Animal Cell Technology
'Developments Towards the 21st Century'
September 12-16, 1994

CONFERENCE PROGRAM

MONDAY, SEPTEMBER 12, 1994

18.00 - 18.10 h **Conference Opening and Welcome**
Coen Beuvery
National Institute of Public Health and
Environmental Protection (RIVM), Bilthoven,
The Netherlands

18.10 - 19.00 h **Opening Lecture**

Are We Prepared for Animal Cell Technology in the 21th Century? (0.1)

Charles Cooney
Massachusetts Institute of Technology, USA
(invited speaker, sponsored by JRH Biosciences Inc)

19.00 - 20.00 h Welcome Reception

20.00 h Dinner

TUESDAY, SEPTEMBER 13, 1994

08.15 h I **ESTABLISHMENT OF PRODUCTION CELL LINES; CLONING AND SELECTION STRATEGIES**

08.15 - 09.55 h **Chinese Hamster Ovary (CHO) Cells Proliferate Rapidly in Suspension in Protein-Free Medium Following Transfection with a Cyclin E Expression Vector (1.1)**
James Bailey
ETH, Switzerland
(invited speaker)

Controlled Proliferation of Mammalian Cell Lines (1.2)
Hansjörg Hauser
GBF, Germany
(invited speaker)

Oncogene Activated Production System to Enhance Cellular Recombinant Protein Productivity (1.3)
Sanetaka Shirahata
Kyushu University, Japan

Transient Expression of Recombinant Proteins at the Level of Productive Stable Cell Lines - Optimizing DNA Transfer and Expression in Mammalian Cells (1.4)
Martin Jordan
Genentech Inc., USA

09.55 - 10.25 h Coffee Break

VII **NEW DEVELOPMENTS: CLINICAL STUDIES**

10.25 - 11.25 h **Monitoring of Cytokine and Monoclonal Antibody Therapy (7.1)**
Erik Hack
CLB, The Netherlands
(invited speaker)

MDR Bone Marrow Gene Therapy (7.2)
Arthur Bank
Columbia University, USA
(invited speaker,
sponsored by Microbiological Associates Ltd)

TUESDAY, SEPTEMBER 13, 1994 (continued)

11.25 h II	**CONDITIONS FOR HIGH PERFORMANCE CULTURES FOR THE PRODUCTION OF BIOLOGICALS**
11.25 - 12.35 h	**Effects of Ammonia Removal on Cell Growth and MAb Production (2.1)** Masatoshi Matsumura University of Tsukuba, Japan *(invited speaker)*
	Cell Cycle Phase Analysis in Glucose-Limited Continuous Cultivation for the Effective Production of Antibody (2.2) Takeshi Omasa Osaka University, Japan
	Kinetic Analysis of the "Grow or Die" Cell Cycle System in Perfusion Culture of Hybridoma Cells (2.3) Bernard Massie Institut de Recherche en Biotechnologie, Canada
12.35 - 14.00 h	Lunch
14.00 - 15.20 h	**Continuous Fermentation Consumption Kinetics for Basal Media Design-(2.4)** Carlos Figueroa Miles Biotechnology, USA
	Productivity Enhancement Using Liquid Medium Concentrates (2.5) David W. Jayme Life Technologies, Inc., USA
	Culture Conditions for High Performance Production of Monoclonal Antibody (2.6) Yoshihito Shirai Kyushu Institute of Technology, Japan
	Comparison of Nutrient Requirements of Mammalian Cell Lines (2.7) Heino Büntemeyer University of Bielefeld, Germany
15.30 - 17.00 h	**Poster Session A**
17.00 - 18.30 h	Dinner
18.30 h	Departure for Museum

WEDNESDAY, SEPTEMBER 14, 1994

08.15 h **III** **PROTEIN PROCESSING**
(session sponsored by Genentech Inc)

08.15 - 10.15 h **Protein Folding in the Secretory Pathway of Animal Cells (3.1)**
Robert Freedman
University of Kent, England
(invited speaker)

Erythropoietin Processing in Erythropoietic System and Nervous System (3.2)
Ryuzo Sasaki
Kyoto University, Japan
(invited speaker)

Effects of Lipids on Recombinant Interferon-gamma Glycosylation, and Improvements in Product Monitoring (3.3)
Nigel Jenkins
University of Kent, England

Glycosylation of Recombinant Therapeutic Proteins (3.4)
Raj B. Parekh
Oxford GlycoSystems Ltd., England

Effects of Ammonia and Glucosamine on the Glycosylation Pattern of Recombinant Proteins Expressed from BHK-21 Cells (3.5)
Martin Gawlitzek
GBF, Germany

10.15 - 10.45 h Coffee Break

10.45 h **IV** **PROTEIN (MONOCLONAL ANTIBODY) ENGINEERING**

10.45 - 12.15 h **Functional Display of Proteins, Mutant Proteins, Fragments of Proteins and Peptides on the Surface of Filamentous (Bacterio)Phages (4.1)**
Hans Pannekoek
University of Amsterdam, The Netherlands
(invited speaker)

PCR Amplification of the Variable Regions of Immunoglobulin Heavy and Light Chain Genes on Genomic DNA from Mouse Hybridoma Cells (4.2)
Jose Berdoz
Swiss Institute for Experimental Cancer Research, Switzerland

B Cell Activation and Differential Immunoglobulin Isotype Switch Induced by Plasma Membranes of Activated Helper T Cells (4.3)
Akio Ametani
University of Tokyo, Japan

	Construction and Expression of Chimeric Protein A-Marine Firefly Luciferase in Mammalian Cells (4.4) Eiji Suzuki University of Tokyo, Japan
12.15 - 13.45 h	Lunch
12.30 - 13.30 h	General Assembly of ESACT Members (including lunch)
13.45 h **V**	**REGULATORY ISSUES, SAFETY AND CONTAINMENT**
13.45 - 15.45 h	**Acceptability of Continuous Cell Lines for the Production of Biologicals (5.1)** Johannes Löwer Paul Ehrlich Institute, Germany *(invited speaker)*
	Multi-Use Biopharmaceutical Manufacturing Facilities (5.2) Mic N. Hamers Chiron BV, The Netherlands *(invited speaker)*
	Facility Design and Validation Considerations for Continuous Cell Culture Processes (5.3) Bernard E. Horwath Cellex Biosciences, Inc., USA
	Validation Strategy for Viral Clearance (5.4) Paula Shadle SmithKline Beecham Pharmaceuticals, USA
	Removal of the Prion Protein Using Validatable Filter (BMM^{R}) (5.5) Jun Tateishi Kyushu University, Japan
16.00 - 17.00 h	Japanese Tea Ceremony
17.00 - 19.00 h	Dinner
19.00 - 20.00 h	*HyClone Lecture* **The Acceptability of Continuous Cell Lines: an Historical Perspective (0.2)** John C. Petricciani Genetics Institute, USA *(invited speaker)*
20.00 h	Drinks Offered by Trade Exhibitors

THURSDAY, SEPTEMBER 15, 1994

08.30 h VI **ANIMAL-CELL BIOREACTORS: DESIGN AND SCALE-UP CONSIDERATIONS**

08.30 - 10.00 h **Oxygen Gradients in Small & Big Animal-Cell Bioreactors (6.1)**
Hans Tramper
Wageningen Agricultural University, The Netherlands
(invited speaker)

Bubble Bed Aeration for Animal Cell Cultures (6.2)
Martin Trocha
ETH, Switzerland

Finite Element Analysis of 3-D Flow in an Agitated Bioreactor (6.3)
Kazumori Funatsu
Kyushu University, Japan

Optimal Operation of Forced-Flow Membrane Bioreactors for High-Density Mammalian Cell Cultures (6.4)
Jean-Marc Engasser
Institut National Polytechnique de Lorraine, France

10.00 - 10.30 h Coffee Break

10.30 - 11.30 h **Immobilization of Anchorage-Independent Cells to Porous Glass Beads: Mass Transfer Phenomena in Long-Term Packed Bed Reactor (6.5)**
Lidia M.D. Gonçalves
Instituto de Biologia Experimental e Tecnológica (IBET), Portugal

Model-Based Monitoring and Control of a Monoclonal Antibody Production Process (6.6)
Richard K. Biener
Institut für Systemdynamik und Regelungstechnik, Germany

Evaluation of Process Strategies for Efficient Cultivation of Hybridoma Cells Based on Mathematical Models (6.7)
Ralf Pörtner
TU Hamburg-Harburg, Germany

11.30 - 12.00 h *Toon van Wezel Memorial Lecture*

Microcarrier Technology, present status and perspective (0.3)
Tiny van der Velden-de Groot
RIVM, The Netherlands
(sponsored by Pharmacia)

12.00 - 13.30 h Lunch

13.30 - 15.00 h **Poster Session B**

15.00 h Departure for Social Program

FRIDAY, SEPTEMBER 16, 1994

08.30 h VII **NEW DEVELOPMENTS: TISSUE ENGINEERING, APOPTOSIS AND NOVEL APPLICATIONS**

08.30 - 10.10 h **Hematopoietic Tissue Engineering (7.3)**
Bernhard O. Palsson
University of Michigan, USA
(invited speaker)

***Ex Vivo* Expansion of Primitive Hematopoietic Cells under Perfusion Conditions for Cellular Therapies (7.4)**
Terry Papoutsakis
Northwestern University, USA
(invited speaker)

Rapid and Large-Scale Preparation of Porcine Hepatocyte Spheroids and Their Functions in Continuous Suspension Culture (7.5)
Yasuyuki Sakai
University of Tokyo, Japan

Analysis of Physiological Functions of Food Using Mammalian Cell Culture (7.6)
Hiroshi Shinmoto
National Food Research Institute, Japan

10.10 - 10.40 h Coffee Break

10.40 - 12.00 h **Alteration of Apoptotic Cell Death by Environmental and Genetic Modulations (7.7)**
Mohamed Al-Rubeai
University of Birmingham, England

Apoptosis and Nutrition: Involvement of Amino Acid Transport System in Repression of Hybridoma Cell Death (7.8)
Frantisek Franek
Institute of Molecular Genetics, Czechia

Using Computerised Systems in Modern Vaccine Production Plants (7.9)
Gerhan Wieten
RIVM, The Netherlands

On-Line Monitoring and Control of Fed-Batch Culture of Hybridoma with the Aid of an Expert System (7.10)
Yoshiomi Yoshida
Osaka University, Japan

FRIDAY, SEPTEMBER 16, 1994 (continued)

12.00 - 12.30 h	**Evaluation** Caroline MacDonald, University of Paisley, Scotland
12.30 - 13.00 h	**Conclusions** Coen Beuvery, Meeting Secretary of ESACT Hiroki Murakami, Meeting Secretary of JAACT
	Announcement ESACT 14 Portugal Manuel Carrondo
13.00 h	Lunch, Adjournment

ABSTRACTS

ORAL PRESENTATIONS

Are We Prepared for Animal Cell Technology in the 21st Century?

Prof. Charles L. Cooney, Department of Chemical Engineering,
Massachusetts Institute of Technology, Cambridge, MA 02139 USA

One of the great contributions to pharmaceutical manufacturing in the 20th century is the use of large scale animal cell culture for production of complex therapeutic proteins. As we look forward to new opportunities for therapeutic intervention with animal cell technology we see even more change on the horizon. Gene therapy is in clinical trials; transgenic animals are used for production of therapeutically important proteins; tissue and cellular matrix engineering is recognized as a new discipline and patient specific cell and cell products are considered as viable options for treatment of disease. Although animal cell technology is common to these strategies, the research, education, business and regulatory infrastructure needed to nurture and support these technologies is diverse. Surely there must be lessons from the past that will guide us to the future, but what are they? How do we prepare ourselves for the challenges of the 21 st Century in animal cell technology?

0.1

1.1

CHINESE HAMSTER OVARY (CHO) CELLS PROLIFERATE RAPIDLY IN SUSPENSION IN PROTEIN-FREE MEDIUM FOLLOWING TRANSFECTION WITH A CYCLIN E EXPRESSION VECTOR

Wolfgang A. Renner, Hans M. Eppenberger*, Vassily Hatzimanikatis, Kelvin H. Lee and James E. Bailey, Institute of Biotechnology and *Institute of Cell Biology, ETH-Hönggerberg, CH-8093 Zürich, Switzerland

Wild-type CHO K1 cells grow well in protein-free medium supplemented with either insulin or basic fibroblast growth factor (bFGF). Cells in the bFGF-stimulated cultures exhibit a rounded-up morphology often associated with growth in suspension. Immunoblots of SDS-PAGE gels of total cell protein from bFGF-stimulated cultures showed much higher cyclin E expression than in unstimulated cells in the same basal medium. Reasoning that constitutive expression of cloned cyclin E in CHO K1 cells could directly activate the cells to proliferate without any external growth factor stimulation, the cyclin E gene was inserted behind the immediate early human cytomegalovirus enhancer/promoter in the vector pRc. CHO cells transfected with this vector exhibited relatively high-level cyclin E expression and attained specific growth rates of 0.8 day^{-1} in spinner-flask cultivations in protein-free basal medium. Transfections with expression vectors for several other cyclins and transcription factors did not produce a stable, protein-independent CHO cell line. Transient simulation of proliferation obtained by expression of cloned E2F was simulated by a mathematical model which also calculates the sustained cell cycling obtained with cyclin E overexpression. Refinements and applications of this approach will be discussed.

CONTROLLED PROLIFERATION OF MAMMALIAN CELL LINES

M. Köster, S. Kirchhoff and H. Hauser
Genetics of Eukayotes, GBF - Gesellschaft für Biotechnologische Forschung mbH.,
Mascheroder Weg 1, 38123 Braunschweig, F. R. G.

Most cell lines which are used for the production of recombinant proteins show spontaneous high prooliferation rates. In many types of cultivation systems after having reached the desired cell density further proliferation requires the continuous removal of cells. We have attempted to establish a system in which cell proliferation is controlled by physiological regulators of cell poliferation. Interferon-regulatory-factor-1 (IRF-1) is a transcription factor that recognizes a sequence which is present in the Interferon-B promoter as well as in the promoters of IFN-inducible genes. Constitutive overexpression of recombinant IRF-1 leads to the inhibition of cell growth. The extent of this growth arrest depends on the intracellular concentration of IRF-1. Recently it was shown that IRF-1 acts as a tumor suppressor. In order to control gradually the growth of mammalian cell lines, we have established two different systems for conditional IRF-1 transcription and activation, respectively. In one case, and inducible promoter was used, in the other case fusion proteins composed of IRF-1 and the hormone-binding domain of the human estogen receptor was used. Both systems allow to control the proliferation of various cell lines by the concentration of estradiol and tetracycline, respectively, in the medium. The use of these systems for controlling cell proliferation in long-term cultures and the simultancous expression of recombinant proteins in these cells will be shown.

1.2

1.3

ONCOGENE ACTIVATED PRODUCTION SYSTEM TO ENHANCE CELLULAR RECOMBINANT PROTEIN PRODUCTIVITY

Sanetaka Shirahata, Kiichiro Teruya, Takahiro Yano, Junko Watanabe, Yuichi Inoue, Hirofumi Tachibana, Hideya Ohashi* and Hiroki Murakami

*Laboratory of Cellular Regulation Technology, Graduate School of Genetic Resources Technology, Kyushu University, 6-10-1 Hakozaki, Higashi-ku, Fukuoka 812, Japan and *Pharmaceutical Laboratory, Kirin Brewery Co. Ltd., Souja-machi, Maebashi, Gunma 371, Japan*

We have introduced the oncogene activated production (OAP) system in which cellular recombinant protein productivity is enhanced upon by specific promoter activation with oncogenes. To demonstrate the effectiveness of the OAP system, recombinant BHK-21 cells producing human interleukin-6 (hIL-6) under the control of the CMV promoter were cotransfected with the *ras* oncogene and dihydrofolate reductase gene, then selected with 50 nM methotrexate to co-amplify the *ras* oncogene. We found that stable highly productive clones exhibiting about 35 times higher productivity compared to the control value could rapidly be established. Other oncogenes such as *myc*, *myb*, *fos*, and *jun* did not enhance cellular productivity. To rapidly establish high producing recombinant protein cell lines, we created host cell lines from BHK-21 cells which were 'primed' with amplified *ras* oncogene expression units prior to the introduction of a producing gene. The primed BHK cell lines were demonstrated to produce many stable highly hIL-6 productive cells achieving about 15 times higher productivity as compared to original BHK cells upon introduction of the producing gene.

TRANSIENT EXPRESSION OF RECOMBINANT PROTEINS AT THE LEVEL OF PRODUCTIVE STABLE CELL LINES - OPTIMIZING DNA TRANSFER AND EXPRESSION IN MAMMALIAN CELLS

Martin Jordan and **Florian M. Wurm**; Genentech Inc., Cell Culture and Fermentation Research and Development, South San Francisco, California 94080

Two widely used methods for DNA transfer into cultured cells, calcium phosphate co-precipitation and DEAE-Dextran mediated transfection, have been analyzed thoroughly in our lab. The latter method appeared more reproducible in transient transfections experiments, however calcium phosphate/DNA co-precipitation yielded higher maximum expression levels. Also, the variability observed between independent experiments gave indications of ample opportunities for improving the widely used calcium phosphate method. The precipitation of calcium/DNA with phosphate was assessed for critical parameters leading to maximum DNA uptake and expression levels both in hamster (CHO) and human cells (293 HEK) cells. Besides pH and DNA concentration, a number of other parameters during the preparation of the DNA precipitate and the treatment of cells prior and after transfection have major effects on DNA uptake and consequent protein expression. With optimized protocols we have observed transient expression levels similar to those seen in cell lines selected for stable expression. We hope to expand our knowledge of gene transfer mechanisms and to shorten from months to days the time requirements needed to obtain significant quantities of recombinant proteins from mammalian cells.

1.4

7.1

MONITORING OF CYTOKINE AND MONOCLONAL ANTIBODY THERAPIES

C.Erik Hack, Central Laboratory of the Netherlands Blood Transfusion Service, Amsterdam

Recent advances in immunology have provided novel approaches for the treatment of a number of diseases. In particular, recombinant cytokine and monoclonal antibodies(mAb) are increasingly used. For example, the cytokine interleukin-2 and the mAb OKT3 are clinically used for the treatment of cancer and rejection of renal transplants, respectively. These novel therapies sometimes have severe side effects ranging from an increase of body temperature and headache to vascular leakage and shock. These side effects appear to be induced by the release and activation of endogenous infammatory mediators following administration of the therapeutic agent. Monitoring of these mediators in clinical trials seems, therefore, to be important to assess potential toxicities. In addition, analyzing the involvement of mediators may provide clues of how to reduce toxicity. Recent studies illustrating the issues raised above, will be discussed.

MDR BONE MARROW GENE THERAPY

A.Bank, C.Ward, C.Richardson and C. Hesdorffer. Columbia Univ. U.S.A.

The human multiple drug resistance (MDR) gene produces a transmembrane p-glycoprotein which pumps natural subtances including anti-cancer drugs like taxol, vinca alkaloids, anthracyclines and etoposide out of cells preventing their toxicity. Human bone marrow (BM) cells only express low levels of MDR and are preferentially killed by these drugs. Safe and efficient MDR gene transfer can be used to (1) prevent BM toxicity of MDR-responsive drugs, and (2) enrich the BM for cells containing and expressing a second non-selectable gene such as the human beta globin gene in addition to the MDR gene. We have previously reported successful transfer and expression of the human MDR gene in the BM cells of live mice (Podda et al PNAS 89:9676, 1992). Now, we have transferred and expressed the human MDR gene in human BM cells using supernatants from MDR-producer cells (A12M1) which are free of replication-competent retrovirus. In experiments with CD34+ cells isolated from BM, PCR analysis of the cells is positive; up to 50% of individual BFU-E are also PCR positive. Up to 11% of the cells express increased MDR by FACS. In addition, exposure of transduced cells to taxol results in the preferential survival of these cells as compared to untransduced controls. These results indicate that MDR gene transfer can be used to enrich human BM for cells containing the transferred gene.

7.2

2.1

EFFECTS OF AMMONIA REMOVAL ON CELL GROWTH AND MAb PRODUCTION

Masatoshi Matsumura

Institute of Applied Biochemistry, University of Tsukuba, Tsukuba, Ibaraki 305, Japan

Production of monoclonal antibody against hepatitis surface antigen (HBs-MAb) by mouse hybridoma TO-405 has been reported to be higher in serum-free cultures than in serum supplemented cultures, and to be proportional to the viable cell concentration. This cell line, however, was found to be especially susceptible to ammonia inhibition in serum-free cultures. It was therefore thought that if the ammonia could be selectively removed from the culture broth, then the maximum viable cell density as well as the productivity in serum-free cultures could be improved. Serum perfusion cultures of TO-405 cells were carried out in a tank reactor coupled with zeolite A-3 packed beds. Ammonia was selectively removed from the culture broth by passing the cell free permeate from ceramic cross flow filtration through the zeolite packed bed. Ammonia concentration was effectively maintained between 1 and 4 mmol/l which was below the inhibitory concentration for cell growth. The MAb accumulated to 2.63×10^6 IU/l, which was almost half of the amount produced *in vivo*. Pondering that it takes about two weeks for one mouse to produce an average of 3 ml ascetic fluid, a 10 liter reactor would yield MAb equivalent to the amount obtained from roughly 1,500 mice in the same period of time.

CELL CYCLE PHASE ANALYSIS IN GLUCOSE-LIMITED CONTINUOUS CULTIVATION FOR THE EFFECTIVE PRODUCTION OF ANTIBODY

Takeshi Omasa, Suteaki Shioya and Ken-ichi Suga
Department of Biotechnology, Faculty of Engineering, Osaka University, Suita, Osaka 565 Japan

From the synchronous culture experiment, it was found that the specific antibody secretion rate increased at a period from late G1 phase to early S phase on 3A21 hybridoma. The effects of specific growth rate on the cell cycle phase distribution and antibody secretion were investigated in glucose-limited continuous cultivation. With decrease of specific growth rate, the ratio of G1 phase cell increased, hence the specific antibody secretion rate increased. In continuous cultivation, a relative intracellular content of heavy and light (H+L) chain of antibody in each cell cycle phase was also measured by flow cytometer. From these results, the intracellular H+L chains accumulation rate was calculated in each cell cycle phase based on Kromenaker and Srienc's analysis[1]. As a result, the relative intracellular H+L chain accumulation rate increased in G1 phase.

1)Kromenaker,S.J.&Srienc,F.C.(1991) Biotechnol.Bioeng.38:665-677

2.2
2.3

KINETIC ANALYSIS OF THE 'GROW OR DIE' CELL CYCLE SYSTEM IN PERFUSION CULTURE OF HYBRIDOMA CELLS

[1]Sylvain Mercille,[1]Mark Johnson,[2]Stéphane Lanthier,[2]Lucie Bourget and [2]Bernard Massie
[1]Biomira inc., Montréal; [2]Groupe d'ingénierie des cellules animales, Institut de recherche en Biotechnologie, Conseil National de Recherches du Canada, 6100 Avenue Royalmount, Montréal, P.Q., Canada, H4P 2R2

A detailed kinetic analysis was performed on filtration-based perfusion cultures of D5 hybridoma cells in protein-free medium. Significant changes occured as cells progressed from the exponential phase to the steady-state plateau phase. At steady-state, we observed a decrease in glucose and glutamine specific consumption rates concomitant with a decrease in growth rate and to an increase in the proportion of cells in the G_1 phase of the cell cycle. An increase in monoclonal antibody (MAb) specific production rate was also concomitant with the decrease in growth rate and with the increase in the proportion of cells in the G_1 phase of the cell cycle. Such a phenomenon was also observed with the same cell line cultivated at decreased growth rates following the addition of a cytostatic agent in pulsed-batch culture. These results suggest that increased specific MAb production rates in slow growth conditions are related to the prolongation of the G_1 phase. Steady-state viable cell concentrations were found to be proportional to perfusion rates from 0.5 up to 1.5 vol/day. While glutamine was found to limit the steady-state viable cell concentration in this range of perfusion rates, glucose became limiting at the point where viable cell number was no longer proportional to perfusion rate. Indeed, direct oxygen sparging was required to maintain viable cell concentrations above 15×10^6 cells/mL, resulting in a significant increase in the specific glucose consumption rate. Through glucose supplementation, the proportionality was extended to 5 vol/day, allowing for a concentration of 50×10^6 viable cells/mL. Continuous accumulation of non-viable cells was observed under steady-state perfusion conditions. This phenomenon, described as the "grow or die" phenotype was associated with the induction of programmed cell death under nutrient limitation. Indeed, in separate experiments, glutamine or glucose deprivation were found to induce apoptotic cell death in D5 hybridoma cultures.

CONTINUOUS FERMENTATION CONSUMPTION KINETIC FOR BASAL MEDIA DESIGN

Carlos Figueroa, Craig Rice, Mokhtar Mered and David Naveh
Miles Biotechnology, Berkeley, California USA

A kinetic approach to media design has been developed based on providing cells with the precise amounts of consumed basal medium components in a continuously perfused culture. Efficiently designed media minimizes the liquid volume required to produce a given amount of protein, reduces raw media costs from wasted unused components, and improves purification yields due to higher protein titers and less volume to process. This technique is applicable to most consumed components such as amino acids, lipids and nucleotides. The technique involves analyzing the kinetic utilization of each component versus the product specific productivity. At some point, increasing component utilization does not increase rppoduct specific productivity. This component utilization value defines the kinetic zero order breakpoint. The breakpoint values are divided by the desired operating specific perfusion thus defining the final media component concentrations. This paper covers one such medium development for a IgG secreting murine hybridoma cell line. For this case, a two fold increase in titer was realized.

2.4

2.5

PRODUCTIVITY ENHANCEMENT USING LIQUID MEDIUM CONCENTRATES.

David W. Jayme, James M. Kubiak & Richard M. Fike, Cell Culture Research and Development, GIBCO-BRL, Life Technologies, Inc., 3175 Staley Road, Grand Island, New York 14072 USA.

Qualitative and quantitative differences in nutrient utilization have been observed within high density mammalian cell culture bioreactors. Classical responses to depletion of critical nutrients are to increase the rate of total medium replenishment or to recirculate spent medium. An alternative is to maintain the exchange rate for basal medium components while adding rapidly-consumed nutrients as a supplemental concentrate. Composition and formulation of supplemental nutrient concentrates required quantitative analysis of spent biological fluids. Nutrient balance according to pseudo first order nutrient consumption kinetics, rather than post batch culture exhaustion profiles, yielded superior cell culture performance. Knowledge of nutrient biochemistry from partial supplements facilitated preparation of concentrated components to reconstitute complete media, including serum-free and protein-free formulations. Concentrated (50X) media exploited native properties to increase nutrient component solubility, sequester reactive materials, and permit biochemical co-stabilization. Liquid medium (1X) reconstituted from concentrated 50X sub-groups exhibited quality and performance advantages compared with identical formulations produced by classical options. This paper examines three key productivity indicators (cell yield, biological product yield, and overall media manufacturing cost).

CULTURE CONDITIONS FOR HIGH PERFORMANCE PRODUCTION OF MONOCLONAL ANTIBODY

Yoshihito Shirai, Masaaki Yamaguchi, and Hiroki Murakami*
Department of Biochemical Engineering and Science, Kyushu Institute of Technology
Iizuka, Fukuoka 820, Japan. *Graduate School of Genetic Resources Technology, Kyushu University, Hakozaki, Higashi-ku, Fukuoka 812, Japan.

We examined growth and monoclonal antibody production kinetics of the human-human AE6F4 hybridoma under various culture conditions, which produces monoclonal antibody binding with a specific antigen site on lung cancer cells. The culture conditions strongly affected the cell growth and the monoclonal antibody production. For instance, the cell growth rate was remarkably reduced at pH levels less than 6.7. Moreover, the monoclonal antibody production rate at pH 7.3 increased 60 times that at pH 6.7. The best yields of cells and monoclonal antibody were found at pH 7.0 and they were reduced at other pH levels. These suggest that the production performance of the monoclonal antibody which recognizes lung cancer cells strongly depends on the culture condition of the AE6F4 hybridoma.

2.6

2.7

COMPARISON OF NUTRIENT REQUIREMENTS OF MAMMALIAN CELL LINES

Heino Büntemeyer and Jürgen Lehmann
Institute of Cell Culture Technology, University of Bielefeld, P.O. Box 100131, 33501 Bielefeld, Germany

For the design of production processes with mammalian cell lines not only cultivation strategies but also nutrient requirements and waste production are of particular importance. The determination of the specific uptake rates of all relevant substrates including oxygen, glucose, glutamine and amino acids is necessary to optimize the feeding rates for processes such as fed batch or perfusion. The concentration of waste products such as ammonium and lactic acid and other inhibitors also play an important role for growth rate and cell death. We investigated a wide varity of cell lines including CHO, mouse hybridomas, rat hybridomas and human lymphocytes. Data from those cells were collected to calculate the characteristic uptake rates. Similarities and differences between cell lines of different origin are presented and discussed in the paper.

PROTEIN FOLDING IN THE SECRETORY PATHWAY OF ANIMAL CELLS

Robert B. Freedman, Carole Greenall, Nigel Jenkins & Mick F. Tuite
Research School of Biosciences, Biological Laboratory,
University of Kent, Canterbury CT2 7NJ, Kent, U.K.

The exit of newly-synthesized proteins from the lumen of the endoplasmic reticulum (ER) is the rate-determining step in protein secretion. Only correctly-folded and fully-assembled proteins exit the ER and progress along the secretory pathway. Folding and assembly in the ER are mediated by a variety of factors including folding catalysts and molecular chaperones. The properties of these factors, and the nature of their interactions with folding substrates, are beginning to be clarified. Little work has been done to characterize these processes and these factors in cell lines employed for large-scale cell culture. Manipulation of this process may permit improvements in yield or productivity of recombinant proteins by cultured mammalian cells.

3.1

3.2

ERYTHROPOIETIN PROCESSING IN ERYTHROPOIETIC SYSTEM AND NERVOUS SYSTEM

Ryuzo Sasaki, Seiji Masuda and Masaya Nagao
Dept. Food Sci. & Technol. Faculty of Agric. Kyoto Univ. Kyoto 606, Japan

We will summarize processing of cytokines during or after translation, concentrating on erythropoietin (EPO). EPO is modified in two ways; glycosylation and removal of carboxyl-terminal arginine residue. The mature EPO consists of 165 amino acids, one *O*-linked and three *N*-linked sugars. To know significance of the glycosylation, we took three ways; enzymatic removal of sugar chains from recombinant EPO, removal of glycosylation sites by site-directed mutagenesis, and production of EPO in plant cells and procaryotic cells. In conclusion, *N*-linked sugars are important for proper biosynthesis and/or secretion in animal cells, and expression of the *in vivo* activity by enhancing survival in the circulation. *N*-Linked sugars affect binding affinity to the receptor but do not play a key role in expression of the *in vitro* activity. *N*-Linked sugar at a specific site is critical for polarized secretion when EPO is expressed in epithelial cells. Our finding that EPO acts on neurons in paracrine fashion and properties of EPO produced in brain will be presented.

EFFECTS OF LIPIDS ON RECOMBINANT INTERFERON-γ GLYCOSYLATION, AND IMPROVEMENTS IN PRODUCT MONITORING.

Nigel Jenkins, Paula Castro, Andrew Ison*, Alan Bull, Robert Freedman & David James
Research School of Biosciences, University of Kent, Canterbury, CT2 7NJ. U.K. and *SERC Advanced Centre for Biochemical Engineering, University College, London, U.K.

