

Hybridoma 세포의 세포성장, 항체생산 및 세포대사에 미치는 Glucose의 영향

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Glucose Effects on Cell Growth, Antibody Production, and Cell Metabolism of Hybridoma Cells

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ABSTRACT

The effects of glucose on cell growth kinetics, monoclonal antibody productivity, and cell metabolism of hybridoma cells were investigated. The mouse-mouse hybridoma cell line VIII H-8 producing mouse IgG_{2a} was used as a model system. Glucose showed substrate inhibition type dependence on specific growth rate. The maximum cell density increased as initial glucose concentration increased up to 4 g/ℓ. Glucose showed a strong influence on cell death kinetics, and an inverse relationship between specific death rate and glucose concentration was found. Cell viability and monoclonal antibody production increased as initial glucose concentration increased. The specific glucose consumption rate increased with glucose concentration, and cumulative specific lactate production rate increased with increasing initial glucose concentration. The overall kinetics of ammonium ion production was almost invariant with respect to initial glucose concentration, while the cumulative specific ammonium ion production rate was dependent on initial glucose concentration.

INTRODUCTION

Glucose is one of the major carbon and energy sources in animal cell culture as well as glutamine(1-7). Many cultured cells show higher rates of aerobic glycolysis than quiescent normal cells (8,9). The specific rate of glucose uptake can be influenced by its concentration(1, 2, 6, 10).

Usually glucose consumption rate increases with increasing glucose concentration(11-13), with decreasing glutamine concentration(14-16), and with replacement of some glucose with fructose and galactose(13,16).

Glucose undergoes glycolysis or pentose-phosphate pathway to cell mass. According to Reitzer et al.(17), 80 to 100% of glucose used goes through glycolysis to lactate in He-La cells and only 2 to 10% of glucose is completely oxidized

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to carbon dioxide. However, only up to 8% of glucose is assimilated into cell mass in the form of nucleotides and lipids. The relative distribution of carbon flux through each of the two pathways (glycolysis and pentose pathway) also varies with types of sugar(17,18), and relative concentration of glucose(1, 2, 6, 11, 12, 17) and glutamine used (1,13). An increase in glucose consumption rate usually stimulates the flux of glucose to lactic acid via glycolytic pathway and causes a decline in metabolizing glucose through pentose-phosphate pathway(1, 2, 6, 10-13, 17, 18). Consequently, the increase of glucose concentration (11,12,17), the replacement of glucose with slowly utilized carbohydrates such as fructose and galactose(17, 18) and the reduction of glutamine concentration(14-16) tend to drive carbon metabolism through glycolysis and away from pentose-phosphate shunt.

Accordingly, it is predicted that glucose will strongly influence cell metabolism which governs cell growth and MAb productivity. Therefore, understanding the role of glucose on the cell growth and metabolism is a prerequisite for the development of rational strategy for the antibody production from hybridoma cells. The effects of glucose on the cell growth kinetics, monoclonal antibody productivity, and cell metabolism of hybridoma cells were investigated in this paper. We intended to find out the optimum strategy for medium design or glucose feeding schedule for cultivating mouse-mouse hybridoma cell line VIII H-8 which produces mouse immunoglobulin, IgG_{2a}.

MATERIALS AND METHODS

The mouse-mouse hybridoma cell line VIII H-8 used in this study was kindly given by New Brunswick Sci. Co. This cell produces mouse immunoglobulin, IgG_{2a} which is specific for the whole cell of bacterium *Rhizobium japonicum* NR-7. The low glucose DMEM medium (Gibco 31600-091, former 430-1600) containing 1g/ℓ of glucose supplemented with NCTC (National Cancer Tissue Culture) 135 (0.94g/ℓ, Gibco), oxaloace-

tate (150mg/ℓ, Sigma), insulin (75.5 μg/ℓ, Sigma), mercaptoethanol (3.5mg/ℓ, Sigma), sodium bicarbonate (40mM, Gibco), streptomycin (100 μg/ml, Sigma), penicillin (100U/ml, Sigma), and 5%(v/v) calf bovine serum (Gibco 16170-011) was used as a basal medium. For maintaining cells, 3.5g/ℓ of glucose was added to the basal medium, making maintenance medium of 4.5g/ℓ glucose concentration. A designed amount of sterile glucose was added to the basal medium described above to prepare experimental medium. The cells were maintained in 75cm² T-flasks. The maintained cells were centrifuged, washed with experimental medium, and cultivated in 250ml spinner flasks (Corning, working volume: 150ml) which were incubated at 37°C in 7% CO₂ atmosphere. Since cells were not well adapted to new environments of low or high glucose levels, every effects of various concentrations of glucose may involve a transient response of the glucose pulse.

