*Journal of Microbiology and Biotechnology*
Vol. 2, No. 2, 141-151, 1992

# Characterization of Physiological Changes in S3H5/γ2bA2 Hybridoma Cells During Adaptation to Low Serum Media

**LEE, GYUN MIN[1]*, JOANNE SAVINELL[2] AND BERNHARD O. PALSSON[3]**

[1]**Department of Biotechnology, Korea Advanced Institute of Science and Technology*
*373-1, Kuseong-Dong, Yuseong-Gu, Taejon 305-701, Korea*
[2]*Department of Chemical Engineering, Cleveland State University, Cleveland, OH 44115, USA*
[3]*Department of Chemical Engineering, University of Michigan, Ann Arbor, MI 48109, USA*

Received 7 May 1992 / Accepted 5 June 1992

**Physiological changes of the murine hybridoma cell line S3H5/γ2bA2 during adaptation to RPMI 1640 medium with 1%(v/v) fetal bovine serum were characterized in terms of cell growth, antibody production, morphology, and metabolic quotients. Cells adapted to 1% serum medium in T-flasks became sensitive to shear induced by mechanical agitation and required at least 5% serum in the medium or spent medium for cell growth in spinner flasks, while cells adapted to 10% serum medium in T-flasks could grow in 1% serum medium in spinner flasks. Consequently, long-term adaptation to low serum media may not give the expected growth enhancement. After adaptation to 1% serum medium, changes in cell morphology were observed. The cells in 10% serum medium were uniform and circular, while cells in 1% medium were irregularly shaped. The DNA contents, which were measured by flow cytometry, were almost constant among the cells in the range of 1% to 10%. Further, no significant changes in energy metabolism and specific monoclonal antibody production rate were observed among these cells.**

Large-scale *in vitro* cultivation of hybridoma cells is required to meet the increasing demands for monoclonal antibodies (MAbs) (26, 34). One of the main economic drawbacks in large-scale culture is the requirement for expensive serum in the growth media (12, 27). Consequently, it is desirable to grow the cells in low serum media. To grow the cells in low serum media, cells from 10% serum media in which they are normally maintained, are usually adapted to low serum media by the so-called weaning procedure (1, 6, 21, 33, 38).

If hybridoma cells improve their cell growth and MAb productivity by gradual adaptation to low serum media, such adaptation is desirable for large-scale MAb production. However, the requirements for adaptation appear to depend on cell line and media. Some hybridoma cells need adaptation for the growth in low serum or serum-free media while some hybridoma cells do not need adaptation (2, 14). Although adaptation is routinely performed, there are few data available which show in detail the changes in hybridoma cells during adaptation to low serum media (25). Furthermore, some hybridoma cells fail to grow in low serum media or serum-free media despite careful adaptation over 2~3 weeks (12, 14, 15).

The goal of this work is to determine whether improvements in growth and MAb production are possible after long-term (ca. 8 months) adaptation for a cell line that is unable to show growth improvement after a 2 week adaptation period. Here we will also characterize the effects of the adaptation of hybridoma cells (S3H5/γ 2bA2) to various serum levels in terms of cell morphology, intracellular MAb and DNA content, and metabolic rates of major nutrients and waste products.

## MATERIALS AND METHODS

### Cell Line and Culture Maintenance

The murine hybridoma used was S3H5/γ2bA2, provided by Dr. Mark Kaminski from the University of the

*Corresponding author
Key words: hybridoma, adaptation, specific monoclonal antibody productivity, morphology

Michigan Medical Center. The antibody produced by this cell line is γ2b anti-idiotype antibody, directed against the isotype of the surface immunoglobulin of 38C13 mouse lymphoma cells. The fusion partner of hybridoma used was P3/x63/Ag8.653. The cell culture medium for stock cells was RPMI 1640 (Sigma, St. Louis, MO) supplemented with 10%(v/v) fetal bovine serum (FBS, Gibco Laboratories, Grand Island, NY) and 100 unit of penicillin and 100 μg/ml of streptomycin (Sigma). The cells were maintained in T-25 $cm^2$ cell culture flask (Bellco Glass, Inc., Vineland, NJ) at 37℃ in a humidified $CO_2$ incubator (VWR Scientific, San Fransisco, CA). The cells were diluted 1:5 with fresh medium every other day.

**Adaptation to Reduced Serum Media**

Cells were subjected to stepping-down procedure from 10% to 1% serum media over a 6 month period using T-flasks for cultivation. Cells grown in 10% serum medium for approximately 6 months (designated as 10 %-cells) were inoculated into 5% serum medium. These cells were cultured for 2 months in 5% serum medium (5%-cells), and then inoculated into 2.5% serum medium. Likewise, these cells were cultured in 2.5% serum medium for 2 months (2.5%-cells), and then inoculated into 1% serum medium, where they were cultured for another 2 months before the experiments were begun (1%-cells).

The cells in 10%, 5%, and 2.5% serum medium were diluted 1:5 with fresh media every other day. The cells in 1% serum medium were diluted 3:10 with fresh medium every two or three days depending on cell growth.

**Cell Cultures**

Cells adapted to different serum concentration media (10, 5, 2.5, and 1%) were used as inocula. Exponentially growing cells were centrifuged at 1000 rpm for 10 min. The cells were washed with fresh media, and then inoculated into spinner flasks (Bellco) containing 50 ml of RPMI 1640 media with different serum concentrations at initial cell density of $1\times10^5$ cells/ml. The agitation speed used in the spinner flasks was 100 rpm. The cultures were kept at 37℃ in a humindified $CO_2$ incubator (VWR Scientific, San Fransisco, CA).

**Sample Analysis**

Cell growth was monitored by counting viable cells with a hemocytometer. The viable cell population was distinguished form dead cells by the trypan blue dye exclusion method. The cell culture suspension was centrifuged and supernatant was aliquoted and kept frozen at −80℃.

Glucose and lactate were measured using glucose/lactate analyzer (Yellow Spring Instruments, Model 2000, Yellow Spring, Ohio); ammonia was measured using an ammonia electrode (Orion Research Inc., Model 95-12, Cambridge, MA). Glutamine was determined by reverse-phase HPLC column (Rainin Instrument, Inc., Microsorb, short-one C18, Woburn, MA) as described by Seaver *et al.* (31).

The antibody concentration was quantified using an enzyme linked immunosorbent assay (ELISA) as described previously (17).

The intracellular antibody and DNA contents were quantified simultaneously using flow cytometry. The procedures used were modified from those described previously (4, 5, 32). Cells were washed with phosphate-buffered saline (PBS) twice and were fixed in 70% ethanol for at least 18 hrs. After fixation, cells were incubated at 4℃ with 0.4 ml of a 1:30 dilution of fluorescein-isothiocyanate (FITC)-conjugated goat antimouse IgG2b (Southern Biotechnology, Associates, Inc., Birmingham, AL) for 30 min. After washing, cells were stained for DNA with propidium iodide (PI) over a concentration range of 10 to 20 μg/ml in PBS containing RNase (Sigma). The cells were incubated for at least 20 min at 37℃. After centrifugation, cells were resuspended in PBS. Prior to flow cytometric (FCM) analysis, cell suspensions were filtered through a 40-μm nylon-mesh filter. Green and red fluorescence were simultaneously monitored in a Coulter EPICS 751 flow cytometer (Coulter corporation, Hialeah, FL).