The role of lipids in the glycosylation of recombinant human interferon-γ (IFN-γ) expressed in a Chinese Hamster Ovary cell line were investigated in batch culture. Lipids of the dolichol class form an essential part of the N-glycosylation pathway, and have been shown to improve cell viability. The commercial lipoprotein supplement "ExCyte" was shown to minimise the IFN-γ glycosylation deterioration shown by control serum-free cultures. Partially substituting the normal bovine serum albumin content (5g/l) of our cell culture medium with a fatty acid-free preparation also improved IFN-γ glycosylation, suggesting that oxidised lipids carried on albumin may damage the glycosylation process. Inclusion of serum also compromised this process. Improvements in glycosylation monitoring by capillary electrophoresis (CE) and matrix-assisted laser desorption mass spectrometry (LDMS) will also be described. CE provides high resolution separations of more than 30 IFN-γ glycoforms in under 2 hours. LDMS, in combination with fast array exoglycosidase digests, can be employed to determine glycan structures at each of the two N-linked IFN-γ glycosylation sites, using <500μg of pure protein. Significant differences were observed between the oligo-saccharide structures found at each site. Both techniques have great potential for monitoring glycoprotein heterogeneity in animal cell cultures.

3.3

3.4

GLYCOSYLATION OF RECOMBINANT THERAPEUTIC PROTEINS

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Biotechnology is being used to develop numerous proteins as therapeutic agents. The majority of these are in their native state glycosylated, and glycosylation of the recombinant product is generally necessary for full therapeutic efficacy. A consideration of glycosylation by direct analysis is relevant during several stages of the development and production of therapeutic recombinant glycoproteins. First, glycosylation is cell type-specific and recombinant proteins are usually derived from heterologous systems. Consequently, the recombinant form of the protein is almost invariably glycosylated differently to the native form. The differences in glycosylation can include differences in both the number of attached glycan chains and in the precise sequences of glycans at an individual glycosylation site. Glycosylation can influence the specific bioactivity, pharmacokinetics, and immunogenicity of a glycoprotein, and different glycosylation patterns are in several cases associated with differences in the therapeutic profile of a glycoprotein. It is therefor useful to analyse at as early a stage as possible the glycosylation pattern of the recombinant form, to compare this to that of the native form where this is available, and to screen for carbohydrate determinants known to interact with the immune system and with endogenous lectins. Second, the pattern of protein glycosylation is extremely sensitive to both culture method and to variations in the extracellular environment during cell culture. It is therefore important in developing a 'scaled-up' process for cell culture to ensure that the specific glycosylation pattern of the material initially prepared and analysed is maintained after a scale up in the production process. Further, a production process can only be sonciderded valid if it reproducibly allows isolation of protein with a constant glycosylation pattern. Third, given the sensitivity of glycosylation pattern to cell culture conditions, it is useful to assess the glycosylation of each production batch. These points will be elaborated and illustrated during this presentation through the use of specific examples of recombinant therapeutics including monoclonal antibodies.

Effects of Ammonia and Glucosamine on the Glycosylation Pattern of Recombinant Proteins Expressed from BHK-21 Cells

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In order to elucidate culture conditions influencing the glycosylation of polypeptides, recombinant hu-IL-2 variant proteins, bearing different artificial glycosylation sites and expressed by BHK-21 cells were used as model proteins. The effects of ammonia and glucosamine on the N-glycosylation of recombinant glycoproteins were examined. Cultivations were carried out in perfused 2-liter double membrane bioreactors under defined culture conditions. Cell growth was monitored by intracellular nucleotide pool patterns. Glycoprotein products from the various cell culture supernatants were characterized by western blotting, peptide mapping, amino acid sequence analysis as well as by high pH anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD), methylation analysis, matrix assisted laser desorption ionization (MALDI) and fast atom bombardment mass spectrometry (FAB-MS). Significant and reproducible alterations in the glycosylation patterns of proteins produced in the presence of ammonia or glucosamine were observed. Results will be presented and discussed.

3.5

4.1

Functional display of proteins, mutant proteins, fragments of proteins and peptides on the surface of filamentous (bacterio)phages

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In the eukaryotic cell, newly synthesized polypeptides are transported from the cytoplasm to the lumen of the endoplasmic reticulum (ER). During the translocation across the ER membrane, the signal peptide is proteolytically removed, and simultaneously or subsequently, the polypeptide is folded into its proper conformation, which is sustained by correct disulfide-bond formation. The translocation of polypeptides across the inner membrane of a prokaryotic cell (e.g. for *Escherichia coli*) into the periplasm is functionally equivalent to transport of a protein to the lumen of the ER. Initially, these findings were exploited to express heterodimeric Fv and Fab fragment of human and murine immunoglobulins (IgGs) in the periplasm of *E.coli*. Such truncated proteins display virtually identical antigen-binding affinities as their native, intact counterparts.
The routing of proteins involved in the assembly and morphogenesis of filamentous (bacterio)phages, such as M13, largely resembles that of periplasmic proteins. Consequently, proteins fused either to the gene VIII or gene III products of M13 are routed through the periplasm and are ultimately exposed in a functional conformation on the surface of phage particles. This concept has formed the basis for the design of the 'combinatorial Ig repertoire cloning technique' as well as for the functional display of other 'wild-type' proteins, mutant proteins or peptides on the surface of phages. The practical feasibility of these novel developments for studies on structure and functional of eukaryotic proteins will be illustrated by data from recent experiments from our laboratory.

PCR AMPLIFICATION OF THE VARIABLE REGIONS OF IMMUNOGLOBULIN HEAVY AND LIGHT CHAIN GENES ON GENOMIC DNA FROM MOUSE HYBRIDOMA CELLS

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Chimeric or isotype switched antibodies (Abs) bearing a murine Fv fragment of given antigen specificity are combined with a constant (C) domain of human, mouse or rat origin providing the desired effector functions. The first step of the chimerization, or isotype switching process, is the cloning of the murine genes encoding the V regions for the Heavy (H) and Light (L) chain of the hybridoma. The classical approach is based on the amplification by PCR of the V regions using a collection of specific consensus primers targeted at the sequences of the more conserved V region domains in the cDNA template. This approach results in V region gene fragments that may be altered due to an imperfect match of the PCR primers with the template, potentially modifying the binding properties of the antibody. In addition, PCR amplification generates either truncated V genes or V genes linked to part of the C region that need to be adapted when associating V and C region genes. To circumvent both of these problems, we have designed a novel strategy for the preparation of genomic fragments encoding the V_H-D-J_H and V_L-J_L regions of mouse immunoglobulin (Ig) genes. This strategy is based on the PCR amplification of genomic DNA from mouse hybridomas using specific primers chosen in the 5' untranslated region and in the intron downstream of the rearranged J_H/J_L sequences. Variable regions with native coding sequences, including full length leader peptides, are obtained without previous DNA sequencing. Thus, chimeric mouse-human Ig and isotype switched mouse Ig can be easily produced.

4.2

4.3

B CELL ACTIVATION AND DIFFERENTIAL IMMUNOGLOBULIN ISOTYPE SWITCH INDUCED BY PLASMA MEMBRANES OF ACTIVATED HELPER T CELLS

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B cell stimulation of proliferative response and differentiation into antibody-secreting cells is mediated by direct contact with T cells and capture of soluble factors from the same T cells as well as surface Ig-antigen interaction. In this study, first we evaluated a role of a class II-mediated signal on B cell stimulation. Resting and activated B cells fractionated from bovine β-lactoglobulin (β-LG)-immunized BALB/c mice were incubated with anti-A^d mAb and plasma membranes (PM) obtained from an activated T cell clone of D10.G4.1 (D10) specific for an irrelevant antigen. The results indicated that the mAb did not affect activation of both resting and activated B cells. These suggest that a signal through a class II molecule is not required directly for noncognate B cell activation. However, PM from a cognate T cell clone of H1.1 specific for β-LG/A^d stimulated resting B cells in the presence of β-LG more strongly than D10 PM, suggesting that TCR in H1.1 PM interacted with antigen/class II complexes on those resting B cells and that this interaction enhanced B cell stimulation. It is likely that a signal through class II molecules augments B cell stimulation only in the presence of a signal through surface Ig. We further examined antibody secretion by B cells stimulated with D10 PM and culture supernatant of activated D10 cells (D10 sup). The results indicated that IgM and IgG_{2a} was secreted more in the presence of both D10 PM and D10 sup than either one of them, while IgG_1 secretion was reduced in the presence of both, comparing that in the presence of either one of them. These indicate that immunoglobulin isotype switch is regulated not only by the presence of some lymphokines such as IL-4, but also by more complicated manners.

CONSTRUCTION AND EXPRESSION OF CHIMERIC PROTEIN A-MARINE FIREFLY LUCIFERASE IN MAMMALIAN CELLS

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We have developed a chimeric protein of the IgG binding domain of *Staphylococcus aureus* protein A and the luciferase from the marine ostracod *Vargula hilgendorfii* (Umi Hotaru in Japanese) which will have wide applicability in bioluminescent immunoassay. The protein has been transiently expressed and efficiently secreted from cultured COS-1 cell. The chimera with no linker peptide showed luciferase activity, but no IgG binding activity. When peptide sequence of $(Gly)_4$-Ser was added between these two moieties, significant binding activity to both rabbit and human IgGs was observed. The concentration of secreted protein increased gradually after transfection and as a result, up to 100μg/l of protein was detected after purification with human IgG-agarose. The results of further investigation to widen the binding specificity of the antibody binding domain by employing the synthesized protein G Fab binding domain will also be shown.

4.4

5.1

ACCEPTABILITY OF CONTINUOUS CELL LINES FOR THE PRODUCTION OF BIOLOGICALS

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For decades, it was intensively discussed whether or not continuous cell lines can be accepted for the production of biologicals. The major concern came from the fact that most if not all continuous cell lines cause tumours in laboratory animals. It was feared that some tumorigenic principle could be transferred with material produced on these cell lines. It is now well known that as carrier for this principle only DNA has to be regarded. Calculations performed by an expert group gathered by WHO in 1986 show that 100 pg. residual cellular DNA per dosage may be harmless. However, the conditions for this calculation, e.g. lack of incorporation of DNA into a product like a live viral vaccine have still to be considered. A re-evaluation may be necessary because of recent reports which describe the use of naked DNA for vaccination. A second issue is the possible contamination of products with viruses which may replicate in the cell lines used for production. Rules have been put together, especially by expert groups of the European Union, which describe how to prove the absence of viruses in a product derived from cell lines. The practical experience with the performance as well as with the evaluation of such studies discovers a series of pitfalls. To obtain valid results a balance has to be performed if viruses are removed by distribution into different compartments and kinetics have to be done if viruses are claimed to be inactivated by a given procedure.

MULTI-USE BIOPHARMACEUTICAL MANUFACTURING FACILITIES

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The concept of a multi-use biopharmaceutical plant will be discussed from three perspectives.

Economic Perspective
Market value of biopharmaceuticals; cost involved from concept to marketing authorization; cost involved in capital investment.

Regulatory Perspective
Containment rules, Good Manufacturing Practice, Contamination Control.

Design Perspective
"Open" versus "closed" systems, "live" versus "dead" areas, "concurrent" versus "segregated" manufacturing, logistics.

5.2

5.3

FACILITY DESIGN AND VALIDATION CONSIDERATIONS FOR CONTINUOUS CELL CULTURE PROCESSES

Bernard E. Horwath and Mark D. Hirschel, Ph.D., CELLEX BIOSCIENCES, Inc.

There is growing interest in the use of continuous processes for the production of mammalian cell derived products. Although batch and fed batch operations still predominate, an increasing number of firms are looking to continuous production techniques as a means of decreasing facility and equipment costs, and the eventual cost of production. This presentation will address the aspects that distinguish a continuous production process from batch and fed batch modes of operation. Facility design considerations and cost implications will be discussed for continuous versus batch processes. The validation issues that are unique to a continuous production process will be covered and illustrations presented on how they can be effectively handled.

VALIDATION STRATEGY FOR VIRAL CLEARANCE

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Regulatory agencies worldwide require, for products derived from continuous cell lines, validation of the capability of a purification process to clear or inactivate potential viruses and virus-like particles. New guidance suggests a requirement for statistical evaluation of these studies. The industry has yet to develop standards for the statistical evaluation of viral clearance validation. In addition, the task of estimating excess capacity is complicated by a number of issues including variable and expensive assays, accumulation of variability in clearance estimates over several unit operations, dependence of clearance capacity on critical operating parameters, selection of model viruses, and expense of full-scale experiments. We propose an experimental strategy to determine the excess clearance capacity of a biopharmaceutical process and to provide statistical estimation of this excess capacity in an efficient way. This strategy requires a minimum number of full scale runs for risk assessment, and small scale runs for validation of clearance. Information accumulated from process development runs is used to determine variability in the feed stream and in the clearance capacity estimates and to quantify the dependence of clearance on critical process parameters. Clearance estimates and their variances are calculated for each unit operation based on a Poisson model. Estimates from "orthogonal" unit operations are combined along with their variances to form an interval estimate of overall process clearance capacity. We believe that this approach should meet regulatory guidelines in a cost effective way.

5.4

5.5

REMOVAL OF THE PRION PROTEIN USING VALIDATABLE FILTER(BMM^R)

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Infectious isoform of the prion protein($PrP^{Sc, CJD}$) is commonly detected from brains and other tissues with prion diseases, including Creutzfeldt-Jakob disease (CJD) in humans, scrapie in sheep and bovine spongiform encephalopathy (BSE). PrP^{CJD} is highly resistant to chemical and physical inactivations, such as formalin fixation for many years or heating at 121℃ for 4h, but is eliminatable by membrane filtration. The supernatant of the centrifuged homogenate obtained from the mouse brains infected with CJD was filtered through BMM^R, that is cuprammonium regenerated cellulose hollow fibers with various mean pore size $2\overline{r}_f$ ranging between 10 and 75nm, and injected into brains of NZW mice. The infectivity of the filtrate decreased when $2\overline{r}_f$ decreased, and for filters less than 35nm, the infectivity disappeared completely. The treatment of homogenate with a surfactant (Sarkosyl), revealed infectivity even after filtration with 10 ± 2nm filter, probably due to separation of PrP^{CJD} molecules into smaller size. Aggregations of PrP^{CJD} were observed, by electron microscopy, in the wall of the BMMs, but drastically decreased after being treated with Sarkosyl.

THE ACCEPTABILITY OF CONTINUOUS CELL LINES: AN HISTORICAL PERSPECTIVE

John C. Petricciani, M.D.

In the 1950s, only primary cell cultures were acceptable for the production of human biological products. This position was challenged in the late 1960s by human diploid cells (HDCs), and again in the 1980s by continuous cell lines (CCLs). The history of the HDC controversy is reviewed and lessons from that era that are relevant to the use of CCLs are pointed out.

It became apparent in the early days of recombinant DNA technology in the 1980s that CCLs were needed for the development of some products. CCL acceptability therefore became more urgent, and several attempts were made to reach a consensus on regulatory issues. In 1986, the World Health Organization convened a Study Group to review the safety issues related to products derived from CCLs. The Study Group made a clear recommendation to pursue CCLs in product development because of the demonstrated capability of modern manufacturing processes to cope with contaminants.

Issues such as acceptable levels of cellular DNA in products and the relationship of purity to safety are discussed in the context of the need for regulatory authorities, industry, and the general biomedical community to cooperate in addressing problems in a rational scientific manner.

0.2
6.1

OXYGEN GRADIENTS IN SMALL & BIG ANIMAL-CELL BIOREACTORS

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The oxygen gradients in animal-cell bioreactors are estimated using straightforward engineering calculations. Bioreactors considered are stirred vessel, bubble column, airlift, packed and fluidized bed, and membrane reactor. Except for the latter, the sizes analyzed are 0.01 and $10 m^3$. First, the gradient is estimated in the stagnant layer surrounding a cell of 15 μm, a microcarrier (185 μm) with 300 cells attached to it, a macroporous support (1.25 mm) with 185,000 cells in it and one (6 mm) with 4.25 million cells in it, assuming an oxygen consumption of 10^{-16} mole $O_2.cell^{-1}.s^{-1}$, and using mass transfer coefficients obtained from Sherwood relations. To estimate if oxygen gradients will exist in the bulk-liquid phase, circulation and liquid-retention times are compared with exhaust times for suspensions with 10^{12}, 10^{13} and 10^{-14} cells/m^3. Finally, the gradient in the liquid film surrounding air bubbles is estimated using k_LA-values obtained from empirical correlations.
It is clear from all these estimations that in many situations severe gradients can be expected. The question remains, however, whether gradients should be avoided as much as possible, or that they can be tolerated to a certain extent and even created by purpose because of beneficial effects.

BUBBLE BED AERATION FOR ANIMAL CELL CULURES

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3. Cell Fermentation Group, Sandoz Pharma Ltd

Previous work at the ETH Zurich has shown the destruction of mammalian cells by sparging gas bubbles. A special designed reactor with very high oxygen uptakes up to 90% has been developed on a laboratory scale (2.4ltr). Sparging monodisperse bubbles results in high oxygen transfer while the superficial gas velocity remains one to two orders of magnitude below typical values of air -lift reactors. This advantageous reactor type, bubble bed reactor (Swiss patent CH681808A5), is currently scaled-up to industrial scale (250ltr). Oxygen transfer, impeller dissipation, shear stress, and residence time distribution will be further analyzed. Long time perfusion experiments with animal cells will lead to reliable data of the industrial potential of this system.

6.2

6.3

FINITE ELEMENT ANALYSIS OF 3-D FLOW IN AN AGITATED BIOREACTOR

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We have developed the simulation technique for 3-D flow of a Newtonian fluid in agitated bioreactors using finite element method on a super-computer. We assumed an isothermal and stationary flow of an incompressible Newtonian fluid, MEM supplemented with FBS. The 3-D finite element simulation of flow in agitated vessels has succeeded in obtaining flow patterns, stress patterns, tracking path lines which are very useful for optimal design of agitated bioreactors. The most significant stress is the shear stress on the r- θ plane, $\tau_{r\theta}$, whose maximum values usually occur at the tip of the impellers. The reliability of numerical results was confirmed by comparing them with our data of velocities measured by the Laser Doppler Velocimeter. A commercial spinner flask of 250 ml in volume with 2 bladed paddle impellers, for example, was used in the present study. The agitation speed was controlled at 60 rpm which was often used in actual animal cell culture. The maximum shear stress of 95 mPa which is nearly equal to the critical separation force from a substratum for a species of weakly adherent cells, for example, human embryonic kidney cell (293), is occurring at the impeller tip for this rotation. For evaluation of the simulation results, the culture of 293 cells using cytodex-1 microcarriers (1.4g/l) was performed, and the cells were found to be rapidly detached under this value of shear stress from the microcarriers in the stationary phase of the growth. Agreement between the simulation and experimental results shows that the simulation technique can be applicable to obtain hydrodynamic and mass transfer data for optimal design of agitated culture vessel. Further study are being done on various shapes of the agitated vessel and impeller.

OPTIMAL OPERATION OF FORCED-FLOW MEMBRANE BIOREACTORS FOR HIGH-DENSITY MAMMALIAN CELL CULTURES

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Recently developed perfusion bioreactors with cells confined between flat microfiltration membranes and aerated by an integrated system of tubular membranes have great potential in the area of protein production, cellular therapy and tissue engineering. High dilution rates of nutrients combined to homogeneous flow through the cellular compartment and reduced shear stress allow the obtainment of high cell densities (exceeding 10^8 cells/ml with high viability) and extended cultures of several months without membrane clogging. The additional possibility to partially recirculate the culture medium can also yield an improved utilisation of the nutrients.

An analysis of the rate limiting steps determining the performance of such forced-flow membrane bioreactors is presented. It includes the cell growth limiting effects as well as the death promoting effects of glucose, amino acids and oxygen limitations, the kinetics of cell metabolism and protein production, the kinetics of oxygen transfer through the membrane, shear stress effects, as well as the influence of local concentration gradients.

The rate limiting processes have been integrated into a simulation model which is used to propose an optimal operation of forced-flow membrane reactors. Special attention is given to the selection of the appropriate dilution and recirculation rates, and of the medium composition during the growth and stationary phase. The reactor optimisation in terms of cell densities inside the perfusion chamber is also discussed.

6.4

6.5

IMMOBILIZATION OF ANCHORAGE-INDEPENDENT CELLS TO POROUS GLASS BEADS: MASS TRANSFER PHENOMENA IN LONG - TERM PACKED BED REACTOR

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Porous glass beads (Siran, Schott Glasswerke, Mainz, Germany) with particle diameters of 1-2 mm, pore diameters in the range of 60-300μm and porosity of 60% were used in a packed bed reactor as an immobilization matrix for the SP2/O hybridoma cell line. The work reported here considers the mass transfer phenomena.
A stimulus-response tracer technique (RTD, Residence Time Distribution) was used to characterize the packed bed reactor. The cell content on the porous support and the flow rate were the parameters changed during the experiments of RTD to evaluate their effect on the various transport mechanisms. Sodium Chloride was used as the tracer. Liquid phase dispersion, interphase mass transfer, and intraparticle diffusion coefficients were determined for packed beds with and without cells.
The long-term packed bed reactor was operated for up to one month and the dilution rates have been changed between 0.025 h^{-1} and 0.25 h^{-1} at constant recirculation rate. Cell distribution along the packed bed reactor was evaluated and has shown the homogeneity of the system for the tested recirculation rate.

MODEL-BASED MONITORING AND CONTROL OF A MONOCLONAL ANTIBODY PRODUCTION PROCESS

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For the efficient design of biotech processes, tools are required which allow an optimal performance of the process. Mathematical models in connection with model-based methods of monitoring and control are powerful tools to meet this requirement. A structured mathematical model describing the dynamics of Hybridoma growth and monoclonal antibody production in suspension cultures is presented. Key elements of the model are the energy metabolism and the dynamic description of an intracellular compound which represents the function of cyclins. Inhibitory effects of waste products are part of the kinetic expressions. The model fits well to experimental data obtained from batch, fed-batch and continuous cultures with Hybridoma cells. Applications of the model for process control will be demonstrated: 1) An Extended Kalman Filter (EKF) was designed to estimate the state of the process. The oxygen consumption rate of the cell culture is monitored on-line and is used as the only measurement information for the EKF. This EKF is able to provide good estimates for living and dead cell densities and the medium composition. 2) The mathematical model was applied for the optimization of fed-batch cultures. The possibilities of improving the process by optimized feeding strategies will be discussed.

6.6
6.7

EVALUATION OF PROCESS STRATEGIES FOR EFFICIENT CULTIVATION OF HYBRIDOMA CELLS BASED ON MATHEMATICAL MODELS

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Efficient production of biologicals with animal cells, e. g. monoclonal antibodies, requires a high process yield. Kinetic models regarding cell growth and productivity enable a preliminary evaluation of different process strategies.
For a hybridoma cell line extensiv kinetic studys were performed to develop a model describing cell growth with respect to nutrient limitation and ammonia inhibition, nutrient consumption (glucose, glutamine), production of metabolites (lactate, ammonia) and antibodies. Based on this model different process strategies for suspended hybridoma cells (fed-batch, chemostat, high density cultivation in a membrane dialysis reactor) have been compared regarding cell density, MAb productivity, MAb concentration and medium usage. The model predictions were validated by experimental data.

Microcarrier Technology, present status and perspective.

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Only a decade after Van Wezel introduced the first product produced in microcarrier cultures namely Inactivated Polio Vaccine (IPV) produced on industrial scale at economically acceptable costs, interest was taken in this revolutionary type of cell growth system. The basic idea was to develop a culture system with equal potentials for control of environmental culture conditions and scaling up as the sytems used in industrial microbiology.
Although initially only positively charged beads were used it soon became clear that negatively charged or amphoteric materials such as proteins or amino acids polymerized to the surface were equally usefull. This resulted in the development of numerous different microcarriers.
The second generation of microcarriers exists of macroporous beads providing increased surface area by external and interior space for cell attachment and growth. These microcarriers offer enormous potential for high cell densities and enhanced productivity for certain production systems, especially recombinant CHO-cells. These carriers offer not only possibilities for anchorage dependant cells but also for suspension growing cells and can be used in homogeneous bioreactors as well as in fluidized or fixed bed systems.
Despite considerable investments in research on development and improvement of microcarriers the question is still open: Is microcarrier technology still in its infancy or is it full-grown and is the basic idea realized?
In this lecture a general view will be given of the present status of microcarrier technology and the perspective for tomorrow.

0.3

7.3

HEMATOPOIETIC TISSUE ENGINEERING

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Circulating blood cells all originate from a common pool of stem cells and are formed by a process called hematopoiesis. Considerable interest has developed in recent years to reconsitute functional human hematopoiesis *ex vivo*. Such a system would have a broad range of applications to both basic biological and clinical problems. The approach of mimicking the dynamic *in vivo* environment has led to prolific culture conditions that expand total cells, progenitor cells, and cells with long-term culture initiating capability. A bioreactor perfusion system that provides the simulated physiological environment has proven to be scalable and capable of producing a clinically meaningful number of cells. A series of studies addressing a variety of hematopoietic tissue engineering challenges will be presented. These include the kinetics of extended growth, kinetics of erythropoiesis, growth factor production and consumption, and clinical applications to transplantation and gene therapy.

***EX VIVO* EXPANSION OF PRIMITIVE HEMATOPOIETIC CELLS UNDER PERFUSION CONDITIONS FOR CELLULAR THERAPIES**

E. Terry Papoutsakis, Craig E. Sandstrom, William M. Miller, Dept. of Chemical Engineering, Northwestern University, Evanston, IL 60208, USA, & James G. Bender, Immunotherapy, Baxter Healthcare, Round Lake, IL 60073, USA.

Sources of hematopoietic cells for bone marrow transplantation are limited by the supply of compatible donors, the possibility of viral infection, and autologous (patient) marrow that is depleted from prior chemo- or radiotherapy or has leukemic involvement. An *in vitro* system to amplify hematopoietic progenitor cells could increase the number of patients eligible for autologous transplant, allow use of cord blood hematopoietic cells to repopulate an adult, reduce the amount of bone marrow required for transplantation, and allow collection of mobilized peripheral blood stem and progenitor cells to replace the bone marrow harvest process. In addition, such a system is likely to become useful in the development of effective large-scale gene therapies. Present methods for hematopoietic cultures (HC) on stromal (i.e., accessory cells that support hematopoiesis) layers in flasks lack a well-controlled growth environment. The prospects for use of HC in bone marrow transplant are very good if an efficient culturing system can be developed. Our research suggests that perfusion conditions improve substantially the performance of hematopoietic reactors. We have designed and tested a perfusion bioreactor system which is suitable for the culture of non-adherent cells and readily scaleable for clinical therapies. We have also identified conditions that allow rapid expansion of hematopoietic progenitors in our perfusion bioreactor without stromal cells. Eliminating the stromal layer eliminates the need for a stromal cell donor, reduces culture time, and simplifies the culture system. In addition, we have compared the expansion characteristics of both mononuclear and CD34+ cells, since the latter are frequently assumed to give a superior performance for likely transplantation therapies.

7.4

7.5

RAPID AND LARGE-SCALE PREPARATION OF PORCINE HEPATOCYTE SPHEROIDS AND THEIR FUNCTIONS IN CONTINUOUS SUSPENSION CULTURE

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Spheroidal aggregates (spheroids) of hepatocytes are expected to be utilized as good biocatalysts for a bioartificial liver module because their functional expressions are two or three times higher as compared to conventional monolayer culture on collagen-coated dishes. We previously reported that rat hepatocyte spheroids can be successfully formed by rotational tissue culture methods and large-scale suspension culture in a spinner flask within 24 hours. In the present study, we have isolated porcine hepatocytes and tried to prepare a large number of spheroids in the same manner. Porcine hepatocyte spheroids were formed in a 1 L-scale spinner flask within 24-36 hours by using the same medium as previously established for rat hepatocytes. The spheroids thereby formed were very stable in their morphology and functions for 1 weeks even when continuously cultured in suspension. Detoxification rate of ammonium added to the culture media and urea synthesis rate were almost the same, respectively, as expressed by the spheroids cultured in static condition in dishes or collagen gel. No significant decrease in cell number was observed during the culture period in terms of the cellular DNA amount. Rotational culture in hormone-supplemented human or porcine sera was also tried. Though gradual cell necrosis occurred in the spheroids, approximately a half the level of ammonium detoxification rate at the initiation of culture was sustained after 1 week of exposure to both the sera. Due to good mass transfer and easiness to scale up, continuous suspension culture of porcine hepatocyte spheroids would be one of promising bioartificial liver modules.

ANALYSIS OF PHYSIOLOGICAL FUNCTIONS OF FOOD USING MAMMALIAN CELL CULTURE

Hiroshi Shinmoto,* Masuko Kobori,* Tojiro Tsushida,* Kazuki Shinohara,* and Hiroki Murakami**
*National Food Research Institute, The Ministry of Agriculture, Forestry and Fisheries, 2-1-2 Kannondai, Tsukuba 305, Japan, **Graduate School of Genetic Resources Technology, Kyushu University, 6-10-1, Hakozaki, Higashi-ku, Fukuoka 812, Japan

We assessed cancer-preventive functions of food using cultured mammalian cell lines. Addition of non-dialyzable extracts from several vegetables to U-937 human leukemia cells induced macrophage differentiation markers, such as NBT-reducing activity and CD11b expression. The differentiation factor from spinach was a high molecular weight glycoprotein. Some vegetable extracts and extract from miso, a Japanese traditional fermented food, induced suppression of melanin production of B16 mouse melanoma cells. Immunoglobulin production stimulation activity was found in extracts from several vegetables and fruits. In the case of immunoglobulin production, use of a protein-free medium improved a sensitivity of the analysis.

7.6

7.7

ALTERATION OF APOPTOTIC CELL DEATH BY ENVIRONMENTAL AND GENETIC MODULATIONS

M. Al-Rubeai, R. P. Singh, A. N. Emery
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In seeking to implement high-performance bioreaction strategies it is necessary both to consider the response of cells to environmental insults and also the possibility that such cellular response may be genetically modified. Having already demonstrated that apoptosis responses can result from nutrient limitation and cytotoxic product accumulation (Singh et al., ESACT, Wurzburg, 1993), it has now been shown that similar responses can also be invoked by stressful hydrodynamic conditions in bioreactors. When hybridoma cells were subjected to an intensive energy dissipation regimes in a bioreactor a sub-population of smaller-sized apoptotic-like cells emerged. Analysis of the cell cycles of small and normal size populations indicated that greater proportions of S and G2 cells had become apoptotic and there was evidence of preferential survival of G1 cells. The possibility of beneficial genetic modification is suggested by the knowledge that the *bcl*-2 has been shown to be involved in the regulation of apoptosis in B lymphocytes and many other cell lines. A lymphoblastoid cell line which had been transfected with *bcl*-2 (Millner et al., Int. J. Cancer, 52, 636-644) was compared with non-transfected cells in a range of culture conditions and systems. Results indicated that the expression of this gene extended the life span of cells and prolonged culture duration following nutrient limitations. Transfected cells were significantly less susceptible to the induction of apoptosis by the absence of glutamine or the addition of Camptothecin (a DNA topoisomerase inhibitor).

