon embryo cell culture at different glucose and glutamine concentrations. Q_{Glc}, Q_{Gln}, Q_{Lac} and Q_{Amm} are specific consumption or production rates of glucose, glutamine, lactate and ammonia, respectively, mmol $cell^{-1}$ d^{-1}. $Y_{Lac/Glc}$, $Y_{Amm/Gln}$ and $Y_{Ala/Gln}$ are the yield coefficients of lactate to glucose, ammonia and alanine to glutamine, respectively, mmol $mmol^{-1}$.

Medium	Q_{Glc}	Q_{Lac}	$Y_{Lac/Glc}$	Q_{Gln}	Q_{Amm}	$Y_{Amm/Gln}$	$Y_{Ala/Gln}$
Glc (0 g l^{-1}) Gln (0 g l^{-1})	–	2.31	–	–	0.45	–	–
Glc (0 g l^{-1}) Gln (0.1 g l^{-1})	–	0.63	–	0.37	0.69	1.86	0.24
Glc (1 g l^{-1}) Gln (0 g l^{-1})	3.10	3.45	1.11	–	0.31	–	–
Glc (1 g l^{-1}) Gln (0.1 g l^{-1})	3	4.18	1.39	0.31	0.49	1.58	0.31

Table 2. The specific consumption rates (10^{-10} mmol cell^{-1} d^{-1}) of amino acids in CHSE cell culture with different concentrations of glucose and glutamine. "–" indicates production. Q_{AA} is specific consumption rates of amino acids, and AA includes 20 amino acids.

Q_{AA}	Glc (0 g l^{-1}) Gln (0 g l^{-1})	Glc (0 g l^{-1}) Gln (0.1 g l^{-1})	Glc (1 g l^{-1}) Gln (0 g l^{-1})	Glc (1 g l^{-1}) Gln (0.1 g l^{-1})
Q_{Gln}	–	3.70	–	3.10
Q_{Ser}	3.86	3.42	2.68	1.29
Q_{Asn}	0.11	0.07	0.09	0.07
Q_{Glu}	0.36	0.42	0.20	0.21
Q_{Gly}	3.15	3.19	2.81	1.97
Q_{Thr}	2.85	0.44	0.42	0.49
Q_{Ala}	1.55	−0.87	1.90	−0.60
Q_{Arg}	1.95	1.31	1.34	1.09
Q_{Val}	0.71	0.59	0.30	0.41
Q_{Met}	1.67	0.73	0.50	0.56
Q_{Ile}	1.23	1.09	0.39	0.50
Q_{lys}	2.88	3.40	0.48	0.75
Q_{Asp}	0.28	0.13	−0.05	0.16
Q_{His}	0.17	0.06	0.10	0.09
Q_{Tyr}	−0.35	0.06	0.23	0.38
Q_{Cys2}	−0.30	0.01	−0.13	0.02
Q_{Try}	0.17	0.10	−0.10	0
Q_{Phe}	−0.09	0.15	−0.15	0.17
Q_{Leu}	−0.67	1.07	−1	0.07

Table 3. Specific activities [μmol min^{-1} (mg protein)$^{-1}$] of intracellular enzymes (see Methods) involved in glucose and glutamine metabolism of CHSE cells at different glucose and glutamine concentrations. Each value represented an average of quadruplicate determinations.

Medium	HK	LDH	GA	GDH	GPT	GOT
Glc (0 g l^{-1}) Gln (0 g l^{-1})	0.015 ± 0.002	2.23 ± 0.01	0.036 ± 0.011	0.086 ± 0.008	4.54 ± 0.26	4.12 ± 0.65
Glc (0 g l^{-1}) Gln (0.1 g l^{-1})	0.015 ± 0.002	1.80 ± 0.30	0.056 ± 0.010	0.310 ± 0.015	4.37 ± 0.60	5.93 ± 0.27
Glc (1 g l^{-1}) Gln (0 g l^{-1})	0.021 ± 0.001	2.79 ± 0.19	0.025 ± 0.008	0.096 ± 0.012	4.17 ± 0.17	4.69 ± 0.41
Glc (1 g l^{-1}) Gln (0.1 g l^{-1})	0.022 ± 0.003	3.49 ± 0.20	0.045 ± 0.004	0.250 ± 0.004	4.31 ± 0.40	5.79 ± 0.24

acids, except for Tyr, Cys2, Phe and Leu, increased greatly. Catabolism of amino acids produced pyruvate, acetyl-CoA, succinyl-CoA and α-ketoglutamate to enter TCA cycle and supply energy for cell growth. Every amino acid molecule could produce one ammonia molecule in catabolism, so Q_{Amm} only decreased 8%. Because only a few kinds of amino acids could produce pyruvate in catabolism with very low proportion of pyruvate ending up in lactate, Q_{Lac} decreased greatly by 44%.

