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현탁배양 하이브리도마 세포의 속도론적 모델링

†정 연 호 · 박 현 규 · *최 정 우
강원대학교 식품생명공학부 · *서강대학교 화학공학과

A Kinetic Modeling for the Dynamics of Hybridoma Cells in Suspension Culture

Yeon-Ho Jeong†, Hyun-Kyu Park, and Jeong-Woo Choi*
Division of Food Science and Biotechnology, Kangwon Nat'l Univ., Chuncheon, Kangwon 200-701, Korea
*Department of Chemical Engineering, Sogang Univ., C. P. O. Box 1142, Seoul 121-742, Korea

ABSTRACT

Batch suspension cultures of hybridoma cell were performed with various initial glutamine concentrations to investigate the effects of glutamine on cell growth and death, monoclonal antibody production, glucose and glutamine consumption, and the production of lactate and ammonium ion. An mathematical kinetic model was formulated to describe the kinetics of cell growth, the consumption of nutrients (glucose and glutamine), and the production of monoclonal antibody and waste metabolites (lactate and ammonium ion) based on experimental data. An equation for the specific growth rate was developed such that superimposed Monod equation in glucose and glutamine, with non-competitive type inhibition relations in ammonium ion and lactate. The inhibition constant for lactate was inversely proportional to the lactate concentration. The specific death rate was considered to be a function of glucose, glutamine, ammonium ion and lactate concentration.

INTRODUCTION

The cultivation of animal cells has been used for the production of many complex proteins of phamacological interest such as enzymes, hormones, viral vaccines, monoclonal antibodies, and lymphokines. This is mostly due to the requirement for post-translational modification of these proteins. One of the most important products of animal cell culture has been monoclonal antibodies (MAbs) produced by hybridomas. MAbs have been used for diagnostic assay, therapeutics, and biological separations (for example, affinity chromatography) (1). MAbs have been used to determine well over 100 drugs, toxins, vitamins, and other biological compounds as diagnostic agents (1). MAbs are also used for purification of protein molecules such as interferon by affinity chromatograph Hybridomas obtained by fusing lymphocytes with myeloma cells are non-anchorage dependent, so they can proliferate in a liquid suspension culture. Suspension cultures of hybri-

† Corresponding Author

domas have been performed by a number of investigators to elucidate culture kinetics and cell metabolism for hybridomas(2-5).

A growth medium for mammalian cells contains various component such as carbohydrates, amino acids, salts, vitamins, hormones and growth factors. The medium is usually spplemented with expensive serum to provide the cells with necessary growth factors, trace nutrients, and transport proteins, and to protect cells from shear and oxidizing environment. Unlike fermentation of bacteria and yeast, mammalian cells cultured *in vitro* require two primary substrates, i.e. glucose and glutamine(5). Glucose and glutamine are the major carbon and energy sources in animal cell culture(2, 3). Glutamine is used also for the synthesis of amino acids, cell proteins, nucleotides, and lipids(5, 6). In animal cell culture, waste by products from substrate metabolism are excreted into the culture medium. These waste by -products can give inhibitory effects to cell growth and product production at their high levels. The principal wastes considered in this study are ammonium ion and lactate. Ammonium ion is one of the major metabolic by-products from glutamine metabolism and it is also generated in cell culture medium during storage by spontaneous first order deamination of glutamine (7, 8). Ammonium ion has been shown to be toxic for most animal cells, but the critical inhibitory ammonium ion concentration differs between cell types (2, 7, 9). Lactate is a by-product from both glucose and glutamine metabolism(5). The accumulation of lactate can lower the pH of the culture medium. This decrease of pH may cause inhibition of cell growth(2, 7). In order to predict the behavior of hybridoma cell cultures and optimize their performance, a kinetic model to describe these environmental effects on cell growth and MAbs production is required.

The objective of this study is to develop a biochemical kinetic model of cell growth and MAbs production. Batch suspension cultures of hybridoma cell were performed with various initial glutamine concentration to investigate the effects of glutamine on cell growth and death, MAbs production, glucose and glutamine consumption, and lactate and ammonium ion production. The model cell line used for this study was a mouse-mouse hybridoma cell line producing immunoglobulin, IgG_{2a} which is specific for the whole cell of bacteria *Rhizobium japonicum* NR-7 (10, 11). The mathematical kinetic model is formulated to describe the cell growth, the consumption of nutrients (glucose and glutamine), and the production of MAbs and waste metabolites (lactate and ammonium ion) based on experimental data. This kinetic model will be used for the development of strategies to achieve high cell density and MAbs production.

MATERIALS AND METHODS

Cell Line

A mouse-mouse hybridoma cells (VIII H-8) used in this study was kindly given by New Brunswick Scientific Co. (Edison, NJ). These cells produce immunoglobulin, IgG_{2a} which is specific for the whole cell of bacteria *Rhizobium japonicum* NR-7. These cells were obtained by fusion of mouse myeloma cells and spleen lymphocytes from BALB/C mice immunized with whole cells of *Rhizobium japonicum* NR-7. The antibody reacted specifically with a lipopolysacchride fraction isolated from the culture broth of *Rhizobium japonicum* NR-7.