APOPTOSIS AND NUTRITION: INVOLVEMENT OF AMINO ACID TRANSPORT SYSTEM IN REPRESSION OF HYBRIDOMA CELL DEATH

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Association of the extent of spontaneous apoptosis with the level of basal medium components (Franěk,F., Dolníková,J.(1991) Cytotechnology 7, 33-38) was investigated using mouse hybridoma cells cultured on the verge of starvation-induced apoptosis (Franěk,F. in: Animal Cell Technology (R.E. Spier et al., eds.) Butterworth-Heinemann 1994, pp.192-196), i.e. in basal medium diluted to 40-50% by saline. Investigation of the capacities of selected additives to the diluted media revealed different roles of individual groups of medium components. The starvation-induced death could be prevented to a significant degree by amino acid mixtures (MEM essential and non-essential amino acids) or by glutamine, while MEM vitamin mixture was quite ineffective. Except for glutamine, the optimum concentrations of amino acid additives exceeded the conventional concentrations by one order of magnitude, being in the range of 1 to 2 mM. The specific monoclonal antibody production rate in cultures supplemented with amino acid mixtures was strikingly low, whereas supplementation with glutamine alone or simultaneously with other amino acids resulted in antibody production rate comparable to the rate in undiluted medium. Testing of the effects of individual amino acids revealed that starvation death was prevented by alanine, serine, proline, asparagine, glutamine, glycine, histidine, and by some analogues, such as ß-alanine. Amino acids with strongly hydrophobic or charged side chains did not display this kind of activity. Most of the active amino acids belong to those classified as non-essential ones. The same amino acids have been frequently reported to be secreted by the cells into the medium. The specificity of the death-repression effect points to the involvement of the adaptively regulated amino acid transport systems A and possibly also N. The present findings indicate that the extracellular concentrations of certain amino acids may control, through the linkage with adaptive transmembrane transport system(s), the repression and de-repression of the apoptotic death programme. The work was supported in part by the Grant Agency of the Czech Republic (No. 503/93/2292).

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USING COMPUTERISED SYSTEMS IN MODERN VACCINE PRODUCTION PLANTS

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In a modern vaccine production plant controlling processes is almost synonymous to controlling data- and information flows. In 1992 a supervisory production information management system, ProSys, was implemented in our new facility for production of inactivated Poliomyelitis Vaccine. ProSys acquires relevant data in all phases of the production cycle, presents specific information to operators and production managers, generates the formal production batch records and archives the information.
Following the implementation of ProSys and using the same hard- and software lines, we initiated in 1993 the development of Batch Control System for Vaccines (BCSV). BCSV locates directly on the shop floor and functions as a man machine interface to operate bioreactors. The heart of BCSV is a recipe-management module which provides for in-house generation of executable, product-specific control recipes. The recipe template allows for a highly interactive operator approach and implementation of complex control strategies, e.g. based on 'estimators'. Also within BCSV are modules for local batch management, data and information management & presentation and for safety-interlocking. BCSV was developed generically, and is therefore to a large extend independent of the equipment that it monitors and controls.
Both, BCSV and ProSys are compliant to GMP, in addition, BCSV was modelled on the ISA standard for Batch Control Systems SP-88. Both were developed within the context of a QA system for development and maintenance of computer software.
An outline of ProSys and BCSV, including examples of interactive control recipes will be presented.

ON-LINE MONITORING AND CONTROL OF FED-BATCH CULTURE OF HYBRIDOMA WITH THE AID OF AN EXPERT SYSTEM

Toshiomi Yoshida, Tamaki Izuishi, Oh Gyusop, Takeomi Inoue, Mutsumi Takagi, Kazuhito Fujiyama, Tatsuji Seki
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A computer-coupled cultivation system for suspended cells or microcarrier depended cells was established using an on-line expert system, G2 (Gensym). The system could monitore various state variables such as cell density, specific glucose consumption rate, specific oxygen uptake rate, and specific growth rate. Lactic acid accumulation was effectively reduced by suppression of glucose feed resulting in a better cellular yield. Further limitation of glucose supply altered cellular energy metabolism to utilize more glutamine for supplementation of energy. It was found that the ratio of consumption rates of two substrate, glucose and glutamine, was correlated with the specific glucose consumption rate under a certain glucose limitation condition. Hereby, the glutamine consumption rate could be estimated using this correlation. A scheme of dual control of the concentrations of glucose and glutamine have been established. The robustness of the control was increased by use of the data of the specific oxygen consumption rate, which is also useful to estimate the glutamine consumption rate.

TUESDAY, SEPTEMBER 13, 1994

POSTER SESSION A

SESSION 1

1.5 **Establishment and Characterization of a Stable, Amplified Chinese Hamster Ovary (CHO) Cell Line Producing Insulin-like Growth Factor Binding Protein-1 (IGFBP-1)**
Charlotte Dyring, Mats Lake, Karin Mellström

1.6 **High Level Expression of Measles Virus Proteins from a Non-replicating Adenovirus Vector in Mammalian Cell Lines**
A.R. Fooks, A. Warnes, A. Racher, A.B. Dowsett, B.K. Rima, G.W.G. Wilkinson, J.R. Stephenson

1.7 **Cell Engineering to Optimise Protein Secretion: Analysis of Components of the Secretory System**
Carole Greenall, Nigel Jenkins, Mick Tuite, David Robinson, David Cook, Robert Freedman

1.8 **Cultivation of SV40 Large T Transfected Human Endothelial Cell Lines in Serumfree Medium**
Otmar Hohenwarter, Andrea Waltenberger, Christine Schmatz, Hermann Katinger

1.9 **Selection of Useful Physiological Variants from Mouse NSO Cell Populations**
J.R. Birch, H.K. Metcalfe, S.J. Froud

1.10 **Immortalization of Human Endothelial Cells by Simian Virus 40**
Katsuhide Miyake, Hideyo Kirinaka, Shinji Iijima

1.11 **Identification and Analysis of the Hepatocyte Growth Factor Activator**
Yuuki Morimoto, Takeshi Shimomura, Jun Kondo, Keiji Miyzazwa, Naomi Kitamura

1.12 **Production of *Spodoptera Exigua* NPV in an Established *SE* Cell Line**
A. van Oorschot, B. Möckel, J.M. Vlak, H.G. Miltenburger, J. Tramper, C.D. de Gooijer

1.13 **Bioprocess Applications of Cell Cultures Stimulated by Recombinant Cyclin E Expression**
Wolfgang A. Renner, Ellen A. Nollen, Vassily Hatzimanikatis, James E. Bailey, Hans M. Eppenberger

1.14 **A Reliable Strategy for the Achievement of Cell Lines Growing in Protein-free Medium**
Klaus Scharfenberg, Roland Wagner

1.15 **RAS Oncogene Enhances the Various Recombinant Protein Productivities of BHK-21 Cells Regulated by the CMV Promotor**
Kiichiro Teruya, Sanetaka Shirahata, Takahiro Yano, Junko Watanabe, Kiyohiko Seki, Kazuhiro Osada Hirofumi Tachibana

1.16 **Production of Recombinant Prourokinase by CHO Cells in a Perfusion Biosilon Microcarrier Culture System**
Chengzu Xiao, Zicai Huang, Zhengguang Zhang, Zhaolie Cheng, Fengzhi Li, Jianxin Ye, Zhixia Guo

SESSION 2

2.8 Improvement of the Performance of Commercially Available Insect Cell Culture Media for the Baculovirus-directed Production of Recombinant Proteins in Bioreactors
Marcus Ackermann, Dietmar H.J. Hellenbroich, Volker Jäger

2.9 The Effect of Unsaturated Fatty Acids on the Growth, Metabolism and Productivity of a Murine Hybridoma
M. Butler, N. Huzel

2.10 Effect of Environmental Conditions on Intracellular pH of Hybridoma Cells Cultured in Bioreactors
M. Cherlet, P. Franck, J.M. Engasser, A. Marc

2.10.1 Effect of Sodium Butyrate on Protein Production in Different culture systems
I. Chevalot, M. Dardenne, M. Cherlet, J.M. Engasser, A. Marc

2.11 Human Therapeutic Monoclonal Anti-D Antibody Produced in Long-term Hollow-fibre Culture. I. The Relationship Between Antibody Productivity and Metabolic Parameters
J.M. Davis, C.M. Lavender, K.J Bowes, B.S. Combridge, S.L. Kingsland

2.12 Metabolic Studies of a Hybridoma Cell Line
J. Dempsey, D.H. Glass, F.B. Ward

2.13 Expression of the ER Stress Proteins: Relationship to Productivity and Nutrient Availability
M.R. Downham, W.E. Farell, H.A. Jenkins

2.14 High-density Cytostat Cell Cultures with Homeostasis of Glucids and Amino Acids
D.A. Dubois, M.A. Mouyart, V. Degouys, A.O.A. Miller

2.15 Increase of Productivity in Recombinant CHO-cells by Enchanced Glucose Levels
J. Fieder, P. Schorn, R. Bux, W. Noé

2.16 Effect on Cultured Human Cell Lines of Polysaccharides in the Stem of Kiwi Fruit
Y. Fuke, Y. Yoshidome, K. Shinohara

2.17 Serum Free Monoclonal Antibody Production in a Hollow Fibre Bioreactor
Heidi Gerber, Kenneth McCullough

2.18 Media Additives for High Performance Cell Culture in Hollow Fiber Bioreactors
Randal A. Goffe, Joseph Y. Shi, Anna K.C. Nguyen

2.19 Specific Growth Rate as a Parameter for Tracing Growth Limiting Substances
Lena Häggström, Jan Ljunggren

2.20 Control of Growth and Metabolism of Animal Cells in Bioreactors
Lena Häggström, Jan Ljunggren, Kristina Martinelle, Lars Öhman

2.21 ***In Vitro* Conversion of Serine to Formate: Determination of Millimolar Quantities of Formate in Chinese Hamster Ovary Cell Cultures**
Thomas J. Ihrig, Matin A. Maulawizada, Benjamin D. Thomas, Fredric S. Jacobson

2.22 **Serum-free Media: Influence of the Definition of the Cell Culture Environment on Stability and Antibody Productivity of Mouse Hybridomas**
N. Kessler, G. Thomas, S. Bertrand, M. Aymard

2.23 **Influence of the Temperature on Process Optimization**
G. Kretzmer, R. Weidemann, B. Rössler, H. Lübben

2.24 **Production Kinetics of Interferon-gamma by CHO Cells in Different Culture Systems**
V. Leelavatcharamas, B. Amos, A.N. Emery, M. Al-Rubeai

2.25 **Catabolic Control of Hybridoma Cells by Glucose and Glutamine Limited Fed Batch Cultures**
Jan Ljunggren, Lena Häggström

2.26 **Influence of Glucose, Glutamin and Oxygen Supply on the Metabolism of Hybridomas and Primary Lymphocytes**
S.-T. Maier, R. Buchholz, U. Marx

2.27 **Ammonium Transport in Myeloma and Hybridoma Cells**
Kristina Martinelle, Lena Häggström

2.28 **Evaluation of the New Protein-free Powder Medium (MDSS1-Powder) for the Production of Monoclonal Antibodies**
O-W. Merten, H. Keller, E. Couve, S. Petres

2.29 **Cell Growth Regulatory Peptides from Bovine Caseins**
Shin-ichi Nagaune, Shuichi Kaminogawa

2.30 **Optimizing Vendor Proprietary Serum-free Media**
Anne Newell, Beth Sutton, Mike Glacken

2.31 **Hyperosmotic Hybridoma Cell Cultures: Mechanisms of Osmoprotection**
K. Oyaas, T.E. Ellingsen, N. Dyrset, D.W. Levine

2.32 **The Effect of Medium Composition on Growth and Monoclonal Antibody Production of Hybridoma Cells**
A. Sanfeliu, J.J. Cairó, C. Casas, F. Gòdia

2.33 **Investigations of High Cell Density Baculovirus Infection Using Sf9 and High Five Insect Cell Lines in the Low-cost SF-1 Medium**
E-J. Schläger, J. Stricker, J. Wippler, M. Foggetta

2.34 **Large Scale Production of Mammamodulin (MM). Development of a Low-cost Large Scale Production Process for Adherent Cell Growth**
E-J. Schläger, K. Christensen W. Küng

2.35 Transcription Level of CHO Cells Influenced by Amino Acids
T. Seewöster, F. Korioth, J. Frey, J. Lehmann

2.36 Inhibitors of Cell Growth: Accumulation and Concentration
S. Siwiora, M. Fingberg, H. Büntemeyer, J. Lehmann

2.37 In situ Ammonium Extraction in a Continuous Medium Recycling Bioreactor for the Production of Cell Growth Inhibitors
Claus Wallerius, Heino Büntemeyer, Jürgen Lehmann

2.38 Growth of Anchorage-dependent Mammalian Kidney Cells in Serum-free Media
D.E. Wyatt

2.39 Performance Characteristics of a Protein-free Medium for Mammalian Suspension Cells
D.E. Wyatt, J. Doak, R. Festen

2.40 Effect of pH on the Growth and Monoclonal Antibody Production in Batch Culture of a Human-human Hybridoma
Masaaki Yamaguchi, Yoshihito Shirai, Masahiro Shoji, Shuichi Hashizume, Hiroki Murakami

2.41 Simultaneous Prediction of the Concentrations of Glucose, Glumatine, Lactic Acid, Ammonia and Antibody in the Culture Broth of Mouse-mouse Hybridoma by Near Infrared Reflectrance Spectroscopy
Takuo Yano, Masami Harata, Tandanori Aimi, Yasuhisa Nakano

2.42 Control of Cell Metabolism in High Density Fed-batch Cultures by Dynamic Nutrient Feeding
Weichang Zhou, Jutta Rehm, Anna Europa, Wei-Shou Hu

SESSION 6

6.8 Hybridomas in a Bioreactor Cascade
Wilfried A.M. Bakker, Hendrik H. Beeftink, Johannes Tramper, Cornelis D. de Gooijer

6.9 On-line Immunoanalysis of Antibodies During a Long-term Continuous Fermentation
M. Biselli, M. Machnik, J.J. v.d. Pol, O. Stelling, U. Erhardt, C. Wandrey

6.10 Evaluation of a Cell Separator in Large Scale Perfusion Culture
Torsten Björling, Ulrich Dudel, Christel Fenge

6.11 Scale-up of Transient Gene Expression with the Semliki Forest Virus System
Horst D. Blasey, Kenneth Lundström, Alain R. Bernard

6.12 Respiration Quotient in Bicarbonate-buffered Mammalian Cell Culture
H.P. Bonarius, C.D. de Gooijer, J. Tramper, G. Schmid

6.13 Production of Monoclonal Antibodies in a Pilot Scale Fluidized Bed Reactor
C. Born, M. Biselli, C. Wandrey

6.14 A Systematic Approach for Modelling Animal Cell Cultures: Experimental Validation
V. Chotteau, G. Bastin

6.15 The Use of Software Sensors for Measurement and Control of Animal Cell Cultures
R.C. Dorresteijn, C.D. de Gooijer, J. Tramper, E.C. Beuvery

6.16 Scale-up of Ultrasonic Resonance Field Cell Separation Devices Used in Animal Cell Technology
Th. Gaida, O. Doblhoff, H. Katinger, W. Burger, M. Gröschl, E. Benes

6.17 Implication of Controlled Glycosylation and Apoptosis in Novel Hollow Fiber Bioreactor
Randal A. Goffe, Joseph Y. Shi, Anna K.C. Nguyen

6.18 The Effect of Flow Rate on the Immobilisation of Anchorge-independent Cells to Porous Glass Beads
L.M.D. Gonçalves, J.M. Rodriques, P.M. Alves, J.G. Aunins, M.J.T. Carrondo

6.19 Continuous Biotransformation by Immobilized Microorganism on the Surface of Cuprammonium Regenerated Cellulose Hollow Fiber (BMM)
C. Hatanaka, T. Haraguchi, S. Ide, G. Ishikawa, S. Manabe

6.20 Determination of Ks-values (Monod Constants) for Essential Amino Acids Using CHO and Hybridoma Cells in Batch and Chemostat Cultures
R. Heidemann, D. Lütkemeyer, H. Büntemeyer, J. Lehmann

6.21 Cell Culture Application of a Nutating Disk Pump
T. Heng, A. Allee, D. Weuster-Botz, M. Biselli, C. Wandrey

6.22 Small Microcarrier Aggregates Yield High Cell Density
William Hilligas, Dennis Inman, James Varani

6.23 Testing Computer Predictions of Viral Effects on Hybridoma Productivity
L. Johnson, P.J. Philips, C. Harbour, J.P. Barford

6.24 The Sensitivity of Uninfected and Baculovirus Infected Spodoptera Frugiperda Cells to Hydrodynamic Forces
N. Kioukia, M. Al-Rubeai, Z. Zhang, A.N. Emery, A.W. Nienow

6.24.1 MAB Production and Treatment of Rheumatoid Desease Using Encapsulated Anti-idiotypic Hybridoma Cells
C. Kloth, C. Riese, M. Cho, R. Buchholz, U. Marx

6.25 Expression of Recombinant Human Interleukin-2 in Baby Hamster Kidney Cells and Cultivation on Different Carriers
M. Knezevic, O. Hohenwarter, R. Grabherr, G. Blüml, R. Predl, F. Rüker, C. Shmatz, B. Felipic, P. Raspor, H. Katinger

6.26 Detachment and Mitosis of Adherent Cells under Shear Stress Conditions
G. Kretzmer, A. Ludwig, J. Tomeczkowski

6.27 Patterns of the Specific Productivity of the Murine Hybridoma, AFP-27, in Batch Culture
C.P. Marquis, J.P. Barford, C. Harbour, A. Fletcher

6.28 Bioreactor with a Radial-flow Non-woven Fabrid Bed for Animal Cell Culture
M. Motobu, M. Matsumura, S. Matsuo, H. Kataoka

6.29 Production of Human Growth Hormone by Protein-free Cultivation of Anchorage-dependent Cells with Porous Cellulose Carrier
N. Nomura, M. Matsumura, K. Saijo, T. Ohno, H. Kataoka

6.30 VERO Cell Growth During Different Modes of Cultivation
M.C. Philippi, B. Romein, R.X. van der Meer, K. Harbrink Numan, E.C. Beuvery

6.31 Application of Dialysis Techniques for Cost Effective Production of Biologicals with Immobilized Animal Cells
Ralf Pörtner, Armin Bohmann, Herbert Märkl

6.32 High Density Fixed Bed Culture of Animal Cells Using Macroporous Cellulose Carriers
Ralf Pörtner, Kentaro Shimada, Mastoshi Matsumura, Herbert Märkl

6.33 Comparative Study for the Propagation of Anchorage-dependent Cells Using Different Forms of Macroporous Carriers
A. Preissmann, R. Bux, P. Schorn, W. Noé

6.34 Control of Cell Concentration in Fed Batch Hybridoma Cultures
B. Romein, A.K. Shrivastava, C. Hellinga, J.P. van Dijken, K.Ch.A.M. Luyben

6.35 Monitoring and Modelling Hybridoma Cultures
B. Romein, I.Q.I.O. Melchy, C. Hellinga, J.P. van Dijken, K.Ch.A.M. Luyben

6.36 Improvement of Mammalian Fed Batch Culture
E-J. Schläger, K. Christensen

6.37 On-line Monitoring and Control of Bioprocesses Using Object Oriented Languages and Graphical User Interface
Michael R. Schuppenhauer, Irving J. Dunn

6.37.1 Non-invasive On-line Investigations of Industry Style Bioreactors
Micheal R. Schuppenhauer, Steffen Zeng, Irving J. Dunn

6.38 Aggregate Animal Cell Culture by Using Microbeads
Naohiro Shiragami, Kei Nishii, Hajime Unno

6.39 IL-4 Production Using Macroporous Microcarrier
J. Shirokaze, K. Yanagida, K. Shudo, K. Konomoto, K. Kamiya, K. Sagara

6.40 **Application of Cellcul-20A Bioreactor and Microcarriers for Large Scale Cultivation of VERO Cell**
Jia Li Song, Lie Shen Chen, Wen Lan Chen, Zhi Hui Chen, Shu Pei Dong, Xiao Hua Gu, Yin Liang Chen

6.41 **Cultivation of Animal Cells in a New Modular Minifermenter**
H. Weichert, F.W. Falkenberg, M. Krane, I. Behn, H.O. Nagels

6.42 **Subculture Method for Large Scale Culture Using Macroporous Microcarrier**
Koichiro Yanagida, Kiyoshi Kamiya, Junichi Shirokaze

6.43 **Large Scale Suspension Perfusion Culture Process with a Compact Centrifuge for Mammalian Cell Recycle**
Seiichi Yokoyama, Hiryuki Takamatsu, Kimihiko Hamamoto, Masamichi Motoki, Takami Arai, Kenji Ishimaru, Masahiro Kimura, Akira Tanokura, Shoji Ono, Kenji Nagura, Michiyuki Tokashiki

6.44 **Hybridoma Culturing in Aqueous Two-Phase Systems**
G.M. Zijlstra, L.A. van der Pol, J.J. van Weperen, C.D. de Gooijer, J. Tramper

ESTABLISHMENT AND CHARACTERIZATION OF A STABLE, AMPLIFIED CHINESE HAMSTER OVARY (CHO) CELL LINE PRODUCING INSULIN-LIKE GROWTH FACTOR BINDING PROTEIN-1 (IGFBP-1)

Charlotte Dyring, Mats Lake and Karin Mellström
Pharmacia BioScience Center, S-112 87 Stockholm, Sweden

Expression of human Insulin-like growth factor binding protein-1 (IGFBP-1) at high levels has been achieved in Chinese Hamster Ovary (CHO) cells by cotransfection and subsequent coamplification of expression vectors containing the IGFBP-1 cDNA and a dihydrofolate reductase (DHFR) cDNA gene into CHO DHFR-deficient cells. Stepwise selection of the $DHFR^+$ transformants in increasing concentrations of methotrexate generated cells which had amplified IGFBP-1 genes to around 100 copies. IGFBP-1 expression was found to increase with increasing gene amplification. These stably amplified cell lines expressed elevated levels of intact IGFBP-1 as well as one IGFBP-1 decomposition product. A biosensor, the BIACore, was used to determine the amount of IGFBP-1 secreted into the culture medium while a flow cytometric method was used to measure the intracellular level of IGFBP-1.

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HIGH LEVEL EXPRESSION OF MEASLES VIRUS PROTEINS FROM A NON-REPLICATING ADENOVIRUS VECTOR IN MAMMALIAN CELL LINES.

A.R. Fooks[1], A. Warnes[1], A. Racher[1], A.B. Dowsett[1], B.K. Rima[2], G.W.G. Wilkinson[3] and J.R. Stephenson[1].

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2. Queen's University of Belfast, UK.
3. University of Wales, Cardiff, UK.

The WHO has estimated that measles is responsible for about 1.6 million infant mortalities each year in developing countries. In addition, vaccine failures in developed countries have contributed to a renewed interest in measles vaccines. Among the candidates are recombinant defective adenoviruses expressing measles virus proteins. Our aim is to investigate the use of recombinant adenovirus vectors for large scale virus growth and protein production. In this study, a replication deficient adenovirus containing the measles virus N gene (nucleoprotein) was propagated on a specially constructed mammalian helper cell line (293 cells), and then used to infect a target cell population where expression of the cloned gene only is obtained from the strong CMV IE promoter. The recombinant (RAd68) produces up to 25% total cell protein as N protein in non-permissive human fibroblasts (MRC5 cells), increasing with time up to 96 hours post-infection. Expression is accelerated by stimulating the promoter with a chemical inducer (forskolin). TEM and IF techniques showed that the N protein accumulates in both the cytoplasm and nucleus of infected cells. Further studies will investigate the growth of recombinant adenoviruses in 293 cells.

CELL ENGINEERING TO OPTIMISE PROTEIN SECRETION: ANALYSIS OF COMPONENTS OF THE SECRETORY SYSTEM

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A series of recombinant clones, derived from the mouse NS/0 cell line, have been studied. These three clones secrete varying high levels of h1B4, a humanised monoclonal antibody. The secretion levels of protein have been measured by ELISA and are between 40 and 60 pg/cell/day. The clones and a series of wild-type cell lines have been analysed by western and northern blotting, using antibodies and cDNA probes against various chaperone proteins and components of the protein secretory system. The activity of protein disulphide isomerase, the enzyme which catalyses protein folding, has been measured in these clones and wild-type cells. Pulse-chase and subcellular fractionation experiments, using the high-secreting clone, have been done to monitor protein synthesis, secretion, and glycosylation. Further studies of the clones will be aimed at identifying possible rate- or yield-limiting steps in protein secretion.

1.7

1.8

CULTIVATION OF SV40 LARGE T TRANSFECTED HUMAN ENDOTHELIAL CELL LINES IN SERUMFREE MEDIUM

Otmar Hohenwarter, Andrea Waltenberger, Christine Schmatz and Hermann Katinger
Inst. of Applied Microbiology, University of Agriculture, Vienna, Austria

Human endothelial cells normally need a content of 10-15% serum and endothelial cell growth factor in the culture medium for longterm cultivation. After expression of SV40 largeT protein the serum requirement is reduced. We have developed a serumfree medium which allowed us to study the need for growth factors. SV40 largeT positive cell lines were found to be able to proliferate without any growth factor in the culture medium. Endothelial cell growth factor, epidermal growth factor and insulin can enhance the growth rate and prolong the in vitro life span. Endothelial cells capable of growth under serumfree conditions can be used for different kinds of in vitro tests which often require the use of defined media.

SELECTION OF USEFUL PHYSIOLOGICAL VARIANTS FROM MOUSE NS0 CELL POPULATIONS

J.R. Birch, H.K. Metcalfe, S.J. Froud
Celltech Biologics plc,

The mouse NS0 cell line is extensively used for the expression of recombinant antibodies. We have used glutamine synthetase as a selectable marker. In addition to efficient expression this also confers a nutritional advantage namely glutamine independence and reduced ammonia accumulation. The NS0 cell line (ECACC 851LO503) has an absolute requirement for cholesterol. The insolubility of cholesterol is a disadvantage in the development of serum free and particularly protein free media. We will describe the isolation and characteristics of a cholesterol independent variant of NS0. In addition to altering the nutritional requirements of the NS0 cell line we have also found that growth characteristics of transfected cell lines vary greatly from clone to clone and we have taken advantage of this variability to isolate clones with growth profiles suited to our production process.

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IMMORTALIZATION OF HUMAN ENDOTHELIAL CELLS BY SIMIAN VIRUS 40

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Endothelial cells, which cover inside of the vascular wall, play very important roles in angiogenesis, blood vessel-related disease and cancer metastasis. Stable and long term *in vitro* cultivation of endothelial cells is still difficult. Therefore, establishment of stable human endothelial cell lines is very important for development of new therapeutical agents for blood vessel-related disease and cancer metastasis. To obtain immortalized human endothelial cells without losing their original characteristics, human endothelial cells derived from umbilical cord vein was transfected with an origin defective SV40 virus based plasmid and two clones were obtained. These clones showed prolonged life span, typical cobble stone morphology and endothelial cell specific characteristics such as production of fibrinolysis related factors . One of the clones acquired infinite life span and could pass more than 300 generations but was morphologically transformed. The clone produced tissue plasminogen activator(tPA) and tPA inhibitor(PAI-1) but not factor VIII related antigen.We also tried to obtain cell lines with mutated SV40 with a temperature sensitive largeT antigen. We are now investigating endothelial cell specific phenotypes of the clone at 33°C and 37°C.

IDENTIFICATION AND ANALYSIS OF THE HEPATOCYTE GROWTH FACTOR ACTIVATOR

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The hepatocyte growth factor(HGF) is synthesized and secreted as an inactive single-chain precursor and then converted to an active two chain form by endoproteolytic processing. The recombinant human HGF(r-hHGF) produced by Chinese hamster ovary cells transfected with hHGF cDNA(r-CHO) was the two chain form in fetal bovine serum(FBS) containing culture. However, in serum-free culture the non-processed r-hHGF, single chain form was detected with two chain form r-hHGF. We purified the protease that proteolytically processed single chain r-hHGF to two chain form r-hHGF. A protease was purified to give a single peak from the culture supernatant by use of several column chromatographies. When this protease was added to serum-free culture of the r-CHO cells, the proteolytic processing of single chain r-hHGF to two chain form r-hHGF was completely achieved. Then we purified from human serum a novel serine protease(HGF activator) responsible for this process, and cloned cDNA from human liver c DNA library based on partial amino acid sequences. The nucleotide sequence showed the HGF activator to consist of multiple domains homologous to those in the blood coagulation factor XII.

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PRODUCTION OF *SPODOPTERA EXIGUA* NPV IN AN ESTABLISHED *SE* CELL LINE

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Baculoviruses are potential biological control agents. Traditionally, baculoviruses are produced in insect larvae, which is a laborious process and difficult to scale-up. Modern cell-culture technology facilitates the growth of insect cells in suspension in bioreactors. The cells can be infected with the extracellular form of the virus (ECV) and produce the occluded form of the virus (polyhedra) found in nature, which can then be easily purified. The existing *Spodoptera exigua* cell-line USC-1 (Gelernter and Federici, 1986, Journal of Invertebrate Pathology **48**: 199-207), was adapted to growth in suspension and investigated for the production of *Spodoptera exigua* nuclear polyhedrosis virus (SeNPV). The effect of different MOI (multiplicity of infection) was studied in Erlenmeyer flasks. The yield in $TCID_{50}$ units varied between 1E6 and 4E6 per ml, the polyhedra concentration reached 5E7 per ml. In conclusion suspension cultures of Se-cells can be infected with SeNPV producing relatively low amounts of ECVs but high amounts of polyhedra.

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Bioprocess Applications of Cell Cultures Stimulated by Recombinant Cyclin E Expression

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James E. Bailey and Hans M. Eppenberger

Recombinant cyclin E overexpression can overcome the requirement of wild type CHO K1 cells for external growth factor stimulation. The properties of this approach, its suitability for the production of recombinant proteins, and its application in bioprocessing have been further explored. Production cell lines were engineered to grow in serum- and protein-free medium, and the productivities and the growth patterns of such cultures after the transfer into the new protein-free medium were investigated. Bioreactor cultivations were performed in order to demonstrate the applicability of this approach.

A RELIABLE STRATEGY FOR THE ACHIEVEMENT OF CELL LINES GROWING IN PROTEIN-FREE MEDIUM

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The reduction of costs for the production of recombinant proteins with mammalian cell lines is dependent on an efficient growth in serum- or better protein-free medium. In particular, protein-free formulations have the advantage to lower the burden on downstream processing by minimizing the total protein content. However, good growth in protein-free medium is not always easy to achieve. Therefore, we developed a reliable strategy to adapt permanent mammalian cell lines to a growth in protein-free media. The adaptation process from the serum dependent growth to a complete medium-protein-independent culture takes 6 to 12 weeks. The method is based on a directed strategy consisting of a sequential reduction of the serum content by using the substrate dependance and the altered adherance characteristics of the cell lines.

RAS ONCOGENE ENHANCES THE VARIOUS RECOMBINANT PROTEIN PRODUCTIVITIES OF BHK-21 CELLS REGULATED BY THE CMV PROMOTER

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When applying the oncogene activated production (OAP) system to enhance recombinant protein production rates in BHK-21 cells, we found that a single gene amplification of the *ras* oncogene using the dihydrofolate reductase gene and selecting with 50 nM methotrexate resulted in a remarkable enhancement of the human interleukin-6 (hIL-6) production rate. Furthermore, we investigated the effectiveness of the OAP system for enhancing the productivities of various recombinant proteins such as hIL-6, human monoclonal antibodies, human interleukin-5, human erythropoietin and human granulocyte colony stimulating factor. The recombinant protein productivities of BHK cells under the regulation of the CMV promoter were enhanced by the transfection and amplification of the *ras* oncogene, suggesting that the *ras* oncogene is useful for rapidly establishing highly productive cell lines that produce various recombinant proteins.