Enzyme activities

Table 3 gives the key enzyme activities of glycolysis and glutaminolysis. In glycolysis, glucose was first converted to glucose 6-phosphate by hexokinase, and metabolized to pyruvate which, then, either entered the TCA cycle or was converted to lactate by LDH. When glutamine was absent, hexokinase activity was constant, which approved of unaltered Q_{Glc}. Meanwhile, the decrease of LDH activity by 20% indicated a shift away from lactate production, offering an explanation for the decrease of $Y_{Lac/Glc}$.

When both glucose and glutamine were lacking, hexokinase and LDH activities decreased by 29% and 36%, respectively. Under the condition, the bulk of pyruvate from amino acid catabolism may enter TCA cycle instead of lactate production to increase energy metabolism efficiency and remedy short of energy. In glutaminolysis, glutamine

was first converted to glutamate catalyzed by glutaminase. Glutamate can be either served as a substrate for anabolic pathway, such as amino acid biosynthesis, or further metabolized to α-ketoglutamate for entering into TCA cycle through the deamination route and the transaminase (TA) route. Analyses of flux (Street *et al.* 1993, Bonarius *et al.* 1998) and enzyme activities (Vriezen & van Dijken 1998, Neerman & Wagner 1996) suggested that the TA route appear to be the main pathway in continuous and rapidly proliferating cell lines. In the TA route, glutamate/oxaloacetic aminotransferase (GOT) seems to be the main enzyme, with a low activity of glutamate/pyruvate aminotransferase (GPT). In CHO and BHK cell cultures, GOT activity was approx. eight times higher than GDH and ten times higher than GPT (Yallop *et al.* 2003). However, in CHSE cell culture, GOT activity and GPT activity were almost equal and approx. fifteen times higher than GDH activity.

When lacking of glucose, glutamine was chosen as an alternative energy source. The increase of GA activity by 24% prompted an increased flux into the glutaminolysis pathway. The unchanged activities of GOT and GPT and increased activity of GDH by 24% meant an increased flux via the GDH route, which matched to the increase of $Y_{\mathrm{Amm/Gln}}$ above mentioned. A similar result was reported (Martinelle *et al.* 1998) that GDH flux increased under glucose-limited conditions in hybridoma cell culture.

When lacking of glutamine (regardless of glucose), the activities of glutaminase, GDH and GOT decreased greatly, but GPT remained nearly unaltered because an amount of alanine was converted to pyruvate in the catalysis of GPT.

ATP levels and energy charge

Except for supplying energy for cell growth, ATP plays a major role on regulation of cell metabolism (Fitzpatrick *et al.* 1993), cell cycle, and nucleates synthesis (Ryll & Wagner 1992). Research on the contribution of glucose and glutamine to energy was always a hot spot (Schneider *et al.* 1990). However, the contribution of other amino acids to energy generation was usually overlooked. The most common indicator of energy state of a cell is the adenylate energy charge (EC). EC is defined as

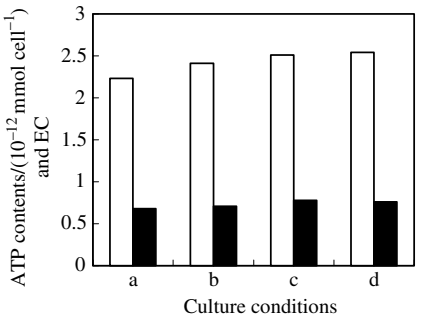

Fig. 2. ATP contents (□) and energy charge (EC, ■) at 6 d in CHSE cells culture with different glucose and glutamine concentrations. (a) Glc (0 g l^{-1}), Gln (0 g l^{-1}); (b) Glc (0 g l^{-1}), Gln (0.1 g l^{-1}); (c) Glc (1 g l^{-1}), Gln (0 g l^{-1}); (d) Glc (1 g l^{-1}), Gln (0.1 g l^{-1}).

$$\mathrm{EC} = \{[\mathrm{ATP}] + 0.5 \times [\mathrm{ADP}]\}/\{[\mathrm{ATP}] + [\mathrm{ADP}] + [\mathrm{AMP}]\}.$$

For CHSE cells, ATP contents and EC were almost unchanged when lacking of glutamine or glucose, and only decreased by 11% even when lacking of both glucose and glutamine (Figure 2). The ATP contents and EC were always maintained at about 2×10^{-12} mmol cell^{-1} and 0.72, much lower than that of mammalian cells (Ana *et al.* 2002). This may represent a special feature of marine fish cell line itself.

