

Serum-Free Media in Hybridoma Culture and Monoclonal Antibody Production

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Accepted for publication November 30, 1987

The replacement of serum in hybridoma cultures is considered. The focus is on the effects of serum-free media on hybridoma growth and monoclonal antibody secretion. Comparative literature data with serum supplemented cultures are discussed with an analysis of serum-free formulations and selection rules for the serum-free ingredients. In general, serum-free media which are "lipid rich" can sustain cell growth rates approaching that of serum supplemented cultures. Specific antibody secretion rate, however, is usually higher in serum-free media, irrespective of the lipid content.

INTRODUCTION

Serum is traditionally added to cell cultures to provide hormones, growth factors, binding and transport proteins, and other supplementary nutrients. However, serum compositions are subject to variation and are seldom well defined.

Proliferation and differentiation of lymphocytes and subsequent secretion of immunoglobulins are mediated by chemical signals which interact with cell surface receptors. Serum proteins can complicate the biology of these effector molecules. One class of these proteins, immunoglobulins,

may interfere with the reactions of monoclonal antibodies or elicit immunoresponses with *in vivo* applications. Although serum contains several hundred proteins, it is now apparent that surprisingly few supplements are necessary in a serum-free medium.^{1,2} Table I lists additional advantages and disadvantages of serum-free media. The protein concentration in serum is usually much higher than the concentration of secreted protein products. Serum proteins can easily mask the presence of secreted proteins of similar size which makes simple purification strategies such as gel permeation difficult. Thus, industrial downstream recovery and purification processes can be facilitated by the elimination of serum. With the removal of serum hormones and antibodies, well-defined culture conditions are possible for studies of cell physiology, cellular immunology, cell-cell interaction, and product formation kinetics. Utilization of serum-free media reduces significantly the risk of contamination. Hence, products harvested from serum-free culture are particularly attractive in many applications, especially *in vivo* human therapeutic protocols.

Reviews and monographs have been compiled on the subject of serum-free media.³⁻¹⁰ Nevertheless, most treatments

Table I. Advantages and disadvantages of serum-free media for hybridomas.

Advantages

1. Consistent and chemically defined composition.
2. Improves reproducibility of cell culture growth and product yield.
3. Decreases potential of contamination.
4. Reduces difficulty and cost of product purification.
5. May provide cost savings with a low protein serum-free formulation.

Disadvantages

1. Requires optimization for each hybridoma line.
2. Applicable serum-free media have not been developed for all cell lines.
3. May require serum hormones and growth factors which are difficult to isolate and purify.
4. Frequently results in longer lag periods.
5. Cell growth rate, maximum cell density and cell viability are often lower.
6. Protease inhibitors in serum may help protect cells from enzymes such as trypsin.

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are confined to a restricted number of cell lines of interest to individual investigators. The objective of this report is to provide a unified treatment of serum-free media in hybridoma culture and monoclonal antibody production. We need to address the following questions. Are there common features in media developed from different works? How general are the available results? How does the selection of media supplements affect cell propagation and antibody secretion? Problems inherent in the use of commercial serum-free formulations are also addressed.

In the following discussion, there are two points which we would like to clarify. First, most data reported in the literature were obtained in experiments performed in micro-wells, petri dishes or T-flasks. Few of these studies paid particular attention to the extraction of quantitative kinetic data. Despite the qualitative nature, we observed striking similarities in the pattern on the use of serum-free media.

Second, serum-free media are classified as high or low in lipid or fatty acid content. Previous development distinguished between low or high protein serum-free media. The major protein components in serum-free media are usually albumin, insulin, and transferrin. One motivation behind the development of low protein serum-free formulation is to simplify downstream purification. Nonetheless, cell proliferation is improved if an exogenous lipid source is present, due largely on the dependence of membrane and prostaglandin synthesis on phospholipids and fatty acids. Albumin functions mainly as a carrier for the membrane precursors, fatty acids and lipids. Since albumin is normally present in concentrations up to three orders of magnitude higher than other protein supplements, the distinction between low and high protein is really a reflection of the albumin content. Thus classification of serum-free media

on the basis of lipid or lipid precursor availability is more appropriate than the high or low protein classifications. For abbreviation, we will use the terms "lipid rich" and "lipid lean." A medium is considered lipid rich if there are components (crude albumin, delipidated albumin coupled to a fatty acid, cholesterol, lipoprotein, etc.), which could provide precursors for *de novo* synthesis of lipids. Thus, a medium is lipid lean if such additional lipid sources are lacking. We begin our discussion with a brief review of serum and common supplements used in serum-free media.

SERUM

Serum is a very complex mixture.¹¹ A simplified representation¹² of bovine serum is shown in Figure 1. The molecular weight is used as the basis for identifying the components. The actual quantities vary according to the lot and source, but the general pattern and relative quantities of most components, other than regulatory proteins, should remain the same. Table II is a summary of the components in different molecular weight fractions which are grouped as follows:

1. At the lower end of the molecular-weight scale (below 300 D) are the inorganic salts, small vitamins, essential and nonessential amino acids, and carbohydrates which altogether constitute numerically the majority of the components present. Variations in these components are not critical as they are mainly provided by the basal media.
2. The low molecular-weight fraction from 300 to 2000 D contains cofactors, vitamins, hormones, growth factors, and other small regulatory proteins, some of which may be very cell line specific or even cell cycle dependent.
3. The moderate molecular-weight components (2×10^3 – 5×10^4 D) contain larger hormones and growth factors. Many important regulatory molecules are in this fraction.

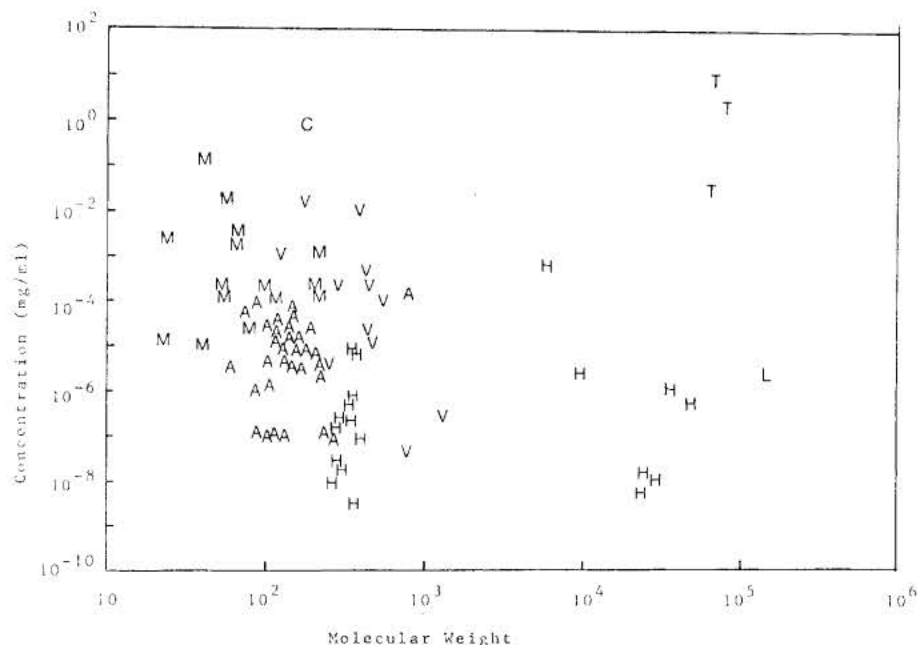


Figure 1. Concentration range of serum components in defined bovine serum (ref. 12): (M) metal cations, (C) glucose, (A) amino acids and derivatives, (H) hormones, (T) transport proteins, (V) vitamins, and (L) lactate dehydrogenase. Components with concentrations below 10^{-9} mg/mL have been omitted.