Cell Culture Media and Chemicals

The cells were maintained in DMEM (GIBCO 430-1600) containing 4.5 g/L of glucose, supplemented with NCTC 135 (0.94g/L, GIBCO, Grand Island, NY), oxaloacetate (150 mg/L), insulin (75.5 μg/L), mercaptoethanol (3.5mg/L), sodium bicarbonate (40mM), streptomycin (100 μg/mL), penicillin (100U/mL), and 5% (v/v) calf bovine serum (maintenance medium). This maintenance medium contained 4 mM glutamine. The medium was filtered with 0.22μm membrane. The

cells were maintained in 25 and 75cm^2 T-flasks. Subculture were carried out every 4 days. For studying glutamine effect, the glutamine free medium of GIBCO 320-1960 supplemented as described above was used as the basal medium for experiment. Then a specific amount of sterile glutamine corresponding to each experimental plan was added to prepare the experimental medium. The maintained cells were centrifuged and washed with experimental medium and cultivated in 250 mL spinner flasks (Corning, working volume; 150mL). The cells were incubated at 37℃ in 7% CO_2 atmosphere.

Serum, glucose and glutamine were purchased from GIBCO (Grand Island, NY), lactic acid from Sigma (St. Louise, Mo), and ammonium chloride from Fisher Scientific (Pittsburgh, PA 15219). All of these chemicals were dissolved in distilled water when they were needed and filtered through 0.22μm syringe filters (MSI Westboro, MA) before use.

Analysis

Two independently taken samples were mixed to average the cell number in each sample. The mixed cell sample was diluted 1:1 with 0.4% trypan blue (GIBCO, Grand Island, NY) in normal saline. A small amount of sample was vortexed and injected under the coverslip of a hemocytometer and examined under the microscope. The trypan blue dye is taken up by only the nonviable cells, so it is possible to differentiate viable cells (transparent) from dead cells (blue stained). An average of two counts was used to determine the viable cell concentration and percent viability.

Glucose was assayed by a Beckman Glucose Analyzer 2 which measures the rate of change in oxygen consumption when a sample is injected into an enzyme solution. When a sample is injected into the enzyme solution containing glucose oxidase, β-D-glucose from the sample combines with dissolved oxygen from the solution, generating gluconic acid and peroxide. At all times during the reaction, the rate of oxygen consumption was directly proportional to the concentration of glucose.

The ammonium ion concentration was measured using a Orion (Cambridge, MA) 9512 ammonium ion electrode and a digital pH/milli volt meter 611 (6). Standard solutions of 8mM, 4mM, 2mM, 1mM, and 0.5mM of NH_4Cl in distilled water were used to generate a linear calibration curve for voltage readings (mV) versus the logarithm of ammonium ion concentrations.

Glutamine concentration was measured with enzymatic method developed by S.S. Ozturk et al. (6, 12). First the concentration of ammonium ion in each sample was measured using the ammonium ion electrode discribed above. Glutamine concentration was then measured by the same electrode after enzymatic reaction with glutaminase (Sigma). Glutaminase converts glutamine to glutamic acid generating one mole of ammonium ion. The enzyme was kept at a 25 unit/mL stock solution in 0.005 M acetate buffer, pH 6. Since the ammonium ion in the samples had been determined before the glutaminase reaction, the additional ammonium ion generated can be directly related to the concentration of glutamine present in the samples.

Lactate concentration was determined by using a Sigma procedure 826-UV (11). Lactate and excess NAD^+ are converted to pyruvate and NADH by Lactate dehyclrogenase. The increose of absorbance at 340nm due to reduction of NAD^+ to NADH becomes a measure of the amount of lactate originally present in the sample. Absorbance was measured with Bausch & Lomb Spectronic 601 spectrophotometer.

A radial immuno diffusion assay which had been described by Mancini et al. was used to analyze MAbs concentratoin in the sample (13). Velez used same cell line and compared two analyzing methods of MAb concentration, namely ELISA (Enzyme-Linked Immuno Sorbent Assay) and RID (Radial Immuno Diffusion) methods (11). Acccording to his results, ELISA showed

large deviation while RID showed less deviation, and values obtained with RID were more accurate and reproducible. He reported that the greater reliability of RID method compared to the ELISA is related to the physical nature of diffusion which is a very reproducible phenomenon, while the ELISA method depends on an antigen-antibody binding which is very specific but also very sensitive to external conditions such as pH, type of buffer, and antigen concentration. Therefore RID method was selected for measuring MAb concentration in culture supernatants throughout this study.

MODEL DEVELOPMENT

Model Equations

A. Cell Growth Kinetic Equation

As mentioned before, gluwse and glutamine are essential for hybridoma growth but ammonium ion and lactate inhibit hybridoma growth. Hence, the following equation can be suggested for specific growth rate.

$$\mu=\frac{\mu_{max}\, G\, Gl}{(K_G+G)(K_{Gl}+Gl)(1+A/k_A)(1+L^2/k_L)} \quad (1)$$

Where, G, Gl, A and L are the concentrations of glucose, glutamine, ammonium ion and lactate, respectively ; μ_{max} is the maximum specific growth rate ; K_G and K_{Gl} are the monod type constants for glucose and glutamine, respectively ; k_A and k_L are the inhibition constants for ammonium ion and lactate, respectively.

The specific death rate of cells decreases as the concentrations of glucose and glutamine increase and reaches a minimum plateau, but it increases as the concentrations of ammonium ion and lactate increase (14). Therefore, the specific death rate can be represented by the following equation.

$$K_D=\frac{D_{el}\, exp(A/\kappa_{DA}+L/\kappa_{DL})}{(1+G^{0.5}/k_{DG})(1+Gl^{0.5}/k_{DGl})} \quad (2)$$

Where, D_{el} is the specific death rate without activations by ammonium ion and lactate, and depressions by glucose and glutamine ; K_{DA} and K_{DL} are the constants which represent the degree of death activation by ammonium ion and lactate, respectively ; K_{DG} and K_{DGl} are the constants which represent the degree of death depression by glucose and glutamine, respectively.