For transmission electron microscopy, cells in late exponential phase were fixed with 2.5% glutaraldehyde in cacodylate buffer at pH 7.4, postfixed with 1% osmium tetroxide and buffered with s-collidine (7).

**Determination of Specific Growth Rate and Metabolic Quotients**

The specific growth rate, μ, was calculated using data collected during the exponential growth phase and defined as follows:

$$\mu=\frac{1}{X_v}\frac{dX_v}{dt} \tag{1}$$

where $X_v$ denotes the concentration of viable cells and t denotes the cultivation time.

The specific metabolic quotient calculations for substrate consumption and product formation ($q_s$ and $q_p$, respectively) were also based on data collected during the exponential phase of the growth. They are defined as follows:

$$-q_s=\frac{1}{X_v}\frac{dS}{dt} \tag{2}$$

$$q_p=\frac{1}{X_v}\frac{dP}{dt} \tag{3}$$

where S and P are the substrate and product concentrations, respectively.

Glutamine spontaneously decomposes following first order kinetics to pyrrolidone-carboxylate and ammonia (20, 35). The sepcific glutamine consumption and ammonia production rates ($q_{gln}$ and $q_{NH_4}$, respectively) were determined by accounting for degradation of glutamine at 37℃ (10, 24):

$$-\frac{d[GLN]}{dt} = k[GLN] + q_{gln}X_v \quad (4)$$

$$\frac{d[NH_4]}{dt} = k[GLN] + q_{NH_4}X_v \quad (5)$$

where [GLN] is the glutamine concentration (mM); $[NH_4]$ is the ammonium ion concentration (mM); k is the first-order rate constant for glutamine decomposition ($hr^{-1}$). Since the first-order decomposition rate varies with serum and media components (20, 22, 24), we measured experimentally the values of k. The values of k were 0.0040~0.0043 $hr^{-1}$ in RPMI 1640 media at 37℃ and pH 7.4 under 5% $CO_2$ atmosphere, in the different serum concentrations used in this experiment. This value is consistent with that obtained by Ozturk and Palsson (24).

Assuming that the specific MAb production rate, $q_{MAb}$, is constant, one can calculate it from

$$q_{MAb} = C_{MAb}(t)\frac{1}{\int_0^t X_v(t)dt} \quad (6)$$

where $C_{MAb}(t)$ denotes the concentration of MAb over time (28, 30).

## RESULTS AND DISCUSSION

### Cell Growth

Cells adapted to specific serum concentrations (10%, 5%, 2.5%, and 1%, respectively) in T-flasks were inoculated into spinner flasks containing media with different serum concentrations (10%, 5%, 2.5%, and 1%). As shown in Fig. 1(a), serum requirements for cell growth did not decrease after the weaning of cells, and in fact, increased. When 10%-cells and 5%-cells were used as an inoculum, they grew well in a spinner flask containing 1% serum medium. However, when 2.5%-cells were used, they required at least 2.5% serum concentration in medium for cell growth. Furthermore, 1%-cells could grow neither in 1% nor 2.5% serum medium. Cell growth rate was not enhanced after the adaptation to low serum media and in fact, deteriorated during the weaning procedure. Cells adapted to media with high serum concentrations showed higher maximum cell densities than cells adapted to media with low serum concentrations in all growth conditions (Fig. 1(b)).

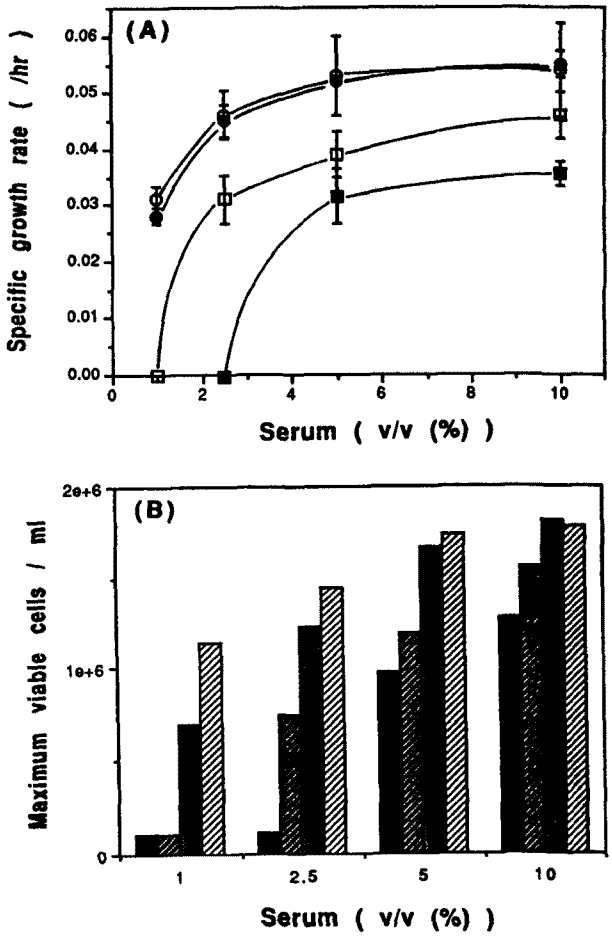


**Fig. 1. (a) Specific growth rate and (b) maximum cell densities as functions of serum concentrations using inocula that were adapted previously to four different serum concentrations.**
(a) 10% −○−, 5% −●−, 2.5% −□−, 1% −■−; (b) 10% −▨−, 5% −▩−, 2.5% −▨−, 1% −■−.

These observations indicate that the cells become mechanically weak and unhealthy during adaptation to low serum media. These changes could be related to the changes in the fatty acid composition in the cell membrane after adaptation to low serum media (30). Changes in cell morphology were also observed by transmission electron microscopy (TEM). The 10%-cells were uniform and circular, while the 1%-cells were irregularly shaped (Fig. 2).