Table II. Molecular weight fractions of serum and their biological functions.

Components	Molecular weight range (D)	General biological function
Inorganic salts, Amino acids	0–300	essential and nonessential amino acids, nutrients, and cofactors
Amino acids, vitamins, carbon sources, hormones, growth factors	300–2000	nutrients, growth factors and hormones
Calcitonin, Insulin, Sex and growth hormones	2000–50000	regulation of carbohydrate, phosphate and calcium metabolism, regulation and stimulation of cell or tissue growth
Hemoglobin, albumin and transferrin	50000–100000	transport proteins for oxygen, fatty acids and iron
Serum immunoglobulins, lactate dehydrogenase	>100000	no useful purpose in hybridoma culture

4. While the majority of the components are in the low-molecular-weight range, transport proteins, albumin and transferrin, in the ($5\text{--}10 \times 10^4$ D) range account for the largest weight fraction in serum.

5. The high-molecular-weight fraction above 10^5 contains large macromolecules including the immunoglobulins and lactate dehydrogenase. These proteins are usually not useful to hybridoma culture. In fact, they may be deleterious to antibody production and should be removed.

Dialysis of serum has been attempted to better define the liquid media.^{13–15} In addition to dialysis, the use of serum fractions^{16,17} has been suggested as a replacement for complete serum in cell culture, although this would be only a partially defined medium. However, delineation of the biological functions of serum components on the basis of physical size is not necessarily a wise approach for two reasons: (1) the large number of proteins present in serum, and (2) the large variations in size for the different growth factors and hormones.

In general, with the exception of specific growth factors and hormones, most of the serum components are relatively well-defined. Only a few of the components are necessary for a given cell line. A comparatively simple formulation can be devised to supplement the basal medium in the development of a serum-free medium. Nevertheless, the process of optimizing a chemically defined medium for a cell line can be extremely labor intensive and time consuming.

SERUM-FREE MEDIA SUPPLEMENTS

In the mid-1950s, Eagle^{18,19} initiated the development of modern cell culture media. Ham²⁰ had developed a completely synthetic medium by 1965. These developments were continued in the laboratories of Ham²¹ and Sato.^{22–28}

The rationale behind Sato's initial development was that of an endocrinologist. He discovered that the addition of various hormones, growth and attachment factors, transport proteins and nutrients to a basal medium could promote cell growth and differentiation *in vitro*.²⁵

Thus, earlier developments made extensive use of hormone supplements such as epidermal growth factor, fibroblast growth factor, nerve growth factor, progesterone, prostaglandin and other steroid hormones.^{24,25} Attachment factors such as fibronectin or collagen were added to facilitate cell attachment and spreading of anchorage-dependent cells.²⁶ These additions were necessary in the control of clonal development from primary tissues. However, the use of hormones is prohibitively expensive in mass cell culture. More recently, the trend has moved toward formulations which are simple alterations of common basal media and have little, if any, hormonal additions.

The successful culture of hybridomas in serum-free medium was reported as early as 1978.²⁹ The state of development has reached the point where the fusion and selection of hybridomas have been conducted in serum-free media *in vitro*.^{9,27,30} Various supplemental components serve different biochemical functions. A brief introduction of the more important supplemental components follows.

Albumin and Lipids

Exogenous lipids or their precursors are required for the proliferation, differentiation, and antibody secretion of B-lymphocyte derivatives.^{31–33} Albumin serves as a carrier for essential fatty acids and some trace minerals. Albumin also appears to have other secondary functions; it may transport hormones and peptide growth factors, act as a detoxifying agent for H_2O_2 ³⁴ and excess trace elements,^{35–37} and provide cells with protection against mechanical shear dam-

age.⁴ The addition of albumin helped the proliferation and antibody secretion of lymphocytes and hybridomas.^{38,39} The albumin concentration in different serum-free media ranges from 0.5 to 5 mg/mL.

Two different forms of albumin have been used. Crude serum albumin extract is associated with fatty acids of undefined nature. More refined media use delipidated albumin coupled to either linoleic, oleic, or other fatty acids. However, such preparations are expensive and not feasible for large-scale monoclonal antibody productions. An increasing number of researchers have cultured cells in the absence of albumin.^{28,40} These results underscore the unique response of different cell and hybridoma lines.

Insulin

Insulin serves as a growth and maintenance factor⁴¹⁻⁴⁴ and is considered to be essential in the serum-free culture of hybridomas.^{45,46} The functions served by insulin include the stimulation of uridine and glucose uptake, and the syntheses of RNA, protein, and lipid.⁴⁴ Increased fatty acid and glycogen synthesis has been noted by many investigators.⁴⁷⁻⁴⁹ Insulin is included in most serum-free media at concentrations ranging 5–10 μ g/mL.

Transferrin

Transferrin is considered one of the essential growth promoting supplements in serum-free media for hybridomas^{27,50,51} and in some circumstances, its omission has led to severe inhibition of cell growth.^{46,52,53} Transferrin is an iron binding glycoprotein, and typical concentrations in serum-free media range 1–30 μ g/mL. Transferrin interacts with specific cell surface receptors and has an intimate role in the facilitation of iron transport across the plasma membrane.^{54,55} There may be additional *in vitro* functions such as the chelation of deleterious trace metals.^{4,37} The function provided by transferrin is unlikely to be replaced by other components.

Ethanolamine

Ethanolamine was first reported as the active component in peptone and a necessary component in the culture of rat mammary carcinomas.^{56,57} The exact function of ethanolamine in serum-free culture is not fully understood, but is likely associated with lipid synthesis. Ethanolamine was shown to be an essential growth factor for hybridomas in serum-free culture. A concentration of 10 μ M–20 μ M was found to be optimal and the stimulatory effect is saturated above 40 μ M.⁴⁶ Phosphoethanolamine could be a substitute but required a tenfold concentration increase.⁵²

Selenium

Selenium is a trace element essential or beneficial to mammalian cell cultures.^{58,59} The mechanism of cell growth enhancement by selenium is poorly understood. It has been

suggested that selenite is a cofactor for glutathione peroxidase and possesses H₂O₂-destroying ability.^{60,61} There was evidence that sodium selenite enhances the hybridoma growth rate in serum-free cultures.^{34,52,62} Nevertheless, not all results favored selenite; selenium may either be not necessary⁵³ or toxic.⁶³ Selenite concentration is commonly in the 10nM–60nM range.