Mass balance equations for viable cell, dead cell and total cell are described as equations (3)-(5).

$$\frac{dX_V}{dt}=(\mu(1-exp(-\frac{t}{t_{lag}}))-K_D)X_V \quad (3)$$

$$\frac{dX_D}{dt}=K_D X_V \quad (4)$$

$$X_{tot}=X_V+X_D \quad (5)$$

Where, t_{lag} is the lag time which is required for cells to adapt change of environment ; X is the concentration of cell and the subscript V, D and tot designate viable cell, dead cell and total cell, respectively.

On the right-hand side of equation (3), the first term represents the cell growth and the second term represents the cell death. As shown in equation (5), the total cell concentration is the sum of the viable cell concentration and the dead cell concentration. Differentiating equation (5) and substituting equation (3) and (4) on the right side yields the following equation if lag time effect is neglected.

$$\frac{dX_{tot}}{dt}=\mu X_V \quad (6)$$

B. Product Production Kinetic Equation

Monoclonal antibody production is not totally related to the rate of cell proliferation; rather, it is proportional to the number of live cells present (15). Therefore, usually the following nongrowth associated model has been used by several investigators (16, 17).

$$\frac{dP}{dt}=m_P X_V \quad (7)$$

$$m_P=\frac{1}{X_V}\frac{dP}{dt}=\frac{\Delta P}{\int_0^t X_V dt} \quad (8)$$

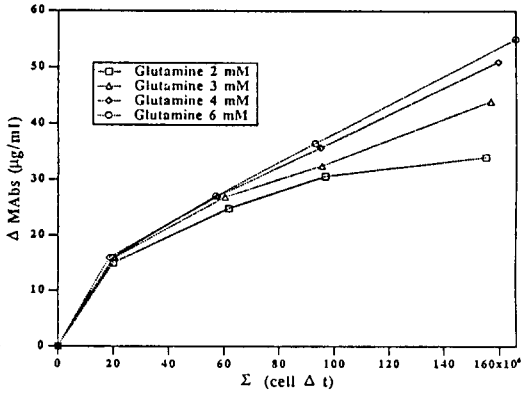

Fig. 1. Relation between antibody production and cumulative live cell-hrs in Ⅷ H-8 hybridoma cells WQ.

Where, P is the concentration of MAbs and m_P is the specific productivity of MAbs.

This model implies that antibodies are synthesized and secreted at a constant specific rate by the viable cell population throughout the culture period. The plot of ΔP versus cumulative cell-hours available for antibody production is illustrated in Fig. 1. If nongrowth associated model is available, a straight line is expected form equation (8). However, the specific antibody productivity, the slope of the curve in Fig. 1, shows high at low cumulative cell-hours and it decreases with increasing cumulative cell-hours. This implies that the specific antibody productivity is also related with specific growth rate (18) because the specific growth rate of hybridomas is usually high in the early phase when the cumulative cell-hours is low and the specific growth rate decreases as cumulative cell-hours increases. Therefore, following mixed growth associated model of Leudeking and Piret could be available in the production of IgG_{2a} from VIII H-8 hybridoma cells (19, 20).

$$Q_P=\beta_1\frac{\mu_{max}\,G\,G1}{(K_G+G)(K_{G1}+G1)(1+A/k_A)(1+L^2/k_L)}\times\left(1-\exp\left(-\frac{t}{t_{lag}}\right)\right)+\beta_2\exp(A/K_{PA}) \quad (9)$$

$$\frac{dP}{dt}=Q_PX_V \quad (10)$$

Where, β_1 is the the growth associated constant of Leudeking-Piret mixed growth associated model, β_2 is the nongrowth associated constant of Leudeking-Piret mixed growth associated model and K_{PA} is the constant which represents the degree of antibody production activation by ammonium ion.

In the second term on the right-hand side of equation 9, the term, $exp(A/\kappa_{PA})$, represents the activation of antibody produciton due to increase of ammonium ion concentration. Many researchers reported that hybridomas increased antibody production under harsh conditions such as serum depletion, oxygen limitation and high concentration of ammonium ion (3, 14, 21).

C. Waste By-product Production Rate Equation

Ammonium ion is excreted into the cell culture medium as a metabolic by-product from glutamine metabolism during cultivation of hybridomas and it is also generated by spontaneous first order deamination of glutamine.

Mass balance for ammoninum ion is as follows.

$$Q_A=\Lambda_A\frac{G1}{(C_{AG1}+G1)}\exp(A/\kappa_{AA}+L/\kappa_{AL}) \quad (11)$$

$$\frac{dA}{dt}=Q_AX_V+c\,G1 \quad (12)$$

Where, Λ_A is the maximum production rate of ammonium ion without activations by ammonium ion and lactate ; C_{AG1} is the saturation constant for dependence of ammonium ion on glutamine ; K_{AA} and κ_{AL} are the constants which represent the degree of activation of ammonium ion productoin by ammonium ion and lactate, respectively ; c is the first-order spontaneous decomposition rate of glutamine.

On the right-hand side of equation (11), the term, $exp(A/\kappa_{AA}+L/\kappa_{AL})$, represents the activations of ammonium ion production by ammonium ion and lactate. It is reported that ammonium

ion production rate increased with increasing ammonium ion concentration and lactate concentration (14). The harsh conditon caused by the high inhibitory concentration of ammonium ion would require higher nutrients consumption rate (22). So higher specific glutamine and glucose consumption rates are expected. Since ammonium ion is a major product from glutamine metabolism, higher specific ammonium ion production rate is expected as ammonium ion concentration increases (14). The lactate production is more closely related to glucose metabolism than to glutamine metabolism. Under the conditoin of high lactate concentratoin, glycolysis is inhibited and especially the pathway from pyruvate to lactate is inhibited, so less lactate is produced via glycolysis (14). The inhibiton of glucose consumption by high lactate concentratoin shifts cells to proceed toward glutamine metabolism (9, 23, 24). So higher specific ammonium ion production rate is expected as lactate concentration increases.