When 1%-cells were inoculated into spinner flasks containing spent medium (the mixture of cell culture supernatant of 1% serum medium from T-flask culture and fresh 1% serum medium at the ratio of 3 to 7), they grew as well as 10%-cells (Fig. 3). This improvement in

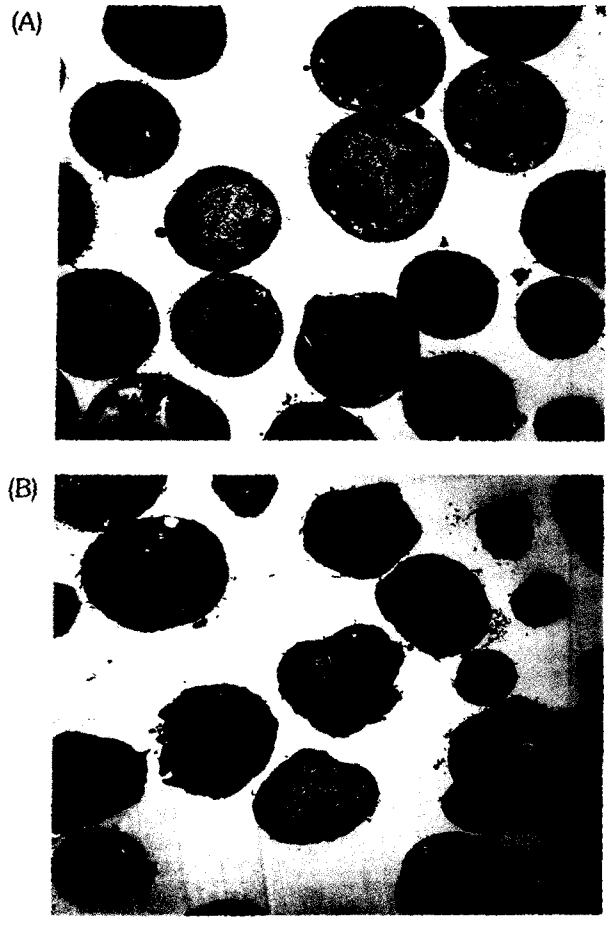


**Fig. 2. Transmission electron microscopy pictures of (a) cells adapted to 10% serum medium and (b) cells adapted to 1% serum medium.**

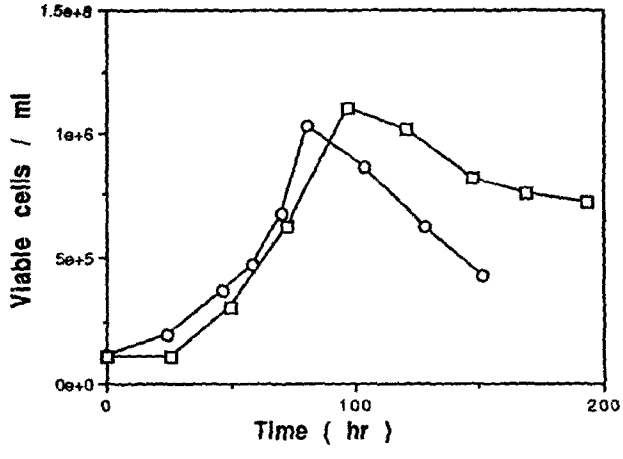


**Fig. 3. Effect of spent medium for cell growth.**
−□− the growth of cells adapted to 1% serum medium in spent medium; −○− the growth of cells adapted to 10% serum medium in 1% serum medium.

cell growth may result from autocrine growth factors which may present in the spent medium. These factors may stimulate cell growth, especially when cells are unhealthy. No effect of spent medium was observed when the 10%-cells were used as an inoculum (data not shown). The spent medium also may have stimulated cell growth by binding toxic metals in the fresh medium. Serum is known to detoxify the media by binding toxic metals (3). Cells inoculated into fresh medium may be exposed to toxic levels of certain metals due to the low amount of serum, and 1%-cells may be more sensitive to this toxicity than 10%-cells. The addition of the conditioned medium improved the growth of the 1%-cells because it may contain the same detoxifying factors found in serum.

### Metabolite Concentrations

Glucose and glutamine, which are the major carbon and energy sources in most cell culture media, and which are required for cell growth, were measured during the cultivation. The major byproducts, lactate and ammonia were also measured. The glucose and lactate concentrations during the cultivation are shown in Fig. 4. The glucose level decreased markedly during the exponential growth and glucose utilization was accompanied by a corresponding accumulation of lactate. Glucose was not a limiting nutrient for cell growth in all culture conditions tested. The glucose consumption and the complementary production of lactate ceased shortly after maximum cell densities were achieved. The ratio of lactate produced to glucose consumed was almost constant throughout the culture in all growth conditions, as shown in Table 1.

When the cells adapted to the specific serum concentrations were inoculated into the media with different serum levels, the cells grown in low serum media showed higher glucose consumption rates than in high serum media, even though their growth rates were lowest at low serum media. Cells grown in high serum media appear to utilize glucose more efficiently than in low serum media. Dalili and Ollis showed similar results for effect of serum on specific glucose and glutamine uptake rates (6). This relationship between glucose uptake and serum concentration in the medium is the same regardless of inocula used, indicating that there was no significant effect of adaptation to low serum media on the glucose uptake in the cells.

Glutamine, unlike glucose, was completely depleted at maximum cell density, which suggests that glutamine is a limiting nutrient in this medium (Fig. 5). The observation that cells adapted to low serum media showed lower maximun cell density than cells adapted to high serum media in all growth conditions can be explained by glu-

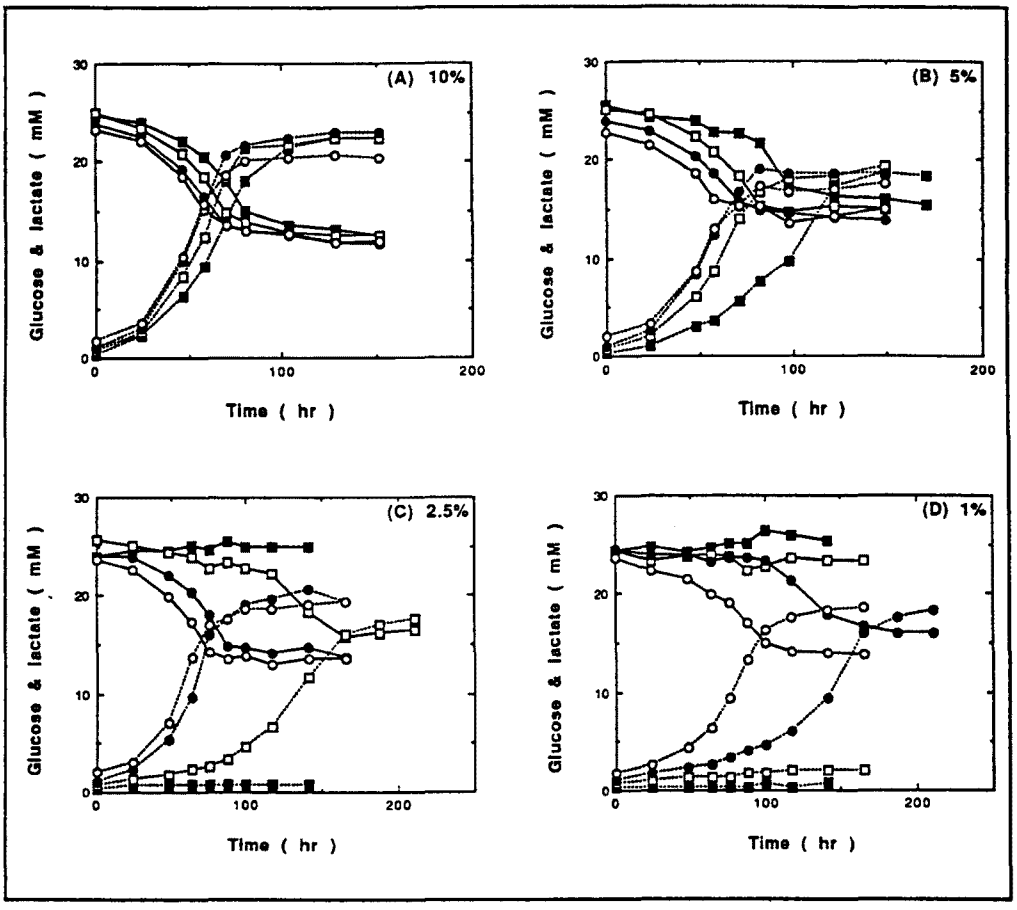


**Fig. 4. Glucose and lactate profiles using inocula that were adapted to four different serum concentrations.**
(A) 10%, (B) 5%, (C) 2.5%, (D) 1%. Serum concentration in culture media: −○− 10%, −●− 5%, −□− 2.5%, −■− 1%.