Cells were enumerated using the microscopic examination after a small amount of sample was injected into a hemocytometer. Cell viability was determined by trypan blue exclusion method. The mouse immunoglobulin, IgG_{2a} was analyzed by using RID(Radial Immuno Diffusion) method (19). Anti-IgG was immobilized in agar on the RID plates. Five μl of centrifuged sample was injected into each sample hole on RID plates and incubated at 37°C for 36 hours. After incubation, ring was visualized by first staining with a buffalo-black stain solution and then rinsing with RID washing solution (5% v/v glacial acetic acid, 0.5% v/v glycerol).

Glucose concentration was measured using Beckman Glucose Analyser 2 which measures the rate of oxygen consumption when a sample is injected into an enzyme solution containing glucose oxidase. Lactate concentration was determined by using a Sigma procedure 826-UV. Lactate and excess NAD⁺ are converted to pyruvate and NADH by lactate dehydrogenase. The increase of absorbance at 340nm due to reduction of NAD⁺ to NADH becomes a measure of the amount of

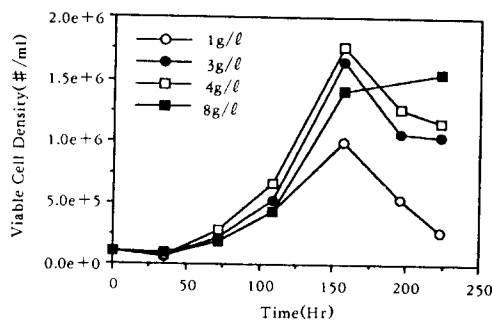


Fig. 1. Effect of initial glucose concentration on viable cell growth kinetics of VIII H-8 hybridoma cells. Cultured in 150ml spinner reactor at 37°C and 7% CO₂ atmosphere with variation of initial glucose concentration (g/l).

lactate originally present in the sample. Absorbance was measured with Bausch & Lomb Spectronic 601 spectrophotometer. The ammonium ion concentration was measured using an Orion (Cambridge MA) 9512 ammonium ion electrode and pH/millivolt meter 611.

RESULTS AND DISCUSSIONS

Cell Growth

Fig. 1 shows the effect of initial glucose concentration on viable cell kinetics. The increase of initial glucose concentration shows relatively little effect on initial cell growth rate in the exponential growth. Miller (5) and Low et al. (18) also reported that the specific growth rate was not sensitive to differences in glucose concentration within the range evaluated. On the other hand, maximum cell density increases substantially when initial glucose concentration increases from 1g/l to 3g/l while further increase to 4g/l does not show significant increase in maximum cell concentration. Further increasing glucose to 8g/l just extends the duration of stationary phase without further increasing in cell density. The cell growth patterns of all runs show stoichiometric limitation (20), but this limitation is no longer applicable beyond 4g/l of glucose because

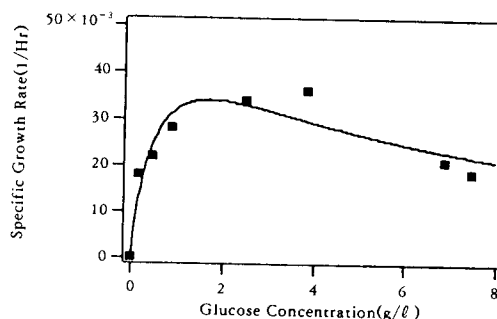


Fig. 2. Effect of glucose on the specific growth rate of VIII H-8 hybridoma cells. Closed square shows experimental data and solid line shows theoretical values from substrate inhibition type growth model as represented by Eq. 2.

maximum cell density does not increase further here possibly due to the depletion of other nutrients. Above results suggest that cell growth is not kinetically limited (20) by glucose at glucose concentration higher than 1g/l but still under stoichiometric limitation at initial glucose concentration of less than 4.0g/l.

The cell number begins to drop after 160hr except cells with 8g/l of glucose, generating a large difference in viable cell number at later stage (Eventhough data after 305 hrs were not shown in the figure, the viable cell number with 8g/l of glucose still increased to 1.6×10^6 /ml while that with 1g/l of glucose decreased to 9×10^4 /ml). The viable cell number with 1g/l of glucose drops most drastically at later phase because glucose is most seriously limited eventhough other nutrients are still mostly available due to a very low cell growth (Fig. 7). This phenomenon suggests that cell death rates may be a strong function of the availability of glucose. In other words, one of the main reasons of declining viable cell number at later phase seems to be the depletion of glucose.