Lactate is a metabolic by-product from both glucose metabolism and glutamine metabolism. Therefore, lactate production rate equation can be represented by following equation.

$$Q_L = \Lambda_{LG}\frac{G}{(C_{LG}+G)(1+L/k_{LL})} + \Lambda_{LGl}\frac{Gl}{(C_{LGl}+Gl)} \quad (13)$$

$$\frac{dL}{dt} = Q_L X_V \quad (14)$$

Where, Λ_L is the maximum production rate of lactate and the subscript G and Gl designate glucose and glutamine, respectively ; C_{LG} and C_{LGl} are the saturation constants for dependence of lactate on glucose and glutamine, respectively ; k_{LL} is the constant which represents the degree of depression of lactate production by lactate.

On the right-hand side of equation (13), the first term represents the lactate production via glucose metabolism and the second term represents the lactate production via glutamine metabolism. As mentioned before, high lactate concentration inhibits glycolysis and it results in less production of lactate via glucose metabolism. So the first term on the right-hand side of equation (13) comprises the inhibition term, $1/(1+L/k_{LL})$.

D. Substrate Consumption Rate Equation

Glucose is consumed for the growth of cells, the maintenance of cells and the syntheses of MAbs and lactate.

Mass balance for glucose is as ;

$$\frac{dG}{dt} = -\left(\frac{1}{Y_{X/G}}\mu\left(1-exp\left(-\frac{t}{t_{lag}}\right)\right)+m_G+\frac{1}{Y_{P/G}}Q_P+\frac{1}{Y_{L/G}}\frac{G\,\Lambda_{LG}}{(C_{LG}+G)\left(1+\frac{L}{k_{LL}}\right)}\right)X_V \quad (15)$$

Where, Y is the yield coefficient and m_G is the maintenance coefficient of glucose.

Glutamine is consumed for the growth of cells, the maintenance of cells and the syntheses of MAbs, ammonium ion and lactate.

Mass balance for glutamine is as ;

$$\frac{dGl}{dt} = -\left(\frac{1}{Y_{X/Gl}}\mu\left(1-exp\left(-\frac{t}{t_{lag}}\right)\right)+m_{Gl}+\frac{1}{Y_{P/Gl}}Q_P+\frac{1}{Y_{L/Gl}}\frac{Gl\Lambda_{LGl}}{(C_{LGl}+Gl)}+\frac{1}{Y_{A/Gl}}Q_A\right)X_V - c\,Gl \quad (16)$$

Where, m_{Gl} is the maintenance coefficient of glutamine.

Parameter Estimation

The complete model comprised with differential equations ; equaiton (3), (4), (10), (12), (14), (15) and (16). The parameters which are involved in model equations were estimated by using nonlinear parameter estimation technique (25, 26). While the parameter estimation was done, model equations were being solved simultaneously with numerical integration of differential equations using Runge-Kutta-erner fifth-order method. The experimental dat a are compared to the model predictions by choosing parameters that give a best fit of the model to the data. The estimated values of parameter are shown in Table 1.

Table 1. List of the mode parameters

Parameter	Dimension	Value	Parameter	Dimension	Value
c	1/Hr	1.25×10^{-3}	C_{AGl}	mM	2.54
C_{LG}	g/L	1.7	C_{LGl}	mM	2.
Det	1/Hr	9.4893×10^{-3}	k_A	mM	14.1
k_{DG}	$(g/L)^{0.5}$	1.43	k_{DGl}	$mM^{0.5}$	0.263
k_L	$(g/L)^2$	2.6	k_{LL}	g/L	1.6
K_G	g/L	0.79	K_{Gl}	mM	0.42
m_G	g/L/Hr-(#/ml)	8.95×10^{-12}	m_{Gl}	mM/Hr-(#/ml)	0.
t_{lag}	Hr	8.	$Y_{X/G}$	(#/ml)(g/L)	9.5×10^5
$Y_{X/Gl}$	(#/ml)/mM	5.8×10^7	$Y_{P/G}$	(μg/ml)(g/L)	2.4×10^2
$Y_{P/Gl}$	(μg/ml)/mM	47.	$Y_{L/G}$	g/g	2.9
$Y_{L/Gl}$	g/mmol	25.4	$Y_{A/Gl}$	mmol/mmol	0.8
β_1	μg/#	1.64×10^{-5}	β_2	μg/#-Hr	0.08×10^{-6}
K_{AA}	mM	48.	K_{AL}	g/L	55.
K_{DA}	mM	2.7862	K_{DL}	g/L	14.
K_{PA}	mM	6.	Λ_A	mM/Hr-(#/m)	2.45×10^{-8}
Λ_{LG}	(g/L)/Hr-(#/ml)	6.2×10^{-8}	Λ_{LGl}	(g/L/Hr-(#/ml)	2.5×10^{-9}
μ_{max}	1/Hr	0.061			

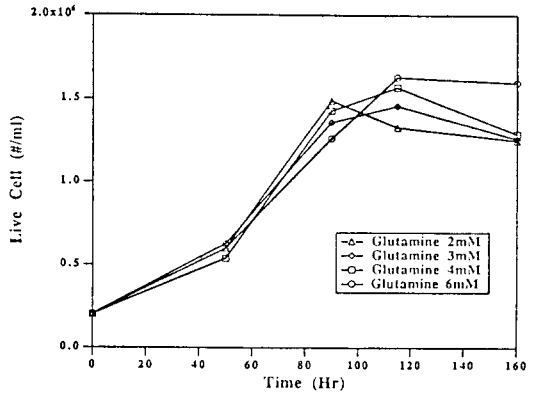

Fig. 2. Effect of glutamine on live cell growth kinetics of Ⅷ H-8 hybridoma cells.