β -Mercaptoethanol

The addition of thiols, which are easily oxidized and can prevent peroxide damage, have proven effective in hybridoma and lymphoma cultures.⁶⁴ The best growth supporters were β -thioglycerol and β -mercaptoethanol.⁹ β -mercaptoethanol is involved in the stimulation of cystine uptake,^{65,66} in restoring the reduced form of glutathione which in turn helps to prevent peroxide damage,^{67,68} and facilitates the transition from the G₁ to S phase in the cell cycle.⁶⁹ In the presence of serum, β -mercaptoethanol also works in conjunction with serum cofactors.^{66,70-72} A common concentration of β -mercaptoethanol is 10 μ M.

Additional Supplements

In addition, other components have been used in various serum-free formulations. In place of albumin, other supplements such as cholesterol,^{31,63} soybean phospholipids or low density lipoproteins,²⁸ and casein^{34,73} have been used. Other substances, including testosterone, catalase, dilinoleoyl phosphatidylcholine, dipalmitoyl lecithin, ascorbic acid, hydrocortisone, and manganese chloride have all been used in various capacities.^{34,46,50} Ferrous salts have also been used in lymphoid cultures, but there is no evidence that they actually can replace transferrin.^{74,75}

Protein-Free Media

The complete elimination of macromolecules appears attractive. However, the design of completely protein-free media is difficult and the scope of its application has been limited. Under most circumstances, its success depends heavily on the development of clonal derivatives with very low requirements for albumin, transferrin and insulin.

Hamilton and Ham⁷⁶ first demonstrated the growth of Chinese Hamster cell lines in a protein-free medium which was made up solely of amino acids, vitamins, organic compounds, and inorganic salts. More recently, protein-free media have been used for the culture of hepatoma,⁷⁷ normal human peripheral blood lymphocytes,⁷⁸ and murine hybridomas.⁷⁹ However, these media do make use of steroid hormones and lipid precursors. Protein-free media are not necessarily more cost effective than simple low protein (under 100 μ g/mL) serum-free media.

LYMPHOID CELL CULTURES

The development of serum-free media for hybridomas is closely related to that of lymphoid cell cultures. A brief

discussion of various serum-free lymphocyte derived cultures is warranted. Pokeweed mitogen (PWM), phytohemagglutinin (PHA), or bacterial lipopolysaccharide (LPS) are commonly used to stimulate the proliferation of normal human peripheral blood leukocytes (NPBL). Table III lists the compositions of serum-free media used in various lymphoid cell cultures. These media are relatively simple and, with few exceptions, can be designated as TABIE(S), ITES, or BITES, where T stands for transferrin, A for albumin, B for β -mercaptoethanol, I for insulin, E for ethanolamine, and S for selenium. The CITTL medium (Table III) is an exception. CITTL relies heavily on testosterone to enhance growth, and catalase is added to help reduce hydrogen peroxide buildup. Moreover, vitamin-free crude casein, a relatively undefined component, is used as a low-molecular-weight replacement for albumin.

Several investigators have studied the optimal concentra-

tions of various media components. These results are summarized in Table IV. There is generally a relatively flexible concentration range between saturation of the component and inhibition due to overdose. The compositions used in Table III mostly fall within the ranges in Table IV. The implication is that it is not too difficult to search for a near-optimal value for each component, and reduces the concern that a suboptimal serum-free medium may adversely affect the culture.

Conditioned medium, harvested from an exponentially growing lymphoma culture, has been used to enhance growth promoting properties.^{34,75} It was speculated that a factor in the 10^3 – 10^4 D molecular-weight range in the conditioned medium helped the clonal growth.⁷³ Such conditioned medium was also extracted from the MLA144 ape lymphoma to provide interleukin-2 for stimulating proliferation of normal human peripheral leukocytes.⁷⁵ However,

Table III. Composition of serum-free media for lymphocyte-derived cells.

Cell lines (Reference)	Basal medium	Albumin (mg/mL)	Ethanolamine (μ M)	Insulin (μ g/mL)	2-ME ^a (μ M)	Selenium (nM)	Transferrin (μ g/mL)	Additional supplements
<i>Lymphocyte cell lines</i>								
S49 (34)	DME:Ham's F12	0.0	0.0	15.0	1.0	20.0	1.5	casein, 0.5 mg/mL ^c testosterone, 2nM linoleic acid, 1 μ g/mL
	DME:Ham's F12	0.5	0.0	15.0	1.0	20.0	1.5	BSA coupled to linoleic acid
	DME:Ham's F12	0.0	0.0	15.0	1.0	20.0	1.5	catalase, 20 μ g/mL
	DME:Ham's F12	0.0	0.0	15.0	1.0	20.0	1.5	catalase, 20 μ g/mL dilinoleoyl phosphatidylcholine, 0.5 μ g/mL
	DME:Ham's F12	0.0	0.0	15.0	1.0	20.0	30.0	catalase 50 μ g/mL testosterone, 2nM dilinoleoyl phosphatidylcholine, 0.5 μ g/mL
SK-L7 (63)	Ham's F12	30.0	0.0	5.0	0.0	0.0	5.0	cholesterol, 25 μ g/mL
MLA144 (75)	IMDM or RPMI-1640	4.0	0.0	5.0	0.1	0.0	1.0	cholesterol, 20 μ g/mL soybean lecithin, 80 μ g/mL ferrous chloride, 7×10^{-11} M
S49, WEH17, BE5147, R1.1, EL4 (80)	DME:Ham's F12	0.0	0.0	5.8	0.0	0.0	1.0	casein, 750 μ g/mL testosterone, 1.7nM
MOLT-4f, CEM MOLT-3, HSB-2 JURKAT-77	IDMEM	0.4	0.0	0.0	0.0	0.0	1.0	
WIL-2, RAJI, BJAB (81)								
UC 729-6 (82)	RPMI-1640	0.5	10.0	10.0	10.0	0.0	5.0	
<i>Normal lymphocytes^b</i>								
NPBL (38)	IMDM:Ham's F-12	4.0	0.0	5.0	44.3	0.0	5.0	progesterone, 6.5 ng/mL
MNC (63)	RPMI-1640	10.0	0.0	5.0	0.0	0.0	5.0	
NPBL, B-Lymphocytes (74)	DMEM	5.0	0.0	10.0	0.0	23.1	5.0	ferrous sulfate, 0.8 μ g/mL
NPBL (78)	CFBI 1000	0.0	0.0	0.0	0.0	0.0	0.0	phytohemagglutinin, 2 μ g/mL Adenine, 1.35 μ g/mL Choline chloride, 14 μ g/mL Inositol, 18 μ g/mL
NPBL (83)	DME:Ham's F-12	0.0	20.0	5.0	0.0	2.5	35.0	
NPBL, LGL (84)	IMDM	4.0	0.0	5.0	0.1	0.0	1.0	interleukin-2 from conditioned medium of MLA 144 ferrous chloride, 7×10^{-11} M

^a β -mercaptoethanol. ^bNPBL: Normal Peripheral Blood Lymphocytes; MNC: Mononuclear Cells, LGL: Large Granular Lymphocytes; information on mitogens is in Table III. ^cDesignated CITTL (casein, insulin, testosterone, transferrin and linoleic acid).