Fig. 2 shows the plot of the specific growth rate versus glucose concentration in the media. For the calculation of specific growth rate, data

points only within the early exponential phase(34-109 hr) were used because usually the effects of waste metabolites and other nutrients are not significant in the range. The specific growth rate between t_1 and t_2 (hr) range was calculated as follows.

$$\mu = \frac{\ln\left(\frac{X_2}{X_1}\right)}{t_2 - t_1} \quad (1)$$

where X_1 = cell concentration at t_1 hour,

X_2 = cell concentration at t_2 hour.

The specific growth rate at glucose concentration below $4\text{g}/\ell$ follows Monod type dependence but it decreases as glucose level increases further. This pattern represents a substrate inhibition type relationship between glucose and specific growth rate. The following growth model can be suggested for this relationship.

$$\mu = \frac{\mu_{\max} [G]}{K_1 + [G] + \frac{[G]^2}{K_2}} \quad (2)$$

where $[G]$ is the concentration of glucose(g/ℓ)

Taya et al. also suggested a similar model for HL-60 human promyelocytic leukemia cells(21). The solid line of Fig. 2 shows the result of nonlinear regression of experimental data to the proposed growth model. The estimated values for μ_{\max} , K_1 , and K_2 are $0.0582(1/\text{Hr})$, $0.6009(\text{g}/\ell)$, and $4.8178(\text{g}/\ell)$, respectively. The K_1 value estimated here seems to be small so that there is no appreciable influence of glucose on the specific growth rate at glucose level higher than $1\text{g}/\ell$. This phenomenon was also observed by several investigators. Miller(5) reported that the specific growth rate was independent of glucose concentration over the range commonly employed in cell cultures(He proposed Monod type relationship and the estimated K_1 value was $0.09\text{g}/\ell$). The result of Ray et al.(22) showed not only that specific growth rate was almost invariable with glucose level higher than $1\text{g}/\ell$ but also that cell growth might be slightly reduced as glucose level increased further.

The phenomenon of a slight decrease of specific growth rate at high glucose level(substrate inhibition) is noticeable here and this was also observed by several investigators(18, 22, 23). Ray et al. observed that switching from $3.2\text{g}/\ell$ to $2.0\text{g}/\ell$ of glucose in the continuous culture of hybridoma cells slightly increased cell density(22). Low & Harbour(18) observed that the increase of glucose level from $2\text{g}/\ell$ to $4\text{g}/\ell$ caused the increase of doubling time from 15 hrs to 17.1 hrs(This means a decrease of specific growth rate) while it showed no effects on cell yield and antibody yield. They explained this phenomenon as a result of pH toxicity due to increased lactate production or limitation of other substrates. However, the increased production of lactate with increasing initial glucose concentration is not observed at initial glucose concentration higher than $4\text{g}/\ell$ according to Fig. 9. It shows almost negligible difference in final lactate concentration between $4\text{g}/\ell$ run and $8\text{g}/\ell$ run even though the cumulative specific lactate production rate increased with initial glucose concentration(Fig. 10). In addition to that, the negligible effect of lactate on cell growth was observed by several researchers(21, 24, 25). Miller applied a lactate pulse from 25 to 44 mM to a continuous culture and did not observe any change in cell concentration and even observed that viability increased slightly after one day after pulsing(24). Hassell studied the effect of added lactate at the concentration range up to 20 mM for BHK, McCoy and Vero cells and found that lactate addition had a negligible effect on cell yields for those three cells(25). He also reported that the addition of 4.9mM lactate in batch culture did not affect the growth of human hybridoma cells. According to Taya et al., the lactate inhibition constants for human HL-60 and RPMI 8226 cells are two to three times those for ammonium ion(21). Consequently lactate does not seem to be inhibitory and might require much higher lactate concentration before inhibition begins. Therefore it is hard to say that increased lactate production causes reduction of cell growth rate at high glu-

ucose concentration.

Another plausible explanation for substrate inhibition type dependence is the change of cell's metabolism with the increase of glucose concentration(11-13). The increase of glucose uptake directs carbohydrate carbon chains toward glycolysis and away from pentose-phosphate pathway. It is worthwhile to recall Reitzer's result(17) that the increase of glucose concentration from 0.2mM to 10mM results in not only an increase of the conversion rate of glucose to lactate but also a 50% decline in assimilation of glucose into pentose-phosphate pathway. Miller(5) also reported the deviation of specific growth rate from a modified Monod model and suggested that the change of metabolic pathway caused the deviation because the amount of glucose going into each of the metabolic pathways depended on the glucose concentration. Therefore, the decrease of specific growth rate with further increase of glucose may be caused not by pH toxicity but by the reduction of glucose assimilation into cell mass.