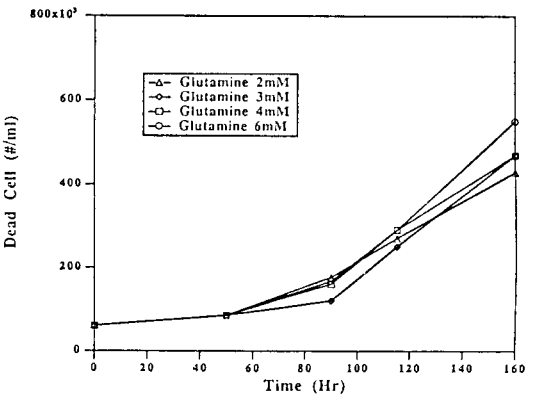

Fig. 3. Effect of glutamine on cell death kinetics of Ⅷ H-8 hybridoma cells.

RESULTS AND DISCUSSION

Experimental

Glutamine is an essential amino acid which provides a major energy source in mammalian cells. It plays a role of both carbon and nitrogen source for the synthesis of other amino acids, lipids, cell proteins and antibodies, nucleotides, purine and pyrimidine. Therefore it plays a significant role in overall culture performance. To investigate the role of glutamine, batch suspension cultures were carried out in spinner reactors of 150ml working volume with various initial glutamine concentrations.

Fig. 2 displays the live cell growth kinetics of culture with varous initial glutamine concentrations in media. And Fig. 3 shows the change of the dead cell number according to time. The initial specitis growth rate in the Fig. 2 is almost same

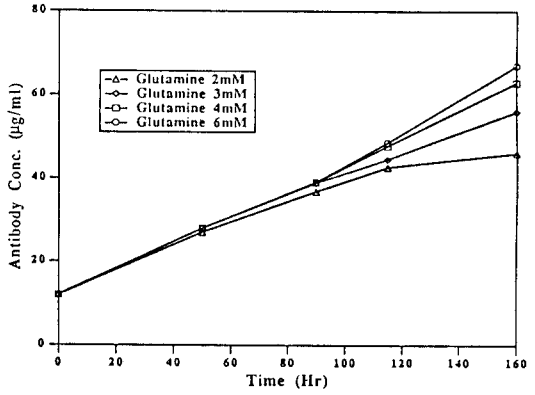

Fig. 4. Effect of glutamine on MAbs production kinetics of Ⅷ H-8 hybridoma cells.

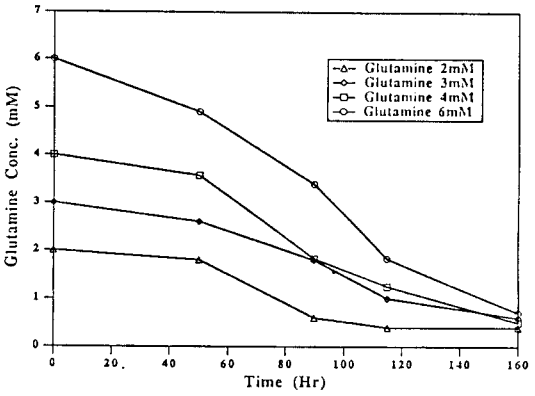

Fig. 5. Effect of glutamine on the glutamine consumption kinetics of Ⅷ H-8 hybridoma cells.

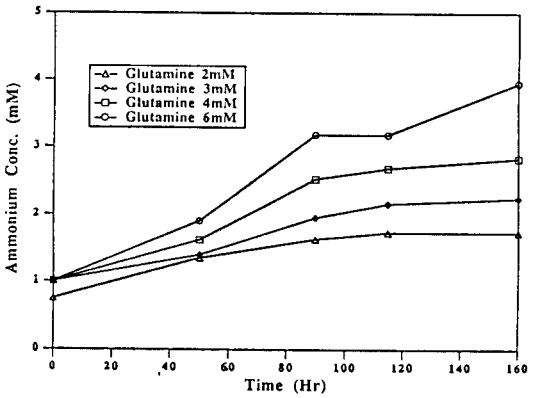

Fig. 6. Effect of glutamine on the ammonium production kinetics of Ⅷ H-8 hybridoma cells.

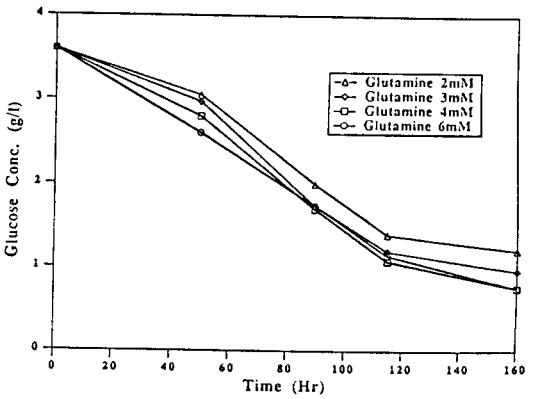

Fig. 7. Effect of glutamine on the glucose consumption kinetics of Ⅷ H-8 hybridoma cells.

for all runs before 50 hours. This means that the initial specific growth rate is independent of initial glutamine concentration in the concentration range investigated (27). The cell growth is under stoichiometric limitation of glutamine because the maximum cell density is a strong function of initial glutamine concentration.