Table IV. Summary of concentration effects in serum-free cultures.^a

Cell lines (Reference)	Albumin (mg/mL)	Ethanolamine (μ M)	Insulin (μ g/mL)	2-ME (μ M)	Selenium (nM)	Transferrin (μ g/mL)	Other components
<i>Lymphocyte cell lines</i>							
S49 T-lymphoma (34,80)	0.2–0.5	–	6–9	–	2–60	1.5–30	vitamin-free crude casein, 75–750 μ g/mL testosterone, 0.5nM–5nM linoleic acid, 0.01–1 μ g/mL
NPBL (83)	–	0.8–20	1–25	–	0.5–12.5	7–35	
MLA144 (75)	–	–	1–25	0.1–5	–	0.2–5	FAF-BSA, ^b 0.08–4 mg/mL cholesterol, 4–40 μ g/mL lecithin, 16–160 μ g/mL ferrous chloride, (1.4–350) $\times 10^{-11}$ M
NPBL (74)	–	–	–	–	–	1–100	PWM, ^d 0.5–50 μ g/mL
MOLT-4f (81)	–	–	–	–	–	0.1–1	
LPS B-lymphocytes ^c (37)	–	–	–	–	–	1–360	soybean lipid, 50–100 μ g/mL
<i>Hybridomas</i>							
MPC11-BL (52)	–	10–50	–	–	–	–	phosphoethanolamine, 100 μ M–500 μ M
PGC-02 (46)	–	20–40	–	–	30–60	–	hydrocortisone, 2–3 ng/mL
T3-03 (50)	–	–	–	–	–	0.5–20	
1116NS-19 (85)	–	–	5–50	–	–	1–10	

^aThe composition ranges in which the cell propagation is relatively insensitive to concentration changes in the component. The lower limit represents the condition when the effect of the component becomes saturated. The upper limit is chosen when the component appears to exert inhibitive effects. ^bFatty acid free bovine serum albumin. ^cLipopolysaccharide activated B Lymphocytes. ^dPokeweed mitogen.

since conditioned medium can no longer be identified as well-defined, it lacks the basic benefit associated with serum-free media.

Without more complete cell kinetics data, doubling time is used as the basis for growth rate comparison in cultures with and without serum (Table V). Figure 2(a) combines the information in Tables III and V, with the further classification of the serum-free media as either lipid lean or rich. It is apparent from Figure 2(a), that the doubling times in serum-free media compare reasonably well with those in serum supplemented media. In several cases, the doubling times of lipid rich media are actually lower than serum supplemented media.^{2,74,75} On the other hand, the values for lipid lean media are generally at least 25% higher than those with serum supplement, indicating a slower growth rate in these media. The only two points which deviate from the general trend are that from a long term fed batch culture.⁷⁵ With peripheral blood leukocytes, the propagation remains the same after 27 days in serum-free medium, while the doubling time of the control in serum supplemented medium increases to 18 days. Conversely, a similar experiment utilizing large granular lymphocytes had the reverse result; the growth rate diminishes drastically in serum-free medium after 26 days.

Serum-free media can also be used to maintain long-term cell cultures. The growth of a murine T-lymphoma S49 in the CITTL medium was maintained for 47 days. An ape lymphoma MLA144 was maintained for 34 days⁷⁵ and several T- and B-lymphoid cell lines were maintained up to 8 months.⁸¹ Resting human mononuclear lymphocytes were maintained for as much as 8 weeks.² The number of plaque-forming cells in hemolytic plaque assays in serum-free media is comparable or superior to that observed in

serum supplemented cultures.⁷⁴ Finally, the secretion of immunoglobulins is reported to be enhanced in serum-free media.^{38,74,87}

HYBRIDOMA CULTURES

The compositions of serum-free media successfully applied to hybridomas are not significantly different from that for lymphoid cell cultures (Table VI). A comparison of doubling time in serum supplemented and serum-free media for various hybridomas is given in Table VII. Analogous to Figure 2(a), a better perspective on the differences between lipid rich or lean media for hybridoma cultures is presented in Figure 2(b). There is no obvious difference in behavior between murine or human hybridomas. The ability of serum-free media formulations to sustain a comparable growth rate as compared to a serum supplemented medium is varied. In most cases, the doubling time is higher for growth in serum-free media.

Nevertheless, growth behavior of hybridomas in serum-free media at rates comparable to serum supplemented media can be attained.⁴⁶ For example, two human hybridomas, VLN3G2 and VLN1H12, had virtually identical growth curves with the serum-free and serum supplemented media.^{87,90} In a detailed study using lipid lean ITES medium, most murine hybridoma lines had higher doubling times than the serum supplemented culture.⁵² Only several murine hybridomas (AS100-2, ROB57.2.1 and ABM202.5) had lower doubling times in the ITES medium (Table VII). Also there are no apparent discernable differences on the choice of myeloma fusion partner.

As is the case with lymphoid cultures, hybridoma growth rates generally are comparable to those seen in serum sup-

Table V. Comparison of doubling times for lymphoid cells in serum supplemented and serum-free media.

Cell lines (Reference)	Doubling time (day)			Ratio of serum-free/serum
	Mitogen	Serum supplemented	Serum-free	
<i>Lymphocyte Cell Lines</i>				
S49 (34)		0.73	— ^a	—
		0.73	0.60 ^b	0.82
		0.73	2.18 ^c	2.97
		0.73	0.83 ^d	1.14
		0.73	0.82 ^e	1.11
		0.73	0.99 ^f	1.35
S49 (80)		0.71	1.45	2.04
SK-L7 (63)		1.25	1.61	1.29
MLA 144 (75)		1.07	1.11 ^g	1.04
		1.07	1.18 ^h	1.11
		1.08	1.33 ⁱ	1.23
		1.08	1.25 ^j	1.16
MOLT-4f (81)		1.49	1.81 ^k	1.22
NC-37		3.20	4.30 ^l	1.34
6410		3.00	5.00	1.67
Raji (86)		4.00	4.80	1.20
UC 729-6 (87)		1.06	1.09 ^m	1.03
<i>Normal lymphocytes</i>				
NPBL (83)	PHA	2.96	3.71	1.25
PBL ⁿ (84)	PHA	4.58	4.13 ^o	0.90
		18.30	4.51 ^p	0.25
LGL ⁿ (84)	PHA	5.67	5.86 ^q	1.03
		2.75	16.90 ^r	6.13
IgG-PFC (74)	PWM	1.83 ^s	1.14	0.62
		1.16 ^t	1.14	0.98
IgM-PFC (74)	PWM	3.58 ^s	1.18	0.33
		1.16 ^t	1.18	1.02
B-lymphocytes (37)	LPS	1.17 ^u	3.03	2.59

Notes: NPBL: Normal Peripheral Human Lymphocytes, PBL: Peripheral Blood Leukocytes, LGL: Large Granular Lymphocytes, PFC: IgG, IgM secreting Plaque Forming Cells. PHA: Phytohemagglutinin, PWM: Pokeweed mitogen, LPS: Lipopolysaccharide.