The other explanation for substrate inhibition type dependence is the increase of medium osmolarity at high glucose concentration. The medium osmolarity of 8g/l glucose run is about 22mOsm higher than that of 4g/l glucose run. According to Ozturk(26), increasing osmolarity from 290 to 435 mOsm(50% increase of osmolarity) depressed the specific growth rate of hybridoma cells by a factor of about 2. Although the increase of osmolarity is very small here(7.5%) compared that of Ozturk's, still some depression of cell growth is expected due to the increase of osmolarity at high glucose concentration.

Cell Death and Cell Viability

Fig. 3 shows the changes of dead cell number according to time. As time goes by, the differences on dead cell number between various runs become more significant, indicating that glucose level influences more strongly on dead cell kinetics rather than the viable cell kinetics. Fig. 4 shows the specific death rate versus glucose concentration. The specific death rate decreases as

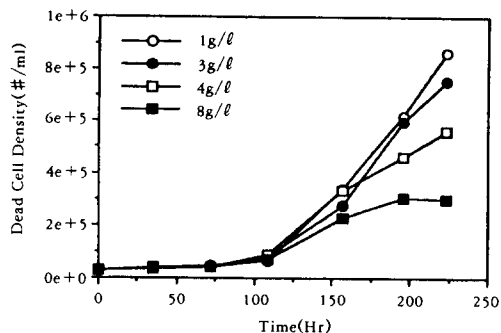


Fig. 3. Effect of initial glucose concentration on cell death kinetics of VIII H-8 hybridoma cells. Cultured in 150ml spinner reactor at 37°C and 7% CO₂ atmosphere with variation of initial glucose concentration (g/l).

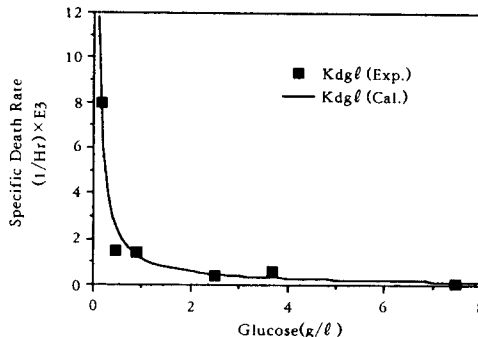


Fig. 4. Effect of glucose on the specific death rate of VIII H-8 hybridoma cells. Experimental data were compared with theoretical values from Eq. 3. The parameter value of 1.18×10^{-3} was used as D_{gl} in calculation.

glucose level increases, showing the pattern of an inverse relationship between specific death rate and glucose concentration(27).

The specific death rate for glucose depletion can be represented by using the following simple inverse relationship.

$$k_{Dgl} = \frac{D_{gl}}{[G]} \quad (3)$$

where k_{Dgl} = specific death rate for glucose depletion (1/Hr),

$[G]$ = glucose concentration (g/l),

D_{gl} = reciprocal constant (g/l/Hr), specific death rate when $[G]$ is 1g/l.

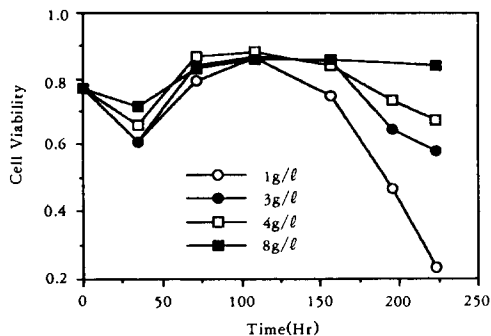


Fig. 5. Effect of initial glucose concentration on cell viability change of VIII H-8 hybridoma cells with respect to time. Same culture conditions as Fig. 1.

The comparison between experimental data and calculated values using Eq. 3 with D_g of 1.18×10^{-3} was shown on Fig. 4. This Fig. shows good agreements between experimental data and calculated values.

One of the most promising strategy for enhancing MAb productivity is to keep high cell density and high cell viability as long as possible. Fig. 5 shows the change of cell viability according to time with variation of initial glucose level. The run with $1g/l$ glucose shows the lowest viability and an abrupt drop in viability especially at later phases, while $8g/l$ run shows high cell viability throughout the culture period because glucose is not depleted even at later stages (Fig. 7). Therefore, maintaining a sufficient glucose level throughout the culture period is a very important strategy for enhancing cell viability and thus MAb productivity. The best strategy is to initially provide $4-5g/l$ of glucose and next provide additional glucose by fed-batch before glucose is depleted to maintain sufficient glucose level at the later phase. The reason why initially $4-5g/l$ of glucose is recommended is that it gives the highest specific growth rate and does not show stoichiometric limitation.