Fig. 4 displays the effect of initial glutamine concentration on MAbs pxroduction kinetics. Both the initial production rate after lag phase and the final MAbs concentration increase as the initial glutamine level increases. Therefore it can be concluded that the monoclonal antibody production is strongly dependent on the glutamine concentration in the cell culture media.

Fig. 5 shows glutamine consumption kinetics with variation of initial glutamine concentration. It is noticeable that still considerable quantity of glutamine remains at the later phase in every run as shown in Fig. 5. This indicates that glutamine may be not a limiting substrate at the later phase in the concentration range investigated because other limiting nutrients get depleted faster than glutamine. As a whole, high level of initial glutamine concentration maintains high glutamine concentration throughout the culture period.

Fig. 6 shows the production of ammonium ion at various initial glutamine concentrations. As shown in Fig. 6, it is clear that ammonium ion production is stimulated by high level of gluta-

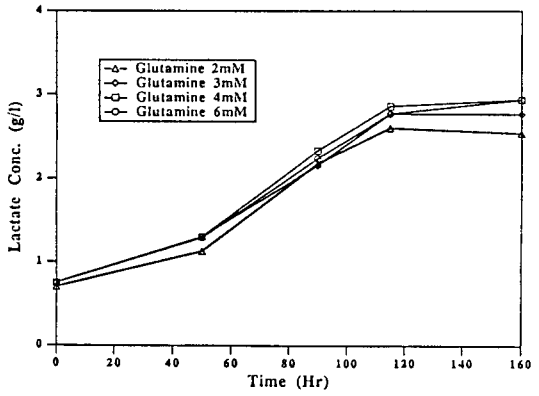

Fig. 8. Effect of glutamine on the lactate production kinetics of Ⅷ H-8 hybridoma cells.

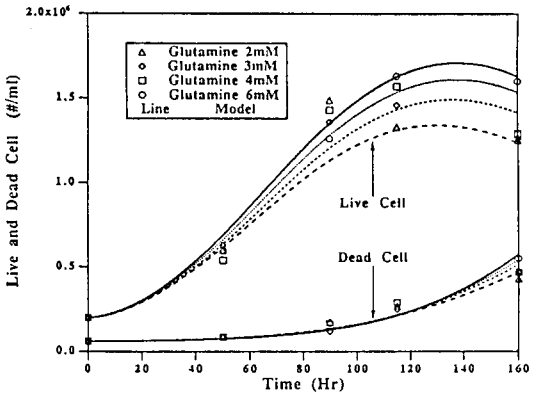

Fig. 9. Observed and model predicted time course changes of live cell and dead cell with various initial glutamine concentrations.

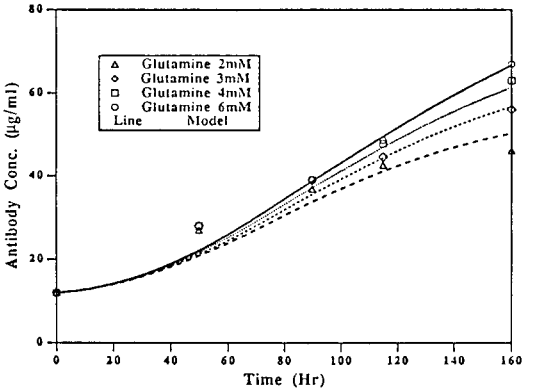

Fig. 10. Observed and model predicted MAbs production with various inital glutamine concentrations.

mine. These results show that the increase of glutamine concentration results in the increase of glutamine uptake rate and consequently results in the increase of ammonium ion production. During the culture, 3.5 mM of glutamine was consumed and 1.9 mM of ammonium ion was produced with an initial glutamine of 4 mM. This demonstrates that high portion of glutamine (54% for run with 4 mM and 58% for run with 6 mM of initial glutamine) has been converted to ammonium ion. Since ammonium ion is toxic to both cell growth and antibody production, it is desirable to reduce the ammonium ion production. The ammonium ion production can be reduced by lowering glutamine uptake because glutamine is the major source of ammonium ion production. The glutamine uptake rate can be lowered by keeping glutamine concentration low.

Fig. 7 shows the effect of initial glutamine concentration on glucose consumption kinetics and Fig. 8 shows the effect on lactate production kinetics. As mentioned above, the increase of glutamine concentration has been shown to stimulate in cell growth, cell viability and MAbs production. Therefore higher substrate consumption rate is expected with increase of glutamine concentration. Howeves the glucose consunption rate is not seriously affected by initial glutamine concentration in the concentration range investigated. Since the lactate production is more closely related to glucose metabolismm than glutamine metabolism, it is closely related to the glucose consumption. Therefore it is natural that the lactate production rate is not seriously affected by initial glutamine concentration as shown in Fig. 8.

Model

Fig. 9 shows the observed and model-predicted time course changes of live cell and dead cell with various initial glutamine concentrations. It can be concluded that the solution of the model equations represents the experimental data fairly accurately. The cells undergoes growth lag and

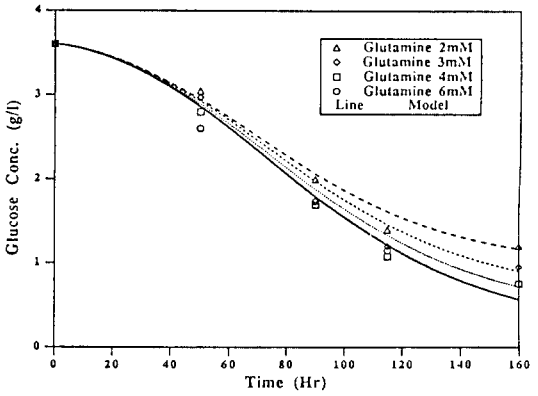

Fig. 11. Observed and model predicted glucose consumption with various initial glutamine concentrations.