^aCITTL Medium. ^bITT (insulin, testosterone and transferrin) + Bovine Serum Albumin. ^cITT + Catalase + 2ME. ^dITT + Catalase. ^eITT + Dilinoleoyl Phosphatidylcholine. ^fRPMI, days 0–3. ^gIMDM, days 0–3. ^hRPMI, days 31–34. ⁱIMDM, days 31–34. ^jSerum supplemented is RPMI-1640 + 10% FCS, Serum-free in IDE + albumin + transferrin. ^kSerum supplemented is RPMI-1640 + 10% FCS, Serum-free in modified DME. ^lSerum supplemented is RPMI-1640 + 10% FCS, serum free is HB102 (Hana, Berkeley, CA). ^mBoth serum supplemented and serum-free are diluted with conditioned medium as a source of Interleukin-2. ⁿDays 0–6. ^oDays 20–27. ^pDays 0–12. ^qDays 19–26. ^rSerum supplemented Growth in RPMI-1640 + 10% FCS, with Pokeweed mitogen. ^sSame as p, without Pokeweed mitogen. ^tSerum and serum-free are based on MDEM.

plemented media when a lipid rich serum-free medium is used. Figure 2(b) indicates that hybridoma cultures are even more sensitive to the absence of lipid precursors. The doubling times of hybridomas increase much more drastically than lymphoid cultures in lipid lean media. The importance of lipid precursors is underscored by the protein-free media of Shive et al.⁷⁸ (Table III) and Cleveland and co-workers⁷⁹ (Table VI), both of which rely on alternate sources.

Irrespective of lipid conditions, the removal of transferrin or insulin results in much higher doubling times. For example, the hybridoma line N-146 has a doubling time of 11.3 h in newborn calf serum supplemented media and

11.4 h in Iscove's serum-free medium.⁹³ When amino acids, vitamins and transferrin are eliminated, the doubling time increases to 34.5 h. As one optimizes a serum-free medium, the doubling times in serum-free and serum supplemented media approach one another, indicating that sufficient growth supporting substances are being provided.

Without more complete data and analysis with kinetic models, it should be emphasized that our discussion is qualitative. Doubling time cannot be the sole index for cell kinetics. As an illustration, Baker and associates⁶² reported that doubling times were 20% higher for growth in serum-free media, although the maximum cell densities were comparable. On the contrary, Yen and Duigou,⁶³ culturing

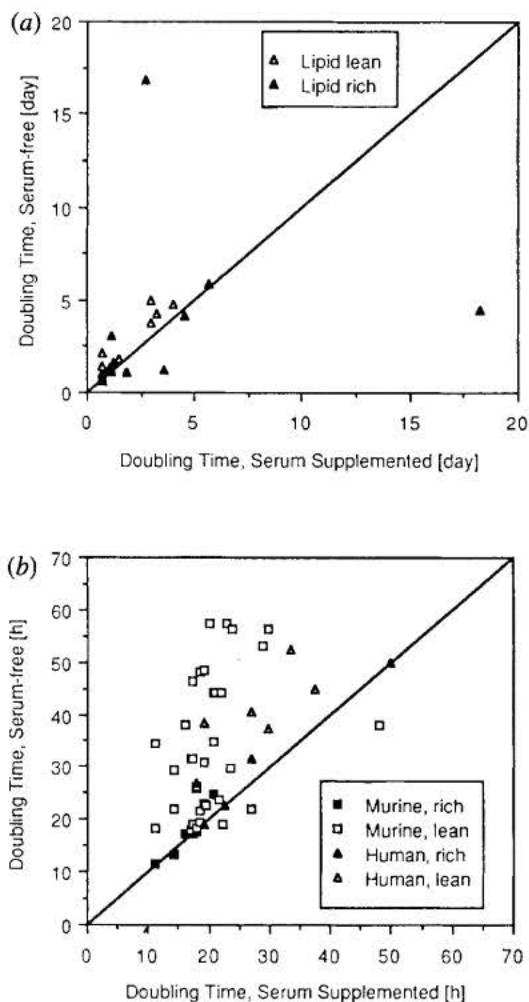


Figure 2. Comparison of doubling times in serum supplemented and serum-free cultures: (a) doubling time (days) of lymphoid cell cultures, (b) doubling time (hours) of murine and human hybridomas. Media are separated into lipid lean and lipid rich.

human lymphocytes, found that despite lower stationary phase cell densities in serum-free media, the doubling times were comparable.

Although most hybridoma cell lines have viabilities comparable to those seen in serum supplemented media, Cole and co-workers³⁹ found that their hybridomas had very low viability under reduced serum conditions. The viability was improved in a serum-free medium with 1% serum albumin and 10 $\mu\text{g/mL}$ transferrin added. However, the viable cell density remained 30% lower than that in a 10% serum supplemented medium after one week. The growth rates of two of the hybridomas in their study were similar to those seen in serum supplemented medium, while the other two were lower. Cole and co-workers used the same serum-free medium on all four hybridoma lines. Thus their result reaffirmed the fact that nutritional requirements can be very cell line specific.

In addition to slower growth, the necessary seeding density or inoculum level of serum-free cultures is usually higher than that in serum supplemented media.^{34,52,88} It is not uncommon for the required seeding density to be

10^5 cells/mL. Hybridomas do secrete non-immunoglobulin factors,⁹⁴ and these factors are speculated to have a growth stimulatory function. With a serum supplemented medium, it is probable that certain serum components provide the stimulatory activity necessary for growth. However, since these factors are not present in a serum-free formulation, promotion of cell growth likely depends on self-secreted autocrine factors and the accumulation of these factors only becomes effective at a fairly high cell density. We feel that this subject is not well understood and further investigation is necessary.

Serum-free media have also been used in the hybridization and isolation of hybridomas.^{9,27,62} Kawamoto et al.²⁷ demonstrated that fusion with NS-1-503, a myeloma clonal derivative of NS-1 which was adapted to a serum-free medium, provided 7.5 times more hybridoma secretors than fusion with normal NS-1 cells. The fusion with NS-1-503 also gave rise to hybridomas which were more adaptable to serum-free media without lipid additions.

SECRETION OF IMMUNOGLOBULINS

Monoclonal antibody production at high titers is a major goal of hybridoma technology. The most important factor in determining the utility of serum-free media is whether immunoglobulin secretion is impaired by the removal of serum components. Other key features are the secretion patterns, the stability of immunoglobulin secretion and the specificity of the hybridoma cell line requirements under serum-free conditions.