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PRODUCTION OF RECOMBINENT PROUROKINASE BY CHO CELLS IN A PERFUSION BIOSILON MICROCARRIER CULTURE SYSTEM

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CL-11G, a new genetically-engineered CHO cell line, which was transformed with a transfer vector pMTSV-du containing pro-UK and dhfr genes, controlled by MT and SV40 promoter respectively. The expression level of pro-UK was much higher than previous CD-1 cell line. When cultivated with DMEM/F12 media, plus 5% newborn calf serum, 1 μmol/L MTX and 10 KIU/ml aprotinin, The pro-UK yield was about 500 IU/10^6 cells/day. The growth rate of CL-11G was a little bit fast than CD-1, its doubling time was about 37h. Using orthogonal design we have developed a low serum medium supplement-BIGBEF. So we could reduce serum to 1% and without affecting growth rate and pro-UK secretion of CL-11G cells. With 1.5L celligen bioreactor and a modified perfusion system, when the concentration of microcarrier was 50 mg/ml, the cell inoculum wad 1.7×10^5 cells/ml, and the perfusion rate was gradually increased from 0.5 to 1.5 working volume/day, the cell density could reach 2.06×10^7 cells/ml after 10 days cultivation, and the pro-UK production was also increased along with increasing cell density stably.

With CM-PECC membrane radial flow chromatography, MPC chromatography, HPLC Sephacryl S-100 gel chromatography and p-aminobenzamidine Sepharose chromatography, the pro-UK was purified to pruity of 95% and with specific activity of 1.3×10^5 IU/mg of protein approximately.

Improvement of the Performance of Commercially Available Insect Cell Culture Media for the Baculovirus-directed Production of Recombinant Proteins in Bioreactors

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During the past decade the baculovirus expression system has been established as a rapid and simple tool for the expression of heterologous recombinant proteins. The culture media normally used for propagation of the lepidopteran host cell lines contain high concentrations of amino acids but relatively low concentrations of glucose (0.7 to 2.5 g l^{-1}). Using TC-100 or serum-free EX-CELL 401 media to cultivate IPLB-SF21-AE or Sf9 cells in membrane-aerated stirred tank perfusion bioreactors and airlift bioreactors glucose became the growth limiting component at cell concentrations of 2 to 5×10^6 ml^{-1}. Feeding of the culture with additional glucose (and glutamine) facilitated a prolongation of exponential cell growth in batch culture up to cell concentrations of 1×10^7 ml^{-1}. In continuous perfusion culture glucose (and glutamine) concentrations of the feed medium could be increased significantly allowing a markedly reduced perfusion rate without exhaustion of nutrients in the culture medium and resulting in a more economical production process. Results of fed-batch and perfusion cultures will be presented and discussed.

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THE EFFECT OF UNSATURATED FATTY ACIDS ON THE GROWTH, METABOLISM AND PRODUCTIVITY OF A MURINE HYBRIDOMA

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The addition of linoleic acid or oleic acid (10-50 μM) improved the cell yield of a murine hybridoma (CC9C10) grown in serum-free media. The maximal cell yield was obtained in an oleic/linoleic mix (25 μM). The fatty acids also caused a significant increase (78%) in the volumetric Mab titre. However, continued growth of the cells in the fatty acid supplemented media over several passages resulted in a gradual deterioration of the Mab yield concomitant with the appearance of lipid inclusions in the cytosol. Incorporation studies with ^{14}C-linoleic acid showed the distribution of the fatty acid into cell fractions was as follows: polar lipid (80%), non-polar lipid (16%) and free fatty acid (4%). The metabolic effects of the fatty acid addition were studied by measurements of rates of oxidation of radioactively labelled substrates. Cells grown in fatty acids showed significant changes in energy metabolism with a decreased (x2) rate of oxidation of glutamine and an increased (x3) rate of oxidation of glucose. The flux of glucose through all three measured metabolic pathways (glycolysis, pentose phosphate pathway and TCA cycle) was enhanced in cells that had been grown in the fatty acids. The significance of the use of unsaturated fatty acids on hybridoma growth, metabolism and productivity will be discussed.

EFFECT OF ENVIRONMENTAL CONDITIONS ON INTRACELLULAR PH OF HYBRIDOMA CELLS CULTURED IN BIOREACTORS

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For a better understanding of the effect of environmental parameters on cell behaviour in mass cultures, kinetic studies have to be extended at the intracellular level. One interesting parameter is the intracellular pH (pHi) which is implicated in the control of vital cellular functions. The pHi monitoring has been mainly performed on cells in laboratory static flasks, without control of the cell metabolism. In this study, the pHi has been monitored during hybridoma cultures in batch and continuous reactors, using different controled operating conditions. The pHi measurements have been performed by flow cytometry analysis of cells loaded with the pH-sensitive dye BCECF-AM. The procedure has been adapted and validated to limit the interferences from the cell state on the pHi analysis all over the cultures.

When the extracellular pH (pHe) is maintained constant at 7.0, results in batch culture and in continuous culture operating at various dilution rates, point out an increase of the pH_i from 7.35 to 7.60 when the actual specific growth rate increases from 0.018 to 0.044 h^{-1}, indicating the possible relation between cell growth and pH_i. Elsewhere, other experiments performed with various pHe values or NH4Cl levels showed a decreasing of the growth rate for extrem values, pHe < 6.7 or NH4 > 13 mM, while the pHi is maintained at a near constant value. Then, when the cells are in a growing state, the negative effect of these two parameters on the cell proliferation could not be explained by an effect on the pHi.

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EFFECT OF SODIUM BUTYRATE ON PROTEIN PRODUCTION IN DIFFERENT CULTURE SYSTEMS

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Sodium butyrate has been reported to increase the production of secreted proteins in small scale culture systems. In this study, we present the butyrate effect on the production of a membrane-bound enzyme and of an antibody in different culture modes. In a first step, we have tested the butyrate influence on the production of the non-secreted GGT enzyme by r-CHO cells growing in exponential phase in T-flasks. The optimal sodium butyrate level, allowing an increase of the GGT activity from 19 to 52 mU.10^{-5} cells, was found to be around 5 mM. Neither the catalytic constants nor the molecular masses of the two GGT sub-units were affected.

In a next step we have compared the induction level with the CHO cells growing on microcarriers or as aggregates inside spinner flasks. Despite the fact that the GGT synthesis was strongly reduced in the absence of an appropriate cell support, the specific GGT activity has been similarly induced by a factor 2 in both systems.

In a last step, we have investigated the sodium butyrate effect on mass cell cultures in bioreactors. When the butyrate was added after 125 h of fed-batch culture, the induction level of GGT was found lower than in previous results. This can be explained by the 3 times lower specific growth rate (μ) at the induction time. The same observation has been done for the induction of antibody production by hybridoma cells. While the induction was effective in batch culture, at a μ of 0.02 h^{-1}, it was unapparent in fed-batch mode, when the μ was near zero. Then the cell physiological state seems to be predominant for the induction of protein production by sodium butyrate.

HUMAN THERAPEUTIC MONOCLONAL ANTI-D ANTIBODY PRODUCED IN LONG-TERM HOLLOW-FIBRE CULTURE I. THE RELATIONSHIP BETWEEN ANTIBODY PRODUCTIVITY AND METABOLIC PARAMETERS

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Polyclonal Rhesus anti-D antibody obtained from immunised volunteers is currently given to Rhesus-negative women at parturition to prevent Haemolytic Disease of the Newborn in their second and subsequent children. However, problems in maintaining a sufficient base of immunised volunteers has limited supplies, and monoclonal anti-D is being developed as a replacement. This is produced *in vitro* by Epstein-Barr virus transformed lymphoblastoid cells in long-term serum-free, antibiotic-free hollow-fibre culture. In this paper we examine the metabolic characteristics of anti-D producing cell lines in this culture system, and the relationship between these parameters and antibody productivity during the course of the culture. Glucose, lactate, glutamine and ammonia concentrations were measured off-line in medium samples from the intracapillary circuit, whilst dissolved oxygen tension was measured on-line. Antibody concentration was measured off-line in the medium harvested from the extracapillary circuit. The relationship between various derived parameters was examined, and correllations with antibody productivity sought.

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METABOLIC STUDIES OF A HYBRIDOMA CELL LINE

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The understanding of energy utilisation of antibody producing hybridoma cells is important for the optimisation of antibody secretion. A secreting and a non-secreting murine hybridoma cell line were grown in continuous culture at a variety of dilution rates. The limiting nutrient was determined and the metabolic quotient of the limiting nutrient was plotted against the specific growth rate for each dilution rate to determine the growth yield and metabolic coefficient of both cell lines. The maximal specific growth rate was also determined for each cell line. The proportion of energy devoted to antibody production was determined from this. It was shown that the amount of energy devoted to cell maintenance was lower for the non-producing cell line with the growth yields being similar. The secretion of antibody and the production of waste metabolites at varying flow rates were also determined and compared for both cell lines. The results obtained from this work can be used to improve the feeding of hybridoma cells in continuous and batch culture and may offer a means to increase total and specific antibody secretion.

EXPRESSION OF THE ER STRESS PROTEINS: RELATIONSHIP TO PRODUCTIVITY AND NUTRIENT AVAILABILITY.

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Eukaryotic cells respond to particular conditions of stress, such as glucose starvation or treatment with calcium ionophore, by inducing the expression of the endoplasmic reticulum (ER) proteins GRP78, GRP94 and ERp72. These proteins appear to influence the folding and processing of nascent polypeptides in the ER. Controlling the expression of these stress proteins may provide the potential to enhance recombinant protein yield. The expression of GRP78, GRP94 and ERp72 was investigated during batch growth of a recombinant myeloma (NSO) secreting the chimeric antibody, B72.3. All three ER proteins were expressed throughout growth, but were further induced at a point that was coincidental with depletion of glutamate from the medium and a halt or marked slowing in the uptake of glucose. Reducing the initial concentrations of glucose or glutamate in the medium resulted in a more pronounced induction of these ER proteins, particularly GRP94. Feeding the cells with glucose, glutamate or a mixture of amino acids during batch growth altered both the productivity of the cells and the pattern of ER protein expression.

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HIGH-DENSITY CYTOSTAT CELL CULTURES WITH HOMEOSTASIS OF GLUCIDS AND AMINO ACIDS

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Continuous production of biologicals from high-density cultures requires that cell physiology be maintained in high-performance conditions for several weeks. In the perfused bioreactors used in this study, continuous supply of the main nutrients is adapted to the culture demand, by closed-loop control of their concentrations in the culture. For biochemical analyses, a sample processor ensure intermittent sampling of ultrafiltered supernatant from the bioreactor. On-line determination of amino acids and ammonia is achieved by reversed-phase HPLC after derivatization with 9-Fluorenylmethyl chloroformate. Glucids and organic acids are on-line determined by isocratic HPLC on a size exclusion /ion exclusion column. The actual values for glucids and amino acids are used to automatically adapt their supply to the culture. Efficient withdrawal of waste metabolites depends on the adequacy of perfusion rate to cell concentration. For cultures in perfused cytostat mode, cell outflow is controlled to keep the culture in a growth state at high and constant cell concentration. The resulting homeostasis is shown to be helpful in sustaining cellular metabolism for continuous operation at high regimen.

INCREASE OF PRODUCTIVITY IN RECOMBINANT CHO-CELLS BY ENHANCED GLUCOSE LEVELS

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Mammalian cells are the preferred production organisms, which can produce recombinant proteins in their native, fully glycosylated form. However, the overall cellular productivity is limited, when compared to yeast and procaryotes. Therefore the main target in bioprocess development for mammalian cell cultures is the enhancement of product yield to achieve economical and competitive product titers.
In the outlined presentation we investigated the effects of various compounds on the specific productivity of serum-free growing recombinant CHO cells, with special regard to the effects of the carbohydrate sources. We observed a strictly proportional expressed product formation when compared to glucose consumption. On the other hand, the glutamine metabolism seems to be down regulated during enhanced product formation phases. The growth rate seems to be 'inverse' regulated depending on the availability of the main carbohydrate (glucose).
The data which will be presented and discussed in terms of an 'easy to perform' process optimization by controlled addition of glucose / glutamin.

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EFFECT ON CULTURED HUMAN CELL LINES OF POLY-SACCHARIDES IN THE STEM OF KIWI FRUIT

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The water extract fraction of the stem of kiwi fruit greatly accelated the growth of cultured human-human hybridoma cells. The growth-promoting substances were isolated by precipitation with different alcohol concentrations. Growth-promoting effect on human hybridoma HB4C5 was greatest with the polysaccharide fraction precipitated by 60% EtOH. The polysaccharide fraction precipitated by 60% EtOH was treated at 100°C for 10 min in order to eliminate the effects of protein components present in only small quantities, and because polysaccharides are extremely stable at high temperature. The growth-promoting activity of heat-treated samples was 3 times that observed in human-human hybridoma cell lines, 5 times greater than in macrophages, and double that in U-937. In contrast, hardly any growth-promoting activity was observed in the human intestinal epithelial cell line or in human fibroblasts a form of anchorage dependent cell. The growth rate of MKN-28 (human stomach cancer cell line) decreased slightly with an increase in the concentration of added polysaccharide. The growth-promoting substance was a polysaccharides of high molecular weight.
The authors are currently attempting to both identify the active substance and clarify the mechanism underlying the growth promotion.

SERUM-FREE MONOCLONAL ANTIBODY PRODUCTION IN A HOLLOW FIBRE BIOREACTOR.

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The routine application of monoclonal antibodies (Mab) in diagnostic tests requires large batches of hybridoma cell supernatants containing Mab in high concentrations. It is also favourable to produce these under serum-free conditions to facilitate antibody purification. HC26, an anti-classical swine fever virus hybridoma producing an IgG2b antibody, was therefore cultured both in flasks and the Tecnomouse bioreactor. DMEM/F12 medium containing 10%(v/v) FBS, 1%(v/v) FBS plus 1%(v/v) Nutridoma SR or only 1%(v/v) Nutridoma SR were compared for their relative efficiency at supporting growth and antibody productivity of this hybridoma. With all three media, the HC26 hybridoma readily adapted to growing in the Tecnomouse. It was noted that the Mab concentration increased to approximately 2mg/ml - that is at least 100 times higher than in flask cultures - by 5 to 8 weeks regardless of the level of serum in the medium. Once the cells were producing high Mab concentrations, these were consistently higher in medium with lower serum concentrations (2.3mg/ml maximum in "high serum" medium, compared to 7.9mg/ml in "low serum" medium). Current work is investigating the growth of other hybridoma lines in serum-free medium in the Tecnomouse, in order to determine if the above observations are generally applicable or particular to HC26. One part of this work is concentrating on the growth of cells from fusions following *in vitro* primary immunizations; such hybridomas are often IgM-producing. Overall, the main aim of our work is to have the maximum replacement of animal usage in hybridoma technology. In this context, it is essential to identify what, if any, differences exist between hybridomas secreting different isotypes in terms of their adaptability to serum-free conditions in a hollow-fibre bioreactor.

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MEDIA ADDITIVES FOR HIGH PERFORMANCE CELL CULTURE IN HOLLOW FIBER BIOREACTORS

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Oxygen mass transfer has long been recognized as a major limiting factor in hollow fiber bioreactor cell culture. A variety of strategies have been explored to overcome this pervasive problem. Recently, a novel engineering design has resulted in a high performance bioreactor (HPBr) that has proven to be highly successful [1,2]. In this study the Unisyn Micro Mouse™ commercial small scale bioreactor has been investigated with a new approach. Blood substitutes, developed for human use, have been employed as media additives to enhance oxygen transport to cells in culture. An extensive investigation has been completed. This involved three different blood substitutes: two types of stabilized bovine hemoglobin; and, a perfluorochemical (PFC) emulsion. Extraordinarily high performance have been achieved with these additives. The most impressive results were obtained within the first week after inoculating the bioreactor. Operating costs were equivalent to, or lower than when fetal bovine serum (FBS) media additive was used.

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SPECIFIC GROWTH RATE AS A PARAMETER FOR TRACING GROWTH LIMITING SUBSTANCES

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Substrate limited fed batch cultures were used to study growth of hybridoma cells. In a strict sense, fed batch means the continuous feeding of a nutrient, to a stirred tank reactor, at a rate that is lower than the culture's maximum consumption rate of that nutrient according to: $\frac{F}{V} \cdot S_0 < q_{S_{max}} \cdot N_v$ where $\frac{F}{V} \cdot S_0$ is the amount of the fed substrate in mM h^{-1} (F=feed flow rate, V=volume in the fermenter, S_0=substrate concentration in the feed, q_S=specific substrate consumption rate, N_v=viable cell concentration). This procedure usually reduces the specific growth rate (μ) compared to an unlimited batch culture. If a constant flow rate is applied, μ decreases successively as the culture grows while an exponentially increased flow rate can result in a constant μ, although lower than μ_{max}. In this work a constant flow rate of the feeding solutions was applied in our studies on glucose, glutamine and combined glucose and glutamine limited fed batch cultures of hybridoma cells. Surprisingly, our results show that μ was not markedly reduced in the fed batch cultures. In all cultures μ reaches its maximum early during growth and decreases thereafter so that no exponential growth occurs. The decline in μ occurs when the cell density still is low and none of the major substrate components are depleted. Neither glutamine nor glucose seemed growth rate limiting although the glutamine concentration (>0.085 mM) was lower than reported K_S-values and glucose was below 0.9 mM. The data show that the decrease in μ does not depend on inhibition by metabolic by-products or energy source limitation but some other factor(s) become growth rate limiting in all the cultures. The shape of the μ curve reveals further that it appears to be potential for further cell growth in the dual substrate limited fed batch culture as judged by a comparison of μ in the different cultures.

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CONTROL OF GROWTH AND METABOLISM OF ANIMAL CELLS IN BIOREACTORS

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Bioprocess control for the future is a much broader concept than the mere control of parameters as pH, temperature and dissolved oxygen. It is the designed control of cellular metabolism and physiology in order to attain a specific goal of a process. To do this, key parameters have to be identified and the underlying mechanisms understood. One parameter that has received little attention is the specific growth rate (μ) of cultured animal cells. We found that in batch cultures of myeloma, hybridoma and insect cells μ reaches its maximum early during culture and declines thereafter so that no exponential growth phase occurs. If the reasons for the decline in μ could be understood it should be possible to improve cell growth. Hybridoma cell growth was studied in substrate limited fed batch cultures, a technique used to control growth rate in microbial cultures. The results showed that the declining μ was not caused by energy source limitation, exhaustion of major medium components or by inhibition of metabolic by-products. In fact, by addition of specific factors, μ could be maintained at its maximum level in batch and controlled in fed batch cultures. The increased growth rate was accompanied by an increase in viability. Further, the energy metabolism of hybridoma cells could be controlled in substrate limited fed batch cultures. In a dual substrate limited fed batch culture the formation of lactate, ammonium and alanine became negligible, while, at the same time, cell and antibody production was slightly increased. This was due to an increased efficiency of the energy metabolism as fluxes in energy yielding pathways were changed.

IN VITRO CONVERSION OF SERINE TO FORMATE: DETERMINATION OF MILLIMOLAR QUANTITIES OF FORMATE IN CHINESE HAMSTER OVARY CELL CULTURES

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Although production of formate has not been previously reported in cell culture, we have measured by ion chromatography concentrations as high as 6 mM. To determine if the generation of formate is folate dependent, cell cultures were set up in 60 mm dishes with media that contained from 0 to 14.8 µM folate, and were evaluated for cell growth by measuring total lactate dehydrogenase (LDH) and for formate production. While media folate concentration had no effect on cell growth, formate levels were reduced in the absence of folate. Since the major source of one carbon fragments mediated by folates is serine, similar experiments were set up where medium serine concentration was varied in the presence of standard folate concentrations. Formate synthesis increased in direct proportion to the amount of serine present up to 1500 µM, with no formate produced in the absence of serine. In these cultures, serine is utilized at a rate proportional to its concentration, and approximately 60% of the serine utilized can be accounted for as formate. To determine if formate is toxic or cytostatic, cells were seeded at low density (20,000 cells/ml) or high density (600,000 cells/ml) with 0 to 20 mM formate and measured for cell growth up to 8 days. At low cell density cultures were about 20% growth-inhibited with 4 mM formate and 60% growth-inhibited with 10 mM formate. There was no effect on growth or productivity of recombinant proteins by formate concentrations up to 10 mM in cultures inoculated at high cell density.

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SERUM-FREE MEDIA : INFLUENCE OF THE DEFINITION OF THE CELL CULTURE ENVIRONMENT ON STABILITY AND ANTIBODY PRODUCTIVITY OF MOUSE HYBRIDOMAS

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Growth of hybridoma cells has been achieved in numerous serum-free formulations but only scarce informations are available as regards the potential variations in antibody productivity and karyological number of cells during the weaning process and further long-term cultivation in defined environment. Using different strategies, three mouse hybridoma lines (IgG1, IgG2a, IgM) were adapted to grow in three different serum-free media (BioWhittaker) : UltraCulture TM, a high-protein medium, UltraDoma TM, a low protein medium and Ultradoma-PF TM, a protein-free medium. The selected cell lines have been shown previously to express differences in terms of stability when cultivated in either FBS supplemented medium or UltraCulture TM (Kessler et al, 1993, In Vitro 29A, 203-207; Kessler et al, 1993, Esact Meeting, Würzburg); as a function of these characteristics, adaptation to UltraDoma TM and UltraDoma-PF TM started from UltraCulture TM and/or FBS-DMEM grown cells. Cell growth, antibody producing activity (Ig secretion on a per cell basis, immunofluorescense reactivity) and karyological analysis were regularly checked all along the adaptations procedure and then over a 4 months period after transferring hybridoma cells to serum-free conditions. Modifications in productivity and karyotype are expressed and discussed with respect to the cell line, the cell generation number and the definition of the cell culture environment.

INFLUENCE OF THE TEMPERATURE ON PROCESS OPTIMIZATION

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Mammalian cell cultivation is ussally performed at 37°C, the temperature of the donor and thus deduced to be the optimal temperature. Nevertheless, our studies showed that the variation of the temperature could led to benefits for example in shear stress restistance of the cells. Concomitantly the glucose consumption and the lactate production rates were reduced with decreasing temperature. Surprisingly the production rate of the product was not affected by the temperature reduction. This led to higher yield according to the substrate. Batch, repeated-batch and perfusion cultivations with adherent baby hamster kidney (BHK) cells will be presented. Batch cultivations were carried out at 37 and 30 °C as well as cultivations with temperature shift (37/30°C) after 90 hours of cultivation. Repeated-batch and perfusion cultures were conducted with temperature shift. The perfusion cultures were monitored by off line and on line analysis. The lactate concentration proved to be growth limiting, therefore, the lactate concentration was the parameter controlling the perfusion rate. Control was performed manually or by a means of a computer. First results obtained with batch cultivations of suspension Chinese hamster ovary (CHO) cells showed similar positive effects. These cells cultivated under serum-free conditions showed a prolonged stationary phase with reducing temperature. With both cell lines the positive effect of low temperature on cultivation time and protein yield is shown.

PRODUCTION KINETICS OF INTERFERON-γ BY CHO CELLS IN DIFFERENT CULTURE SYSTEMS

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Growth, interferon-γ productivity and intracellular content and cell cycle of CHO cells were studied in batch, continuous and dialysis perfusion culture. In batch culture a positive association between growth rate and productivity was found. Intracellular interferon increased with increasing cell size and progression through the cell cycle (i.e. from early G1 to G2/M) indicating similarity to protein accumulation and growth in biomass during the cell division cycle. A positive linear relationship was also found between the proportion of S phase cells and specific growth rate. Such a relationship can be used to predict future culture proliferative capacity. Dialysis culture, with a medium dilution rate of 0.5 volume /day, achieved substantially higher cell density than in batch or continuous cultures (4x10e6 cell/ml). The enhanced cell concentrations, longer operation times and retention of the product in the reactor led to a 14 times increase in interferon concentration compared to batch culture. However, the specific production rate was still similar to that obtained in batch and continuous cultures (about 5.5x10e-3 IU/cell/hr). The use of dialysis culture allowed substantial savings in the use of serum as it was not added to the perfusion medium.

CATABOLIC CONTROL OF HYBRIDOMA CELLS BY GLUCOSE AND GLUTAMINE LIMITED FED BATCH CULTURES

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The energy metabolism of cultured animal cells has been a matter of considerable interest during many years. Nevertheless, the metabolic background to overflow metabolism, that is the excretion of partly oxidised end products, still needs further attention. The following hypothesis was formulated: High glucose and glutamine concentrations lead to overflow metabolism while limiting concentrations provoke the use of more efficient energy pathways and reduce formation of inhibitory by-products. Substrate limited fed batch cultures of hybridoma cells were used to study this hypothesis. A glucose limited fed batch, a glutamine limited fed batch and a combined glucose and glutamine limited fed batch culture were compared to batch culture. The specific rates for consumption of glucose and glutamine were dramatically influenced in fed batch cultures resulting in major metabolic changes. Glucose limitation decreased lactate formation but increased glutamine consumption and ammonium formation. Glutamine limitation decreased ammonium and alanine formation, glucose consumption, and lactate formation. The formation of lactate, alanine and ammonium was negligible in the dual substrate limited fed batch culture. The efficiency of the energy metabolism increased, as judged by the increase in the cellular yield coefficient for glucose by 100% and for glutamine by 150% and by the change in the metabolic ratios lac/glc, ala/gln and NH_x/gln, in the combined fed batch culture. The data indicate that a larger proportion of consumed glutamine enters the TCA cycle through the glutamate dehydrogenase pathway which releases more energy from glutamine than the transamination pathway. We suggest that the main reasons for these changes are decreased uptake rates of glucose and glutamine which in turn lead to a reduction of the pyruvate pool and a restriction of the flux through glutaminase and lactate dehydrogenase.

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INFLUENCE OF GLUCOSE, GLUTAMIN AND OXYGEN SUPPLY ON THE METABOLISM OF HYBRIDOMAS AND PRIMARY LYMPHOCYTES.

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In this work we established an assay for studying the influence of different substrates (glucose, glutamine and oxygen) on the metabolism of a chosen cell line. Based on the fractional factorial analysis the test is useful for determining the influences of selected substrates on cell metabolism. All cells were cultured in 6-well plates and to determine the influence of oxygen supply the cells were cultivated in different medium heights (2, 4, 6 ml).
The cell lines were characterized by consumption or production rates of glucose, glutamine, oxygen, ammonia, lactate and monoclonal antibodies and by their doubling time. Intracellular redox state was measured using a tetrazolium test. Additionally the cells morphology were characterized by light and electron microscopy and by cell size distribution.
The characterization of a mouse hybridoma (αCB03) and human peripheral bood lymphocytes is presented in this paper.
The results showed that using a well designed test, influences of several selected substrates on the cells metabolism can be determined easily and gives useful hints for optimizing and controlling the culture of animal cell lines.

AMMONIUM TRANSPORT IN MYELOMA AND HYBRIDOMA CELLS

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Knowledge about the effects of ammonia and ammonium on the cell metabolism and physiology may provide means for developing control strategies in animal cell processes. Our work with a myeloma cell line has shown that ammonium ions are transported into the cell by certain transport proteins normally transporting K^+. A futile cycle of NH_4^+ transport and NH_3 diffusion across the plasma membrane creates a cytoplasmic acid load, which results in an increased demand for maintenance energy. Addition of K^+ to myelomas and hybridomas inhibits the transport of NH_4^+ into the cell, thus preventing the negative effects of the futile NH_4^+ transport. Experiments indicate significant differences between the myeloma cell line and the hybridoma cell line: i) the transport rate of NH_4^+ is higher in myeloma cells, ii) the concentration of K^+ needed to inhibit NH_4^+ transport over the plasma membrane is lower with myeloma cells. Further, preliminary experiments suggest an increased transport rate of NH_4^+ in hybridomas exposed to ouabain, an inhibitor of the Na^+/K^+-ATPase. This behaviour was not observed in myeloma cells, and it indicates the involvement of different transport proteins in the two cell lines. We speculate that the difference in NH_4^+ transport rate between myeloma and hybridoma cells is one reason for the difference in ammonia and ammonium sensitivity which can be seen in bioreactor cultivations. Myeloma cells that are more sensitive to ammonia and ammonium than hybridomas also show a higher NH_4^+ transport capacity.

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EVALUATION OF THE NEW PROTEIN-FREE POWDER MEDIUM (MDSS1-POWDER) FOR THE PRODUCTION OF MONOCLONAL ANTIBODIES

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The use of serum-free media (SFM) is an important improvement in animal cell biotechnology. Classically, these media are commercialized in a liquid form which poses several technical problems for their use in industrial scale bioreactor cultures. Therefore, the SFM MDSS1 which is originally a liquid medium, was further developed in order to obtain a full powder medium.
The evaluation of this medium consisted in comparing cell growth of three mouse-mouse hybridomas (I.13.17, 9E10, TB/C3) and monoclonal antibody (MAB) production in the original liquid MDSS1 as well as in the powder MDSS1 by using static batch and continuous stirred tank reactor cultures. In continuous cultures, the specific growth rates were equally or better in the powder MDSS1 (0.017-0.039/h) than in the originally liquid MDSS1 (0.016-0.026/h). The steady state cell densities varied for the cell lines tested between 2 and 3*10^6 c/ml mainly as a result of differences in the cell retention by the spinfilters used. The specific IgG production rates varied very much in dependence of the cell line used. For 9E10 we could state a considerable increase from 1.2 to 6.6 pg IgG/c.h, for I.13.17 no statistically significant difference was stated, and for TB/C3 a three-fold reduction from 0.78 to 0.26 pg IgG/c.h was stated when the powder MDSS1 was used instead of the liquid formulation of MDSS1 In absolute values, IgG concentrations ranged from 50 to 300 mg/l.
These results signify that both types of MDSS1 are equally useful for the production of MAB in reactor cultures. However, it is also evident that different hybridoma cell lines perform differently on both SFM and that the choice of the SFM depends finally on the cell line used.

CELL GROWTH REGULATORY PEPTIDES FROM BOVINE CASEINS

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Milk is infant's only provision and various physiologically active substances besides nutrition are included. These physiologically active substances have possibility to function actually in the infant. And in recent years, some physiology active factors were found in digestive peptides of the casein which had been thought to be nutritive substance of milk . These were, for instance, opioid peptides, immunity activating peptides and angiotensin converting enzyme (ACE) inhibitory peptides. Then, we retrieved the peptide which controlled the proliferation of the cell in the β-casein digestion peptides. And it was found that β-casein peptide β-CN(f177-183) stimulated the proliferation of the BALB/c 3T3 cell and had been announced at previous meeting of this society. The research of other casein elements was advanced this time. As a result, we found cell proliferation stimulatory peptides in κ-casein peptides and inhibitory peptides in αs1-casein peptides. The presentation will describe these results in detail.

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OPTIMIZING VENDOR PROPRIETARY SERUM-FREE MEDIA

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In an environment driven by drug cost containment, it may be an efficient use of limited R&D resources to take advantage of the development efforts that media manufacturers have expended in formulating serum-free and/or protein-free media. Although such commercially available media result in adequate cell growth and production, rarely will any one medium satisfy all the rigorous process specifications for each given recombinant cell line required for large scale manufacture. The challenge then becomes how one attempts to modify or augment the vendor media to suit a given production process without having access to the vendor's proprietary formulation. This poster will outline the strategy we undertook to successfully meet this challenge. The keys to this strategy are: (i) establishing a working partnership with the vendor company that allows access to media components without compromising the vendor's proprietary position; (ii) use of statistically designed experiments; (iii) media blends. The example used to illustrate this strategy involves growth and production of a recombinant CHO line in serum and protein-free media developed by JRH Biosciences.

HYPEROSMOTIC HYBRIDOMA CELL CULTURES: MECHANISMS OF OSMOPROTECTION.