**Table 1. Metabolic quotients of cells adapted to various serum levels.**
The first number in column is the mean of 2-4 experiments and the number in parenthesis is standard deviation. Glutamine and ammonia rates/yields have been corrected for the natural decomposition of glutamine.

| Serum conc. which cells are adapted to (%(v/v)) | Serum conc. in media (%(v/v)) | Quoteints ($mM/10^{10}$ cells/hr) glucose | lactate | glutamine | ammonia | Yield (mM/mM) −Δlactate/Δglucose | −Δammonia/Δglutamine |
|---|---|---|---|---|---|---|---|
| 10 | 1 | 3.89(0.305) | 6.92(0.409) | 0.81(0.194) | 0.73(0.246) | 1.80(0.134) | 0.88(0.136) |
| | 2.5 | 3.68(0.350) | 6.46(0.394) | 0.77(0.159) | 0.54(0.085) | 1.75(0.099) | 0.70(0.043) |
| | 5 | 3.08(0.606) | 6.02(0.914) | 0.69(0.091) | 0.43(0.071) | 1.87(0.418) | 0.63(0.020) |
| | 10 | 2.62(0.370) | 4.53(0.588) | 0.52(0.034) | 0.29(0.022) | 1.72(0.078) | 0.55(0.029) |
| 5 | 1 | 4.62(1.814) | 7.13(0.703) | 0.82(0.030) | 0.58(0.167) | 1.64(0.491) | 0.70(0.118) |
| | 2.5 | 3.30(0.203) | 6.67(0.533) | 0.75(0.003) | 0.56(0.082) | 1.88(0.046) | 0.74(0.075) |
| | 5 | 2.84(0.184) | 5.71(0.843) | 0.82(0.033) | 0.57(0.181) | 1.85(0.416) | 0.69(0.154) |
| | 10 | 2.81(0.218) | 4.90(0.623) | 0.64(0.029) | 0.34(0.078) | 1.63(0.100) | 0.53(0.110) |
| 2.5 | 2.5 | 3.68(0.837) | 6.30(0.993) | 0.56(0.085) | 0.57(0.120) | 1.73(0.123) | 1.04(0.371) |
| | 5 | 2.88(0.232) | 6.29(0.320) | 0.54(0.171) | 0.32(0.080) | 2.19(0.288) | 0.60(0.045) |
| | 10 | 2.57(0.126) | 4.60(0.008) | 0.50(0.022) | 0.33(0.084) | 1.80(0.091) | 0.66(0.139) |
| 1 | 5 | 3.65(1.432) | 5.24(0.916) | 0.63(0.183) | 0.41(0.128) | 1.50(0.339) | 0.71(0.313) |
| | 10 | 2.58(0.320) | 4.68(0.638) | 0.50(0.051) | 0.35(0.104) | 1.81(0.224) | 0.70(0.072) |

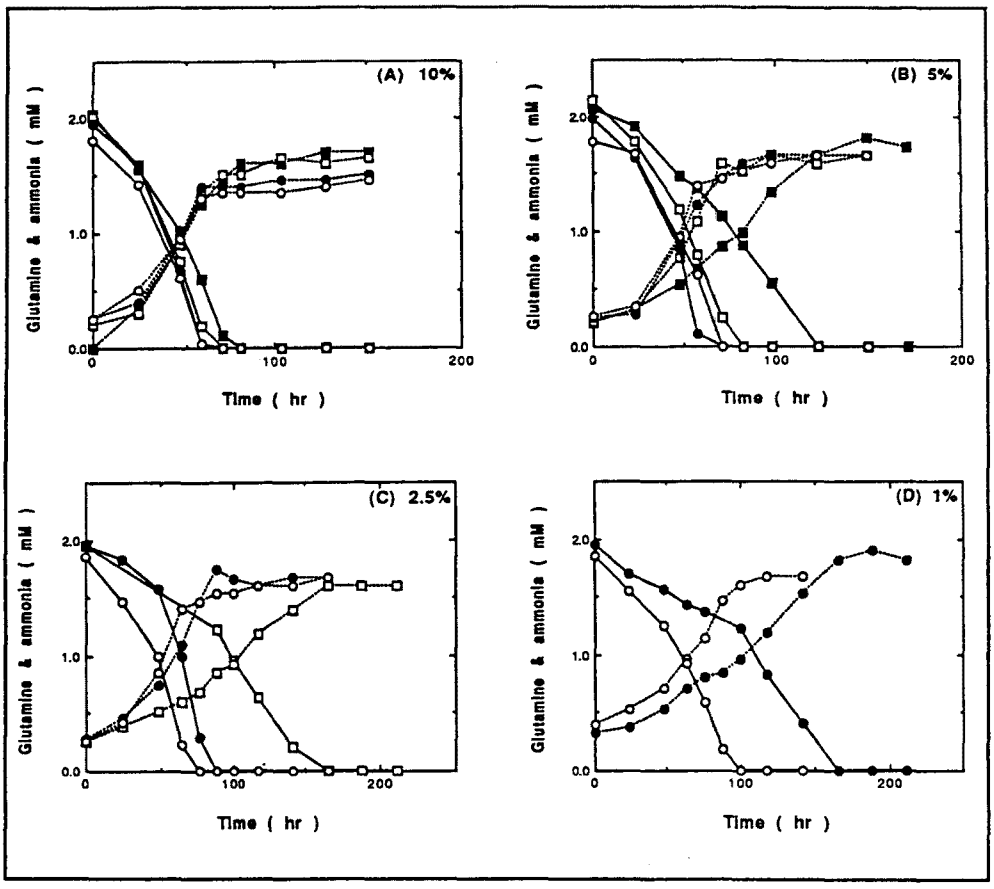


**Fig. 5. Glucose and lactate profiles using inocula that were adapted to four different serum concentrations.**
(A) 10%, (B) 5%, (C) 2.5%, (D) 1%. Serum concentration in culture media: —○— 10%, —●— 5%, —□— 2.5%, —■— 1%.

tamine depletion. When cells adapted to low serum media were cultivated, they had a 2~3 day lag phase and the growth rate was slower than that of cells adapted to high serum media. By the start of their exponential phase, 20~30% of the glutamine in the media was depleted due to decomposition. Less glutamine was available for cell growth, and consequently, a lower maximum cell density was achieved compared to cells adapted to high serum media. The effect of serum on glutamine uptake rate was similar to that of glucose uptake rate (Table 1). With high serum media, the uptake rate of glutamine decreased, although the growth rate was higher, indicating that the cells utilize glutamine more efficiently in high serum media. It has been reported that glutamine uptake is proportional to glutamine concentration at the glutamine concentration used here (13, 23). The cells adapted to low serum media were exposed to 20~30% less glutamine than cells adapted to high serum media, at the start of their exponential phase, as described above. This change in the initial concentrations of glutamine will affect the glutamine uptake rates, and make it difficult to determine the effect of adaptation on glutamine metabolism.