As opposed to what is presented for cell growth, less comparative immunoglobulin secretion data are available. Where results are provided, the accumulation of immunoglobulin in the culture supernatant is often higher in a serum supplemented culture. However, under most circumstances, hybridomas have a higher specific growth rate in a serum supplemented medium. Therefore, a more appropriate basis to assess the ability of hybridomas to secrete monoclonal antibody is the specific secretion rate (Table VIII). Figure 3 further illustrates the difference between lipid rich and lean serum-free media. There are no apparent differences in the secretion of IgG or IgM using various serum-free media.

The specific secretion of antibodies in serum-free media is higher than that in serum supplemented media in most cases. It is apparent that, on average, cultures in lipid lean media have higher Ig secretion rate than lipid rich media. Since doubling times are higher in lipid lean media, Ig secretion appears to be favored by slower growing hybridomas. A quantitative hemolytic plaque assay of a mouse hybridoma showed that the distribution of antibody secreting cells in a serum-free medium is the same as that in a fetal calf serum supplemented medium.⁴⁰ Thus hybridomas are more efficient in antibody production under serum-free conditions. The lower product titer is a consequence of the slower cell growth in serum-free media and certainly not an indication that secretory ability is impaired upon removal of serum.

Table VI. Composition of serum-free media for hybridomas.

Cell Lines (Reference)	Basal medium	Albumin (mg/mL)	Ethanolamine (μ M)	Insulin (μ g/mL)	2-ME ^a (μ M)	Selenium (nM)	Transferrin (μ g/mL)	Additional supplements
B5C, A1H, XY134-1, XY134-2 (39)	RPMI-1640	5.0	0.0	0.0	0.0	0.0	10.0	
SPS.6, RDP 45/20, MPC11-BL ^b MCB1, MCB4, MCB6, MCB17 MCB18, MB4, MB8, AS100-2 N-S.1, N-S.3, 53-7.313 T11D7E2, ROB57.2.1, Abm202.5 (28, 52)	1:1 (v/v) Ham's F-12:DMEM	0.0	20.0	5.0	0.0	2.5	35.0	
PGG-0, PGG-05, HE-24 T3-03, PTF-02 (46)	RPMI-1640 ^d	1.0	20.0	10.0	0.0	45.0	5.0	linoleic acid, 5 μ g/mL coupled to FAF-BSA ^c ascorbic acid, 3 μ g/mL hydrocortisone, 2 ng/mL other trace elements
81M, ARB, 8A1, SP2, 53-7313 OB-298, M1/9.3.4.HL-2 M1/70.15.1 (2, 73)	1:1 (v/v) DME:Ham's F-12	0.0	0.0	15.0	0.0	0.0	1.5	vitamin-free casein, 0.5 mg/mL testosterone, 2nM linoleic acid, 1 μ g/mL
M1/70.15.1, ARB, 8A1 S49 ^e (34)	1:1 (v/v) DME:Ham's F-12	0.0	0.0	15.0	0.0	0.0	1.5	Casein, 0.5 mg/mL ^f Linoleic acid, 1 μ g/mL Catalase 50 μ g/mL Testosterone, 2nM
1116NS-19, 1116NS-33a 1116NS-52a, 1083-17-1A (85)	MEM ^d or RPMI-1640 ^d	0.0	0.0	5.0	0.0	0.0	5.0	1% nonessential amino acids
SS1.1, NS6.3, 455 (40, 88, 89)	DME ^d	0.0	20.0	10.0	50.0	1.0	10.0	
VLN3G2.1, VLN1H12 (87, 90)	RPMI-1640 ^d	0.5	10.0	10.0	10.0	0.0	10.0	
CLNH5, CLNH11 (91)	RDF ^{g,h}	1.0	1.0	10.0	1.0	1.0	10.0	
H-9, H-S60, HB4C5, HB4G7 HF10B4, HF10F7 (92)	RDF ^g	0.0	20.0	5.0	0.0	2.5	35.0	egg yolk lipoprotein, 5 μ g/mL
NS-1, NS-1-503 ⁱ (27)	RDF ^g	0.0	10.0	10.0	10.0	1.0	10.0	low density lipoprotein, 1 μ g/mL oleic acid, 4 μ g/mL coupled to FAF-BSA
N-146, N-266, N-266, N-266 S-6, S-84, 1M/41, C2-23 R33-18-12, R33-24-12, R33-60 (93)	DMEM ^d	1.1	0.0	0.0	4.4	1.6	31.0	soybean lipids, 22.6 μ g/mL cholesterol, 1.6 μ g/mL additional amino acids, vitamins
53-7.313, 53-6.72, Hy1 M1/70.15.11, M1/75.16.4 M1/69.16.11, HO13-4-9 331.12, 2b-187.1 (79)	1:1 (v/v) Ham's F12:IMDM	0.0	0.0	0.0	0.0	0.0	0.0	α -thioglycerol, 8 ng/mL Progesterone, 0.01 μ g/mL Other trace elements

^a β -Mercaptoethanol. ^bThe medium for MPC11-BL was supplemented with 0.5 μ g/mL soybean phospholipids. ^cFatty acid-free bovine serum albumin (FAF-BSA). ^dBasal medium supplemented with glutamine. ^eS49 is a murine T lymphoma. ^fThe transferrin concentration need be 30 μ g/mL in casein-free media. Also used dilinoleoyl phosphatidylcholine (DiLinPC) 0.5 μ g/mL. ^gRDF is a 2:1:1 mixture of RPMI-1640, DME and Ham's F-12. ^hThe RDF is enhanced by unspecified amounts of amino acids, vitamins, glucose and NaCl. ⁱThe hybridomas derived from these myelomas are not identified explicitly.

Table VII. Comparison of doubling times for murine and human hybridomas in serum supplemented and serum-free media.

Cell lines (Reference)	Fusion partner	Doubling time (h)		Ratio of serum-free/serum
		Serum supplemented	Serum-free	
<i>Murine hybridomas</i>				
MPC11-BL ^a	MPC11	18.7	21.4	1.14
RDP 45/20	MPC11	17.4	18.9	1.09
SPS-6	SP2/0	21.6	23.7	1.10
AS100-2	SP2/0	48.0	38.0	0.79
MCB 1	MPC11	19.6	22.6	1.16
MCB 4	MPC11	20.7	44.2	2.14
MCB 6	MPC11	18.6	19.4	1.04
MCB 17	MPC11	28.9	53.1	1.84
MCB 18	MPC11	21.2	44.2	2.08
MB4	MPC11	23.5	29.9	1.27
MB8	MPC11	29.6	56.5	1.91
N-S.1	NS-1	18.0	25.8	1.44
N-S.3	NS-1	18.6	48.0	2.57
53-7.313	NS-1	24.0	56.5	2.35
T11D7E2	NS-1	22.0	44.2	2.01
ROB57.2.1	S194	26.8	22.0	0.82
ABM202.5 (52)	S194	22.3	19.0	0.85

Table VII. (Continued).

