

Proceedings in Life Sciences



Hormonally Defined Media

A Tool in Cell Biology

Lectures and Posters Presented at the
First European Conference on
Serum-Free Cell Culture

Heidelberg, October 7–9, 1982

Edited by
G. Fischer and R.J. Wieser

With 185 Figures

Springer-Verlag
Berlin Heidelberg New York Tokyo 1983

Dr. G. FISCHER
Institut für Neurobiologie
der Universität Heidelberg
Im Neuenheimer Feld 504
6900 Heidelberg 1, FRG

Dr. R. J. WIESER
Institut für Pharmakologie
der Universität Mainz
Obere Zahlbacher Str. 67
6500 Mainz, FRG

ISBN-13: 978-3-642-69292-5 e-ISBN-13: 978-3-642-69290-1
DOI: 10.1007/978-3-642-69290-1

This work is subject to copyright. All rights are reserved, whether the whole or part of the material is concerned, specifically those of translation, reprinting, re-use of illustrations, broadcasting, reproduction by photocopying machine or similar means, and storage in data banks.

Under § 54 of the German Copyright Law, where copies are made for other than private use, a fee is payable to "Verwertungsgesellschaft Wort", Munich.

© by Springer-Verlag Berlin Heidelberg 1983

Softcover reprint of the hardcover 1st edition 1983

The use of registered names, trademarks, etc. in this publication does not imply, even in the absence of a specific statement, that such names are exempt from the relevant protective laws and regulations and therefore free for general use.

2131/3130-543210

Preface

Until some years ago serum or crude tissue extracts were used predominantly or exclusively as media supplements for the cultivation of cells. However, during this time evidence accumulated that these supplements could not provide in an optimal way most of the cultivated cells with all factors necessary for their survival, their proliferation and/or differentiation. Moreover, a variety of cells could not be cultivated at all under these conditions and often the composition of the cultures changed within rather short periods of time by overgrowth of initially present subpopulations of those cells which grow well in these supplements, as for example fibroblasts. Nevertheless, using these supplements (or fractions thereof), insight could be gained into some of the influences of serum or tissue extract constituents with regard to survival, proliferation and differentiation of cells in culture. It became obvious from these experiments that serum or tissue extracts did not only supply cells with nutrients or vitamins (which are now constituents of all basic media), but also with hormones as well as growth-, differentiation-, and attachment-factors.

In course of time experiments were performed in which serum enriched with hormones and other growth factors was used to successfully cultivate those cells which could not survive in serum-supplemented media alone. Under normal conditions in an organism, however, only a small population of cells has direct contact with serum. Serum combines plasma and platelet-derived molecules (among them growth factors) which are set free during injury and blood coagulation. Therefore, most of the cells in an organism will be exposed only to plasma or plasma filtrates. It is interesting also from this point of view that often high serum concentrations were used when proliferating cells were studied and low serum concentrations for quiescent cells.

The knowledge gained from these experiments led to the search for methods by which serum is replaced by mixtures of hormones, growth factors, transport proteins, and attachment factors. Inspiring experiments by G. Sato and colleagues made it soon possible not only to cultivate established cell lines under the new "defined" conditions - by adapting the cells from serum to serum-free, hormonally defined conditions - but also successfully to establish culture conditions for a variety of primary cell cultures. It was not surprising that now, under these "defined" conditions, even those cells could be cultivated that had failed to survive in serum-supplemented media.

The quickly expanding influence of this cultivation technique on different aspects of cell biology, including research on cell proliferation and differentiation, cell-cell interactions, tumor growth as well as endocrinological and pharmacological investigations, had made it desirable to organize a conference in Europe which concentrated on this new tool in cell biology and to provide a forum for direct exchange of experience.

Gerd Brunner was instrumental in the organization of this conference. He died a few months before the conference took place. We will always remember him as a good friend and enthusiastic scientist who has contributed his almost unlimited resources on ideas and energy to make this conference a success.

This conference was generously supported by Bethesda Research Laboratories. We are also grateful for the endorsement of the Gesellschaft für Biologische Chemie and the Gesellschaft für Zellbiologie.

We wish to thank our co-organizers T. Leenen and T. Wäli for their excellent cooperation, and J. Becker and I. Makowiecki for their help in preparing these proceedings.

July, 1983

G. FISCHER
R.J. WIESER

Contents

OPENING LECTURE

Divining the Role of Serum in Cell Culture G.H. SATO	2
---	---

NUTRITIONAL REQUIREMENTS

Reducing the Requirement for Serum Supplements in High Yield Microcarrier Cell Culture J. CLARK	6
Growth of Normal Human Cells in Defined Media R.G. HAM	16
Investigations on the Cultivation of Insect Cell Lines in Serum-Free Media H.G. MILTENBURGER	31

CELL PROLIFERATION AND GROWTH FACTORS

Different Signalling Systems Control the Initiation of DNA Replication in Cultured Animal Cells L. JIMENEZ DE ASUA and A.M. OTTO	46
Ectopic Auto-Stimulation of Growth R.R. BÜRK	57
The Growth and Differentiation of Human Endothelial Cells T. MACIAG and R. WEINSTEIN	68
3T3 Cell Growth Initiation and Multiplication in Defined Medium Supplemented with Growth Factors and Hormones That Replace Serum D. PAUL	79
Growth-Promoting Activity of Thrombin in Fibroblasts: Temporal Action and Early Biochemical Events in the G ₀ → G ₁ Transition J. POUYSSÉGUR, J.C. CHAMBARD, A. FRANCHI, G. L'ALLEMAIN, S. PARIS, and E. VAN OBBERGHEN-SCHILLING	88
Pure Platelet-Derived Growth Factor Stimulates Human Fibroblasts to Proliferate in Plasma-Free Culture C. BETSHOLTZ, C.-H. HELDIN, Å. WASTESON, and B. WESTERMARK	103
Synergistic Effect of Retinoic Acid on DNA Synthesis of Prostaglandin F _{2α} Stimulated Swiss 3T3 Cells M. ESCHENBRUCH, A.M. OTTO, and L. JIMENEZ DE ASUA	107