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Animal cells are sensitive to changes in medium ionic strength and osmolality. In animal cell processes, medium osmolality is a largely uncontrolled and ignored process parameter, which is defined mainly by medium composition and allowed to vary freely during culture growth. In designing processes for high cell density culture one can imagine changes in the current growth medium formulations, involving increased levels of essential nutrients. Thus, we feel that it is important to see medium osmolality as a process variable which could be used in process design.
Hybridoma cells may utilize exogenously added osmoprotective compounds for protection against dehydration when grown under conditions of hyperosmotic stress. Glycine betaine, sarcosine, proline and glycine have all been shown to be effective osmoprotective compounds for hybridoma cell line 6H11, giving increased growth rates and cell densities when added to hyperosmotic culture media. The protective compounds have been shown to accumulate inside the osmotically stressed hybridoma cells, so as to balance the increased extracellular osmolality and thus avoid unfavorable pressure differences across the cell membrane. In the present work we hope to provide further information about the mechanisms of osmoprotection in hybridoma cells.

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THE EFFECT OF MEDIUM COMPOSITION ON GROWTH AND MONOCLONAL ANTIBODY PRODUCTION OF HYBRIDOMA CELLS

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One of the major challenges in the process for the obtention of monoclonal antibodies in animal cell culture is the low cell growth and product concentration levels usually obtained. The complexity of the culture medium and of the interaction between nutrients consumption, accumulation of possible toxic metabolites, operational conditions and culture strategies, demands the sequential study of many factors, usually having quite different influence for each cell line, in order to achieve a basic knowledge of the culture. With this knowledge further optimization is then possible.
In this work, the effect of medium composition on the cell growth, monoclonal antibody accumulation and cell metabolism is discussed, for batch and fed-batch cultures of hybridoma cell line KB-26.5, previously adapted to grow at low-serum concentration (1%). The results given focus on the influence of glucose and glutamine feeding, lactate and ammonia accumulation, suplementation of the medium with vitamins and amino acids, and also on the addition of foetal calf serum. Finally, a fed-batch culture taking into account all the previous experiments has been performed, obtaining a longer life span for the cells and a two-fold increase in final monoclonal antibody concentration in the culture.

Investigations of high cell density baculovirus infection using Sf9 and High Five insect cell lines in the low-cost SF-1 medium.

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Many recombinant proteins are well expressed in the baculovirus expression system using the insect cell line Spodoptera frugiperda Sf9 in the low-cost SF-1 medium[1]. Recently we have adapted the High Five cell line (Trichoplasia ni) to grow in a single cell manner in suspension culture with SF-1 medium. The expression of several different recombinant proteins was tested in High Five cells. For some proteins, increased expression levels of up to 4-6 fold compared to Sf9 cells could be achieved, for other proteins similar or poorer expression levels were obtained. Besides the differences in metabolismn (glucose, amino acids, O_2 consumptions) both insect cell lines revealed reduced activities with increasing cell concentrations during the infection period. Using the soluble recombinant TNF receptor - p 55, which was 5-6 times higher expressed in the High Five cells compared to Sf9, we have studied the high cell density infection in more detail.
Here we will present prelimnary results of High Five infection experiments at lab scale as well as at fermenter scale. Furthermore, high cell density infection investigations of High Five and Sf9 will be discussed.

[1] E.-J. Schlaeger et al., 1993. Biotechnology Techniques 7, 183-188.

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Large scale production of Mammamodulin (MM).
Development of a low-cost large scale production process for adherent cell growth.

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MM,a novel heparin-binding factor which influences breast cancer behaviour, was purified from cultured medium conditioned by the highly tumorigenic estrogen receptor negative human MDA-MB-231 cells. MM is a potent stimulator for estrogen receptor positive cell lines and , according to the amino acid sequences obtained, the factor is a member of the heregulin protein family.
In order to produce sufficient amount of MM, the cells were grown in HL-medium, which was recently developed for high density growth of HL60 cells,using Raschig-rings(glass,later stone-ware) as an immobilization carrier. The cells were grown first in the presence of serum (5%) followed by the serum-free production phase with fortified medium in a discontinuous manner using a 3L Bioreactor (Bellco). The culture was run almost 12 weeks without any lost of productivity.
In a scale-up experiment several hundred liters production medium have been produced in the discontinuous mode (1 reactor volume per day) using an simply modified airlift fermentor (23L). The outer volume of the draft tube was filled with about 9kg Raschig- rings (8x8x5mm). The fermentor was started with gently trypsinized cells harvested from 3 cell factories and the growth phase lasted 2 weeks . During the production phase the cell mass in the fermentor slowly increased. Information on the metabolic state of the cells were obtained by measuring glucose, glutamine (amino acids) and O_2 consumption ,lactate formation, LDH activity and the osmolality. The yield of the 52 kDa growth factor in the production medium , which was measured in a bioassay, was very low (about 0.1-0.2 ug/L).

TRANSCRIPTION LEVEL OF CHO CELLS INFLUENCED BY AMINO ACIDS

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For the production of pharmaceuticals with mammalian cell cultures, normally cell lines like BHK or CHO are used and transfected with a vector system. The promotors of such heterologous expression systems are often constitutive like the SV40, CMV or adenovirus promotor. To express a protein that is potentially cytotoxic, it is advisable to use an inducible expression system regulated by an external stimulus. But such systems e.g., the heat shock or the metallothionein promotor, causes often problems regarding to the product stability or biosafety. Here we report the search of inducible homologous promotors in the CHO genome regulated by harmless substances. For that, the transcription activity of CHO-DUKX cells under amino acid deficiencies was investigated. The level of transcription was altered by targeted omitting of the nonessential amino acids serine or asparagine in the serum-free culture medium. These changes in transcription activity were accompanied by intra- and extracellular changes of the amino acid concentration and were monitored by measurement of the total RNA and the mRNA concentration during the shift from complete to deficient medium. An identification of a strong serine or asparagine dependent regulated promotor will led to new homologous expression systems with high biosafety and to an economical two-step production process with a growth phase and an induced expression phase of the recombinant product. This work was supported by the EU in the scope of an EU-BRIDGE project.

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INHIBITORS OF CELL GROWTH: ACCUMULATION AND CONCENTRATION

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Although a sufficient supply with nutrients is guaranteed, animal cells cultivated in conditioned medium stop growing. Growth inhibitors secreted by the cells into the cell culture medium are responsible for this effect. The object of this investigation was to find out which components of the conditioned medium cause cell death or low growth rates, and to purify them. An inhibition caused by ammonia or lactate could be excluded. In order to provide considerable amounts of inhibitory cell culture supernatant for characterisation and purification of growth inhibitors, a special fermenter system was used. The supernatant produced in this system was fractionated by gelfiltration. Each fraction was tested in a bioassay for inhibition. This experiment showed a molecular weight of the inhibitor(s) of less than 5000 Da. Several different methods were used to obtain a high concentration of such small substances. Results of inhibitor production and concentration are presented.

IN SITU AMMONIUM EXTRACTION IN A CONTINUOUS MEDIUM RECYCLING BIOREACTOR FOR THE PRODUCTION OF CELL GROWTH INHIBITORS

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Cell growth inhibitors have been found for several cell lines during cultivation. High concentrations of unknown inhibitory substances in cell culture supernatant are useful for their further characterization. Common continuous systems for mass cell culture such as chemostat or perfusion systems seemed to be unsuitable. Supplying these cell culture systems with nutrients requires high dilution rates of fresh medium, which also dilute the inhibitory substances instead of a concentration. Avoiding those disadvantages continuous medium recycling for perfusion systems was proofed as well as fedbatch systems. Negative effects on cell growth by recycling used medium in a perfusion bioreactor system has been shown by Büntemeyer et al. The negative effects on cell growth because of a high ammonia concentration secreted by the cells as well as by spontaneous decomposition of glutamine could be reduced by an online ammonia extraction. A supernatant containing unknown inhibitory substances without an accumulation of lactate or ammonia was produced. The supernatant was fractionated by gel filtration and tested in a cell activity test (MTT). This test showed a significant lower activity for several fractions with low molecular weight.

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GROWTH OF ANCHORAGE-DEPENDENT MAMMALIAN KIDNEY CELLS IN SERUM-FREE MEDIA

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Many vaccines are produced in cell substrates sourced from animal kidneys. These cells are highly susceptible to viral infection and have been shown to provide high titer yields. Traditionally, these cell lines are propagated in minimal basal media supplemented with 2-5% serum. As a result of the increasing concerns from regulatory agencies, the country of origin for animal-sourced components must be tightly controlled. Additionally, the potential for introducing adventitious agents must be closely monitored with each new lot. EX-CELL™320 serum-free medium and modifications provide a consistent, reproducible growth environment for propagating kidney cells as well as for specific viral replication. Data will be presented for the growth of several kidney cell lines from various animal sources, including MDCK, Vero, CrFK, MDBK, and BHK. The scale-up of these cells to roller cultures will be discussed. Many similiarities have been identified among the studied cell lines which will streamline the development process for other kidney cell lines.

PERFORMANCE CHARACTERISTICS OF A PROTEIN-FREE MEDIUM FOR MAMMALIAN SUSPENSION CELLS

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Long term culture of CHO-K1 cells has been achieved in a protein-free medium, EX-CELL™PF CHO. Previously reported data demonstrated that these cells could be transferred directly from a serum-free medium without extensive weaning procedures. This paper will present data on the effects of changing culture parameters on growth and viability. These modifications include adjustment of the buffer system in closed spinner culture, the effect of osmolality on the health of cells, addition of antioxidants, and addition of increased concentrations of amino acids. In addition, the transition of variant anchorage-dependent CHO cells to protein-free suspension growth have indicated unique adaptation protocols may be required for each cell type. These procedures are significantly different from those observed previously with CHO-K1 cells. The compatibility and potential applications of this simple protein-free formulation with other suspension cell types will be also presented.

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EFFECT OF pH ON THE GROWTH AND MONOCLONAL ANTIBODY PRODUCTION IN BATCH CULTURE OF A HUMAN-HUMAN HYBRIDOMA

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The growth and monoclonal antibody production kinetics of a human-human hybridoma AE6F4 which produces a monoclonal antibody specifically binding with an antigen site on lang cancer cells were examined in a narrow pH range between 6.7 and 7.6. The specific growth rate at pH 6.7 was reduced half compared with that at pH 7.0. The maximum cell concentration reached more than 3 x 10^6cells/ml at pH less than 7.0, while the summits of the cell concentration at higher pH decreased with increasing pH. Little lactate was produced at the early stage of the batch culture at pH 6.7, while much ammonium was accumulated in the culture broth in the same period. On the other hand, lactate was produced at the late stage of the culture with a similar lactate yield from glucose to those at other pH levels.However, the ammonium accumulation was stopped at the late stage of he culture. During the batch culture at pH 6.7, the cell growth rate was unchanged, suggesting that the metabolic pathways would be changed during the exponential growth phase, and that glutamine was mainly used as an energy source at the early stage of the culture and glucose was converted to lactate at the late stage of the culture.Moreover, the monoclonal antibody production is more markedly affected by pH. The best and more than 60 times of the monoclonal antibody productivity was observed at pH 7.3 compared with that at pH 6.7. The best growth and monoclonal antibody production was observed at pH 7.0 and 7.3, respectively. This suggests that some optimization strategies should be applied to maximize the monoclonal antibody productivity in a bioreactor.

SIMULTANEOUS PREDICTION OF THE CONCENTRATIONS OF GLUCOSE, GLUTAMINE, LACTIC ACID, AMMONIA AND ANTIBODY IN THE CULTURE BROTH OF MOUSE-MOUSE HYBRIDOMA BY NEAR INFRARED REFLECTANCE SPECTROSCOPY

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For the control of animal cell culture, it is very important to be able to easily and rapidly measure the concentrations of antibody produced, glucose, glutamine, lactic acid and ammonia. Near infrared spectroscopy (NIR) was applied in the prediction of the concentration of these nutrients and products in the culture broth. The correlation between the concentration obtained by the conventional method and that obtained by NIR was studied by multiple regression analysis. NIR values predicted were in good agreement with those by the conventional method. The multiple correlation coefficients were over 0.98 in all factors. The operation procedure involved in NIR is simple with the operation time required to predict the concentrations of these substances being only 5 min. The results of the present study suggest that NIR is a useful method for the control of animal cell culture.

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CONTROL OF CELL METABOLISM IN HIGH DENSITY FED-BATCH CULTURES BY DYNAMIC NUTRIENT FEEDING

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A computer controlled bioreactor with on-line oxygen uptake rate (OUR), base addition and turbidity measurements was used to obtain a high viable cell concentration in fed-batch hybridoma cultures. Cell concentration and specific rates were estimated on-line. These on-line parameters were used to determine the metabolic activities. The ratios among glucose, glutamine and other medium components in the feeding solution were adjusted stoichiometrically to provide balanced conditions for cell growth. Through on-line control of nutrient feeding rates, glucose and glutamine concentrations were maintained at low levels while exponential growth continued. The metabolism of hybridoma cells was altered, resulting in a reduced glucose consumption, a lower conversion of glucose to lactic acid and an increased oxygen consumption. A very high cell concentration (1.36×10^7 cells/ml) was achieved accompanying a high viability (> 90 %) in the fed-batch cultivation. Such a high cell concentration with a high viability was previously only attainable in a perfusion culture with cell retention. The control of cell metabolism by manipulating nutrient concentration at low levels minimized metabolite accumulation, and thus eliminated the need of perfusion. However, with the high metabolic demand at such a high cell concentration, maintaining high viability would not have been possible without an automated dynamic feeding system. Our results demonstrate the value of process control and automation in mammalian cell cultivation.

HYBRIDOMAS IN A BIOREACTOR CASCADE

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Various modifications of hybridoma cultivation can enhance the overall bioreactor monoclonal antibody (MAb) productivity. For example, a better substrate conversion and a higher product concentration can be obtained in a serial bioreactor instead of a single vessel. In large-scale animal-cell culture, oxygen supply may become limiting. For scale-up, the cascade of aerated bioreactors can be a promising alternative. Therefore, the Multiple Air-lift Loop bioreactor (MAL) was developed. The MAL is a series of air-lift loop reactors within one vessel.
The use of a reactor cascade may be particularly advantageous when product inhibition plays an important role in cell growth and product production. A well known example is ethanol production by yeasts. Also hybridomas are known to be inhibited by two metabolical by-products: lactate and ammonia.
Hybridomas were cultured in a series of two continuously operated stirred tank reactors (CSTRs), using a serum-free medium. The CSTRs are seen as a first approximation of a MAL for animal-cell culture, and gave useful information about hybridoma growth and production kinetics. Steady states were determined at different dilution rates. A comparison will be made between the reactor cascade and a single vessel of the same overall volume using substrate conversion and overall MAb productivity as criteria.

6.8

6.9

On-line Immunoanalysis of Antibodies During a Long-term Continuous Fermentation

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The application of a computer-controlled analysis system for on-line monitoring of monoclonal antibodies during a continuous fermentation over 850 hours is presented. The system was applied for steady steaty measurements of antibody titer at different culture temperatures.
The immuno assay is based on disposable affinity columns (ABICAP) with immobilized anti-mouse IgG. Antibody detection was performed by direct protein assay using a spectrofluorometer. A fully automated autoanalyser system (ASPEC, Gilson) performed the entire liquid handling steps (pipetting of sample, washing solutions, elution buffers, standards).
A fluidized bed bioreactor was used allowing high cell density fermentation of hybridoma cells immobilized in porous microcarriers. Basing on a pneumatically actuated inline membrane valve, reliable sampling of cell containing culture medium was possible. Separation of cells from the sample stream was performed by a glass fibre filter, contamination and cell blocking could effectively be prevented by intermittend chemical rinsing.
Basing on the reliablilty of the sampling system and the flexibility of the analysis system on-line process control of those metabolites accessible to ELISA-methods, should be possible with this approach.

EVALUATION OF A CELL SEPARATOR IN LARGE SCALE PERFUSION CULTURE

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Large scale perfusion culture of suspended animal cells necessitates an efficient cell separation system capable of delivering cell free harvest at a high flow rate. Few data are published on the performance of cell separating equipment in large scale (>100 l).
The aim of this study was to evaluate a disk stack centrifuge (Westfalia CFA-01) in a simulated large scale perfusion culture.
CHO cells were grown in perfusion in a 100 l reactor. In order to simulate cell separation from a much larger reactor in perfusion, a by-pass was used to allow the concentrate and most of the cell free supernatant to be returned to the reactor.
The separation efficiency was studied at feed flow rates between 25 and 250 l/h, varying bowl speed and feed/concentrate flow ratio. Despite high g-forces and high flow rates, no negative effects on cell viability or growth rate could be detected. The maximum capacity that would allow 100 % cell retention was 1,3 m^3 of cellfree harvest per day.
The long term performance was strongly dependent on high cell retention and appropriate concentrate flow rate.

6.10

6.11

SCALE-UP OF TRANSIENT GENE EXPRESSION WITH THE SEMLIKI FOREST VIRUS SYSTEM

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A new expression system based on the Semliki Forest Virus (SFV) has recently been developed. In vitro transcribed RNA encoding the viral replicase and the recombinant protein is electroporated into BHK cells, together with helper RNA encoding the viral structural proteins. This allows in-vivo packaging of the recombinant RNA into infectious but non replicative virus particles. The stock can then be used to infect almost any animal cell. Because of the high level expression of recombinant proteins with the SFV system (up to 25% of cellular protein), we investigated the issues associated with scale-up and process development.
We compared CHO and BHK cells grown in T-flasks and stirred systems. Bacterial ß-Galactosidase was used as a reporter gene allowing analysis of enzyme activity by flow cytometry.
We found that CHO cells grown and infected in a spinner (suspension culture) expressed a reduced level of ß-Galactosidase compared to T-flask experiments. BHK was expressing at a significantly higher level without any difference between cells infected in T-flasks or spinner systems. For the infection phase we identified pH and FCS optima, investigated the influence of stirring, virus concentration (at constant MOI) and the effect of different MOIs.
Based on these studies, we are discussing a scale-up strategy which also takes into consideration the important aspect of biological safety of large scale productions.

Respiration Quotient in Bicarbonate-Buffered Mammalian Cell Culture

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The determination of the respiration quotient (RQ = CER/OUR) has so far not been used as a tool for understanding animal cell metabolism. This has been due to problems in measuring the carbon dioxide evolution rate (CER) rather than the oxygen uptake rate (OUR). The determination of the CER is complicated by the use of bicarbonate in the medium. Using liquid and gas balances we derived an equation for a continuous culture to quantitate the amount of CO_2 that comes from the bicarbonate in the feed. Under cell-free conditions it was proven that this equation is valid. In a continuous culture using hybridoma cells the CO_2 from the feed was found to be a significant amount of the total measured CO_2 in the off-gas (51 and 29 % in a suboptimal and a high-growth medium, respectively), which was determined using a IR-gasanalyzer. Further, the problem of CO_2 loss from the medium tank was solved using a theoretical and experimental approach.

6.12

6.13

PRODUCTION OF MONOCLONAL ANTIBODIES IN A PILOT SCALE FLUIDIZED BED REACTOR

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The immobilisation of animal cells in porous glass carriers is of growing importance. These carriers allow high cell density culture of adherent and suspension cells. Fluidized bed reactors are the preferred cultivation systems in this field, having good mass transfer properties and a high space time yield.

For the production of a commercially relevant monoclonal antibody a fluidized bed bioreactor of 10 l total and 3 l settled carrier volume has been applied. A mouse-mouse hybridoma with a low productivity of app. 25 mg/l in batch culture of suspended cells at 3% Fetal Calf Serum was used. The reactor was aerated with silicone membranes, which are integrated in the fluidized bed.

The reactor has been operated continuously for 450 hours reaching a steady state after 250 hours. After reaching the steady state 48 l/day of cell culture supernatant with a concentration of 16 mgMAb/l (0.8 gMAb/day) was harvested. The viable cell concentration was 10^6/ml in suspension and $2x10^7/ml_{\text{settled carrier}}$ or $7x10^{10}$ viable cells in total. For 80 hours the reactor has been operated with two concentrates and water, permitting a 85% volumetric reduction in total media preparation. The concentrates and the water were introduced unmixed into the recycle loop of the reactor, exploiting the good mixing properties of the system.

The results of this fermentation suggest this concept as alternative to other systems for continuous high density cell cultures, especially for hybridoma and adherent cells. A commercial version of the reactor will be available soon.

A SYSTEMATIC APPROACH FOR MODELLING ANIMAL CELL CULTURES : EXPERIMENTAL VALIDATION*

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During the last few years, we have developed a systematic method for modelling animal cell cultures. This modelling is oriented for control purpose in an automatic control understanding. A good model able to describe the behaviour of the culture in different experimental conditions is the keypoint for an efficient control.
Our modelling method has achieved now good results for the simulation of cultures in different experimental conditions including renewed cultures with different renewal rates, batch cultures with different initial concentrations of glucose and amino acids. The same model with the same coefficients is used for these various conditions, showing fairly good fitting of the simulation with the experimental data. This study is developed for VERO cells adherent on microcarriers. The method is organized in 2 steps: The first step concerns the identification of an underlying reaction network, that describes the main reactions occuring in the culture. This first identification step in based on biological knowledge of metabolic pathways, observation of experimental data and mathematical knowledge by singular value decomposition. We present here new results of this technique that gives an upperbound to the number of independent reactions occuring in the culture. These results allow to improve the determination of the reaction network. The second step concerns the identification of the kinetics involved in the growth. Polynomial models are selected to describe the kinetics. We present here new results of the simulation model resulting from these 2 steps merged together, showing a good agreement between the simulations and the experimental data in various experimental conditions as mentioned previousely.

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THE USE OF SOFTWARE SENSORS FOR MEASUREMENT AND CONTROL OF ANIMAL CELL CULTURES

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Measurement and control of the cultivation process of animal cells are important to improve the quality and quantity of the final product and for the consistency of production. A problem that still arises is that only a few cultivation parameters can be measured on-line. On-line determination of important parameters like the glucose and glutamine concentration or the cell density is still difficult to accomplish. Analysis techniques like Flow Injection Analysis and biosensors have been developed to overcome this problem, but these developments are still in the research stadium. Moreover, in general these are state of the art techniques and therefore both expensive and prone to failures. Another way to obtain information about parameters that cannot be determined on-line, is to estimate them using models. These on-line models (software sensors) use information that is available on-line as input parameters. Applying this technique, software sensors were developed for the biomass activity and the glucose concentration. Using the glucose software sensor, cultivations of Vero cells have been carried out in which the glucose concentration was controlled at different fixed levels between 0.25 and 10 [mM]. This way, the effect of the glucose concentration on the glucose consumption rate, lactate production rate and the biomass activity could be determined. Next to this application, the software sensors were also employed to determine the glutamine consumption rate as a function of biomass activity and glucose consumption rate. With this additional information, cell cultivations were done in which both glucose and glutamine were controlled at fixed concentrations.

Scale-up of ultrasonic resonance field cell separation devices used in animal cell technology

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Ultrasonic standing waves have been used for the separation of suspended particles, such as animal cells. This technology can be used for cell retention within the fermenter for high density cell culture or as cell removal step in downstream processing. Devices for bench scale applications have been developed and evaluated in experimental set-ups and on laboratory fermenters. To scale-up this promising technology for industrial processes, a number of criteria, such as aseptic and hygienic design, steam sterilisability, sufficient flow rates and separation efficiency have to be taken into account. In this paper we will discuss the theoretical limitations of different resonator designs and will describe experimental results with a new type of resonator with an internal cooling loop. The modular, aseptic, device is mounted on top of the fermentation equipment and can be steam sterilised in-situ. High amplitudes necessary to achieve separation of animal cells cause thermal and acoustic flow within the separation chamber. The internal cooling loop reduces this temperature increase and thus the disrupting forces, which would otherwise limit the achievable flow rate and separation efficiency.

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IMPLICATIONS OF CONTROLLED GLYCOSYLATION AND APOPTOSIS IN NOVEL HOLLOW FIBER BIOREACTOR

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A novel high performance bioreactor (HPBr) based on a hollow fiber format is described. It has been specifically designed for optimal production of both cells and metabolites (e.g. monoclonal antibodies and recombinant proteins). Over a factor of three improvement has been achieved for IgG_1 production with 3C11 hybridoma relative to Micro Mouse™ (Unisyn Technologies, Inc.). The cell mass harvested after a 30 day run under controlled culture conditions has been characterized and compared with the 5×10^8 cells inoculum. Oxygen levels in the extracapillary space (ECS) of the bioreactors were monitored during IgG_1 production. Carbohydrate content was analyzed and compared for the HPBr produced antibody versus that from the Micro Mouse™. The novel engineering design and operating parameters of the HPBr enables microenvironmental control of oxygen, pH and other factors. Both controlled glycosylation and environmental influence on apoptosis processes have been implicated in the results presented. These issues are discussed.

THE EFFECT OF FLOW RATE ON THE IMMOBILISATION OF ANCHORAGE-INDEPENDENT CELLS TO POROUS GLASS BEADS

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The use of passive immobilisation into porous supports has been shown to be suitable for suspension cell lines, which presents the advantages of microcarrier culture techniques (high cell density) and the gel entrapment/encapsulation (protection from shear stress). Since cells must be transported into the porous support a relationship exists between the fluid flow velocity and the number of cells retained in the matrix. In order to study this influence, cell suspensions were passed through a packed bed reactor of porous glass beads at different flow rates. Porous glass beads (Siran, Schott Glasswerke, Mainz; Germany) with different particle diameters, pore sizes and surface properties were used to assess the effect of these parameters on the cell adsorption at different flow rates and cell concentrations. Experiments were conducted at liquid flow rates of 50, 200, 340 ml/h. Improved cell retention was obtained when the fluid flow rate was 200 ml/h. At flow rate 200 ml/h the cell concentrations of 0.5*10^5 cells/ml, 1*10^5 cells/ml and 10*10^5 cells/ml were tested for their effect on the specific cell adsorption, support saturation being obtained at 5*10^5 cells/ml.
The results of cell adsorption on different beads have been useful for the selection of the best support for the continuous long-term fixed bed reactor experiments.

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CONTINUOUS BIOTRANSFORMATION BY IMMOBILIZED MICROORGANISM ON THE SURFACE OF CUPRAMMONIUM REGENERATED CELLULOSE HOLLOW FIBER (BMM)

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The efficient delivery of sterilized oxygen and culture medium is necessary when utlizing immobilized cells in a bioreactor system. We studied the conversion of glucose to ethanol by using the immobilized yeast cell as a model reaction system. Here,we show oxygen diffusion and ethanol productivity for yeast cells immobilized on the surface of hollow fiber modules.

The pore size of the fiber is 15–75 nm and air borne microorganisms are removed by an filtration through this pore. Water and air were introduced to the outer surface and inner side of the hollow fiber respectively and the concentration of dissolved oxygen was measured at intervals with DO meter to obtain capacity coefficient (K_La value).

K_La value increased in proportion to available surface area and value slightly increased with increasing of pore size. When the module's pore size is 40 nm and the available surface area is 0.06 m^2,the obtained K_La value is around 300 h^{-1}. This value is considered to be very high,especially when compared to K_La value acquired using other methods such as bubbling through a perforated pipe or shaking. Continuous and stable ethanol production without infection by microorganisms was carried out for over 600 hours. An ethanol conversion value of 95% and a productivity was 50 g–ethanol / kg–gelxh was attained.

DETERMINATION OF K_s-VALUES (MONOD CONSTANTS) FOR ESSENTIAL AMINO ACIDS USING CHO AND HYBRIDOMA CELLS IN BATCH AND CHEMOSTAT CULTURES

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A balanced supply of nutrients is necessary for both growth and productivity of all animal cell cultures to avoid limitations. Especially the correct supply with amino acids is indispensable to achieve high cell densities and good product formation. A lack of any essential amino acid causes a reduction in specific cell growth and later cell death. Therefore, the kinetic characterization of mammalian cells due to the nutrient composition of the culture medium is important for optimal bioreactor process control. Based on batch and chemostat cultures the K_s values for essential amino acids were determinate and calculated using Monod equations and its simulation on computer. Results of a recombinant CHO cell line, producing human tissue plasminogen activator (t-PA) and a mouse-mouse hybridoma cell line will be presented. The data of these experiments were compared with the results of the computer simulation in order to prove or dismiss the preceding model.

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CELL CULTURE APPLICATION OF A NUTATING DISK PUMP

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To meet the requirements for the use of pumps in cell culture technology like long-term sterile operation and low shear impact on the pumped media, a new pump has been developed using the 'nutating disk principle': This results in a peristaltic-like displacement of the media without any tube. The absence of any mechanical seal assures hermetic leak tightness.
Continuous sterile testing of the nutating disk pump with defined bacterial contamination (10^3 spores /ml of *Bacillus stearothermophilus*) demonstrates reliable in-line sterilization capability due to the pump design without dead volume and continuous contamination free operation over months.
To test the shear impact the nutating disk pump was arranged in the bypass of a cell culture fermenter (volume 15 l). During suspended cell fermentation of a *mouse-mouse hybridoma* cell line for antibody production no reduction of the number of living cells was measured at a delivery rate of 2.4 m^3/h. A conventional peristaltic pump used as reference for the same application led to 70 % decrease in the number of living cells after 40 h of operation.

SMALL MICROCARRIER AGGREGATES YIELD HIGH CELL DENSITY

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A commonly held belief has been that microcarrier-cell aggregates (clumps) lead to necrosis of the cells in the core of the aggregate. We, and others, have research results that contradict that belief. Another commonly held belief is that microcarriers below a given size (approximately 75 microns) deter or disallow cell attachment. In our studies (a) we demonstrated that cell yields can be greatly increased by utilizing very small spheres (38-63µm), (b) we showed further that the high cell densities were achieved by virtue of rapid cell attachment to the microcarriers with concomitant microcarrier aggregation and high cell growth and (c) data indicated that cells growing in high cell density remained metabolically active and produced high levels of at least one commercially-relevant biological. On a per cell basis, cells growing in high-density, small-microcarrier cultures synthesized protein and produced infectious bovine rhinotracheitis virus as efficiently as cells in monolayer culture.

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TESTING COMPUTER PREDICTIONS OF VIRAL EFFECTS ON HYBRIDOMA PRODUCTIVITY

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Large numbers of endogenous A-type and C-type virus particles are produced by many hybridoma cell lines, yet the effects of virus production on the metabolism of the cell are poorly understood. Previous studies using an advanced computer model of cell growth in batch culture indicated that virus production may affect both cell numbers and antibody yield. We have continued to look for viral effects predicted by the simulations experimentally. Virus production by a cell line that was sufficiently well characterised for simulation studies was altered chemically and hormonally. The number of virus particles was measured by electron microscopy to determine if changes in particle numbers correlated with changes in cell number and antibody yield as predicted by the computer simulation. The implications of the findings are discussed.

THE SENSITIVITY OF UNINFECTED AND BACULOVIRUS INFECTED SPODOPTERA FRUGIPERDA CELLS TO HYDRODYNAMIC FORCES

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Spodoptera frugiperda (SF9) insect cells were infected with wild or recombinant (β–gal) types of the *Autographa californica* Nuclear Polyhedrosis Virus (AcNPV) in stirred tank reactors. In small scale cultures, infection at low values of MOI (0.1-1.0) was significantly enhanced by agitation, but at an MOI of 10, there was no difference between stationary and agitated cultures. Agitation without bubble entrainment of such cultures in 1 litre bioreactors over a range of energy dissipation rates at an MOI of 10 did not affect the rate of cell infectivity nor the maximum % infectivity or β–galactosidase production. Low rate sparging of infected culture with wild type virus, however, reduced the maximum infected cell number by 45%, the viral titre by 67% and the volumetric polyhedra production by 40%. Qualitative comparison between uninfected and infected SF9 insect cell cultures suggests though that the level of cell destruction in the infected cultures was similar to or only slightly higher compared with that in uninfected cultures. The mechanical properties of the uninfected and infected cells measured by micromanipulation also support these observations. Finally, the presence of Pluronic F-68 (at 0.1% w/v) was seen to alleviate the damaging effects on growing insect cells in the above reactor conditions and also to offer an enhancement in stationary cultures.