Monoclonal Antibody Production

Fig. 6 shows the effect of initial glucose con-

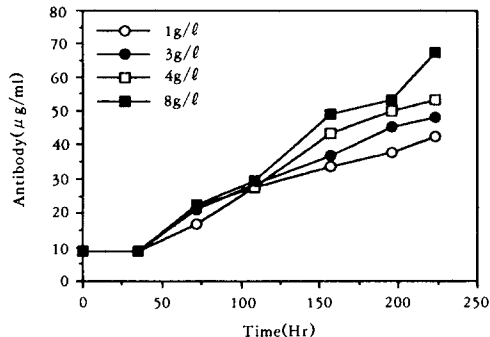


Fig. 6. Effect of initial glucose concentration on monoclonal antibody production kinetics of VIII H-8 hybridoma cells. Same culture conditions as Fig. 1.

centration on MAb production. It shows that the antibody concentration accumulated in the system increases with initial glucose concentration. Only $8g/l$ glucose shows steady increase of MAb concentration in cell culture media while others show levelling-off of MAb concentration with time. This continuous production of MAb in $8g/l$ run is due to the continuous increase of viable cell number as shown on Fig. 1 and the maintenance of relatively high cell viability even in the later phase as shown on Fig. 5. According to Ozturk(26), specific antibody production rates were higher at elevated osmolarities. Thus, the increase of osmolarity may contribute to increase specific antibody productivity in case of high glucose concentration.

Cell Metabolism

The initial variation in pH and DO between four runs would not be significant even though the pH and DO were not rigorously controlled, because four flasks were incubated simultaneously in a CO_2 incubator where pH is controlled by adjusting CO_2 . As time goes by, it is expected that the pH and DO differences between four runs become noticeable due to the variations of cell growth and lactate production. However the actual differences in lactate production (Fig. 9) between four runs are not significant enough to

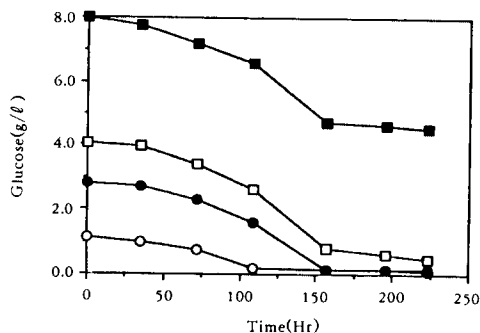


Fig. 7. Effect of initial glucose concentration on glucose consumption kinetics. Same culture conditions and legends as Fig. 1.

cause any significant differences in pH effect on cells, because usually lactate does not significantly affect cells as discussed before. It is also expected that the effect of DO differences is not significant because the major change of cell metabolism can be observed only at the DO level of below 1.2% according to Ozturk(28). In a small culture less than 1 liter, the surface area to volume ratio of culture is high enough to transfer sufficient oxygen from head space of culture vessel(29). Therefore, it is expected that the pH and DO would not seriously affect cell metabolism even though pH and DO were not rigorously controlled here.

Fig. 7 shows the time course of glucose level in the same run. In case of 1g/l of glucose, it decreases to a level of 0.19g/l after 109hr but still shows substantial cell growth from 109hr to 160hr according to Fig. 1. It is noticeable that the glucose consumption (Fig. 7) and lactate production (Fig. 9) of 1g/l run are negligible during this period, whereas other runs show continuous consumption of glucose and continuous production of lactate. This suggests that the cell of 1g/l glucose run mostly obtains growth energy not from glucose but from glutamine because glucose is not much available in the period. This explanation can be supported by Fig. 12 which shows the substantial production of ammonium ion (byproduct of glutamine metabolism) during the period

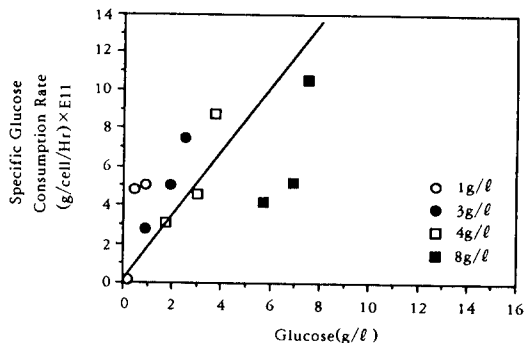


Fig. 8. Effect of glucose on the specific glucose consumption rate of VIII H-8 hybridoma cells. Every data points were obtained from 4 independent runs of different initial glucose concentrations in the exponential phase.

in case of 1g/l run. This kind of joint regulation of glycolysis and glutaminolysis will be discussed again in the last part of this paper.