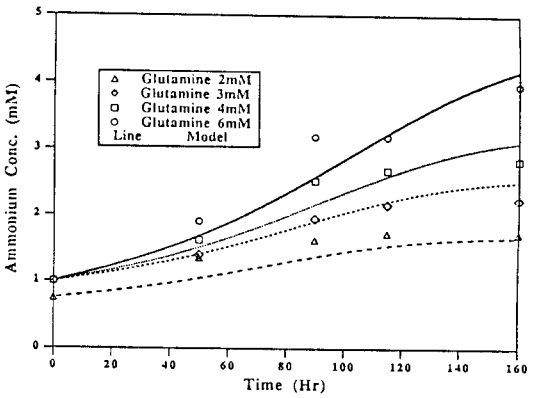

Fig. 13. Observed and model predicted ammonium production with various initial glutamine concentrations.

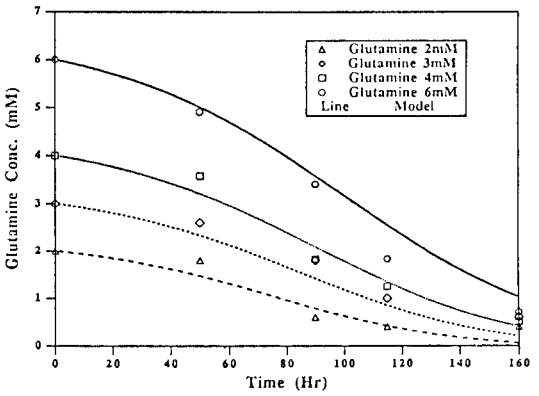

Fig. 12. Observed and model predicted glutamine consumption with various initial glutamine concentrations.

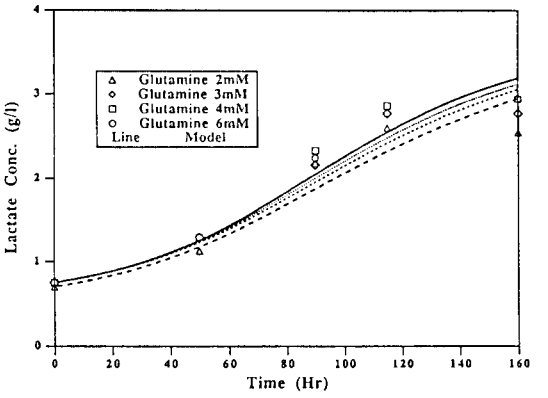

Fig. 14. Observed and model predicted lactate production with various initial glutamine concentrations.

obtains exponential growth. As the initial glutamine concentration increases, the cells grow fast in the exponential phase. After the exponential phase viable cell concentration reaches a maximum and then decreases. At this point the dead cell number abruptly increases and it may be due to the depletion of limiting substrate and accumulation of waste byproduct.

Fig. 10 presents the observed and model-predicted monoclonal antibody synthesis with various initial glutamine concentrations. As shown in this figure the curve predicted by the model equations matches the experimental result very well. The model successfully describes growth associated MAbs synthesis initially followed by a continued increase in MAbs concentration due to nongrowth associated production during stationary phase. The growth associated constant, $\beta 1$, is estimated as two hundred folds larger than nongrowth associated constant, $\beta 2$. So it can be concluded that MAbs production is more closely related to growth associated term.

Fig. 11 and 12 show the observed and model predicted glucose consumption and glutamine consumption, respectively. The solution of model equations represents the experimental data accu-

rately.

Fig. 13 and 14 present the observed and model predicted ammonium ion production and lactate production, respectively. The model accurately follows the experimental data. The estimated value of K_{AA} is much larger than the ammonium ion concentration in cell culture medium. And the estimated value of K_{AL} is also much larger than the lactate concentration in cell cul-ture medium. This means that the activation of ammonium ion production by lactate and ammonium ion is not considerable. The maximum production rate of lactate via glucose metabolism, Λ_{LG}, is estimated as about twenty folds larger than the maximum production rate of lactate via glutamine metabolism, Λ_{LGl}. This is consistent with the fact that the lactate production is more closely related to glucose metabolism than to glutamine metabolism.

NOMENCLATURE

A ammonium ion concentration(mM)
c first order spontaneous decomposition rate of glutamine(1/Hr)
C_{AGl} saturation constant for dependence of ammonium ion on glutamine(mM)
C_{LG} saturation constant for dependence of lactate on glucose(g/L)
C_{LGl} saturation constant for dependence of lactate on glutamine(mM)
D_{el} specific death rate without both activations and depressions(1/Hr)
G glucose concentration(g/L)
Gl glutamine concentration(mM)
L lactate concentration(g/L)
k_A inhibition constant for ammonium ion(mM)
k_{DG} constant which represents the degree of death depression by glucose$(g/L)^{0.5}$
k_{DGl} constant which represents the degree of death depression by glutamine$(mM^{0.5})$
k_L inhibition constant for lactate$(g/L)^2$
k_{LL} constant which represents the degree of depression of lactate production by lactate (g/L)
K_D specific death rate (1/Hr)
K_G monod constant for glucose (g/L)
K_{Gl} monod constant for glutamine (mM)
m maintenance coefficient
P MAbs concentration (μg/mL)
Q specific production rate
t time (Hr)
t_{lag} lag time (Hr)
X cell concentration (cells/mL)
Y yield coefficient