6.24
6.24.1

MAB PRODUCTION AND TREATMENT OF RHEUMATOID DESEASE USING ENCAPSULATED ANTI-IDIOTYPIC HYBRIDOMA CELLS

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A murine hybridoma cell line secreting an anti-idiotypic mab for the treatment of rheumatoid deseases was microencapsulated either using cellulose sulphate (CS) and alginate. For this purposes a new developed 24-jet microencapsulation apparatus was used. We compared cell growth, antibody production, antibody recovery and long term stability in both types of capsules. Densities of 2x10E8 cells/ml, excellent antibody concentrations (max. 2,4mg/ml), high mab recovery and long term stability in vitro could be shown using the CS-capsules.
Ten weeks old female rheumatoid MRL/lpr mice were treated with five CS-capsules per mouse either subcutaneous (sc.) and intraperitoneous (ip.). The used capsules were derived from long term cultures of the encapsulated anti-idiotypic hybridoma cell line. In contrast to the controls a complete remission of the desease could be found in all cases of the ip-treated group over several weeks. Only a mediate loss of typical signs of the desease was detected within the sc-treated group. The clinical use of immortalized microencapsulated cells will be discussed.

EXPRESSION OF RECOMBINANT HUMAN INTERLEUKIN-2 IN BABY HAMSTER KIDNEY CELLS AND CULTIVATION ON DIFFERENT CARRIERS

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Baby Hamster Kidney (BHK-21 C13, ATCC) cells were transfected using pRC/RSV SIGIL2 vector. Interleukin-2 (IL-2) cDNA with original leading sequence was blunt end ligated in the polylinker side of the vector. Rouse Sarcoma Virus (RSV) LTR promoter controlled the transcription of IL-2. BHK cells were transfected using calcium phosphate coprecipitation or electroporation method. Clones were selected using G418 and single colonies were further subcloned. The quantity of the IL-2 was determined using indirect ELISA. The highest producer among several hundred clones, tested after 3 transfections, expressed 5-10 ng/10^6 cells/day. We wanted to determine growth characteristics of BHK-21 cell line on different carriers. We used plastic, glass and collagen coated macroporous carriers and denatured collagen coated microcarriers: Polyethylene PE-1 and PE-2 (IAM), polystyrene PS-80400, Siran (Schott), Microsphere (Verax) and Cytodex-3 (Pharmacia). Shaking flasks and spinners were used as cultivation vessels and DMEM/Ham's F-12 medium containing 5 or 2% of FCS was used. Specific growth, glucose consumption and lactate production rates were measured during 8-22 days long period. The highest cell densities (more than 10^8 cells/ml carriers) were reached using PE-2 carriers and medium containing 5% FCS.

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DETACHMENT AND MITOSIS OF ADHERENT CELLS UNDER SHEAR STRESS CONDITIONS

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The use of anchorage-dependent mammalian cells for production purposes remains challenging to the biotechnologist. Rigorous stirring of the cell's growth liquid, however, creates considerable shear stress on the cell's surface, to which mammalian cells are sensitive due to their lack of cell walls. In previous studies we demonstrated the shear stress effect by measuring marker enzymes, cell viability, and by comparing cell morphology before and after exposure. This rises the question whether the detachment of the cells from the surface is a consequence of cell division. During cell division the cell have to round and, therefore, may just detach in this status when exposed to shear stress. On the other hand the before/after photographs did not tell anything about the mechanism resulting in the observed morphology changes. Using a time lapse film we were able to discern by visual examinations the cell alignment patterns and morphological changes over longer periods of time. The film presented was especially suitable for the observation of morphological alterations caused by different shear rates during mitosis. One conclusion of the evaluation of the film was that cells are able to spread out again after cell division, although, they were exposed to shear stress. Nevertheless, with increasing shear stress level the resulting daughter cells needed more time for spreading. Another conclusion was the observation that cells are able to round opposed to the flow direction. This resulted in piling up the cells forming a wall vertical to the flow direction.

Patterns of the specific productivity of the murine hybridoma, AFP-27, in batch culture

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The overall productivity of a batch process to produce mammalian cell proteins *in vitro*, is determined by the size and longevity of a secreting population of viable cells and the cell specific productivity of those viable cells. In this report, the cell specific productivity of the murine hybridoma, AFP-27 in conventional batch, fed-batch and nutrient enriched batch culture is discussed. In particular, the specific productivity observed in nutrient enriched batch culture is quite unlike conventional specific productivity models previously reported. Conventionally, specific productivity has been described using Leudeking-Piret kinetics with growth and non-growth associated components or alternatively been described using Monod-type models, with accumulating products from catabolism, diminishing the specific productivity from a maximum value. A modified model of specific productivity that incorporates findings in conventional, enriched and butyrate supplemented cultures of the murine hybridoma AFP-27 will be discussed.

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BIOREACTOR WITH A RADIAL-FLOW NON-WOVEN FABRIC BED FOR ANIMAL CELL CULTURE

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Radial-flow bioreactor is effective for reducing concentration gradients because of its large flow area and shallow path for circulating culture broth. As packing material, a non-woven fabric mat made of polyester which gives low pressure drop, was employed. This allows the application of a wide range of shear stress to the cells without depleting the nutrients. In order to enhance cell attachment to the non-woven fabric mat, several kinds of coating agents were tested. Ethylene vinyl alcohol (EVA), an inexpensive coating material, showed almost same effect on cell attachment as compared with atelocollagen which is commonly used for carrier surface modification. In the radial-flow bioreactor with EVA coated non-woven fabric mat, the effect of shear stress on human renin production was investigated. It was revealed that exposure of cells to moderate controlled shear stress enhance the specific human renin production.

PRODUCTION OF HUMAN GROWTH HORMONE BY PROTEIN-FREE CULTIVATION OF ANCHORAGE-DEPENDENT CELLS WITH POROUS CELLULOSE CARRIER

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In the cultivation of anchorage-dependent cells, serum provides the necessary growth factors or attachment factors required for cell growth. However, the addition of serum in the medium makes product purification difficult and increase the cost of medium. In this study, we tried to cultivate Verots-S3, a cell line secreting human growth hormone (hGH), in protein-free medium with two different microcarriers, namely, porous cellulose carrier and Cytodex 3. Microscopic observation revealed that Verots-S3 cells are characterized by aggregation. When the cells were cultivated with Cytodex 3, the cell aggregation induced carrier-bridging and the cells grew just in the void between the carrier particles. In the case of porous cellulose carrier, the cells aggregated inside the carrier and their growth rate and cell density were much superior to those of spinner cultivation with Cytodex 3.

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VERO CELL GROWTH DURING DIFFERENT MODES OF CULTIVATION

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In our laboratory, Vero cells are used as a substratum for the cultivation of poliovirusses for polio vaccine production. In order to obtain maximum cell densities, different modes of cultivation (batch, fed-batch, continuous perfusion, re-circulation and fed-recirculation) were tested on a small scale using standard medium or diluted medium. Recirculation proved to be the most economic alternative. However, since large volumes have to be handled, recirculation is not the favoured mode for production purposes.
In order to compare the results of the different modes on an objective basis, the results of the different modes of cultivation were modelled in order to clarify the bottle-necks which occur during cultivation. A model which was developed for the growth of, anchorage independent, hybridomas was adapted to the growth of anchorage dependent Vero cells.

APPLICATION OF DIALYSIS TECHNIQUES FOR COST EFFECTIVE PRODUCTION OF BIOLOGICALS WITH IMMOBILIZED ANIMAL CELLS

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For large scale cultivation of animal cells special attention has to be paid to the reactor design and the process strategy. Non-porous dialysis membranes offer the possibility for removal of low molecular nutrients, while high molecular nutrients or products are retained. After our experience dialysis membranes do not change their permeability characteristic even during long term cultivation.
A reactor concept will be presented, were a radial-flow fixed bed for cell immobilization is connected with a dialysis modul. This concept enables sufficient oxygen supply even at large scale.
Experiments were performed in a laboratory scale membrane dialysis reactor consisting of two chambers, which are seperated by a dialysis membrane. In the inner chamber the fixed bed containing porous carriers for cell immobilization is introduced.
The performance of this reactor will be discussed and compared to conventionell reactor systems. Based on a mathematical model scale-up criteria and possible process strategies will be discussed.

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HIGH DENSITY FIXED BED CULTURE OF ANIMAL CELLS USING MACROPOROUS CELLULOSE CARRIERS

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Fixed bed reactors are a potential tool for high density cultivation of animal cells. Especially macroporous carriers enable very high cell densities and high productivity. CELLSNOW™ is a newly developed macroporous carrier whose surface is modified with polyethyleneimine (PEI). The surface is slightly positive to enhance attachment of non-adherent cells such as hybridoma through electrostatic forces.
In this investigation two hybridoma cell lines were cultivated in either axial flow or radial-flow fixed beds to evalute the capacity of the carriers (concentration of immobilized cells, MAb productivity) and engineering parameters like the linear flow velocity necessary to support sufficient oxygen supply. The results will be compared with other carrier materials.

COMPARATIVE STUDY FOR THE PROPAGATION OF ANCHORAGE-DEPENDENT CELLS USING DIFFERENT FORMS OF MACROPOROUS CARRIERS

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In order to develop an economic high cell density perfusion culture system for obligatory adherent cells, the availability of surface area is of major importance. Consequently, porous microcarriers have been established recently as advantageous extracellular matrices allowing three- dimensional cell growth.
Suitable materials are expected to offer quasi-homogenuous protective microenvironment conditions which guarantee sufficient mass-transfer for media replenishment. In addition, an ideal support for anchorage-dependent cells should fulfill criterias such as high biomass loading capacity, simple inoculum procedure, mechanical stability and economic aspects. Among a variety of carrier materials mainly macroporous glass carrier and ceramic foam provide these properties.
Thus the following bioreactor systems were chosen to be applied for continuous perfusion cultures of various recombinant CHO cell-lines which produced glycosylated human proteins:
- fluidized bed (using SIRAN® Carrier, 0,4 to 1 mm in diameter)
- fixed bed (using SIRAN® Raschig rings, 2 x 2 mm)
- fixed bed (using a cylindrical monolith, ceramic foam, sintered alumina)

Based on these investigations, general assessments concerning optimal fermentation conditions and handling of the processes are presented. Other features of these culture systems, such as feed- and recycle rates, have been tested to improve overall or specific volumetric productivity. Of special importance is the potential for scale up if larger quantities of proteins are required.

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CONTROL OF CELL CONCENTRATION IN FEDBATCH HYBRIDOMA CULTURES

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The control of cell concentration in fedbatch hybridoma cultures may form one step in the optimisation with respect to product formation, if the relation between growth and product formation is known. In order to realise this, a feed of varying flow containing constant nutrient concentrations can be used. Due to the lack of online biomass measurements a model is needed to predict concentration profiles.
In the present study, an unstructured process model was postulated. Its parameter values were determined from the sampling results of the 40-hour batch period. Offline measurements included viable and non-viable cells, glucose and lactate concentrations. Online measurement of redox potential was used to indicate the process phase and to determine the starting time of the feed.
The feed profile necessary to keep the biomass concentration on a constant level was calculated using the model and applied in a feedforward manner during the fedbatch phase. Sampling results of this period compared well to the model predictions.

MONITORING AND MODELLING HYBRIDOMA CULTURES

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Mathematical models might become a valuable tool in the design, optimisation and control of industrial processes based on animal cells. The development of such models is possible and valuable only if cells can be cultured reproducably. Standardized pre-culturing procedures appear to be a prerequisite for this, but do not guarantee success.
For optimisation of process operation with respect to product formation or cell yield, process performance descriptive models that are valid over a wide range of conditions are required, whereas application in process control puts demands on the short term predictive capabilities of the model. In the postulation and verification of models of the latter class, monitoring all relevant compounds on a relatively high frequency - every 2 or 3 hours - is essential.
In the present study, the above mentioned is shown in the application to fedbatch hybridoma MN12 cultures.

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IMPROVEMENT OF MAMMALIAN FED-BATCH CULTURE

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Typical features of mammalian cells grown in suspension culture are the limitation of growth and very often the initiation of the cell death phase immediately after a critical cell density has been achieved. However, both features can be influenced to a certain extent by fortifying the growth medium as well as by feeding the culture daily with essential nutrients, starting at the end of the active growth phase. Increased cell density and the prolongation of the high cell density state leads to an increase in the volumetric productivity of many mammalian batch cultures like hybridoma cultures.
In order to improve the productivity of fed-batch cultures we have tested several nutrient sources in more detail. Together with informations obtained from our apoptosis studies we have been able to develop a more complex feeding solution which better satisfies the nutrient requirement of growing culture during the so-called stationary phase. Several mouse hybridoma cell lines as well as the recombinant mouse myeloma J558L were tested in serum-free fed-batch cultures under the improved conditions and significantly higher cell densities (ranging from 4-8 X10e6 cells /ml) were obtained. These viable cell numbers could be maintained over 10-20 days leading to substantially increased product titer in lab cultures as well as in 23L fermentors. However, the total cell numbers increased due to a balance of growth and death. In many cases the cell exhibited metabolic activity during the entire culture period whereas with other cell lines metabolic activity dropped off during the late stationary phase although the cells still remained viable.
The study has shown that it is possible to produce large amounts of monoclonal antibodies as well as recombinant proteins from general cell lines by increasing the volumetric productivity of an improved fed-batch culture. The long-term fed-batch production process is ultimately limited by product quality which is affected by the release of cellular proteins. Optimal fed-batch strategies should therefore reduce apoptosis induction during the high cell density state.

ON-LINE MONITORING AND CONTROL OF BIOPROCESSES USING OBJECT ORIENTED LANGUAGES AND GRAPHICAL USER INTERFACE

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Implementation of a high-performance process control system using a graphical programming language (*LabVIEW*) proved successful for comprehensive data acquisition, analysis, control and documentation in the long-term continuos cultivation of animal cells. Flexibility, modularity, and an interactive graphical user interface enhanced transparency and control of the complex bioprocess at every instant for the operator. Multitasking capabilities of the system provides direct interaction with more sophisticated control strategies.

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6.37.1

NON-INVASIVE ON-LINE INVESTIGATIONS OF INDUSTRY STYLE BIOREACTORS

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Gradients for relevant metabolites in fluidized bed reactors used for industrial production of recombinant proteins in high-density animal cell culture were investigated using a new approach. Volume-selective ^{31}P-NMR allowed to non-invasive monitor the influence of pH and oxygen gradients on metabolism and energy state of the cell at different locations within the reactor. Application of step changes gave access to transport pattern and reaction rates. A proven design of the reactor and peripherals as well as first results will show the outlined relationships.

AGGREGATE ANIMAL CELL CULTURE BY USING MICROBEADS

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A microcarrier has been used for cultivating anchorage-dependent animal cells in a stirred vessel. Since animal cells grow on the surface of a microcarrier, cells are damaged due to shear stress around a microcarrier or collisions between microcarriers. Cell attachment to a microbead surface without cell spreading is attempted to avoid the hydrodynamic detrimental effects during cultivation. Microbeads attaching cells contact each other, and then an aggregate of cells and microbeads will be formed. The surface of dextran particle as a microbead was modified with diethylaminoethyl (DEAE). The average diameter of the microbead in phosphate buffered saline was 48μm. Chinese hamster ovary (CHO) cells which produce a granulocyte colony stimulating factor (G-CSF) were cultivated in a 250mL spinner vessel contaning microbeads of 1 g/L. Cell attachment to a microbead surface without cell spreading and the formation of aggregate were observed. The cell density in the microbead culture was almost the same as that in the suspended culture. The cumulative production of G-CSF in the microbead culture was about 1.5-fold greater than that in the suspended culture. These results imply that the productivity was increased by forming aggregates. The reason why the productivity was increased is analysed in both biological and hydrodynamical standpoints.

IL-4 PRODUCTION USING MACROPOROUS MICROCARRIER

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Cytocell™ (distributed as Asahi Microcarrier in Japan) is a new macroporous microcarrier made of cellulose substituted DEAE. This microcarrier is designed to be applied for a high density cell culture especially of recombinant CHO cells. We will address specifications of the microcarrier, subculture, methods for counting total cell numbers attached to the microcarrier and viable cell numbers inside the microcarrier, confirmation of that cells are viable at the center part of the microcarrier by a confocal microscope, and IL-4 production from perfusion culture of recombinant CHO cells immobilized in the microcarrier. Not only recombinant CHO but BHK, Vero, HeLa and Hybridoma cells could be cultivated using the microcarrier.

APPLICATION OF CELLcul-20A BIOREACTOR AND MICROCARRIERS FOR LARGE SCALE CULTIVATION OF VERO CELL

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The Cellcul-20A (20L) and microcarrier (GT-2) used in this study both were made in our country. In addition, the "bead to bead" transfer technique was also adopted aiming at chieving high cell density. Cell density reached $9.0*10^{6}$ cells/ml, (along with 98% attachment rate of all microcarriers), and complete monolayers were formed on at least 82% of the microcarriers 6 days after inoculation.
The concentration of glucose and ammonia in growth environment were kept relatively stable. The results demonstrate that "bead to bead" cell transfer technique offers a viable alternative for scale-up culture of Vero cell.

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CULTIVATION OF ANIMAL CELLS IN A NEW MODULAR MINIFERMENTER

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A new and easy to handle reusable minifermenter for high-density culture of hybridoma cells and other cells is described. It is composed of two modules, separated from each other by a dialysis membrane allowing passage of low molecular mass nutrients and metabolites. The outer part of the production module (the 30 ml cell chamber) is made from a thin gas-permeable silicone rubber membrane allowing passive exchange of gases (oxygen and carbon dioxide). Nutrient media are contained in the larger nutrient container (600 ml). For culturing, the minifermenter is rolled on a roller apparatus in a carbon dioxide-supplied incubator. In the case of hybridoma cells cell densities of more than 10 million cells per ml, monoclonal antibody concentrations in the range of 0.5 - 4.5 mg/ml and antibody yields in the range of 20 - 100 mg were obtained within one to four weeks. The culture of virus infected animal cells is also possible. Depending on the type of virus and the cell line higher amounts of virus can be produced than in classical culture techniques.
Results obtained with other types of cells (human hybridomas, bone marrow cells) will be presented too.

SUBCULTURE METHOD FOR LARGE SCALE CULTURE USING MACROPOROUS MICROCARRIER

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Cytocell™ (distributed as Asahi Microcarrier in Japan) is a new macroporous microcarrier made of cellulose. We have established a new method of subculture to make a large scale cultivation possible, using this microcarrier. Recombinant CHO cells were cultured in a 100 ml spinner flask with the microcarriers. 10 ml of the medium with cells attached to microcarriers in this culture was transferred into another 100 ml spinner flask as seed, then 90 ml of fresh medium containing microcarriers was added into the same flask. The cells moved from the seed microcarriers to fresh ones without trypsinization and continued to proliferate. At last, all the microcarriers were packed with cells. Not only recombinant CHO but BHK, Vero, HeLa and Hybridoma cells could move from seed carriers to fresh carriers. The cell movement was affected by the cell density on seed microcarriers. In case of the ratios of a number of fresh carriers to that of seed carriers were 10 and 100, cells could be serially transferred over 5 times from seed carriers to fresh ones. Even when the ratio was raised to 1,000, CHO cells could proliferate and moved to fresh carriers. So, we tried a large scale culture using this method. We demonstrate that Cytocell is useful for large scale productions of bioactive substances, especially using recombinant CHO cells.

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LARGE SCALE SUSPENSION PERFUSION CULTURE PROCESS WITH A COMPACT CENTRIFUGE FOR MAMMALIAN CELL RECYCLE

Seiichi Yokoyama, Hiroyuki Takamatsu, Kimihiko Hamamoto, Masamichi Motoki, Takami Arai, Kenji Ishimaru, Masahiro Kimura, Akira Tanokura, Shoji Ono, Kenji Nagura, Michiyuki Tokashiki
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We have established an industrially competitive suspension perfusion culture process meeting the commercial production of pharmaceuticals. The system basically consists of usual culture vessel with an agitator and a compact external centrifuge for cell recycle, which facilitates expansion of production with scale-up potential highly increased. The cells separated in the centrifuge can be withdrawn easily from the centrifuge as the cells are kept viable and the system can continuously work for a long period of time with no cell clogging by feeding a liquid carrier such as fluorocarbon into the centrifuge and pushing the cells out with the liquid carrier. Further, it can be widely applied not only to anchorage-independent hybridoma cells but also to 293 cells which are intrinsically anchorage-dependent cells but have been allowed to adapt to the suspension culture. These have been verified by the following results: 1) Maximum separation capacity of the centrifuge attained so far is about 2.6 m^3/d at 100 G in hybridoma cells and larger scale-up can be expected, 2) long operated-perfusion culture of about 4 months has been performed at $(1\sim2)x10^7$ cells/ml with hybridoma cells, 3) perfusion culture of 293 cells producing recombinant Protein C has maintained $(1\sim2)x10^7$ cells/ml over 2 months.

HYBRIDOMA CULTURING IN AQUEOUS TWO-PHASE SYSTEMS

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Aim of this work was to design Aqueous Two-Phase Systems (ATPS) which support the long-term growth of animal cells, as a first step towards extractive bioconversions with animal cells.
It is shown that the polymers needed to form ATPS systems Poly(Ethylene Glycol) (PEG) and dextran (Dx) can affect cell growth because they increase the medium osmolarity. This osmolarity increase can to some extent be compensated by reducing the NaCl concentration of the culture medium.
Besides the osmotic effect the polymers have another 'physical effect' on cell growth. PEG already has a detrimental effect on hybridoma cell growth above concentrations of 0.025 g/g medium, while dextran concentrations up to 0.15 g/g medium can be tolerated. For cell growth in ATPS systems of PEG and dextran therefore systems have to be selected in which the cells partition to the lower PEG-poor phase.
It is shown that using PEG 35.000 and dextran 40.000 ATPS systems can be made in which the hybridoma cells used partition to the lower phase. Furthermore the dextran concentrations in the lower phase of these ATPS systems are tolerable for cell growth.
In two ATPS systems of PEG 35.000 and dextran 40.000 with different 'overall' polymer concentrations hybridoma cells have been cultured over a period of more than two months.

THURSDAY, SEPTEMBER 15, 1994

POSTER SESSION B

SESSION 3

3.6 Serum Content Affects the Structure and Activity of the Antibody Produced by Animal Cells in Culture
D.J. Black, J.P. Barford, C. Harbour, N. Packer, A. Fletcher

3.7 Expression and Secretion of Recombinant Human Tumour Necrosis Factor - beta in Different Insect Cell Lines
H. Chai, M. Al-Rubeai, K.L. Chua, M.G.S. Yap, S.K.W. Oh

3.8 Production of Recombinant Human Thyroid Peroxidase in Chinese Hamster Ovary Cells
Toshihiro Ii, Makoto Murakami, Masahiro Mizuguchi, Ken Matsumoto, Kazukiyo Onodera

3.9 Production and Post-translational Modification of tPA in Namalwa Cells
Mohsan W. Khan, Steve C. Musgrave, Nigel Jenkins

3.10 Role of Physiological CHO Cell Parameters and Culture Conditions on Product Consistency and Glycosylation Pattern of Interferon Omega
K. Kopp, W. Noé, M. Schlüter, R. Werner, F. Götz

3.11 Identification of Carbohydrate Structures of Glycoproteins by a Novel Method Utilizing Lectin-ELISAs
H. Leibinger, U. Marx

3.12 Analysis of Isohormone Profile and Glycosylation Pattern of recCG Produced by Recombinant CHO Cells in Perfusion Culture
Piet R. Levering, John W.M. Mulders, Jan B.L. Damm, Renato de Leeuw

3.13 Human Therapeutic Monoclonal Anti-D Antibody Produced in Long-term Hollow-fibre Culture. II. Influence of Culture Length on Antibody Characteristics
P. Matejtschuk, I. Sellick, E. Tarelli, S.F. Wheeler, J.M. Davis

3.14 Sialidase Activity During Chinese Hamster Ovary Batch Culture and Its Effect on Glycoprotein Product Quality
E. Munzert, J. Müthing, H. Büntemeyer, J. Lehmann

3.15 Analysis of Human Interferon-gamma Glycoforms Produced in Baculovirus Infected *Sf*21 and *Ea* Insect Cells
Olotu Ogonah, Bernadette Rooney, Robert Freedman, Nigel Jenkins

3.15.1 Influence of Bioprocess Conditions on Micro- Heterogeneity of Recombinant Glycoproteins
Michael R. Schuppenhauer, Antje Smala, Irving J. Dunn

3.16 Modifying of Antigen Binding of Human Monoclonal Antibodies Via the Alteration of Glycosylation by Sugar-limited Culture
Hirofumi Tachibana, Kiyotaka Taniguchi, Hiroki Murakami

3.17 **Rapid HPLC Quantification of Recombinant Human Antithrombin III During Production and Purification**
H. Tebbe, H. Büntemeyer, D. Lütkemeyer, J. Lehmann

SESSION 4

4.5 **Clinical Applications of Human Monoclonal Antibodies**
S. Hashizume, S. Sato, M. Kato, M. Kamei, K. Mochizuki, K. Kuroda, K. Kusakabe, K. Kanaya, M. Matsuda,K. Yasumoto, K. Nomoto, H. Murakami

4.5.1 **Monoclonal Antibodies against Human Osteocalcin Made by Using Recombinant GST-rhOc as an Immunogen**
M.-T. Matikainen, S. Virtanen, J. Hellman, M. Karp

4.6 **Establishment of Antigen Primed Two- and Threedimensional Cultures of Human Lymphoid Cells for the Development of Efficient In Vitro Immunization Protocols**
M. Schläfke, W. Döcke, U. Marx

4.6.1.**Production of Specific Monoclonal IgA Antibodies in Different Bioreactor Systems**
T. Stoll, U. von Stockar, I.W. Marison

4.7 **Purification of Monoclonal Antibodies by Fluidized/Expanded Bed Adsorption**
Jörg Thömmes, C. Born, Manfred Biselli, Christian Wandrey, Maria-Regina Kula

SESSION 5

5.6 **Human Therapeutic Monoclonal Anti-D Antibody Produced in Long-term Hollow-fibre Culture. III. Validation of the Chromatographic Purification Process**
R.M. Baker, A-M. Brady, B.S. Combridge, J.M. Davis, L.J. Ejim, S.L. Kingsland, D.A. Lloyd, P.L. Roberts

5.6.1**Process Validation of Continuous Perfusion Cultures**
Berthold G.D. Bödeker

5.7 **Variation in BHK Cell Populations, Examined by Multi-locus DNA Fingerprinting and Isoenzyme Analysis**
J. Clarke, G. Stacey

5.8 **Effective Virus Removal on the Filtration with Large Pore Size Membrane**
S. Fujita, T. Sato, G. Ishikawa, S. Manabe, N. Yamamoto

5.9 **Removal of DNA in Cell Cultured Fluid Using BMMtm**
T. Hirasaki, T. Noda, T. Tsuboi, T. Sato, H. Nakano, Y. Haruta, H. Murakami

5.10 **Virus Concentration Using Virus Removal Filter "Planova"**
G. Ishikawa, T. Sato, H. Nakano, Y. Koyanagi, N. Yamamoto, S. Manabe, K. Yamaguchi

5.11 Significance of Specific Productivity for the Effectiveness and Consistency of Mammalian Cell Cultivation
R. Kempken, W. Berthold

5.12 Advantages and Disadvantages of Glucose Limitation in Perfused Mammalian Cell Cultures: Analysis of a Large-scale, High-density Myeloma Cultivation
Konstantin B. Konstantinov, Mokhtar Mered, Fred Golini

5.13 Stability of Chimeric Antibody Producing Transfectomas During a Long-term Culture
Gyun Min Lee, Sung Won Bae, Hyo Jeong Hong

5.14 Optimization of a Serum-Free Freeze Medium for Mammalian Cells
A.A. Murnane, C. Nagel, J. Mercer, L. May, G. Polastri

5.15 Theoretical Requirements for the Integrity Test Method and Its Practial Application
H. Nakano, T. Noda, Y. Ishizaki, T. Sato, S. Manabe

5.16 Excellent Virus Removal on the Filtration of Biotechnology Product Solution by Planova Filter
H. Nakano, G. Ishikawa, S. Fujita, T. Sato, A. Losikoff, J. Poiley, E. Nelson, S. Manabe

5.17 Development of Procedures for Inactivating Microorganisms from Protein Components
K. Raval, D.E. Wyatt

5.18 Ultrafiltration as a Useful Step for the Purification of Recombinant Proteins During Downstream Processing of Cell Culture Supernatants
Klaus Scharfenberg, Roland Wagner

5.19 Inactivation of Hepatitis A Virus
Patrick Seechurn, David Birch

5.20 On the Acceptability of Biopharmaceuticals
R.E. Spier

5.21 A Simple and Rapid Method for Detection of Mycoplasmas in Mammalian Cell Cultures
Ulrich Valley, Klaus Scharfenberg, Roland Wagner

5.22 Cultivation and Downstream Processing of a Human Monoclonal Anti-alpha Haemolysin Antibody for the Treatment of Septic Patients
R. Waterstrat, R.W. Glaser, H. Tanzmann, Ch. Riese, U. Marx

SESSION 7

7.11 Model System for Apoptotic Death in Cell Cultures
J.H.M. van Adrichem, F.A.M. Asselbergs, C.R. Gandor, H.M. Eppenberger, C.H. Leist

7.12 Bioassay Method for Glycoalkaloids in Food with Animal Cell Cultures
Masahiro Asano, Shiro Yamashoji, Kenji Isshiki

7.12.1 Decrease of Radiation Toxic Effect on Thymus Cytostructure
C. Babak, I. Pavlyuk, H. Samborskaya

7.13 Organotypical Construction of Hybrid Artificial Liver
A. Bader, K. Bork, G. Schumann, K.Fr. Sewing

7.14 A New In Vitro Culture System Developed for Bovine Aortic Endothelial Cell Culture Has Proven Useful for Human Umbilical Vein Endothelial Cell Culture Models
Virgil Caldwell, Tori Mayer, Richard Wilkinson

7.15 Dielectric Spectroscopy of Mammalian Cells. 4. On-line Cell Cycle Analysis of Perfused Cultures of HeLa Cells in Suspension
V. Degouys, D. Petri, J. Harfield, F.D. Menozzi, L. Fabry, A.O.A. Miller,

7.16 Growth and Structure of Three-dimensional Corneal Tissue in Simulated Microgravity
K. Francis, K. O'Connor, D. Caldwell, G. Spaulding

7.17 In Vitro Cultures of B-Chronic Lymphatic Leukaemia (CLL) Cells Derived from Human Bone Marrow (BM) and Peripheral Blood in a Miniaturized Hollow Fibre Bioreactor
S. Hahn, W. Wächter, B. Flath, H. Matthes, H-D. Volk, C. Liebental, U. Marx

7.18 Bovine Embryo Development in a Defined Medium Without Somatic Cell Support
Hroyoshi Hoshi, Keizo Kobayashi, Shoko Yamashita, Takeshi Satoh, Yoshiaki Minato, Katsuhiko Sasaki

7.19 Egg Membrane Extract Damages Cultured Cells by Apoptosis in Serum-free Condition
Masayoshi Iio, Yoshie Yamabe, Mayumi Taniguchi, Reiko Kobata, Keiko Kohno

7.20 Differentiation of Human Leukemia Cells by Bacterial Extracellular Glycolipids
Hiroko Isoda, Hiroshi Shinmoto, Masatoschi Matsumura, Tadaatsu Nakahara

7.21 Cytotoxicity Testing for Food Safety Evaluation
Kenji Isshiki, Masahiro Asano, Shiro Yamashoji

7.21.1 The Function of Glycosphingolipids as Revealed by Endoglycoceramidase Applications
Makoto Ito

7.22 The 31 KD Cytosolic Phospholipase A2, an Antigen Recognized by a Cancer-related Human Monoclonal Antibody AE6F4
Seiji Kawamoto, Shuici Hashizume, Hirofumi Tachibana, Sanetaka Shirahata, Hiroki Murakami

7.22.1 Multicellular Hepatocyte Speheroids for Development of Bioartificial Liver Support System and Drug Metabolism Studies
Arye Lazar, M.V. Peshwa, F.J. Wu, C-M. Chi, T.D. Sielaff, F.B. Cerra, W-S. Hu

7.23 Action of Suppression of Killer T Cell Induction by Prodigiosin 25-C and Concanamycin B
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Serum Content affects the Structure and Activity of the Antibody Produced by Animal Cells in Culture

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Anti-D secreting human lymphoblastoid cell line BTSN4 was grown in two parallel cultures, A and B. In each case, the basal medium was IMDM supplemented with 2mM glutamine. However, to one culture 10% heat inactiviated foetal calf serum was added (culture A) whereas in the other serum-free additives of albumin, transferrrin, insulin, hydrocortisone and linoleic acid were used (culture B). In both cultures anti-D of the IgG1 subclass was secreted. In culture A, the overall and specific anti-D secretion rate was higher than in culture B but the anti-D produced displayed significantly lower functional activity in both monocyte-mediated ADCC and U937 rosetting assays. The glycosylation present in the Fc region of each purified antibody was examined. The observed activity difference between the antibodies from the two culture systems could be solely attributed to changes in this glycosylation.