Lack of glucose prompted cellular dependence on glutamine for the maintenance of a steady ATP level. This coincided with the result that cells shifted to an energy-efficient, deamination metabolism. Similarly, lack of glutamine stimulated cellular uptake of glucose to supply the essential energy. This also supported the result of cell shifting away from lactate production. When lacking of both glucose and glutamine, cells were forced to catabolize amino acids to supply energy for cell growth, in agreement with the marked increase of specific consumption rates of amino acids and decrease of LDH activity. In hybridoma and myeloma cell cultures, depletion of glucose also increased the specific ATP levels of glutamine by 35–40% (Martinelle *et al.* 1998) and flux of deamination metabolism greatly. In continuous culture of BHK cells, when glutamine was as low as 0.14 mmol l^{-1}, the bulk of glutamate was converted to α-ketoglutamate by GDH for energy supply (Cruz *et al.* 1999a, b).

Energy charge (EC) in most mammalian cells is strongly maintained at 0.9 (Schneider *et al.* 1990),

and ATP contents were weakly affected by selective inhibition of nutrient uptake, glucose concentration or its depletion, and the addition of hormones or vitamins (Carvalhal *et al*. 2002, Chapman *et al*. 1971). The energy requirements of anabolism and the energy generation of catabolism should be in equilibrium with each other (Vriezen & van Dijken 1998). Therefore, it was possible to understand the relatively unchanged ATP contents and EC in the cultures with different glucose and glutamine concentrations.

Conclusions

In this work, the metabolism parameters, key glycolytic and glutaminolysis enzymes, and intracellular ATP and EC of CHSE cells were investigated at different glucose and glutamine concentrations, and a possible explanation for CHSE cells to maintain energy balance was proposed when one of the two main nutrients or both was lacking. Once one of the two main nutrients was deprived, energy metabolic efficiency of another nutrient increased. In the culture lacking of both glucose and glutamine, energy metabolic efficiency of other amino acids increased markedly. These results showed that the nutrient requirement of CHSE cell was not rigorous. Combined with the inhibition effect of metabolic byproduct produced under high nutrient content (Chen *et al*. 2004), the results are useful in the design of an optimal medium and the establishment of a process control strategy for mass cultivation of CHSE cells.

However, changes in key enzyme activities can only be used as indirect proof of metabolic flux change. Actual metabolic flux can be exactly quantified by isotope tracer technique and NMR technique. It would be of interest and importance to understand metabolic pathways of amino acids and determine how their pathways are affected especially in the condition lacking of both glucose and glutamine.

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References

Barnabé N, Butler M (2000) The effects of glucose and glutamine on intracellular nucleotide pool and oxygen uptake rate of a murine hybridoma cell line. *Cytotechnology* **34**: 47–57.

Bergmeyer HU (1983) *Methods of Enzymatic Analysis*, Vol III, 3rd edn. Weinheim: Verlag Chemie.

Bols NC, Ganassin RC, Tom DJ, Lee IEJ (1994) Growth of fish cell lines in glutamine-free medium. *Cytotechnology* **16**: 159–166.

Bonarius HPJ, Houtman JHM, de Gooijer CD, Tramper J, Schmid G (1998) Activity of glutamate dehydrogenase is increased in ammonia-stressed hybridoma cells. *Biotechnol. Bioeng*. **57**: 447–453.

Carvalhal AV, Coroadinha AS, Alves PM, Moreira JL, Hanser H, Carrondo MJT (2002) Metabolic changes during cell growth inhibition by the IRF-1 system. *Enzyme Microbial Technol*. **30**: 95–109.

Chapman JD, Webb RG, Borsa J (1971) ATP pools levels in synchronously growing Chinese hamster ovary cells. *J. Cell Biol*. **49**: 229–233.

Chen J, Sun X, Zhang Y (2004) Effects of glutamine on growth and metabolism of Chinook salmon embryo (CHSE) cells. *Chin. J. Appl. Environ. Biol*. **10**: 442–445.

Chen S, Li Q, Chen P, Feng T (1983) Measurement of erythrocytic pyruvate kinase and Chinese normal value. *J. Chin. Med. Exam*. **6**: 214–217.