In case of 8g/l of glucose, there remains still plenty of glucose which can support further growth and high cell viability at later phase. It is noticeable that both runs of 8g/l and 4g/l consume about 4g/l of glucose finally. This indicates that initial concentration of more than 4g/l of glucose does not show stoichiometric limitation caused by glucose, but by running short on other nutrients such as glutamine(1-7, 14, 17, 30, 31). Therefore maximum consumption of glucose is about 4g/l and this result is consistent with the results of other investigators. Hu et al. reported that 3.5g/l of glucose was consumed in the batch culture of HB8178 hybridoma cells(12). Low and Harbour(18) reported that glucose was only slightly limiting at 1g/l for the two murine hybridoma cells of B6 and E2, and that glucose would not be limiting if provided at a high enough initial concentration of 4.5g/l. Tharakan also used 4.5g/l of glucose to avoid glucose limitation(32).

Fig. 8 shows the plot of specific glucose consumption rate versus glucose concentration. The specific glucose consumption rates at each time interval were calculated by integral methods as follows, assuming that the specific glucose consumption rate is

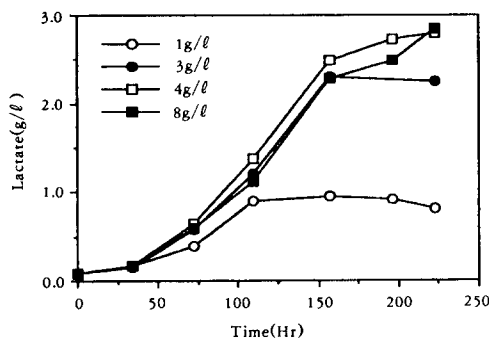


Fig. 9. Effect of initial glucose concentration on lactate production kinetics of VIII H-8 hybridoma cells. Same culture conditions as Fig. 1.

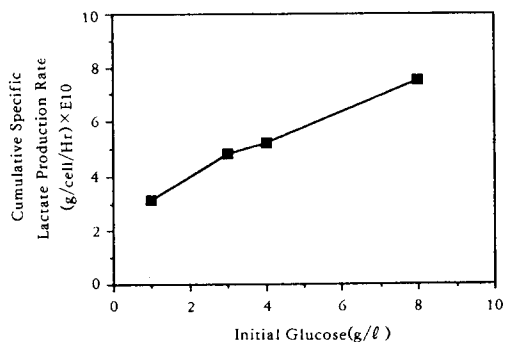


Fig. 10. Effect of initial glucose concentration on the cumulative specific lactate production rate of VIII H-8 hybridoma cells. Same culture conditions as Fig. 1.

constant between time interval t_1 and t_2 .

$$q_{gl} = \frac{[G]t_1 - [G]t_2}{\int_{t_1}^{t_2} X_v dt} \quad (4)$$

where q_{gl} = specific glucose consumption rate (g/cell/hr)

$[G]t_1$ = glucose concentration (g/l) at t_1 hour

X_v = viable cell concentration (cells/ml)

Fig. 8 demonstrates that the increase of glucose concentration stimulates the specific glucose consumption rate and Hu observed exactly the same trend with HB8178 hybridoma cell line from the same type of plot (12). This phenomenon also agrees with the results of Zielke et al. (13), showing that glucose consumption rate increases with increasing glucose concentration (1, 6, 11-13). According to Ozturk (26), specific glucose consumption rate was stimulated by elevated osmolarity. Thus, the increase of specific glucose consumption rate may be accelerated by the increase of osmolarity due to increased glucose concentration.

Fig. 9 shows the lactate production kinetics with various initial glucose concentrations. In some case, a slight decrease of lactate concentration at later phase is observed. Since Reuveny et

al. showed the uptake of lactate during later phase of hybridoma cell culture (23), this phenomenon might be the result of some uptake of lactate by cells. The final lactic acid concentration increased with increasing initial glucose concentration.