The ratio of ammonia produced to glutamine consumed was almost constant throughout the culture at all growth conditions. The yield coefficient of glutamine to ammonia was lowest at high serum media. This result suggests that at high growth rate, a large fraction of glutamine is catabolized through the transamination pathways, in order to provide amino acids for biosynthesis. At low growth rate, the glutamine appears to be diverted through glutamate dehydrogenase, which generates $NH_4^+$, and which can result in energy formation.

At stationary and decline phases, the glucose uptake rate is negligible and glutamine is completely depleted. Since the cells are no longer growing, their needs for energy and biomass source is greatly reduced, and they may obtain their necessary nutrients from residual quantities in the media, from the lysis products of dead cells, or from stores of glycogen within the cells.

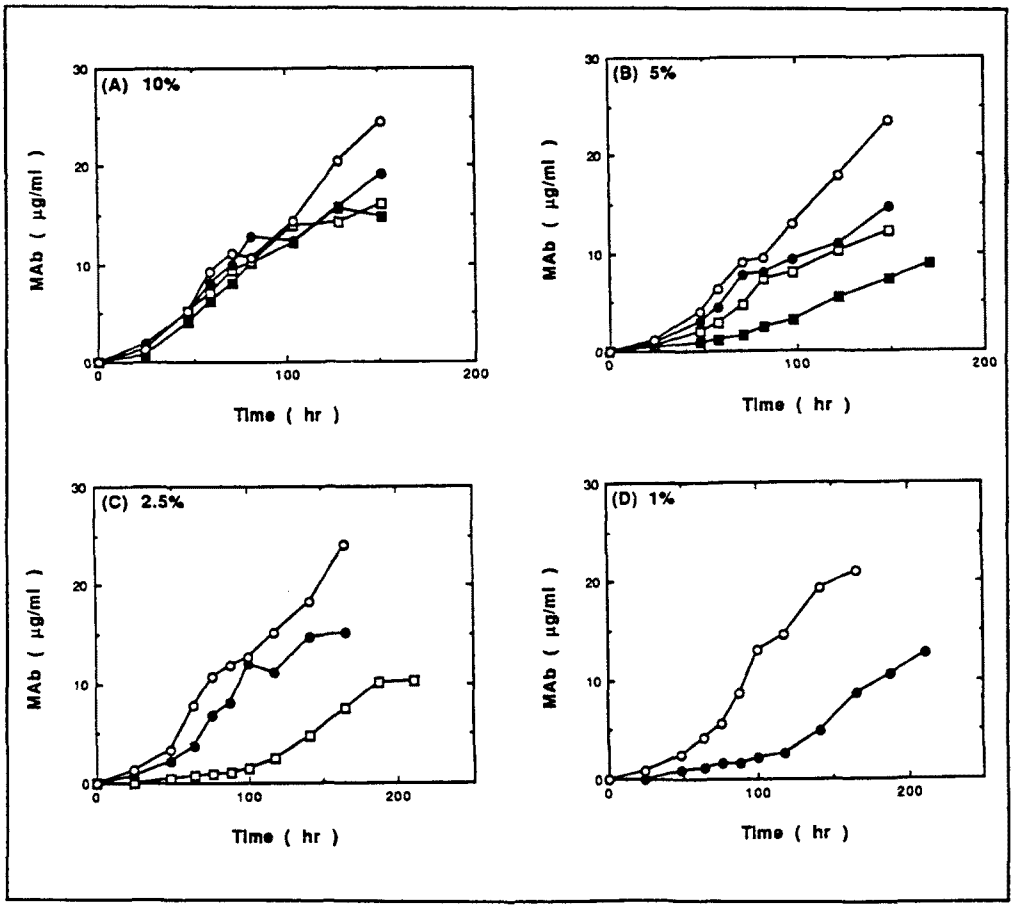


**Fig. 6. MAb production profiles using inocula that were adapted to four different serum concentrations.**
(A) 10%, (B) 5%, (C) 2.5%, (D) 1%. Serum concentration in culture media: −○− 10%, −●− 5%, −□− 2.5%, −■− 1%.

## MAb Production

It has been frequently noted that hybridoma cells secrete MAb throughout batch growth and a significant portion of MAb is produced during stationary and decline growth phases (16, 23, 28, 36). This production does not seem to be due to the release of antibody from dying cells but due to active secretion from viable cells throughout the growth cycle (37). As shown in Fig. 6, cells produced MAb throughout batch growth. It is noteworthy that cells in stationary and decline phases can produce MAb without any glutamine left in the media. The maximum MAb concentration increased as the serum concentration, either in the media or to which cells are adapted, increased. However, cells adapted to different serum levels showed similar specific antibody production rates which are in the range of 0.15 to 0.23 pg/cell·hr. The high titer of MAb obtained with high serum media and inoculum prepared from high serum media was due to the enhanced cell growth, resulting in elevated integral of number of viable cells in those media (see equation 6).

## DNA and Intracellular MAb Contents

The DNA and intracellular MAb contents of cells adapted to different serum media were measured. Cells in the late exponential phase of the growth in T-flasks, which were used as inocula for suspension culture in spinner flasks, were prepared for flow cytometry. DNA histograms, shown in Fig. 8, are plots of a relative DNA content, as measured by fluorescence intensity versus frequency (cell count). There are no significant differences in DNA contents among the cells adapted to different serum levels, indicating that cells might not lose chromosomes during the adaptation to low serum media. Since 1%-cells had lower apparent growth rate than 10%-cells, the percentage of the 1%-cells in G0-G1 phases was higher than that of 10%-cells.

Fig. 9 shows the intracellular MAb content of the cells.

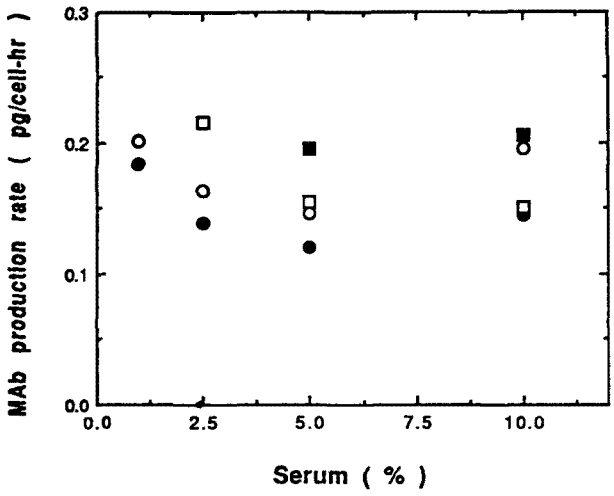


**Fig. 7. Specific antibody production rates using inocula that were adapted to four different serum concentrations.**
—○— 10%, —●— 5%, —□— 2.5%, —■— 1%.