Cell lines (Reference)	Fusion partner	Doubling time (h)		Ratio of serum-free/serum
		Serum supplemented	Serum-free	
MPC11-BL (28)	MPC11	19.3 ^b	22.8 ^c	1.18
		19.3 ^b	31.0 ^d	1.61
		19.3 ^b	48.3 ^c	2.50
N-146	F0	11.3 ^f	11.4 ^g	1.01
		11.3 ^f	18.2 ^h	1.61
		11.3 ^f	34.5 ⁱ	3.05
N-226	F0	20.6 ^f	24.9 ^g	1.21
		20.6 ^f	34.7 ^h	1.68
		20.6 ^f	>60.0 ⁱ	>2.91
N-527	F0	17.5 ^f	17.1 ^g	0.98
		17.5 ^f	31.5 ^h	1.80
		17.5 ^f	46.5 ⁱ	2.66
S-6	F0	16.0 ^f	17.1 ^g	1.07
		16.0 ^f	38.1 ^h	2.38
		16.0 ^f	>60.0 ⁱ	>3.75
R33-18-12	NS-1	14.3 ^f	13.4 ^g	0.94
		14.3 ^f	21.9 ^h	1.53
		14.3 ^f	29.3 ⁱ	2.05
1M/41	SP2/0	17.1 ^f	17.5 ^g	1.02
		17.1 ^f	18.0 ^h	1.05
		17.1 ^f	31.7 ⁱ	1.85
C2-23 (93)	SP2/0	18.0 ^f	17.6 ^g	0.98
		18.0 ^f	18.3 ^h	1.02
		18.0 ^f	26.1 ⁱ	1.45
SS1.1	SP2/0	23.0 ^j	57.4 ^k	2.50
NS6.3 (40)	NS-1	32.0	>60.0	>1.88
455 (89)	NS-1	20.0 ^j	57.5 ^k	2.88
<i>Human hybridomas</i>				
VLN3G2 (82)	UC 729-6	22.6 ^l	22.6 ^m	1.0
		22.6 ^l	22.6 ⁿ	1.0
VLN1H12 (87)	UC 729-6	49.8	49.8 ^m	1.0
		49.8	49.8 ⁿ	1.0
XY134-1	EBV-transformed ^o	37.3 ^p	44.8 ^q	1.20
XY134-2	EBV-transformed ^o	26.1	>60.0	>2.30
AIH	KR-4	33.6 ^p	52.3 ^q	1.56
B5C (39)	KR-4	29.9	37.3	1.25
CLNH11 (91)	UC 729-6	18.00 ^r	>60.0 ^r	>3.33
		18.00 ^r	27.0 ⁱ	1.50
HB4C5	NAT-30	19.2 ^u	19.2 ^v	1.0
		19.2 ^u	38.4 ^w	2.0
H-9 (92)	NAT-30	27.0 ^u	31.5 ^v	1.17
		27.0 ^u	40.5 ^w	1.50

^aSerum supplemented medium is 1:1 Ham's F-12 and DME with 2.5% fetal calf serum and 5% horse serum, the serum free medium is 1:1 Ham's F-12 and DME + ITES. ^b1:1 Ham's F-12 and DME + 2.5% fetal calf and 5% horse serum. ^c1:1 Ham's F-12 and DME + ITES + 0.5 µg/mL soybean phospholipids. ^d1:1 Ham's F-12 and DME + ITES + 0.5 µg/mL egg yolk phospholipids. ^e1:1 Ham's F-12 and DME + ITES. ^fIscove's serum-free medium + 10% newborn calf serum. ^gIscove's serum-free medium, enriched TABIES. ^hIscove's serum-free medium without amino acids and vitamins. ⁱIscove's serum-free medium without amino acids, vitamins and transferrin. When the doubling time becomes so large that the feasibility of the medium is questioned, the doubling time is designated >60 h. ^jDME + 10% fetal calf serum. ^kDME + BITES. ^lRPMI-1640 + 10% fetal calf serum. ^mRPMI-1640 + TABIE. ⁿRPMI-1640 + HB102. ^oHeterohybridoma of EBV transformed lymphocyte fused with KR-4. ^pRPMI-1640 + 10% fetal bovine serum. ^qRPMI-1640 + albumin and transferrin. ^rRDF + 10% fetal calf serum. ^sRDF without supplements. ^tRDF + TABIES. ^uEnhanced RDF + 10% fetal calf serum. ^vEnhanced RDF + ITES + lipoprotein. ^wEnhanced RDF + ITES.

Table VIII. Comparison of antibody secretion in serum supplemented and serum-free media.

Cell lines (Reference)	Ig Class	Antibody secretion ($\mu\text{g}/10^6 \text{ cell} \cdot \text{day}$)		Ratio of serum-free/serum
		Serum supplemented media	Serum-free media	
<i>Murine hybridomas</i>				
NS6.3	IgM κ	8.64 ^a	5.04 ^b	0.6
		8.64 ^a	22.1 ^c	2.6
		4.56 ^d	5.04	1.1
		4.56 ^d	22.1	4.8
		4.08 ^e	5.04	1.2
		4.08 ^e	22.1	5.4
SS1.1 (40)	IgG _{2a} κ	1.68 ^a	3.6 ^b	2.1
		1.92 ^d	3.6 ^b	1.9
455 (89)	IgG _{2a} κ	0.36 ^e	0.48 ^b	1.3
NS-1	— ^f	8.3 ^g	10.0 ^b	1.2
SP2/0 (62)	—	6.7	8.1	1.2
<i>Human hybridomas</i>				
CLNH11 (91)	IgG	0.1 ⁱ	0.27 ^j	2.7
		0.1 ⁱ	0.33 ^k	3.3
		0.1 ⁱ	0.83 ^l	8.3
AIH	IgM	1.34 ^m	2.03 ⁿ	1.5
B5C	IgM	2.36	3.10	1.3
XY134-1	IgM	9.65	9.08	0.9
XY134-2 (39)	IgM	1.9	12.7	6.7
VLN3G2 (82,87)	IgG ₁ κ	0.67 ^o	1.5 ^p	2.2
		0.67 ^o	1.83 ^q	2.7
VLN1H12 (87)	IgM κ	0.1 ^g	0.52 ^p	5.2
		0.1 ^g	0.8 ^q	8.0

^aDME + 10% fetal calf serum. ^bDME + BITES. ^cSame as b without ethanolamine.

^dDME + 10% newborn calf serum. ^eDME + 10% horse serum. ^fUnspecified hybridomas and Ig derived from fusion partners NS-1 and SP2/0. ^gClick's DMEM + 12% fetal bovine serum.

^hKC2000 (K. C. Biological, Lenexa, Kansas). ⁱRDF + 10% fetal calf serum. ^jRDF + TABIES.

^kRDF + BITES. ^lRDF without other supplements. ^mRPMI 1640 + 10% fetal bovine serum.

ⁿRPMI 1640 + albumin and transferrin. ^oRPMI 1640 + 10% fetal calf serum. ^pRPMI 1640 +

TABIE. ^qRPMI 1640 + HB102 (Hana, Berkeley, CA).