Greek letters

β_1 growth associated constant of mixed growth associated model(μg/cells)
β_2 nongrowth associated constant of mixed growth associated model(μg/cells-Hr)
κ_{AA} constant which represents the degree of activation of ammonium ion production by ammonium ion(mM)
κ_{AL} constant which represents the degree of activation of ammonium ion production by lactate(g/L)
κ_{DA} constant which represents the degree of death activation by ammonium ion(mM)
κ_{DL} constant which represents the degree of death activation by lactate (g/L)
κ_{PA} constant which represents the degree of MAbs production activation by ammonium ion(mM)
Λ_A maximum production rate of ammonium ion without activations(mM/Hr-(cells/mL))
Λ_L maximum production rate of lactate ((g/L)/Hr-(cells/mL))
μ specific growth rate(1/Hr)
μ_{max} maximum specific growth rate(1/Hr)

Superscripts and subscripts

A ammonium ion
D dead cell
G glucose
Gl glutamine
L lactate
P MAbs

tot total
V viable cell

요 약

하이브리도마 세포의 성장과 사망, 모노클론 항체의 생산, 포도당과 글루타민의 소비, 그리고 유산과 암모늄 이온의 생산에 미치는 클루타민의 영향을 조사하기 위해 초기 글루타민 농도를 변화시키면서 하이브리도마 세포의 회분식 현탁배양을 실시하였다. 실험 결과에 기초하여 세포의 성장속도, 영양물(포도당과 글루타민) 소비속도, 그리고 모노클론 항체 및 대사 부산물(유산과 암모늄이온)의 생산 속도를 예측할 수 있는 수학적 모델이 제시되었다. 포도당과 글루타민에 대해서는 중첩적인 Monod 형식이며 암모늄 이온과 유산에 대해서는 Non-copmetitive inhibition 관계로 표시되는 세포의 비성장 속도에 관한 방정식이 개발되었다. 유산에 대한 억제 상수는 유산농도에 반비례하였다. 세포의 비사망 속도는 포도당, 글루타민, 암모늄 이온과 유산 농도의 함수로 유도되었다.

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REFERENCE

1. M. L. Shuler and F. Kargi(1992), *Bioprocess Engineering,* Prentice-Hall, N.J.
2. M. W. Glacken, E. Adema, and A. J. Sinskey (1988), *Biotechnol. Bioeng.,* **32**, 491.
3. W. M. Miller, H. W. Blanch, and C. R. Wilke (1988), *Biotechnol. Bioeng.,* **32**, 947.
4. N. G. Ray, S. B. Karkare, and P. W Runstadler(1989), Jr., *Biotechnol. Bioeng.,* **33**, 724.
5. B. C. Batt and D. S. Kompala(1989), *Biotechnol. Bioeng.,* **34**, 515.
6. M. Butler and R. E. Spier(1984), *J. Biotechnol.,* **1**, 187.
7. M. W. Glacken, R. J. Fleischaker, and A. J. Sinskey(1986), *Biotechnol. Bioeng.,* **28**, 1376.
8. A. McQueen and J. E. Bailey(1990), *Biotechnol. Bioeng.,* **35**, 897.
9. M. W. Glacken(1988), *Bio/Technology,* **6**, 1041.
10. S. Reuveny, D. Velez, J. D. Macmillan, and L. Miller(1986), *J. Immunol. Methods,* **86**, 53.
11. D. Velez(1987), *Ph. D. Thesis,* Rutgers University, U.S.A.
12. M. A. Arnold and G. A. Rechnitz(1980), *Anal. Chem.,* **52**, 1170.
13. G. Mancini, A. C. Carbonara, and J. F. Heremans(1965), *Immunochemistry,* **2**, 235.
14. Y. H. Jeong(1992), *Ph. D. Thesis,* Rutgers University, U.S.A.
15. G. L. Altshuler, D. M. Dziewulski, J. A. Sowek, and G. Belfort(1986), *Biotechnol. Bioeng.,* **28**, 646.
16. M. Dalili and D. F. Ollis(1989), *Biotechnol. Bioeng.,* **33**, 984.
17. O. T. Ramirez, G. K. Sureshkumar, and R. Mutharasan(1990), *Biotechnol. Bioeng.,* **35**, 882.
18. D. K. Robinson and K. W. Memmert(1991), *Biotechnol. Bioeng.,* **38**, 972.
19. S. Reddy, K. D. Bauer, and W. M. Miller (1992), *Biotechnol. Bioeng.,* **40**, 947.
20. K. F. Frame and W. S. Hu(1991), *Biotechnol. Bioeng.,* **38**, 1020.
21. E. Suzuki and D. F. Ollis(1989), *Biotechnol. Bioeng.,* **34**, 1398.
22. M. W. Stelling, F. A. Stephenson, and A. D. Rogol(1981), *Horm. Metab. Res.,* **13**, 138.
23. M. Donnelly and I. E. Scheffler(1976), *J. Cell Physiol.,* **18**, 39.
24. H. R. Zielke, P. T. Ozand, J. T. Tildon, D. A. Sevdalian, and M. Cornblath(1978), *J. Cell Physiol.,* **95**, 41.
25. C. M. Metzler, G. L. Elfring, and A. J. McEwen(1974), *Biomeric,* **30**, 562.
26. J. Seinfeld and L. Lapidus(1970), *Process Modeling, Estimation and Identification* p. 383-401, PrenticeHall, N.J.
27. M. Dalili, G. D. Sayles, and D. F. Ollis(1990), *Biotechnol. Bioeng.,* **36**, 74.