There were no significant differences in the intracellular MAb content of the cells adapted to 10%, 5%, and 2.5% serum medium. However, the intracellular MAb content in cells adapted to 1% serum medium was lower than that in the other cells and showed a broad distribution.

Plots of intracellular MAb contents vs DNA content are shown in Fig. 10. It was found that the intracellular MAb content appears to be almost constant throughout the cell cycle. These results suggest that cells produce MAb at a constant rate irrespective of cell cycle. Further, we have shown data suggesting that the intracellular MAb content is proportional to the specific MAb production rate over a range of 0 to 0.4 pg/cell·hr (18).

## CONCLUSIONS

Adaptation to low serum did not change the specific MAb production rate, the DNA contents, and other metabolic quotients of the cells (S3H5/γ2bA2). However,

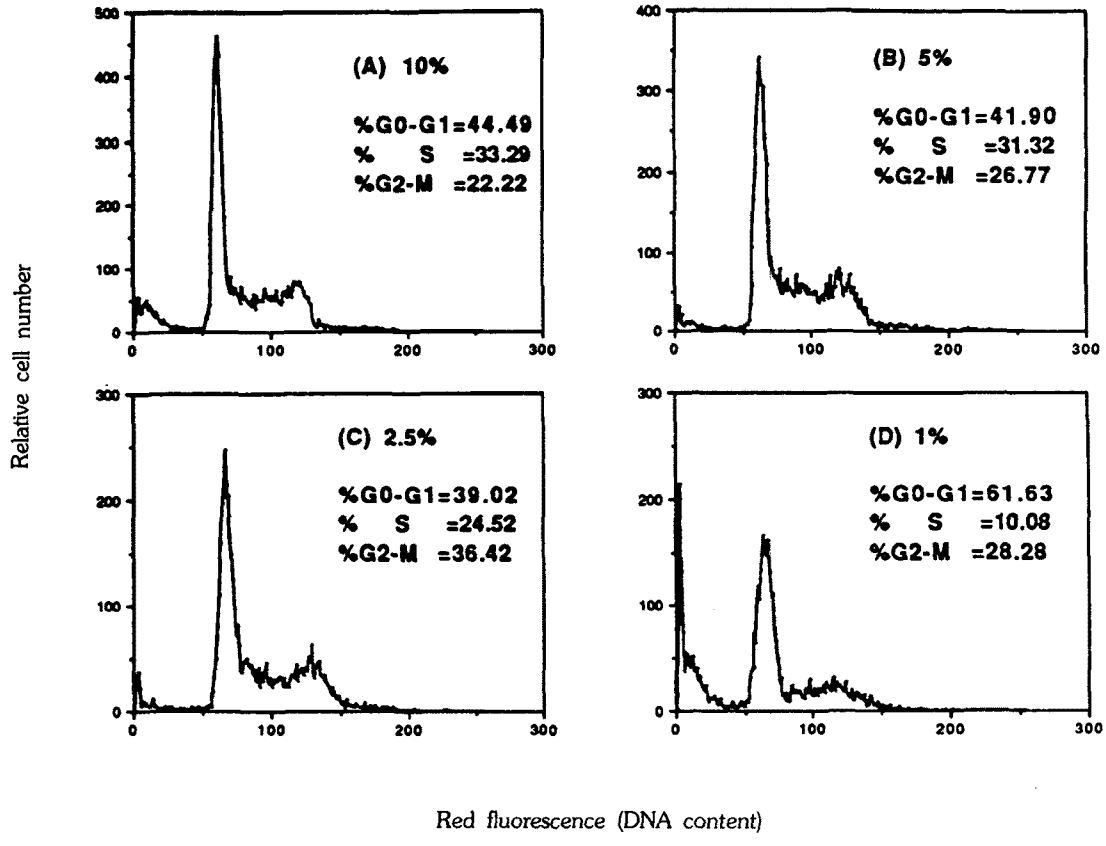


**Fig. 8. DNA histograms of cells adapted to four different serum concentrations.**
(A) 10%, (B) 5%, (C) 2.5%, (D) 1%.

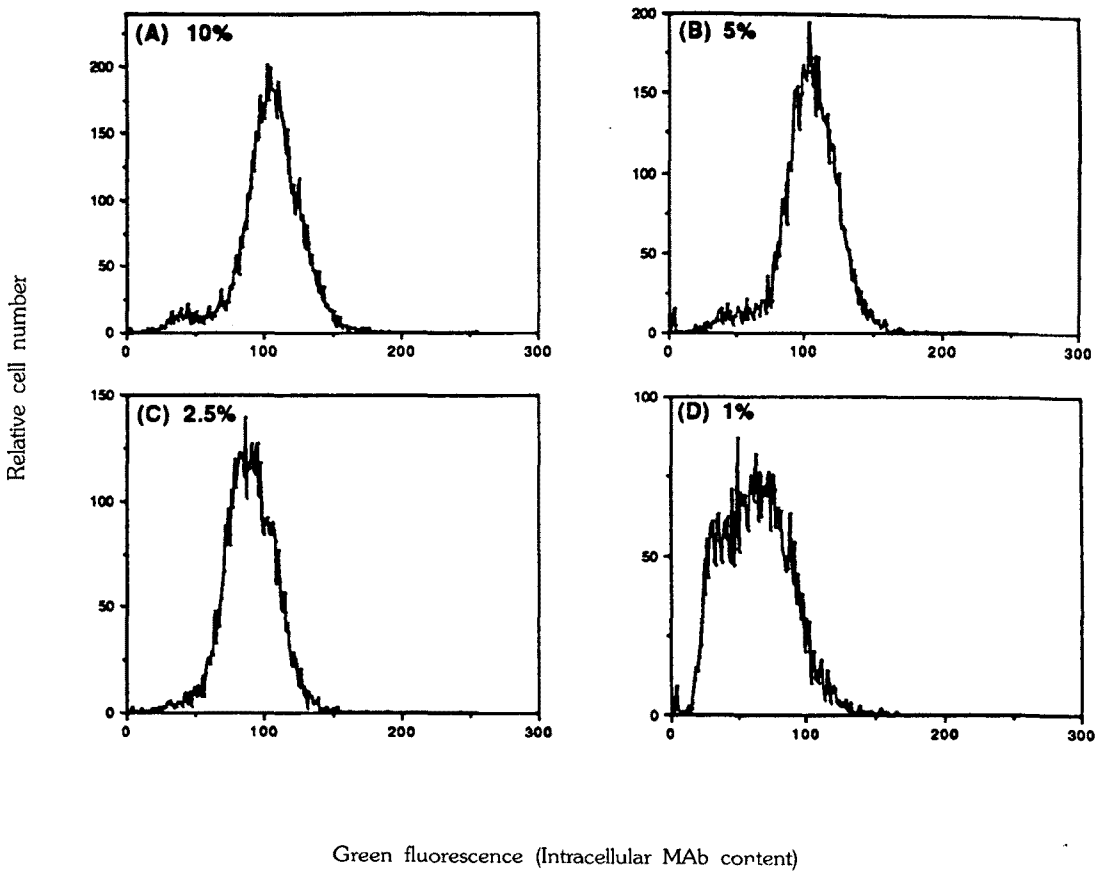


**Fig. 9. Intracellar antibody contents of cells adapted to four different serum concentrations.**
(A) 10%, (B) 5%, (C) 2.5%, (D) 1%.

the morphology of the cell changed during adaptation as viewed by TEM. The growth rate of this cell line was not improved by the weaning procedure. The 1%-cells became shear sensitive, and for growth in a spinner flask, they required media with serum more than 5%. This high serum requirement may be due to the increased sensitivity of 1%-cells to growth factors or toxic compounds in the media.