So far, several variables such as specific growth rate (Fig. 2), specific death rate (Fig. 4), and specific glucose consumption rate (Fig. 8) have been plotted against glucose concentration. These plots represented the instantaneous effect of glucose concentration. However sometimes it is more convenient to plot cumulative metabolic quotient such as cumulative specific lactate production rate in terms of initial glucose concentration to evaluate overall effect of glucose concentration instead of instantaneous effect. Usually the concept of cumulative (overall or averaged) specific productivity has been widely used especially for antibody production (5, 33) to compare overall performance of production when a specific productivity changes significantly with time. The same concept has been applied here to lactate production in order to evaluate overall effect of glucose on lactate production. The cumulative specific lactate production rate can be calculated as follows and it can be used as an index for the overall efficiency of lactate production.

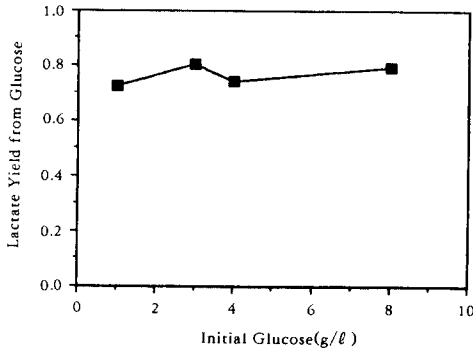


Fig 11. Effect of initial glucose concentration on the lactate yield from glucose. Same culture conditions as Fig. 1.

$$q_1 = \frac{[L]_{t_c} - [L]_0}{\int_0^{t_c} X_v dt} \quad (5)$$

where q_1 = cumulative specific lactate production rate (g/cell/hr)

t_c = total cell culture time (hr)

$[L]_{t_c}$ = final lactate concentration (g/l)

$[L]_0$ = initial lactate concentration (g/l)

X_v = viable cell concentration (cells/ml)

Fig. 10 demonstrates that the cumulative specific lactate production rate increases almost linearly as initial glucose concentration increases, and this trend was observed by several researchers (2, 5, 12, 17, 33). Since lactate is a byproduct of glucose metabolism, specific lactate production rate can be increased by increasing of either specific glucose consumption rate or lactate yield from glucose, or both. The increase of specific glucose consumption rate with glucose concentration was already discussed above. Fig. 11 shows the effect of glucose concentration on lactate yield from glucose. The lactate yield was almost independent of initial glucose concentration even though the increase of lactate yield with glucose concentration was observed by several researchers (2, 5, 12, 17). Therefore the specific lactate production rate increases with glucose concentration not by increasing of lactate yield

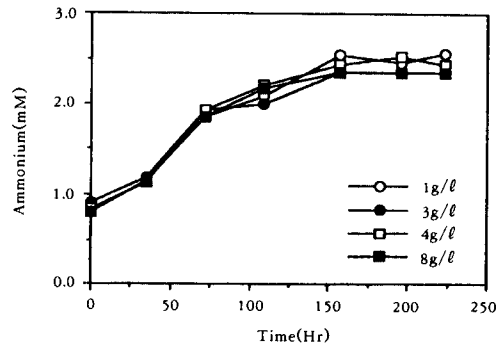


Fig. 12. Effect of initial glucose concentration on ammonium ion production kinetics of VIII H-8 hybridoma cells. Same culture conditions as Fig. 1.

from glucose but mostly by increasing of specific glucose consumption rate. According to Ozturk (26), specific lactate production rates were higher at elevated osmolarities. Thus, the increase of osmolarity due to high glucose concentration may also contribute to increase specific lactate productivity.

Fig. 12 shows the effect of initial glucose concentration on ammonium ion generation kinetics. Usually ammonium ion is produced from glutaminolysis and first order decomposition of glutamine to pyrrolidone-carboxylate (2). The ammonium ion generation kinetics observed in each run were virtually same according to Fig. 12. Therefore it is expected that the glutamine concentration profiles are almost same among four runs, and accordingly glutamine decomposition rates are also almost same between runs. Fig. 13 shows the effect of initial glucose concentration on the cumulative specific ammonium ion production rate. The cumulative specific ammonium ion production rate was plotted against initial glucose concentration to investigate the apparent and overall effect of initial glucose concentration. As expected, lower glucose concentration results in higher specific ammonium ion production rate. Ammonium ion is produced from glutamine metabolism which is also a major source of energy for mammalian cells. It seems that glycolysis and