Cruz HJ, Ferreira AS, Freitas CM, Moreira JL, Carrondo MJT (1999a) Metabolism response to different glucose and glutamine levels in bhk cell culture. *Appl. Microbiol. Biotechnol*. **51**: 579–585.

Cruz HJ, Moreira JL, Carrondo MJT (1999b). Metabolic shifts by nutrient manipulation in continuous cultures of BHK cells. *Biotechnol. Bioeng*. **66**: 104–113.

Du C, Chen L (1987) Measurement of erythrocytic enzyme and Chinese normal value. X. Hexokinase. *Heredity Disease* **4**: 200–202.

Fiorino A, Frigo G, Cucchett E (1989) Liquid chromatographic analysis of amino acids in protein hydrolysates by post-column derivatization with *o*-phthaladehyde and 3-mercaptoproropionic acid. *J. Chromatogr*. **476**: 83–92.

Fitzpatrick L, Jenkins HA, Butler M (1993) Glucose and glutamine metabolism of a murine B-lymphocyte hybridoma grown in batch culture. *Appl. Biochem. Biotechnol*. **43**: 93–116.

Fryer YL, Yusha A, Pilcher KS (1965) The *in vitro* cultivation of tissue and cells of Pacific salmon and steelhead trout. *Ann. NY Acad. Sci*. **126**: 566–586.

Ganassin RC, Bols NC (1992) The effects of purine supplement on the growth of salmond cell lines in different mammalian sera. *Cytotechnology* **8**: 21–29.

Gutmann I, Wahlefeld AW (1974) L-(+)-Lactate-determination with lactate dehydrogenase and NAD. In: Bergmeyer HU (ed), *Methods of Enzymatic Analysis*, 2nd edn. Weinheim: Verlag Chemie, pp. 1464–1468.

Ljunggren J, Häggström L (1994) Catabolic control of hybridoma cells by glucose and glutamine limited fed batch cultures. *Biotechnol. Bioeng*. **44**: 808–818.

Martinelle K, Doverskog M, Jacobsson U, Chapman BE, Kuchel PW, Häggström L (1998) Elevated glutamate dehydrogenase flux in glucose deprived hybridoma and myeloma cells: evidence from $^{1}H/^{15}N$ NMR. *Biotechnol. Bioeng.* **60**: 508–517.

McDermolt RH, Butler M (1993) Uptake of glutamate, not glutamine synthetase, regulates adaptation of mammalian cells to glutamine-free medium. *J. Cell Sci.* **104**: 51–58.

Mercille S, Massie B (1994) Induction of apoptosis in nutrient deprived cultures of hybridoma and myeloma cells. *Biotechnol. Bioeng.* **44**: 1140–1154.

Meister A (1985) *Methods in Enzymology,* Vol. 113. New York: Academic Press Inc.

Neerman J, Wagner R (1996) Comparative analysis of glucose and glutamine metabolism in transformed mammalian cell lines, insect and primary liver cells. *J. Cell Physiol.* **166**: 152–169.

Reitzer LJ, Wice BM, Kennell D (1979) Evidence that glutamine, not sugar is the major energy source for cultured Hela cells. *J. Biol. Chem.* **254**: 2569–2676.

Ryll T, Wagner R (1992) Intracellular ribonucleotide pools as a tool for monitoring the physiological state of vitro cultivated mammalian cells during production processes. *Biotechnol. Bioeng.* **40**: 934–946.

Schneider W, Siems W, Grune T (1990) Balancing of energy consuming processes of rat hepatocytes. *Cell Biochem. Funct.* **8**: 227–232.

Skog S, Tribukait B (1985) Discontinuous RNA, and protein synthesis, and accumulation during cell cycle of Ehrlich ascites tumour cells. *Exp. Cell Res.* **159**: 510–518.

Street JC, Delort AM, Braddock PSH, Brindle KM (1993) A $^{1}H/^{15}N$ NMR study of nitrogen metabolism in cultured mammalian cells. *Biochem. J.* **291**: 485-492.

Sun X, Zhang Y (2004) Glutamine cannot support recombinant CHO cell growth and maintenance in the absence of glucose. *Proc. Biochem.* **39**:719–722.

Vriezen N, van Dijken JP (1998) Fluxes and enzyme activities in central metabolism of myeloma cells grown in chemostat culture. *Biotechnol. Bioeng.* **58**: 28–39.

Yallop CA, Norby PL, Jensen R, Reinbach H, Svendsen I (2003) Characterization of G418-induced metabolic load in recombinant CHO and BHK cells: effect on the activity and expression of central metabolic enzymes. *Cytotechnology* **42**: 87–99.