Some hybridoma cell lines showed improvement in growth after an adaptation period of about 10 generations (14). Changes in cell membrane composition during adaptation to serum-free media were also observed within 10 generations (9). Accordingly, the adaptation period appears to be about 10 generations, so that if a cell line does not show an improvement in growth ability during this time, it is unlikely that there would be significant enhancement over a longer adaptation period, as shown in this study. In fact, deterioration of cell growth during the weaning procedure was observed in our experiments. Another disadvantage of long-term adaptation is the loss of MAb productivity in both high and low serum media (8, 18, 25). Consequently, adaptation to low serum media over a long period (about 8 months) is not recommended for efficient MAb production.

## Acknowledgement

This work was supported by National Science Foundation (BSC-9009389). One of the authors (GML) was supported in part by Korea Advanced Institute of Science and Technology. The authors thank Ms. Kay Brabec for assistance with TEM and Mr. Mark Cameron for his assistance with FCM.

## REFERENCES

1. **Al-Rubeal, M. and R. Spier.** 1989. Hybridoma cultivation in serum supplemented and serum-free media. p. 277-280. *In* R.E. Spier, J.B. Griffiths, J. Stephenne, and P.J. Crooy (eds.), *Advances in Animal Cell Biology and Technology for Bioprocesses.* Butterworth & Co. Ltd., Tiptree.
2. **Baker, P., K. Knoblock, L. Noll, D. Wyatt, and B. Lydersen.** 1984. A serum independent medium effective in all aspects of hybridoma technology and immunological appli-

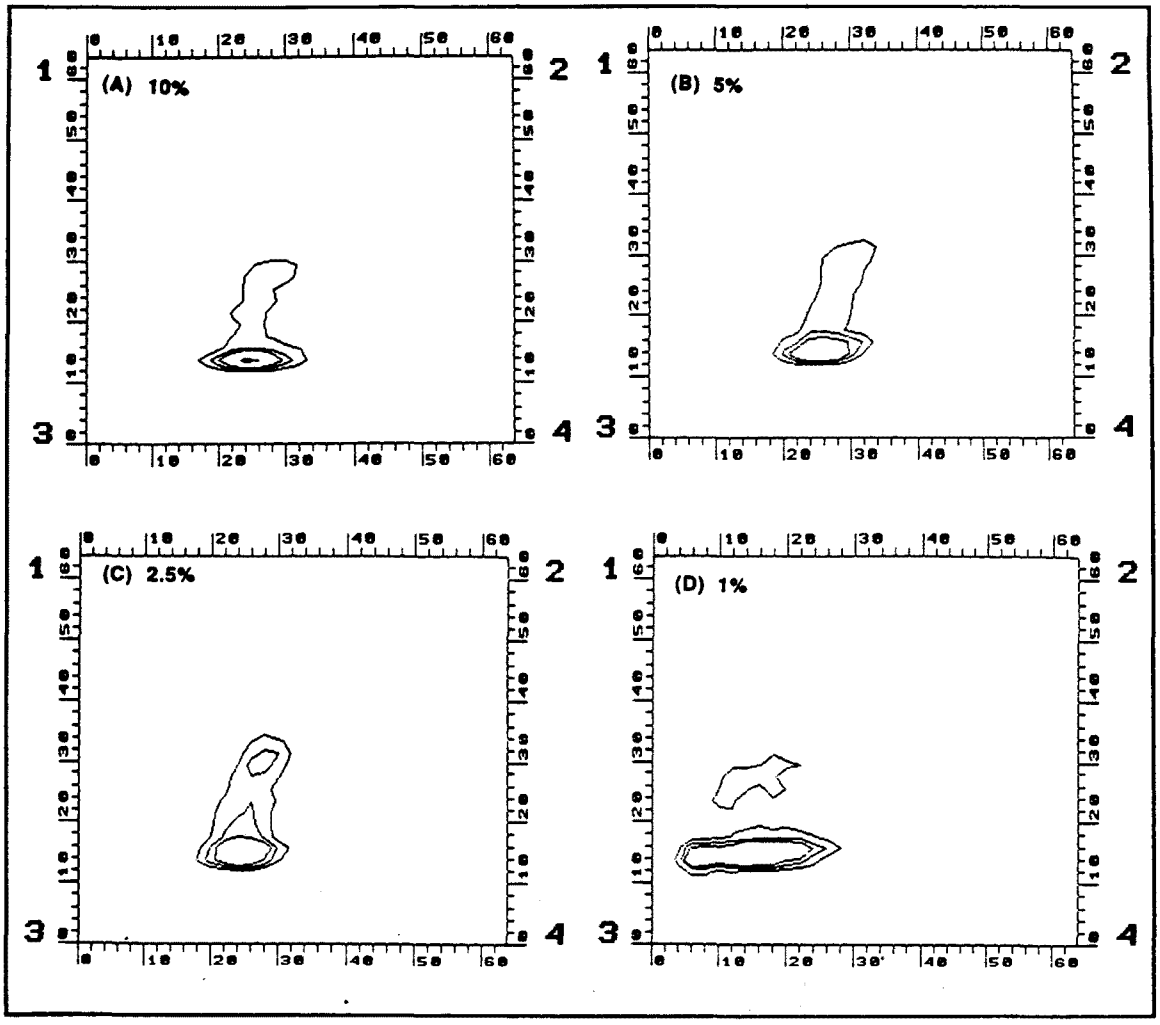


**Fig. 10. Cytogram (DNA (Y axis) vs intracellular MAb (X axis)) of cells adapted to four different serum concentrations.**

(A) 10%, (B) 5%, (C) 2.5%, (D) 1%.

cations. *Develop. Biol. Standard.* **60**: 63-72.