EXPRESSION AND SECRETION OF RECOMBINANT HUMAN TUMOUR NECROSIS FACTOR - β IN DIFFERENT INSECT CELL LINES

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The expression and secretion of recombinant human tumour necrosis factor - β (lymphotoxin) was examined in seven different insect host cell lines, namely, *Spodoptera frugiperda 9 (Sf) - ATCC, Sf 9 - IXP, Sf 21, High - Five*™ (*Trichoplusia ni*), IZD-MB-0503 (*Mamestra brassicae*), *IPLB - HZ1075* and *Antheraea* moth ovarian cells. The highest yield of secreted recombinant human tumour necrosis factor - β was obtained by using the *Sf 21* cells, whereby the maximum protein productivity achieved was 71.7 ug / 1 x 10^6 for *Sf 21* cells, as compared to 32.1 ug / 1 x 10^6 for *Sf 9 - ATCC* cells. This is an increase of 100% over the yields obtained from the *Sf 9 - ATCC* cells. It was also observed that the recombinant human tumour necrosis factor - β expressed and secreted by the different insect cells has the same molecular weight, and hence, a similar glycosylation level. In addition, a new cell clone, *Sf 9 - IXP*, was derived, which was larger in cell size and had a higher tumour necrosis factor - β expression and secretion, in comparison to the native original *Sf 9* cells. Comparing these three cell lines, there is a linear relationship between increased protein production and the increase in cell size and granularity. Intracellular metabolic activities was also higher in productive cells.

PRODUCTION OF RECOMBINANT HUMAN THYROID PEROXIDASE IN CHINESE HAMSTER OVARY CELLS

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We established a recombinant chinese hamster ovary (rhTPO-CHO) cell producing a large amount of human thyroid peroxidase (hTPO) , and examined the possible use of recombinant hTPO (rhTPO) as the antigenic tool in analyzing patients with autoimmune thyroid disease.We developed the suitable culture medium for stable and high-level expression of rhTPO which was similar immunoreactivity to native-hTPO. The culture medium was a basal chemically defined UC203 medium derived from alfa-MEM to support both the cell growth and to enhance the production of biologically active substances encoded by the introduced hTPO gene in CHO cells. The components of culture medium including 11 amino acids, 4 vitamins, 3 inorganic components and 8 others were rearranged. In this study, we report the proliferation of rhTPO-CHO cells in culture system, the high expression system and the purification procedure of rhTPO from rhTPO-CHO cells.

3.8

3.9

PRODUCTION AND POST-TRANSLATIONAL MODIFICATION OF tPA IN NAMALWA CELLS.

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N-glycosylation is of critical importance in determining the therapeutic quality for some proteins, and is affected by the culture environment, genotype and phenotype of the host cells. In order to assess whether human glycoproteins are more authentically glycosylated in human or rodent cell lines, tissue-type plasminogen activator (tPA) expressed in Chinese hamster ovary (CHO) and in Namalwa cells was characterised. tPA is a 66-72KDa glycosylated serine protease, and functions as a fibrin specific activator of plasminogen, which exists as single chain and two chain form. It has four potential glycosylation sites, Asn_{184} is a variably occupied site giving rise to type I and type II tPA. Namalwa cells have endogenous dihydrofolate reductase (*dhfr*) which complicates transfection. Nevertheless tPA has been expressed in Namalwa cells by utilising a dominant selective marker, conferring resistance to G418 (*neo*^{R}), in conjunction with the *dhfr* gene amplification strategy. Transfectants were selected in G418, and clones expressing tPA were transferred intc hypoxanthine and thymidine deficient medium containing progressively higher methotrexate (MTX) concentrations. A clone expressing 5.4 μg/ml tPA after amplification in 400 nM MTX has been obtained. A serum-free medium has also been developed for *dhfr* amplified recombinant cells. Work is under way for the development of tPA purification from serum-free medium using affinity chromatography and results of tPA glycosylation heterogeneity in batch cultures of Namalwa cells will be compared to CHO derived material.

ROLE OF PHYSIOLOGICAL CHO CELL PARAMETERS AND CULTURE CONDITIONS ON PRODUCT CONSISTENCY AND GLYCOSYLATION PATTERN OF INTERFERON OMEGA

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Interferon ω is the most recently discovered member of the human class I IFN superfamily. Its glycosylation site at Asn-78 is carrying complex oligosaccharides. Due to two alternative signal peptidase cleavage sites the N-terminus of interferon ω is heterogenous[1]. In the present investigation a Chinese Hamster Ovary cell line producing human interferon omega was grown in fed-batch cultures under various fermentation conditions. It was used as a model system to study the effects of physiological parameters (e.g. relative cell age of the production cells, growth in suspension vs. adherent growth, serum-containing vs. serum-free production media, NH_4Cl spikes) as well as the effects of other culture conditions (e.g. length of the fermentation period) on the product consistency and identity of interferon ω. Glycoprotein preparations of interferon ω were purified in milligram quantities from the various cell culture supernatants. The product quantities enabled us to compare the glycoprotein variants directly with a reference material of desired product quality. Amongst the investigated parameters NH_4Cl spikes were found to be the most critical factors affecting the product consistency of the glycoprotein. Results of electrophoretic and chromatographic analyses will be shown.

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3.10

3.11

IDENTIFICATION OF CARBOHYDRATE STRUCTURES OF GLYCOPROTEINS BY A NOVEL METHOD UTILIZING LECTIN-ELISAs

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The glycosylation of proteins have a strong influence on their biological functions. In case of IgG antibodies changes in glycosylation patterns may lead to dramatic dysfunctions of the antibody dependent immune response of humans against infections and tumor cells. An ELISA assay was performed to analyse the sugar moieties of nearly native glycoproteins because of the denaturation of the glycoproteins in earlier established lectin-blotting techniques. This test was established on microtiter plates based on lectins. To test the specificity of the lectins to special carbohydrate structures competition test with mono- and disaccharides were used, the influence of blocking reagents was tested. Using these techniques a polyreactive monoclonal human IgG antibody (CBGA 1), its Fc and Fab fragment and λ chain were analyzed for their glycosylation pattern. The Fab fragment contains N- and O-glycosylation types. Different glycosylation patterns were detected at the Fab and Fc fragments and at the λ-chain of the CBGA1 antibody. Most of the glycan structures in the γ chain seems to be sialylated in contrast to the mainly fucosylated λ chain. In addition to the typical biantennary glycan structures terminal mannose which represent hybrid and/or high mannose structures were detected at all fragments.

ANALYSIS OF ISOHORMONE PROFILE AND GLYCOSYLATION PATTERN OF recCG PRODUCED BY RECOMBINANT CHO CELLS IN PERFUSION CULTURE

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Chinese Hamster Ovary (CHO) cells expressing recombinant human Chorionic Gonadotropin (recCG) were grown in perfusion culture using a variety of conditions. Product derived from cells under steady-state conditions was analyzed with respect to isohormone profile (isoelectric focussing), bioactivity (*in vitro, in vivo*), and receptor binding affinity. In addition, the glycosylation pattern of highly purified recCG was determined by monosaccharide composition analysis.
A relationship between isohormone profile and *in vitro/ in vivo* bioactivity was observed. RecCG containing relatively high amounts of basic isoforms were shown to have reduced *in vivo* and increased *in vitro* bioactivities. This corresponded with a low sialic acid content (low NeuNAc:Man-ratio) and also with low N-Acetyl galactosamine amounts (low GalNAc:Man-ratio), the latter being indicative for lower amounts of O-linked glycan structures on recCG. It is concluded that monitoring of the isohormone profile of recCG during a production process may yield important, predictive, information on its biological activity.

3.12
3.13

HUMAN THERAPEUTIC MONOCLONAL ANTI-D ANTIBODY PRODUCED IN LONG-TERM HOLLOW-FIBRE CULTURE II. INFLUENCE OF CULTURE LENGTH ON ANTIBODY CHARACTERISTICS

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For therapeutic biologicals produced in long-term culture, it is important to monitor the product for changes in molecular characteristics as the culture progresses. In this paper we describe studies on the characteristics of human monoclonal anti-D antibodies produced in long-term hollow-fibre culture. The electrophoretic properties of the antibodies were monitored by SDS-PAGE, and the characteristic isoelectric focusing profiles of antibodies obtained from early, middle, and late harvests were compared. The glycosylation of the antibodies was also analysed using high-performance ion-exchange chromatography after chemical cleavage of the glycans.

SIALIDASE ACTIVITY DURING CHINESE HAMSTER OVARY BATCH CULTURE AND ITS EFFECT ON GLYCOPROTEIN PRODUCT QUALITY

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Many recombinant glycoproteins of therapeutical interest are glycosylated in their native state. Variability in the oligosaccharide patterns of glycoproteins affects e.g. their circulation half-time, bio-distribution or biological activity. The labile terminal sialic acids, which are easily cleaved off the oligosaccharide, are of particular importance. Recent studies revealed sialidase activity in cell-free supernatant from chinese hamster ovary (CHO) cell cultures. In order to validate product quality during culture process, we monitored sialidase activity and studied the effect this enzyme in cell-free culture supernatant. Sialidase activity in the cell-free supernatant was determined with 4-methylumbelliferyl substrates, and free and bound sialic acids were detected by RP_{18}-HPLC. Supernatant sialidase activity varied significantly during batch growth. CHO cells secreting the glycoprotein recombinant human antithrombin III were batch-cultivated in aerated super-spinner flasks in serum-free and serum-containing medium. Product quality concerning bound sialic acids was tested using western blot analysis.

ANALYSIS OF HUMAN INTERFERON -γ GLYCOFORMS PRODUCED IN BACULOVIRUS INFECTED *Sf* 21 AND *Ea* INSECT CELLS

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The baculovirus infected insect cells has been used for the production of human therapeutic proteins, many of which are glycosylated in their native form. The type and structure of the oligosaccharide attached can have an important impact on the biological activity, stability and antigenic properties of a protein. Thus, any system used for the in vitro production of recombinant glycoproteins require a complete evaluation of its carbohydrate processing capability. We have assessed the N-glycosylation capability of two insect cell lines, Sf 9 and Ea. Both systems were used to produce human interferon -γ. The micro and macro heterogeneity of the glycoforms produced were analysed by micellar electrokinetic chromatography and matrix-assisted laser desorption time of flight mass spectrometry. The interferon -γ produced exhibited macro-heterogeneity. In addition each glycosylation site exhibits substantial micro heterogeneity, with some sites having undergone fucosylation.

INFLUENCE OF BIOPROCESS CONDITIONS ON MICRO-HETEROGENEITY OF RECOMBINANT GLYCOPROTEINS

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Application of step changes for pH, oxygen, glucose, and glutamine to cultivation of CHO cells expressing a recombinant glycoprotein showed varying patterns of micro-heterogeneity in their carbohydrate chain as determined by electrophoretic and blotting techniques. These results suggest shifts in the metabolic pathways and biosynthesis, influencing the post-translational modification due to changing environmental conditions. Gradients with respect to relevant metabolites in high cell density systems also used in industry may be responsible for previous similar findings.

3.15.1

3.16

MODIFYING OF ANTIGEN BINDING OF HUMAN MONOCLONAL ANTIBODIES VIA THE ALTERATION OF GLYCOSYLATION BY SUGAR-LIMITED CULTURE

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Most of the structural variations seen in the antigen binding sites of antibodies is encoded by the third hypervariable region of heavy chain. By keeping this region intact while modifying other domains via alterations of the carbohydrate moieties on the variable domain, we demonstrate a method to modulate antigen binding without disrupting the key features of the antigen binding site. We have chosen for our investigation to use the human-human hybridoma line HB4C5 and its subline C5TN. Both produce human antibodies specific to lung adenocarcinoma, which possess a *N*-glycosylation in the hypervariable region of their light chains eventhough immunoglobulin light chain usually lacks carbohydrates.

It is well known that the glycosylation of glycoproteins is dependent on the producing-cells and their culture environments. In turn, varied physiological states applied on producing cells can exert major effects on the biological properties of glycoproteins. In the present study, we have characterized the effects of glucose starvation and other saccharides on the glycosylation of the light chains and the resulting biological properties of the antibodies. When the cell lines were grown in the absence of glucose, the antibodies produced showed altered antigen-binding to its original antigen. Analysis of the light chains produced in these conditions revealed that the molecular-mass of the variant light chains varied from about 26 to 33kDa, which is due to the modified glycosylation. Addition of *N*-acetylglucosamine or fetal calf serum to the glucose-free medium lead to the creation of other light chain glycoforms that also exhibited altered antigen binding activity.

RAPID HPLC QUANTIFICATION OF RECOMBINANT HUMAN ANTITHROMBIN III DURING PRODUCTION AND PURIFICATION

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For monitoring of recombinant human antithrombin III (AT III) during cell culture processes and subsequent purification steps a rapid method for quantitative determination was developed. The need for the introduction of this method raised from limited availability and protracted implementation of the ELISA method. The developed method is based on reversed phase high performance liquid chromatography using a C_4 column. The separation in a gradient elution of water and acetonitrile takes less than 20 minutes even when difficult samples, such as serum containing cell culture samples, have to be analyzed. Automation and a high sample throughput are possible with this reliable method. If necessary, insulin, transferrin and albumin can also be quantified with minor changes of the elution profile. AT III concentrations were determined during fed-batch processes by the developed HPLC method and compared to the results obtained by the ELISA method.

3.17
4.5

CLINICAL APPLICATIONS OF HUMAN MONOCLONAL ANTIBODIES

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Human monoclonal antibodies (h-MAbs) are required to have their high specificities and adequate antigen-binding activities for applications of the antibodies to clinical uses. To generate hybridomas producing such h-MAbs, lymphocytes of the regional lymph nodes from cancer patients or peripheral blood lymphocytes from healthy volunteers were highly immunized *in vitro* before fusing the lymphocytes. The h-MAbs thus obtained were applied to a wide range of clinical purposes, including the immuno-cytological detection of cancer cells in sputum, the radioimmunoimaging of lung cancer and the therapies of tetanus and cancer. Cancer cells in sputum can be simply detected by the immunostaining method using h-MAb AE-6. Clear images of lung cancer xenografts in nude mice were obtained with ^{125}I-labeled h-MAb HB4C5. Tetanus was successfully cured by the use of a combination of h-MAbs G2 and G6 with high neutralizing activities in animal tests. Cancer xenografts in nude mice were regressed by the administration of h-MAb S-97 conjugated with *Pseudomonas* exotoxin A. These data indicate that h-MAbs are clinically of potent value.

MONOCLONAL ANTIBODIES AGAINST HUMAN OSTEOCALCIN MADE BY USING RECOMBINANT GST-rhOC AS AN IMMUNOGEN

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Monoclonal antibodies (Mabs) against human osteocalcin (hOC) were made by hybridoma technology using intraperitoneally immunized mice. The immunogen was produced in a bacterial intracellular expression system, recombinant hOC fused to glutathione S-transferase (GST), which acted as a carrier protein. The synthetic double-stranded oligonucleotide insert containing the hOC gene was ligated to plasmid vector pGEX-3X and transformed into *Escherichia coli* XL-1 Blue strain. The soluble GST-rhOC fusion protein was single-step purified by affinity chromatography and cleaved with protease factor Xa releasing the rhOC portion. rhOC was analyzed with LASERMAT mass spectrometry which gave a mass of 6080Da. This equals to the sum of the masses of the individual amino acid residues in this structure. The Mabs produced against this antigen efficiently recognize the native hOC and are applicable to the development of clinical assays for the measurement of hOC in human serum.

4.5.1

4.6

ESTABLISHMENT OF ANTIGEN PRIMED TWO- AND THREEDIMENSIONAL CULTURES OF HUMAN LYMPHOID CELLS FOR THE DEVELOPMENT OF EFFICIENT IN VITRO IMMUNIZATION PROTOCOLS

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In vitro immunization with human immunecompetent cells has been an aim of immunological research for a long time.
Simulation of such processes requires the same conditions as they exist in the human organism. It has been difficult up to now to realize an efficient in vitro immunization , because usual cell culture techniques (flasks, plates, roller bottles, spinners etc.) were not sufficient for reaching this aim.
A new miniaturized hollow fibre bioreactor permits threedimensional culture with high density of cells as well as optimal continuous supply of cultured cells with nutrients and oxygen. The use of this bioreactor will make it possible to built up threedimensional models of human immune organs in vitro , to analyse a large number of immunological phenomena in vivo (e.g. chemotaxis , antigen presentation) and to simulate processes of immunization.
A serumfree antigen primed culture of mononuclear cells from the peripheral blood of a healthy donor was performed in the bioreactor system. Over a period of 8 weeks viable cells could be detected in the bioreactor harvests. An activation of T-cells and macrophages could be shown by FACS - analysis. Regarding adhearence , deficiency of the used perfusion membrane could be proved. For this reason a set of various hollow fibre membranes and other materials was tested in parallel , respecting their properties to enhance cell growth and adhearence. Furthermore different media were investigated concerning the suitability for the optimal culture of these cells.
In order to establish a scheme of in vitro immunization we had to optimize usual culture techniques for raising antigen primed cell populations. Antigen coated beads were used to stimulate interactions between immunecompetent cells , processes of phagocytosis , processing and presentation of the antigen. We tried to establish antigenspecific T-cells being stimulated with lymphokines and thus to bring about an efficient help by these cells for affinity maturation in the bioreactor.

PRODUCTION OF SPECIFIC MONOCLONAL IgA ANTIBODIES IN DIFFERENT BIOREACTOR SYSTEMS

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IgA is the most abundant immunoglobulin in secretions and plays a major role in the protection of mucosal surfaces, which are the main source of antigen material for the body. It has a unique molecular structure, since its active form is a dimer, associated with two proteins, the so-called J-chain and secretory component. We have studied different bioreactor systems (*in vivo*, stirred tank -, hollow fiber and fluidized bed reactors) in medium with and without serum. Each of them was evaluated in terms of cell metabolism and IgA production. For this purpose, various assays were performed : anti α–chain ELISA, specific ELISA and Western blot. We were thus able to characterize each system in terms of the quantity and quality (specificity, dimer/monomer ratio) of the produced IgA.

4.6.1

4.7

PURIFICATION OF MONOCLONAL ANTIBODIES BY FLUIDIZED/EXPANDED BED ADSORPTION

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Fluidized bed adsorption is an integrative technology allowing the combination of three single steps of a purification strategy: Clarification, preconcentration and coarse purification may be accomplished in an integrated operation. In the work presented here the purification of a monoclonal antibody (IgG2a) from whole hybridoma fermentation broth using a fluidized bed procedure is shown . Two different matrices were examined, a commercially available material (STREAMLINE SP) was compared to a self derivatized matrix based on controlled pore glass. Both adsorbents had similar performance, resulting in a cell free eluate, that contained a preconcentrated (up to 4 fold) and coarsely purified (up to 8 fold) product. Scale-up of the process is demonstrated. The results obtained are used to develop a concept of an intergated production/purification process based on fluidized beds. Preliminary results of this concept are also presented.

HUMAN THERAPEUTIC MONOCLONAL ANTI-D ANTIBODY PRODUCED IN LONG-TERM HOLLOW-FIBRE CULTURE III. VALIDATION OF THE CHROMATOGRAPHIC PURIFICATION PROCESS

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The downstream purification of monoclonal anti-D is achieved using an automated three-column chromatographic process. Antibody capture by Protein G is followed by ion-exchange and gel filtration columns. This process must be fully validated in order to demonstrate consistent production of a safe, efficacious product. In this paper we examine certain aspects of the validation of this process, in particular model virus clearance/inactivation, DNA clearance and cytokine clearance.

5.6

5.6.1

Process Validation of Continuous Perfusion Cultures

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Recombinant Factor VIII - Kogenate TM -- from genetically modified BHK cells represents the first FDA licensed recombinant therapeutic protein which is produced from continuous high cell density perfusion culture. The 6 months long fermentation process (1) will be used to outline strategies and performance of process validation in continuous culture and compared to batch. This includes aspects such as batch definition , stability and reproducibility of fermentation, fermenter scale up, product characterisation at different times of fermentation , cell line stability and post production testing as well as challenging of the process to determine robustness. In addition, integration of process validation in the overall product development and validation activities from research to marketing will be addressed.

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VARIATION IN BHK CELL POPULATIONS, EXAMINED BY MULTI-LOCUS DNA FINGERPRINTING AND ISOENZYME ANALYSIS.

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BHK cells are widely used for virus culture and as recombinant vehicles. However, this line is well known to be susceptible to variation. Although descended from the same original culture, populations maintained in different laboratories may differ from each other in growth behaviour, morphology, virus susceptibility, and other features. These cells therefore constitute a model system to investigate changes that may occur during cultivation of a cell line after it has become widely distributed. BHK populations from different laboratories (including those lodged with the European Collection of Animal Cell Cultures), and cloned cell lines derived from some of these populations, have now been investigated using the techniques of multi-locus DNA fingerprinting and isoenzyme analysis. These procedures can provide a refined analysis of the genetic relationships between cell populations, and exclude cross-contamination events. This report represents a timely assessment of the level of standardisation of BHK cells available to the research community.

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EFFECTIVE VIRUS REMOVAL ON THE FILTRATION WITH LARGE PORE SIZE MEMBRANE

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Filtration using novel regenerated cellulose membrane, BMM, is one of the most effective methods for removing many viruses and other infective agents contaminating in bio-drugs and other protein solutions. BMM has large filtration capacity, high rate of component recovery and less inactivation of proteins. Viruses which size is larger than mean pore size of the membrane, are trapped within the membrane. For example, BMM35 (mean pore size 35 nm) can eliminate more than 6.7 logs of SV40 (45 nm), and Polio virus (about 25 nm) is removed more than 5 logs using BMM15 (15 nm), from protein solutions. Although smaller viruses and other agents may be eliminated effectively by smaller pore sized membrane, the recovery rate of some proteins will decrease, because of their own large molecular size.

To resolve this dilemma, we treated the small viruses, such as Polio virus and parvovirus (about 20 nm), to construct the aggregated form with chemical or immunological methods, and then filtration was performed with comparatively large pore size membrane. BMM35 remove non-aggregated parvovirus ineffectively (about 0.2 logs), but virions cluster formed by addition of antibodies against parvovirus can be rejected more than 2 logs at virus DNA amount. We also show effects of chemical treatments to cluster the viruses, pH condition, addition of chemical components and so on, at the filtration.

We propose here effective methods for removing small viruses with high recovery rate of proteins using BMM.

REMOVAL OF DNA IN CELL CULTURED FLUID USING BMM™

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We showed that large amount of cellular DNA contaminated in cell cultured medium.[1] For removal and/or reduction of contaminated DNA in bio-drugs, we want to show the applicability of the BMM virus removal filter. In cell culture step, that is, upstream manufacturing process of bio-drugs, the removability of DNA and the permeability of protein produced by cell were checked. Cell cultured fluid by hybridomas was prepared. Cell cultured fluid was filtered using BMM with mean pore size of 15nm and 35nm.(BMM15 and BMM35) The amount of Monoclonal antibodies (MAbs) (i.e., IgG and IgM) produced by cell were determined by ELISA. The amount of DNA was determined by Threshold[R] total DNA assay system. In early day of culture, the removability of DNA was about 2 logs rejection and decreased with the increase of culture day. It may be caused by the difference of dispersion state of DNA and fragmentation of DNA during culture. The permeabilities of IgG and IgM kept higher level than that of DNA in cultured fluid. DNA in protein solution was easily reduced comparing with naked DNA using BMM.

[1] In JAACT93' presentation

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VIRUS CONCENTRATION USING VIRUS REMOVAL FILTER "PLANOVA"

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Using virus removal filter "PLANOVA", concentrated virus solution was obtained from the original virus solution, and also concentrated gold particle solution was obtained from the original gold particle solution. Concentrated virus solution is useful, for example, when the virus is used as the vector of gene recombination technology.
"PLANOVA" is the best material for this purpose, because the hollow fiber membrane is made of regenerated cellulose, it has the hydrophilic nature, so that it adsorbs little protein or virus.
And of course,"PLANOVA" has exellent virus removability, so that highly concentrated virus solution can be obtained with very high recovery rate. For example, using "PLANOVA35"(its mean pore size is 35nm), for the case of HIV (Human Immunodeficiency Virus) solution, measured with $TCID_{50}$, 100 times concentrated solution was obtained with almost 100% recovery rate. For the case of gold particle solution, which is the substitute particle of virus, 20 times concentrated solution was obtained with about 80% recovery rate.
We found that, for this purpose, the filtration from out side of the hollow fiber membrane to inside is better compared with the filtration from reverse direction.

SIGNIFICANCE OF SPECIFIC PRODUCTIVITY FOR THE EFFECTIVENESS AND CONSISTENCY OF MAMMALIAN CELL CULTIVATION

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During the biotechnological production of therapeutic drugs several parameters are used to assess the performance of mammalian cell lines in culture. Data for cell growth, nutrient consumption, metabolite generation, physical parameters etc. can be applied equally in process development, manufacturing and IND/CPMP/PLA documentation. As well for researchers, engineers, economists, quality assurance and authority members it appears enticing to find inherent parameters to quantify the capability of the cells to generate its product.
The specific productivity is widely considered to be a meaningful parameter to describe the effectiveness of a cell culture. Such productivity data are often generated both in µL-scale multi well plate cultures at the early process development and in large-scale bioreactor cultures at the production process. During the exponential phase of batch cultivations, we could find indeed almost the same cell specific productivity at 100 mL T-flask cultures, at 1 L and 10 L spinner cultures and at 80 L, 400 L and 2000 L bioreactor cultures.
Nevertheless, this parameter can not be regarded as a biological constant and will not give reasonable values for some cell cultivation modes, e. g. at the advanced stage of fed batch cultures. The effect of further principles apart from a constant and constitutional productivity per cell can be illustrated by numerous findings. Enhancement of product formation from ca. 5 mg/L to ca. 200 mg/L was attained in our hands at equivalent cell densities by optimization of media and process conditions without further gene amplification or rise in selection pressure. Besides, the same cell line will often result in different specific productivities when cultivated either in suspended or in adherent modes.
The above-mentioned aspects will be discused in detail and the usefulness of parameters like the specific productivity for manufacturing and regulatory issues will be evaluated.

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ADVANTAGES AND DISADVANTAGES OF GLUCOSE LIMITATION IN PERFUSED MAMMALIAN CELL CULTURES: ANALYSIS OF A LARGE-SCALE, HIGH-DENSITY MYELOMA CULTIVATION

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Feeding strategies based on glucose limitation are becoming increasingly popular in mammalian cell cultivation. While until recently this approach has been limited to small-scale experiments, nowadays there is a considerable interest in its large-scale industrial application. Glucose limitation provides several important advantages, including control of the glucose metabolism, reduced lactate concentration, lower osmolarity, and simplified feeding technique.

Apart from these advantages, however, glucose limitation may provoke other phenomena with profound effect on cell physiology and process efficiency. In order to investigate the overall effect of glucose limitation, we have applied this strategy to the industrial scale perfused cultivation of NS0 myeloma cell line for antibody production. Glucose-limited and non-limited experiments were conducted in a 200 L sparged bioreactor, equipped with an external cell retention device. The NS0 cells were cultured for several months at high-density (20-60x10^6 cells/mL), which enabled the reliable monitoring of phenomena with large time-constants.

A comprehensive set of process variables representing cell physiology and process efficiency were analyzed both in terms of statics and dynamics. The effect of glucose limitation on the following parameters was studied: specific metabolic rates, metabolic rate ratios (including yields), cell viability, cell size and morphology, product glycosylation, and economic parameters (including volumetric productivity, absolute productivity, and titer). It was found that some of these variables were favorably affected by glucose limitation. However, others were changed adversely and irreversibly, resulting in suboptimal overall process performance. We concluded that glucose limited feeding has large potential for industrial application, but should be used cautiously, after careful evaluation of its long-term effect on the cell culture.

STABILITY OF CHIMERIC ANTIBODY PRODUCING TRANSFECTOMAS DURING A LONG-TERM CULTURE

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In the design of large-scale process for the production of chimeric antibodies for in vivo therapy, one of the most important criteria is the stability of transfectomas used. To maintain constant selection pressure for a producing population of transfectoma (P), antibiotics can be added to the culture medium. However, antibiotics such as G418 and hygromycin are so expensive that their use in the medium is impractical. To test the feasibility of the large-scale production of chimeric antibody for the post-exposure prophylaxis of hepatitis B virus (HBV) infection, the stability of transfectomas concerning antibody production was examined during a long-term, repeated-fed culture without selection pressure. Antibody production gradually decreased for the first 8 weeks and thereafter, was stabilized for the next 4 weeks. The specific antibody productivity (q_{MAb}) at the end of long-term culture was 4.63 $\mu g/10^6$ cells/day, which is approximately one half of the initial q_{MAb}. This loss of q_{MAb} was due to the appearance of a nonproducing population of transfectoma (NP) which was monitored during the long-term culture by using both limiting dilution method and flow cytometry. The subclones of NP were further characterized by ribonuclease protection assay of the heavy and light chain mRNA. Taken together, the results obtained here suggest that selection pressure is necessary for a long-term maintenance of stock culture and a continuous culture. However, a large-scale batch production of chimeric antibody may be made without using selection pressure.

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OPTIMIZATION OF A SERUM-FREE FREEZE MEDIUM FOR MAMMALIAN CELLS

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Over the past few years the trend in cell culture has been towards developing serum-free processes. Given the means to grow cells free of bovine serum the need arises for the ability to freeze these serum-free adapted cells in the absence of bovine serum. Currently cells are frozen in basal media supplemented with 20-90% fetal bovine serum (FBS) and dimethylsulfoxide (DMSO). We have been able to freeze successfully, recombinant Chinese hamster ovary (CHO), murine myeloma and hybridoma cells using carboxymethylcellulose (CMC) as a substitute for FBS, as originally reported by Ohno et al (Cytotechnology (1988) 1:257-260). This poster will discuss an evaluation of CMC as a substitute for FBS in freeze medium. Cells frozen in various concentrations and viscosities of CMC were evaluated for cell growth for up to one year after freezing. Results of these experiments indicate optimum freezing conditions using 0.1% high viscosity CMC.