The underlying reason for the higher antibody secretion rate in a serum-free medium is not yet fully understood. Even though antibody is not a primary metabolite,⁹⁴ manu-

facturing a large protein molecule does require energy. Therefore, hybridomas cycling at a lower rate is favored in the production of monoclonal antibodies. It has also been postulated that there may be factors present in serum that could be inhibitory to antibody secretion.⁸⁷ These factors, however, are conjectural and subject to further investigations.

The glucose consumption rate was measured in three different murine hybridoma lines.⁴⁰ It was shown that the specific glucose consumption rate did not have any direct correlations with the antibody secretion rate. The secretion of immunoglobulin is more likely a result of secondary cellular metabolism and not closely coupled to energy metabolic cycles. As long as the hybridomas remain viable the secretion level would remain high.

While the number of studies is limited, there are fair indications that hybridomas which have been adapted to serum-free media can remain productive for long periods. IgG secretion of a murine hybridoma M1/70.15.1 was maintained in a protein-free medium for 22 weeks.⁷⁹ A fed-batch culture of an IgM secreting murine hybridoma, NS6.3, was maintained for 40 days.⁸⁸ The productivity of the fed-batch culture over this period was comparable with that of a serum supplemented culture.

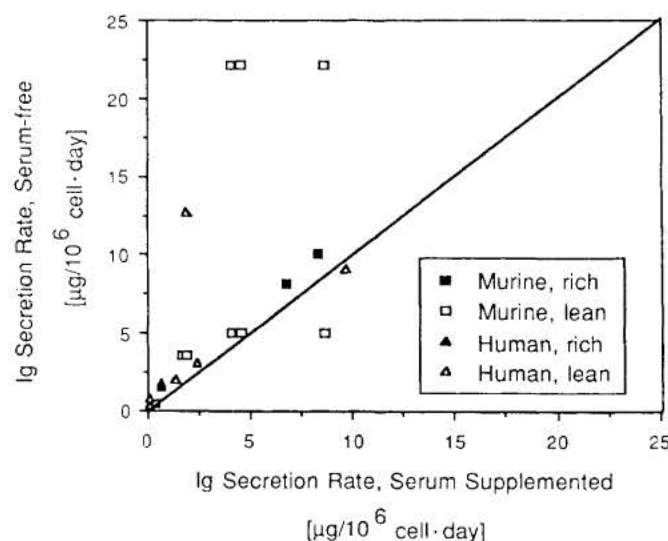


Figure 3. Comparison of specific secretion rate in serum supplemented and serum-free cultures of hybridomas.

It is clear from these results that a high antibody secretion rate can be maintained in a suitably designed serum-free medium. The fact that low lipid level serum-free media often are not as appropriate for cell growth suggests the need to compromise between cell growth and antibody production rate. An attractive approach is the separation of the culture into growth and maintenance (or antibody production) phases. The growth phase would use lipid-rich, serum-free media. The culture could then be shifted to a low lipid maintenance medium in the antibody production phase. The purity of secreted antibody in a serum-free culture is very high as shown by electrophoresis of the supernatants.⁸⁷ The removal of serum from the production processes for monoclonal antibodies would, therefore, facilitate downstream purification operations while eliminating interfering foreign antibodies.

COMMERCIAL SERUM-FREE MEDIA

The demand for more consistent media has led to the establishment of several commercial ventures for the production of serum-free media.^{62,95} Unfortunately, for proprietary reasons, the exact compositions of commercial media are rarely revealed. Based on information released in advertising materials, however, these commercial formulations do not deviate drastically from common serum-free media developed in academic research. These products contain such supplements as albumin or other lipid sources, transferrin, insulin, vitamins, amino acids and other relatively small organic molecules.

Thus, in controlled experiments on cellular physiology or immunology, the use of commercial media defeats the purpose of using a chemically defined medium. These commercial formulations often are optimized for a given list of cell lines tested. However, with each new cell line, any given serum-free medium would not be guaranteed optimal for either cell growth or antibody production.

Despite the current shroud of secrecy, commercial serum-free media will play a definite role in future monoclonal antibody production. The key advantage is that a commercial medium can eliminate the time consuming process of serum-free medium development and preparation. Analogous to basal media, the compositions of serum-free supplements will likely be revealed as the ideas behind designing serum-free media become better known. Furthermore, for optimization studies or more specialized applications, a development contract may be established between the serum-free medium vendor and the antibody production facility.

SELECTION RULES

We propose the following heuristic rules for use in the selection of a hybridoma serum-free medium. While medium optimization is extremely time consuming, one can construct a near-optimal medium relatively easily:

1. Choose a basal medium rich in amino acids and lipid precursors.

2. Add transferrin, insulin, and ethanolamine.
3. Add Selenite and β -mercaptoethanol.
4. For a maintenance or antibody production medium, avoid the use of an exogenous lipid source beyond those contained in basal medium.
5. For a growth medium, an exogenous lipid source is important. Choose either:
 - a. crude albumin;
 - b. fatty-acid-free albumin coupled to a specific fatty acid; and
 - c. an alternate lipid source.

Without serum, it is important to use a basal medium which is rich in amino acids and lipid precursors such as choline or inositol. (Rule No. 1) As is the case with serum supplemented culture, glutamine remains an important Krebs cycle "primer." An adequate concentration of glutamine should be added according to nominal practice. Common basal media are mixtures of DME and Ham's F12, Iscove's modified DME, and RPMI-1640 (Tables III and VI). Cultures not using such basal media should first be adapted to the new basal medium in a serum supplemented culture before being shifted to entirely serum-free conditions.

The presence of transferrin, insulin and ethanolamine is shown to be important in the majority of cases. (Rule No. 2) Thus these components are grouped together. Two other fairly common, but optional, supplements are selenium and β -mercaptoethanol. (Rule No. 3).

There are several choices for a lipid source. Without any exogenous sources beyond the basal medium and ethanolamine (Rule No. 4), the lean lipid medium is suitable for maintenance or antibody production. To enhance cell growth, one can use crude albumin (Rule No. 5a) in which the exact nature of bound fatty acids is not known. For precise experiments in which cell proliferation is important, delipidated albumin coupled to a fatty acid such as linoleic acid (Rule No. 5b) should be used. An alternate route is to use other lipid precursors such as lipoprotein or cholesterol. (Rule No. 5c) Both Rules No. 5b and 5c would incur much higher costs than the first two choices (Rules No. 4 and 5a) and are not feasible for mass hybridoma culture. Appropriate concentration levels for each of these components can be extracted from Tables III, IV, and VI.

CONCLUSIONS

Despite significant differences in the behavior of various hybridoma lines, all serum-free media share a common theme: an appropriate basal medium with the addition of transferrin, insulin, selenium, β -mercaptoethanol, ethanolamine, with or without albumin or exogenous lipid sources. Lipid rich media allow growth rates comparable to that of serum supplemented cultures, while low lipid media are more suitable for maintenance and antibody production. As for the monoclonal antibody secretion, the data are overwhelmingly in favor of serum-free media.

This project was supported by Grants from the National Science Foundation and the University of California Cancer Research Coordinating Committee.

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