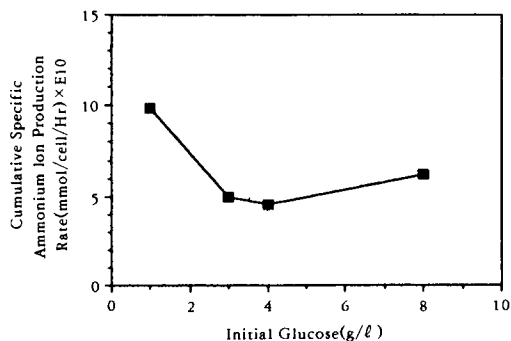


Fig. 13. Effect of initial glucose concentration on average specific ammonium ion production rate of VIII H-8 hybridoma cells. Same culture conditions as Fig. 1.

glutaminolysis are reciprocally regulated. According to Glacken(1), less glutamine need be catabolized, and less ammonium ion produced if more of cell's energy is derived from glucose through glycolysis. It has been shown that the increase of the concentration of glucose reduces glutamine uptake(6, 13, 34, 35) and the decrease of glucose concentration stimulates glutaminolysis(14-16). The result shown on Fig. 8 and other investigators' results(6, 11-13) showed low glucose consumption rate, i.e. glycolysis, at low glucose concentration. Thus, increasing glucose concentration is expected to stimulate glycolysis activity and consequently to depress glutaminolysis with generating less ammonium ion. If glucose concentration increases further, the increase of osmolarity may stimulate specific ammonium ion production rate as Ozturk reported (26). This trend is well depicted in Fig. 13. The combined effect of viable cell number and specific ammonium ion production rate resulted in almost same overall ammonium ion production kinetics as shown on Fig. 12.

CONCLUSIONS

Glucose played a significant role in cell growth stoichiometrically and kinetically. Cell density increased with increasing glucose concentration up

to 4g/l. The stoichiometric limitation was no longer valid beyond 4g/l of glucose due to the depletion of other nutrients. Glucose also showed substrate inhibition type dependence of specific growth rate on glucose. The estimated values for μ_{max} , K_1 , and K_2 were 0.0582(1/Hr), 0.6009 (g/l), and 4.8178(g/l), respectively. This small K_1 value implies little influence of glucose on the specific growth rate with glucose level higher than 1g/l that is under normal growing phase. The decrease of specific growth rate with further increase of glucose beyond 4g/l was observed and this might be caused not by pH toxicity but by the reduction of glucose assimilation into cell mass and by the increase of osmolarity.

Glucose level affected more strongly on dead cell kinetics than on viable cell kinetics. So the depletion of glucose at later phase seemed to be the main reason for declining of viable cell number. An inverse relationship between specific death rate and glucose concentration was observed. Cell viability also increased as glucose level increased. The production of MAb was improved with increasing of glucose concentration due to the increase of viable cell number and cell viability. Since maintaining sufficient glucose level throughout the culture period was very important in enhancing cell viability and MAb productivity, it might be desirable to initially provide 4-5g/l of glucose which could give the highest specific growth rate and next provide additional glucose by fed-batch before depletion of glucose.

Throughout our experiment, the maximum consumption of glucose was about 4g/l of glucose and a high portion of that was converted into lactic acid. The increase of glucose concentration stimulated the specific glucose consumption rate. The cumulative specific lactate production rate increased with initial glucose concentration mostly by increasing of specific glucose consumption rate but the lactate yield from glucose was almost independent of glucose concentration. Initial glucose concentration showed insignificant influence on the overall ammonium ion production kinetics eventhough cumulative specific ammoni-

um ion production rate was dependent on initial glucose concentration.

요 약

Hybridoma 세포의 세포 성장 속도, monoclonal antibody의 생산성 및 세포 대사에 미치는 glucose의 영향이 조사되었다. IgG_{2a}를 생산하는 mouse-mouse hybridoma VIII H-8 세포가 모델로 이용되었다. Glucose 농도에 따른 비성장속도의 변화는 기질저해형식(substrate inhibition type)의 성장모델로 나타낼 수 있었다. 초기 glucose 농도 4g/l까지는 최대 세포밀도의 증가를 보여 주었다. Glucose는 세포사망속도에 큰 영향을 나타내었고 glucose 농도와 비사망속도 간에는 반비례의 관계가 성립됨을 보였다. Glucose 농도가 증가될수록 세포의 생존율과 monoclonal antibody의 생산이 증가되었다. Glucose 농도가 증가될수록 glucose의 비소비속도가 증가되었고, 초기 glucose 농도의 증가는 lactate의 총괄 비생산속도의 증대를 가져왔다. 암모늄 이온의 총괄 비생산속도는 초기 Glucose의 농도에 의존하였지만 암모늄 이온의 전반적인 생성속도는 초기 glucose 농도에 거의 무관함을 보여 주었다.

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