THEORETICAL REQUIREMENTS FOR THE INTEGRITY TEST METHOD AND ITS PRACTICAL APPLICATION

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Virus removability of the virus removal filters should be validated theoretically in the production process of filters. For the case of PLANOVA filters, virus removability of the filters is validated for membrane with same pore structure range, namely membrane with both same mean pore size range and same maximum pore size range. Usually, the structure of the membrane is maintained before filtration, but it changes during filtration.
Although there are various kinds of supposable changes, it was theoretically proved that the worst change for the virus (particle) removal is the formation of extremely large pores compared with the original pore size. Therefore, it must be confirmed that such kind of change did not happen during filtration, and from this viewpoint, leakege test was introduced as one of the integrity test method. When it was confirmed, it will be sufficient enough to make sure the pore size distribution shift is within the acceptable range, by measuring the ratio of larger pores in the membrane, namely, gold particle removability test or liquid forward flow test.
In conclusion, if there is a possibility of any kind of pore size distribution change, then the guaranteed level of the virus removability is theoretically the level of the worst case pore size distribution change, but practically, it is avoidable by combining both leakage test and gold particle removability test or liquid forward flow test.

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EXCELLENT VIRUS REMOVAL ON THE FILTRATION OF BIOTECHNOLOGY PRODUCT SOLUTION BY PLANOVA FILTER

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Novel virus removal filters, PLANOVA15 (15nm mean pore size) and PLANOVA35 (35nm mean pore size) were evaluated their virus removability using protein solutions at TSI Washington Laboratories, showed excellent virus removal results. PLANOVA15 can eliminate more than 4.5 logs of polio virus. The removability of both Parvo virus and SV40 virus will be presented too. On the other hand, PLANOVA35 can eliminate more than 6.2 logs of Murine Xenotrope virus, more than 7.9 logs of Sindbis virus and more than 6.7 logs of SV40, respectively. PLANOVA35 cannot eliminate polio virus effectively(about 0.4 logs), because the mean pore size (35nm) is larger than the virus size (25~30nm).
Both IgG and IgM can easily pass through PLANOVA35 without change in their activities so that this filter is useful for the removal of larger size viruses (larger than 35nm) in the protein solution, whereas IgG can pass through PLANOVA15, so that PLANOVA15 is useful for the removal of smaller size viruses (smaller than 35nm) such as polio virus, Parvo virus and Hepatitis A virus.
These experiments were conducted considering the worst conditions for filtration. Higher protein concentration, maximum filtration capacity, lower filtration pressure and dead-end filtration are the worst case for the filtration using PLANOVA filters. The results showed good reproducibility on virus removal results based on the validated production process of filters.

DEVELOPMENT OF PROCEDURES FOR INACTIVATING MICROORGANISMS FROM PROTEIN COMPONENTS

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Significant progress has been made in the development of validated inactivation procedures for potential extraneous agents often introduced through the use of serum for cell culture applications. Although some inactivation methods have been shown to have a minimal damaging effect on the product itself post-treatment, not all animal-sourced components lend themselves to a reliable means of validation. Protein components which are isolated through a number of purification steps may present validation difficulties. As a step of assurance, many protein manufacturers have developed procedures to qualify starting raw material as well as spiked starting material with key viruses to demonstrate the degree of their removal or inactivation throughout the process of isolating the specific end-product. Although these data are useful, customers are often required internally to show that these proteins do not contribute extraneous agents to culture systems. This poster will outline procedures developed to spike dry protein components with various microorganisms, treatment of the challenged materials by irradiation, and the residual levels of each organism will be shown as well as the overall effect or irradiation on the performance characteristics of individual protein components.

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ULTRAFILTRATION AS A USEFUL STEP FOR THE PURIFICATION OF RECOMBINANT PROTEINS DURING DOWNSTREAM PROCESSING OF CELL CULTURE SUPERNATANTS

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High molecular weight proteins secreted from mammalian cells growing in protein-free medium formulations are efficiently purified by ultrafiltration as a first step in a downstream process. Simultaneous purification of two highly glycosylated EBV surface proteins of 250 and 350 kd, respectively, from recombinant CHO cells is presented. The products were efficiently concentrated using a membrane with a NMWCO of 100000 by removing nearly all low molecular weight proteins (purification factor >2) such that only two additional chromatographic procedures were necessary for achieving homogeneity of the two glycoproteins.

INACTIVATION OF HEPATITIS A VIRUS

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Concerns regarding the transmission of hepatitis A virus in blood products have increased recently with reports of haemophiliacs developing the disease after treatment with high-purity factor VIII. We have recently evaluated a quantitative plaque assay for the detection of a lytic strain of hepatitis A virus. This assay will be of use to manufacturers of blood products since it can be used in the validation of purification processes for virus removal. A rapidly-replicating, lytic strain of the virus is inoculated onto FRhK-4 cells, a foetal rhesus monkey kidney cell line, and incubated for 7-9 days before staining for enumeration. The clearance of hepatitis A virus has been tested in a number of physical and chemical inactivation steps and the data from selected experiments is presented.

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ABSTRACT: On The Acceptability of Biopharmaceuticals

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It is well known that some 90% of the money expended on obtaining a license to market a Biopharmaceutical is used in satisfying the requirements of the regulatory agencies. The amount of money involved is considerable and ranges anywhere between $100 million to $200 million per license; the time required is from 5 to 10 years. This represents a considerable effort to obtain what amounts to the acceptance of a society watchdog to the promulgation of a particular Biopharmaceutical. It is therefore worthwhile to investigate the factors that promote or impede the achievement of such an acceptance.
It is facile and superficial to merely focus on the three key words; safety, efficacy and consistency. We have rather to recognise that safety is relative and different levels of risk are acceptable under different circumstances. Similarly efficacy is can vary between that which is barely detectable to that which is overwheaningly obvious. Consistency too has its problems. A process engineer is well aware that each and every run is a different animal. But between what limits lie the tolerances of the regulatory agency? These conundrums lead directly to a deeper appreciation of the pivotal function of the regulatory agency. This body adjudicates, evaluates, determines relative worthiness, estimates the benefit or goodness of a product in its deliberations; it cannot but be otherwise.
How are these ethical or moral parameters determined? The regulators are humans; they are susceptible to the same sorts of influences that determine how you and I determine what is good and bad; what is acceptable and what is not.Of course they have a job to do in that they have to consider what happens socially were they to make a misjudgement; for such events have personal as well as wider consequences. My paper will address these issues and will examine the psychology and the sociology of the phenomenon of acceptance. It will also explore how both of these issues can link into a basic philosophy of ethics.

A Simple and Rapid Method for Detection of Mycoplasmas in Mammalian Cell Cultures

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A simple and rapid method for the detection of mycoplasmas in mammalian cell cultures has been developed based on the enzymatic activity of adenosine phosphorylase. This enzyme, which has been found in high activity in mycoplasmas in contrast to mammalian cells, catalyses the transformation of 6-methylpurine deoxyriboside (6-MPDR) into the two cytotoxic products 6-methylpurine and 6-methylpurine riboside. Estimation of parasitic incidation relies on the direct visualization of the enzymatic conversion of 6-MPDR by a simple isocratic ion-pair reversed phase high performance liquid chromatography. The final result of the test is available after 3 to 12 hours of incubation of the cells together with the indicator metabolite 6-MPDR and depends on the adenosine phosphorylase activity of the respective mycoplasma species. The direct detection of enzymatic activity results in a high sensitivity allowing the observation of infections that could not be found by the indirect cytotoxicity test.

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CULTIVATION AND DOWNSTREAM PROCESSING OF A HUMAN MONOCLONAL ANTI-ALPHA HAEMOLYSIN ANTIBODY FOR THE TREATMENT OF SEPTIC PATIENTS

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Alpha haemolysin is an important virulent factor of most strains of Staphylococcus aureus. Studies with human hyperimmune serums have demonstrated that neutralizing antibodies against this toxin considerably improved the clinical symptoms of patients suffering from staphylococcal sepsis. However, infectional risks and economical considerations seem to make monoclonal antibodies superior for this application.

By fusion of peripheral blood lymphocytes with the heteromyeloma CB-F7 we developed a human monoclonal IgG antibody that neutralizes alpha haemolysin of Staphylococcus aureus. A stable hybridoma line was established by repeated cloning. The upstream processing and the composition of the cuture medium were optimized for hollow fibre reactors. The antibody was finaly produced in a CellPharm I hollow fibre bioreactor (CD Medical; U.S.A.).

We have developed an on-line purification procedure with particular emphasis on almost pyrogen-free conditions. Protein A chromatography is followed by dialysis in a computer-controled hollow fibre module (Integra Biosciences; Germany) and by a filtration via PLANOVA 75 filter (ASAHI Chemical Industry Co., LTD.; Japan) for virus removal.

An application dose for one patient (134 mg) was manufactured with a neutralizing activity of 38,6 U/mg. The concentration of lipopolysaccharides was below 145 pg/mg.

According to the presently available tests, the human monoclonal IgG antibody resulting from the described on-line purification process was found to be suitable for human in vivo application.

MODEL SYSTEM FOR APOPTOTIC DEATH IN CELL CULTURES

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Keywords: Apoptosis, CHO cell line, protein- and serumfree medium

Abstract
The foundation of bioprocess development and control is the characterization of the morphological and biochemical changes of the cell. Not only proliferation or growth, but also cell death should be considered. In general, cell death may follow two distinct patterns: necrosis and apoptosis. In a model system, apoptotic death is induced in a CHO producer cell line expressing p53. This cell line grows as a suspension in serum- and proteinfree medium. DNA fragmentation, cell morphology and growth rates were analysed.

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BIOASSAY METHOD FOR GLYCOALKALOIDS IN FOOD WITH ANIMAL CELL CULTURES

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Glycoalkaloids, e.g. solanine and tomatine are found in potato and tomato. They are toxic to animals. It is difficult to analyze them in food. In this study, we examined to detect cytotoxicity of tomatine in tomato. Cell lines of NIH3T3, HepG2, HuH-6KK and U937 were used. Detection of tomatine with some methods were compared. The methods were Alamar blue, MTT, WST-1, reduction and chemiluminescent method. These methods were possible to detect cytotoxicity of tomatine. Particularly, a combination of HepG2 cell with chemiluminescent method was more easy to operate and rapid than others.

DECREASE OF RADIATION TOXIC EFFECT ON THYMUS CYTOSTRUCTURE

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By biochemical, electron microscopic and autoradiographic methods it has been established that dystrophic disturbance in cells, inhibition of mitosis processes and albumenum-synthesizing function of cells nuclei are exhibited under a radiation effect. Focused decrease of thymocytos has been expressed and thymus accedental involution has appeared. Thymus-dependent zones in lymphatic nodes, spleen and adrenals have been changed. Natural resistance of animal organisms is decreased. On the 30th day of aethonium peroral injection cyto-architectonics of the gland is normalized. On the 40-60th day thymus-dependent zones of organs are regenerated, unspecific resistance factors are improved. Thus, aethonium neutralizes the toxic effect of radiation on the thymus, adrenals and spleen, increasing the reactiveness of animal organisms.

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ORGANOTYPICAL CONSTRUCTION OF A HYBRID ARTIFICIAL LIVER

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Various design characteristics for hybrid artificial livers have been developed over the last decade paralleling extensive advances in cell culture techniques for primary hepatocytes. It has thus become clear that hepatocytes are anchorage-dependent cells. In order to design an appropriate bioreactor capable of large scale adhesion-dependent cultures, we have reconstructed the liver specific micro- and macro-environment. **Methods:** We used a double gel culture technique which sandwiches haptocyte between 2 layers of collagen. Urea synthesis as well as GLDH and AST leakage (n=10) of rat hepatocytes were measured in Petri dishes by enzymatic measurement up to 3 weeks. Albumin secretion was measured by ELISA. Albumin secretion in the bioreactor was analyzed up to nine days (n=3). **Results:** Urea synthesis was stable over time in culture at 3.78 ±0.84 mmol/l (mean±SEM). GLDH as well as AST leakage decreased with time in culture; GLDH: from 16.2±5 to 3.9 ±0.7 10^6 pmol / min x L at day 21 in culture, AST from 31.8 ± 3 to 13.9 ± 4 10^6 pmol / min x L at day 21 (mean ± S.D.). The rise in albumin secretion in the bioreactor module paralleled that of hepatocytes cultured on the same type of membrane at standard incubator conditions. Membranes used for loading the bioreactor had an initial cell specific production rate of 274 ± 161 µg (8 x 10^6cells) (mean of 9 runs ± S.D.). The amount of albumin produced up to the end of the bioreactor run was 2959 ± 131 µg/d(8 x 10^6cells). Hepatocytes seeded on the membranes continued their albumin production rate, increasing with time in culture, and proceeded up to rates of 578 ± 124 µg/d(8 x 10^6cells) by day 8 in culture. **Discussion:** Previously cell culture techniques used for hybrid artificial livers such as use of micro carriers or hollow fibers have either supported suspension, aggregate, single gel or gel entrapment cultures. The bioreactor arranges hepatocytes in plates like *in situ,* which facilitates similar positional interrelationships with extracellular matrix, sinusoidal analogues and non parenchymal cells as *in vivo*. Bipolar attachment of hepatocytes to extracellular matrix allows stable protein secretory and detoxifying functions such as urea synthesis.

A NEW IN VITRO CULTURE SYSTEM DEVELOPED FOR BOVINE AORTIC ENDOTHELIAL CELL CULTURE HAS PROVEN USEFUL FOR HUMAN UMBILICAL VEIN ENDOTHELIAL CELL CULTURE MODELS.

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In a previous report a new endothelial cell culture model using a plasma-based culture system was shown to be effective for growth and manipulation of low-passage, primary derived bovine aortic endothelial cells. This bovine model has now been shown effective for propogation of a primary human umbilical vein endothelial cell line that was developed in our laboratory (AK315). Enhancement of log phase growth was achieved by addition of simple biochemical components while maintaining normal morphological characteristics. Enhancement of culture growth while maintaining normal cell characteristics closely parallels the results of earlier studies with the bovine model. Most importantly, we were able to very simply regulate the extent of log-phase growth with the addition of these simple biochemicals. The ability to take cells in and out of log-phase growth (while maintaining normal characteristics) offers an improved culture system for gene therapy applications as well as other models for endothelial cell biology.

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Dielectric spectroscopy of mammalian cells. 4. On-line cell cycle analysis of perfused cultures of HeLa cells in suspension

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Abstract

The progression through the S-, G2- and M phases of the cell cycle of HeLa cells in perfused cultures kept blocked at the G1/S interphase following a 24 hours incubation with 1 mM hydroxyurea (HU), has been followed by laser flow cytometry (FCM) and dielectric spectroscopy at 1 MHz.

Although less informative than FCM, the latter method allows unexpensive on-line cell cycle analysis.

GROWTH AND STRUCTURE OF THREE-DIMENSIONAL CORNEAL TISSUE IN SIMULATED MIRCOGRAVITY

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This work concerns the regeneration of three-dimensional corneal tissue. The key to regeneration is the NASA High-Aspect Ratio Vessel (HARV). By simulating microgravity, this vessel provides a gentle culturing environment for cell cultivation in which hydrodynamic forces are minimal. In this environment, cells are free of hydrodynamic damage and interact to form three-dimensional structures that closely mimic intact tissue. The emphasis of our work to date has been to characterize the growth and structure of corneal tissue cultured in the HARV. Tissue structure has been investigated with scanning electron microscopy and immunocytochemistry. Also, initial metabolic data will be presented. Regenerated corneal tissue can be used as a cell model and for transplantation. As such, it can improve the quality of life for individuals with impaired vision through a reduction in transplant complications and development of novel treatments for corneal disorders.

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IN VITRO CULTURES OF B-CHRONIC LYMPHATIC LEUKAEMIA (CLL) CELLS DERIVED FROM HUMAN BONE MARROW (BM) AND PERIPHERAL BLOOD IN A MINIATURIZED HOLLOW FIBRE BIOREACTOR

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B-cells from patients with CLL have always been cultivated for pharmacological tests and cellular studies (eg. surface markers, lymphokine pattern, immunoglobulin). In comparison to the situation in vivo, drastic changes in the condition of cultures in vitro regarding oxygen supply, media composition, cell density, etc. lead to a significant change of the cell physiology (eg. loss of surface markers). Because of an optimal combination of supply of nutrients via hollow fibres and supply of oxygen via silicon membranes directly to the cell growth chamber, a miniaturized hollow fibre system suitable for 3D cultures of animal cells was tested for the culture of B-CLL cells derived from different patients and compartments (BM, Peripheral Blood Lymphocytes). Cellular and metabolic parameters were analyzed in those, as well as in conventional cultures with the aim of a comparative control. Suprisingly, in contrast to the conventional control cultures, viable cells could be grown in the bioreactor moduls over more than 8 weeks. Moreover, different CD-markers (CD 5, 19, 20, etc.) were found on the cells in the harvests using FACS analyses. In addition, maximal concentrations of 1500 ng/ml interleukin 3 were found in the cell growth chamber. The results indicate a self conditioning effect of B-CLLs in optimal perfused 3D cultures. Innovative applications of this type of human B-CLL cultures are being discussed.

BOVINE EMBRYO DEVELOPMENT IN A DEFINED MEDIUM WITHOUT SOMATIC CELL SUPPORT

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Large scale, low cost production of cattle embryos from ovaries at slaughterhouse by in vitro maturation and fertilization of oocytes would provide extensive benefits of embryo transfer technology. However, bovine embryos in culture often fail to develop beyond 8-16 cell stage in conventional culture medium. To overcome the developmental block, bovine embryos are cocultured with oviduct epithelial cells, cumulus/granulosa cells, and other types of cells. The gaseous environment of cultured mammalian embryos is important in their developmental process. In a gas atmosphere of 5% CO_2/95% air (20% O_2) the proportion of embryos developing to blastocyst stage was higher in coculture with bovine granulosa cells (BGC) compared to the absence of BGC in high glucose medium (1 mg/ml). In a gas atmosphere of 5% O_2, however, blastocyst formation was apparently lower in coculture with BGC than in BGC-free control culture. Without BGC, a reduced amount of glucose (0.4 mg/ml) remarkably increased the blastocyst formation under low oxygen culture (5% O_2). In summary, a combination of low oxygen atmosphere (5% O_2) and low glucose medium (0.4 mg/ml) greatly improved bovine embryo development in vitro in a chemically defined medium without somatic cells. The establishment of a defined medium under optimal oxygen supply is useful to study the metabolic and nutritional requirement of bovine embryos and also improve the efficient production of embryos for a transfer.

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EGG MEMBRANE EXTRACT DAMAGES CULTURED CELLS BY APOPTOSIS IN SERUM-FREE CONDITION

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An extract from chicken egg membrane with an ethanolic alkaline aqueous solution damaged a variety of cells including WIL-2NS, HF (Hybridoma) and SFME cells. The cell damaging activity was lost when bovine serum albumin or serum was added to the culture with the egg membrane extract. Ovalbumin did not show a similar protective effect. The cell damaging activity was not affected by the presence of excess amount of insulin or transferrin, indicating that the activity was not manifested by an interaction with these growth factors. Morphological change and DNA electrophoretic pattern suggested that the cell damage was an apoptotic one. The active fraction was precipitated by acid treatment or heat treatment and the activity was maintained even after these treatments. The active fraction produced amino acids and sugars after acid-treatment, suggestive of its being glycoprotein(s).

DIFFERENTIATION OF HUMAN LEUKEMIA CELLS BY BACTERIAL EXTRACELLULAR GLYCOLIPIDS

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The effects of bacterial extracellular glycolipids, succinoyl trehalose lipid (STL) and mannosylerythritol lipid (MEL), on the growth and differentiation of a human monocytoid leukemia cell line, U937 and a human promyelocytic leukemia cell line, HL-60, were examined. STL and MEL were prepared from *Rhodococcus erythropolis* grown on n-hexadacane and *Candida antarctica* on vegetable oil, respectively. Both glycolipids markedly inhibited growth and increased phagocytic activity of U937 and HL-60. Using the non-specific esterase method, STL was found to induce monocytoic diffrentiation while MEL induced granulocytic differentiation.

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CYTOTOXICITY TESTING FOR FOOD SAFETY EVALUATION

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We have been interested in studying the factors affecting the quality of food. It is important to detect toxic substances in food as well as to control food-born disease microorganisms. We have applied in vitro bioassay to evaluate the quality of food. We tried the following assay procedures; Alamar Blue reduction assay, lactose dehydrogenase assay, neutral red inclusion assay, MTT reduction assay, WST-1 reduction assay, and others. We have developed a chemiluminescent cytotoxicity assay and applied it to food component and toxins. This assay was sensible and rapid. The WST-1 reduction assay was easy to operate. We discuss the usefulness of in vitro cytotoxicity testing for keeping food safety.

THE FUNCTION OF GLYCOSPHINGOLIPIDS AS REVEALED BY ENDOGLYCOCERAMIDASE APPLICATIONS

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Endoglycoceramidase (EGCase) can specifically remove the sugar chains of glycosphingolipids (GSLs) from living cells (1-6). In a study using A431 cells, treatment with EGCase resulted in the arrest of cell growth accompanying the suppression of epidermal growth factor (EGF) receptor phosphorylation. With a decrease in cell surface GSLs by EGCase, it was observed that the cell-cell attachment of B16 melanoma become weaker and cobblestone morphology seen in control experiments disappeared, while in contrast, attachment for collagen increased. These abnormalities were eliminated completely by the removal of EGCase from the culture following restoration of GSLs. The results strongly suggest that GSLs may play important roles in the modulation of cell growth and cell attachment.

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THE 31 KD CYTOSOLIC PHOSPHOLIPASE A_2, AN ANTIGEN RECOGNIZED BY A CANCER-RELATED HUMAN MONOCLONAL ANTIBODY AE6F4

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The human monoclonal antibody (MAb) AE6F4 is secreted by a hybridoma line which originated from normal peripheral blood lymphocytes immunized *in vitro* with the human lung adenocarcinoma cell line, A549. This MAb exhibits high reactivity with human lung squamous cell carcinomas. A previous study has indicated that the MAb recognizes a 31 kDa antigen in the cytosolic fraction of A549 cells. A partial amino acid sequence of the affinity-purified 31 kDa antigen exhibits 90% homology to a family of proteins collectively designated 14-3-3 proteins. In order to characterize the 31 kDa antigen recognized by the MAb, we tried to detect the 14-3-3 protein family DNA in A549 cells. PCR analysis indicated that the 14-3-3 protein family mRNA is expressed in A549 cells. One of the PCR products' nucleotide sequences perfectly matches the human placental cytosolic phospholipase A_2 ($cPLA_2$), which is one member of the 14-3-3 protein family. The entire coding region-containing the $cPLA_2$ cDNA was re-cloned and applied to COS cell-expression system. SDS-PAGE analysis indicated that a recombinant 31 kDa $cPLA_2$ protein is expressed in those COS cells. Immunoblotting demonstrated that the 31 kDa protein is recognizable by the MAb. Northern analysis revealed that several 14-3-3 protein family mRNAs are expressed in A549 cells.

MULTICELLULAR HEPATOCYTE SPHEROIDS FOR DEVELOPMENT OF BIOARTIFICIAL LIVER SUPPORT SYSTEM AND DRUG METABOLISM STUDIES

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Xenogeneic hepatocytes have recently been used in a bioartificial liver device as a short-term extracorporeal support of acute liver failure. Scaling up the system requires large quantities of viable and highly active cells. Hepatocytes grown as spheroids manifest higher metabolic activities for longer time periods as compared to hepatocytes cultivated as single cell cultures. A method was developed for preparation of hepatocyte spheroids on a large scale. Pig hepatocytes when cultured under stirred conditions, form multicellular spheroids in hormonally defined culture medium. Spheroids were formed 24 hr after cell inoculation with an efficiency of 80-90%. The spheroids reach a relatively uniform shape with mean diameter of 130-140 μm. Scanning electron microscopy revealed numerous microvilli projecting from the entire surface of the spheroids, and deep holes apparently corresponding to bile canaliculus-like structures. Transmission electron microscopy revealed differentiated hepatocytes which displayed well-developed cytoplasmic structures and bile canaliculus-like structures in between. The morphological studies show a resemblance between cells in the spheroids and in the liver *in vivo*. Spheroid liver functions such as ureagenesis, albumin production and lidocaine metabolism were enhanced for longer culture periods as compared with single hepatocytes. Our simple, highly efficient and reproducible method should allow production of large quantities of spheroids to be employed in a bioartificial liver device, as well as used in drug metabolism studies.

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ACTION OF SUPPRESSION OF KILLER T CELL INDUCTION BY PRODIGIOSIN 25-C AND CONCANAMYCIN B

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Prodigiosin 25-C (PrG), which has been found in the metabolites of *Streptomyces hiroshimensis*, specifically inhibits concanavalin A (ConA)-induced blastogenesis of murine splenocytes. We have shown that PrG suppresses induction of allogeneic MHC-specific killer T cells without affecting production of antibody against a T cell dependent antigen, sheep red blood cells, and that it inhibits the induction of allogeneic MHC-specific CTL through selective inhibition of allogeneic MHC presentation of macrophages. *In vitro,* PrG inhibits proton pumping activity of vacuolar type H^+-ATPase (V-ATPase) and raises pH of intracellular organelles in intact cells. To address the question whether the inhibition of acidification is responsible for the inhibition in the induction of killer T cells, we studied the effect of concanamycin B (CB), which is known to be a specific and potent inhibitor of V-ATPase. It has been revealed that CB also inhibits induction of killer T cells against an allogeneic mastocytoma, P815. The flowcytometric analysis of splenocytes showed a strong reduction in the population of $CD8^+$ cells in those splenocytes treated with PrG and CB following immunization with P815 when compared with P815 immune splenocytes. However results from the experiment of MLR (Mixed Lymphocyte Reaction) showed that CB, in contrast to PrG, has no specific inhibitory activity against the functions of responder (T cell) and stimulator (SAC;spleen adherent cell). These results suggest that PrG and CB act differently in their way of inhibiting the killer T cell induction, even though both of them caused reductions in the population of $CD8^+$ cell in splenocytes. Further experiments are now in progress to elucidate the action of PrG and CB.

THE NEW CATALOGUE OF THE CELL LINE DATA BASE

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Within the Interlab Project, supported by the National Institute for Cancer Research and the Advanced Biotechnology Center, Genoa, the Cell Line Data Base (CLDB) was set up. It is available on-line through Internet, and contains detailed information on human and animal cell lines obtained from both Italian and European cell line collections. The second edition of the Catalogue "Human and Animal Cell Lines" directly extracted from the CLDB, has also been realized. The catalogue, is proposed as a complete source for cell lines retrieval. More than two thirds of cell lines are not described in any commercial catalogue. The catalogue consists of two main sections: one relates to human and animal cell lines and the other gives information on the cell lines from specialized collections. In order to simplify the search of a cell line, a general index is available, as well as special indexes (species/tissue, tumor, pathology, function...). It describes 2650 cell lines, 1274 of which are "pathological", 617 are tumoral, 520 are normal and 215 are transformed. It contains data on availability, extensive characterization (immunological profile, cytogenetic analysis...), quality control (identity and sterility), bibliographic references. A disk version of the catalogue is also available for IBM compatible personal computers; the cell lines can be retrieved on the basis of the most relevant features (name, tissue, species, tumour or pathology). CLDB is also indexed and made available on-line by means of the Wide Area Information Servers (WAIS) system and can be reached and queried by means of the Gopher software, a menu-driven, easy-to-use, public-domain package.

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DETECTION OF MICRO-METASTASIS BY POLYMERASE CHAIN REACTION (PCR).

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One of the important subjects in the cancer therapy is development of new modalities to control metastasis. The available methods, however, have an inherent limitation in detecting and quantifying micro-metastases. Recently developed *r/m*HM-SFME-1 cells* (*r/m*HM-1) provide a good experimental system since they are a clone from human c-Ha-ras1 gene-transfected SFME cells which have been derived from Balb/c mice. Thus, human gene in animal tissues is supposed to be easily detected and quantified. [*In Vitro, 26A, 614, (1993)]. In vitro cultured cells were injected into the hind footpads of Balb/c mice. A PCR and the pathological method were employed to detect metastasized cells. Primers for the PCR were set to amplify a 128 bp exon-1 sequence of the human c-Ha-ras1 gene. Since Hind III digests the exon-1 sequence of mouse but not activated human Ha-ras1, DNA samples from the lungs of tumor-bearing were pre-treated with Hind III and then the digested DNA samples were used as templates of the PCR. The 128 bp PCR products were detected in the samples from the lungs where metastasized tumor cells were histologically observed. A standard curve to quantify metastasized tumor cells was determined as follows: HindIII-treated samples of DNA from *r/m*HM-1 and the lungs of normal mice were mixed at various ratios, and the mixtures were then subjected to the PCR. Resulted spots were densitometrically quantified. By this method, more than 1×10^4 could be detectable in a mouse lung in which almost no metastases were histologically observed. Numbers of tumor cells per lung increased with time after inoculation of *r/m*HM-1. For example, less than 10^3 cells and about 3.5×10^4 cells were estimated in the lungs resected at 14 days and at 28 days, respectively. The 128 bp PCR products were also detectable in the lungs of mice whose tumor-bearing hind legs had been amputated 14 days after an inoculation. Thus, the present PCR products detected in the lungs of tumor-bearing mice appear to be metastasized tumor cells. These results indicate the usefulness of the present *r/m*HM-1-Balb/c mice system to analyze and control metastasis.

A COMPUTER INTEGRATED CONTROL SYSTEM FOR SPECIALIZED CELL CULTURE FERMENTATION SET-UPS

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In the last 10 years, new assignments and the special demands of mammalian cells to the culture conditions caused the development of complex small scale fermentation set-ups. The use of continuous fermentation and cell retention devices requires appropriate process control arrangements. We present a system consisting of a modified personal computer combined with especially developed software rendering the facility to control aeration and pumps, even for unusual fermentation modes like perfusion, medium recycling or detoxification. The possibility of free programming allows the establishment of user-oriented software, designed for the far-reaching requirements in the field of animal cell culture. The time course of pump action for several common fermentation modes including chemostat and perfusion fermentation are implemented in the control program and can be configured for special fermentor set-ups or fermentation configurations. Control of aeration, data-acquisition and -storage are additional components within the program, which beside renders the facilities to automate standard operations like measurement of k_La- or OTR-values. The main advantage of this system is the high adaptability to various experimental set-ups. Complex pump control algorithms, feeding strategies or contoller actions like a shift of pH or pO_2 can easily be programmed in a BASIC compiler language.

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DEVELOPMENT OF A HYBRID ARTIFICIAL LIVER SUPPORT SYSTEM USING PUF/SPHEROID PACKED-BED

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Spherical multicellular aggregates of adult rat hepatocytes (spheroids) which have tissue like structure, were formed and immobilized in the pores of polyurethane foam (PUF) which was used as a culture substratum. These hepatocyte/spheroids, about 100 μm in diameter, have maintained higher differentiated functions than those of hepatocyte/monolayer for about 3 weeks in serum-free medium. Then, we designed a prototype module of an artificial liver support system using a PUF/spheroid packed-bed, in which hepatocyte/spheroids were immobilized at high density($0.5{\sim}1.0x10^7$ cells/cm^3-PUF). The urea synthesis activity of the artificial liver was maintained at least 10 days in 100% rat blood plasma supplemented with hormones (insulin, dexamethasone, glucagon, EGF and aprotinin). We developed the rat extracorporeal circulation system of about 1.4ml in the line volume including blood pressure monitoring system and plasma separator. We start examining the performance of hybrid artificial liver in an *ex vivo* extracorporeal experiment with a D-galactosamine induced acute hepatic failure rat. The ammonia concentration of plasma in hybrid artificial liver circulation line, which was one of toxic causal substances for hepatic coma, decreased at normal level for 3 hours circulation. The ammonia concentration of blood circulation line also decreased at normal level after 13.5hours.