3. **Barnes, D. and G. Sato.** 1980. Methods for growth of cultured cells in serum-free medium. *Anal. Biochem.* **102**: 255-270.
4. **Clevenger, C.V., K.D. Bauer, and A.L. Epstein.** 1985. A method for simultaneous nuclear immunofluorescence and DNA content quantitation using monoclonal antibodies and flow cytometry. *Cytometry* **6**: 208-214.
5. **Crissman, H.A. and J.A. Steinkamp.** 1982. Rapid, one step staining procedures for analysis of cellular DNA and protein by single and dual laser flow cytometry. *Cytometry* **3**: 84-90.
6. **Dalili, M. and D. Ollis. 1989. Transient kinetics of hybridoma growth and monoclonal antibody production in serum-limited cultures.** *Biotechnol. Bioeng.* **33**: 984-990.
7. **Dawes, C.J.** 1988. *Introduction to Biological Electron Microscopy: Theory and Techniques*, Ladd Research Industries, Inc., Burlington, Vermont.
8. **Frame, K.K. and W.S. Hu.** 1990. The loss of antibody productivity in continuous culture of hybridoma cells. *Biotechnol. Bioeng.* **35**: 469-476.
9. **Geyer, R.P.** 1967. Uptake and retention of fatty acids by tissue culture cells. p.33-44. *In* G.H. Rothblat and D. Kritchevsky (eds.) *Lipid Metabolism in Tissue Culture Cells*, The Wistar Institute Press, Philadelphia.
10. **Glacken, M.W., R.J. Fleischaker, and A.J. Sinskey.** 1986. Reduction of waste product excretion via nutrient control: possible strategies for maximizing product and cell yields on serum in cultures of mammalian cells. *Biotechnol. Bioeng.* **28**: 1376-1389.
11. **Griffiths, J.B.** 1986. Can cell culture medium cost be reduced? Strategies and possibilites. *Trends Biotechnol.* **4**: 268-272.
12. **Griffiths, J.B.** 1987. Serum and growth factors in cell cul-

ture media: an introductory review. *Develop. Biol. Stand.* **66**: 155-160.

13. **Himes, V.B. and W.S. Hu.** 1988. Glutamine utilization by chinese hamster overy cells in perfused microcarrier cultures. Presented at the annual ACS Meeting, Los Angeles, CA.
14. **Kovar, J. and F. Franek.** 1986. Serum-free medium for hybridoma and parental myeloma cell cultivation, p. 277-292, *In* J.J. Langone and H.V. Vunakis (eds.), *Methods in Enzymology, Vol.* **121**. Academic Press, London.
15. **Kovar, J.** 1989. Various cells grow in protein-free hybridoma medium. *In Vitro Cell. Dev. Biol.* **25**: 395-396.
16. **Lee, G.M., T.K. Huard, M.S. Kaminski, and B.O. Palsson.** 1988. Effect of mechanical agitation on hybridoma cell growth. *Biotechnol. Lett.* **10**: 625-628.
17. **Lee, G.M., T.K. Huard, and B.O. Palsson.** 1989. Effect of serum concentration on hybridoma cell growth and monoclonal antibody production at various initial cell densities. *Hybridoma* **8**: 369-375.
18. **Lee, G.M. and B.O. Palsson.** 1990. Immobilization can improve the stability of hybridoma antibody productivity in serun free media. *Biotechnol. Bioeng.* **36**: 1049-1055.
19. **Lee, G.M., A. Varma, and B.O. Palsson.** 1991. Production of monoclonal antibody using free and immobilized hybridoma cells. *Biotechnol. Bioeng.* **38**: 821-830.
20. **Lin, A. and P. Agrawal.** 1988. Glutamine decomposition in DMEM: effect of pH and serum concentration. *Biotechnol. Lett.* **10**: 695-698.
21. **Low, K. and C. Harbour.** 1985. Growth kinetics of hybridoma cells: (1) The effects of varing foetal calf serum levels. *Develop. Biol. Standard.* **60**: 17-24.
22. **Miller, W.M., C.R. Wilke, and H.W. Blanch.** 1988. Transient responses of hybridoma metabolism to changes in the oxygen supply rate in continuous culture. *Bioprocess Engineering.* **3**: 103-111.
23. **Miller, W.M., H.W. Blanch, and C.R. Wilke.** 1988. A kinetic analysis of hybridoma growth and metabolism in batch and continuous suspension culture: effect of nutrient concentration, dilution rate and pH. *Biotechnol. Bioeng.* **32**: 947-965.
24. **Ozturk, S.S. and B.O. Palsson.** 1990. Chemical decomposition of glutamine in cell culture media: effect of media type, pH, and serum concentration. *Biotechnol. Prog.* **6**: 121-128.
25. **Ozturk, S.S. and B.O. Palsson.** 1990. Loss of antibody productivity during long-term cultivation of a hybridoma cell line in low serum and serum-free media. *Hybridoma* **9**: 167-175.
26. **Ratafia, M.** 1987. Mammalian cell culture: worldwide activities and markets. *Bio/Technology* **5**: 692-694.
27. **Ratafia, M.** 1989. Serum-free media markets. *Bio/Technology* **7**: 574-575.
28. **Renard, J.M., R. Spagnoli, C. Mazier, M.F. Salley, and E. Mandine.** 1988. Evidence that monoclonal antibody production kinetics is related to the integral of viable cells curve in batch systems. *Biotechnol. Lett.* **10**: 91-96.
29. **Savinell, J.M., G.M. Lee, and B.O. Palsson.** 1989. The effect of fatty acids on hybridoma growth, metabolism, and cell composition. Presented at the annual ACS meeting, Miami, FL.
30. **Savinell, J.M., G.M. Lee, and B.O. Palsson.** 1989. On the orders of magnitude of epigenic dynamics and monoclonal antibody production. *Bioprocess. Eng.* **4**: 231-234.
31. **Seaver, S.S.** 1987. Rapid high-performance liquid chromatography technique for monitoring amino acids in culture fluid. p. 315-318. *In* S.S. Seaver (ed.) *Commercial Production of Monoclonal Antibodies,* Marcel Dekker, Inc., New York.
32. **Sen, S., W.S. Hu, and F. Srienc.** 1990. Flow cytometric study of hybridoma cell culture: correlation between cell surface fluorescence and IgG production rate. *Enz. Micro. Technol.* **12**: 571-576.
33. **Shacter, E.** 1989. Serum-free media for bulk culture of hybridoma cells and the preparation of monoclonal antibodies. *Trends Biotechnol.* **7**: 248-253.
34. **Spier, R.** 1988. Animal cells in culture: moving into the exponential phase. *Trends Biotechnol.* **6**: 2-6.
35. **Tritsch, G.L. and G.E. Moore.** 1962. Spontaneous decomposition of glutamine in cell culture media. *Exp. Cell Research* **28**: 360-364.
36. **Velez, D., S. Reuvey, L. Miller, and J.D. Macmillan.** 1986. Kinetics of monoclonal antibody production in low serum growth media. *J. Immuno. Methods.* **86**: 45-52.
37. **Walker, G.A., W. Davison, and C.A. Lambe.** 1987. The mechanism of monoclonal antibody secretion by hybridomas. *Proc. 4th European Congress on Biotechnology* **3**: 587.
38. **Wilson, R. and R.E. Spier.** 1987. Biochemistry of hybridoma technology. *Develop. Biol. Standard.* **66**: 161-167.