VIII

The Secretion of Growth Regulatory Molecules by PC13 Embryonal Carcinoma Cells
J.K. HEATH and C.M. ISACKE 111

Growth Regulation of L-929 Mouse Fibroblasts by Steroid Hormones and Anti-Hormones in Serum Containing and Serum-Free Media
I. JUNG-TESTAS and E.E. BAULIEU 114

Synergistic Interactions of Specific Prostaglandins in Regulating the Rate of Initiation of DNA Synthesis in Swiss 3T3 Cells
A.M. OTTO, M. NILSEN-HAMILTON, B. BOSS, and L. JIMENEZ DE ASUA 120

Use of Eye Derived Growth Factor from Retina (EDGF) in a Defined Medium for the Culture of Bovine Epithelial Lens Cells
J. PLOUËT, M. OLIVIÉ, J. COURTY, Y. COURTOIS, and D. BARRITAUULT 123

Effect of Partially Purified Preparation of Human Somatomedin A in the Three Cultured Cell Systems
J. STRACZEK, M.H. HEULIN, F. SAREM, A. LASBENNES, M. ARTUR, C. GESCHIER, J.F. STOLTZ, F. BELLEVILLE, P. NABET, F.X. MAQUART, P. GILLERY, J. BOREL, A. HERMAN, M.D. LEBEURRE, E. DE LAVERGNE, and N. GENETET 127

CELL-SUBSTRATUM INTERACTIONS

Role of Cell-Substratum Interactions in the Hormonal Control of Rat Prolactin Cells
N. BRUNET, D. BARRITAUULT, D. GOURDJI, and A. TIXIER-VIDAL 132

Control by the Extracellular Environment of Differentiation Pathways in an Embryonal Carcinoma Cell Line
M. DARMON 143

Interaction of Metastatic and Non-Metastatic Tumor Lines with Aortic Endothelial Cell Monolayer and Their Underlying Basal Lamina
V. SCHIRRMACHER and I. VLODAVSKY 151

Control of Behaviour and Growth by Imitating the Contact Environment
R. WIESER and G. BRUNNER 162

NEURAL CELLS

Cells of the Peripheral Nervous System; Requirements for Expression of Function in Tissue Culture
R.P. BUNGE, D.J. CAREY, D. HIGGINS, C. ELDRIDGE, and D. ROUFA 178

Selection and Cultivation of Astrocytes at Different Developmental Stages
G. FISCHER 189

Cholinergic Differentiation in Serum-Free Aggregating Fetal Brain Cells
P. HONEGGER and B. GÜNTERT 203

DNA Synthesis in Nuclei and Mitochondria Purified from Serum-Free or Supplemented Glial Cell Cultures
E. AVOLA, D. CURTI, B. LOMBARDO, P. RAGONESE, M. RENIS, and G. RICCERI 215

Guanylate Cyclase Activators Hemin and Sodium Nitroprusside Stimulate the Growth of Transformed Cells in Serum-Free Medium P. BASSET, J. ZWILLER, G. ULRICH, and M.O. REVEL	219
Tracing the Astroglial Cell-Lineage in Vitro by Modification of the Microenvironmental Conditions K. LANG and G. BRUNNER	222
Growth of Perfused and Nonperfused Neuronal Cell Lines in Serum Deprived Medium A.L. PETERSON and E. WALUM	225
Primary Rat Astroglial Culture in Serum-Free Supplemented Media and Effect of a Bovine Brain Factor M. WEIBEL, B. PETTMANN, G. DAUNE, G. LABOURDETTE, and M. SENSENBRENNER	229

ENDOCRINE AND EXOCRINE CELLS

Hormone Inducible Specific Gene Expression in an Isolated Whole Mammary Organ in Serum-Free Culture M.R. BANERJEE, P.K. MAJUMDER, M. ANTONIOU, and J. JOSHI	234
A New Culture Technique: An Approach to the in-Vitro Reconstitution of Endocrine Organs E. LANG, K. LANG, U. KRAUSE, K. RACKÉ, B. NITZGEN, and G. BRUNNER	250
Hormonal Regulation of Growth and Function of Insulin-Producing Cells in Culture J.H. NIELSEN	264
Studies on Regulation of Ovarian Steroidogenesis in Vitro: The Need for a Serum-Free Medium J. ORLY, P. WEINBERGER-OHANA, and Y. FARKASH	274
Control of Normal Phenotype and of Thyroid Differentiated Functions in the Epithelial Cell Strain FRTL D. TRAMONTANO, R. PICONE, and F.S. AMBESI-IMPIOMBATO	285
Butyrate-Induced Growth Arrest of GH ₃ -Cells is not Linked to a Distinct Morphological Phenotype G. TSCHANK and G. BRUNNER	294

HEMOPOIETIC AND IMMUNE SYSTEM

Human B Cell Function in 20 μ l Hanging-Drop Microcultures Under Serum-Free Conditions J. FARRANT, Ch.A. NEWTON, Ch. WEYMAN, M. NORTH, and M.K. BRENNER	300
Adipose Conversion of Murine Bone Marrow-Derived Macrophages in Serum-Free Medium I. FLESCH, U.-P. KETELSEN, and E. FERBER	304
Analysis of the Mitogenic Effect of Fibrinogen J.A. HATZFELD and A. HATZFELD	307
Hybridoma Formation in Serum-Free Medium T. KAWAMOTO, J.D. SATO, ANH LE, D.B. McCCLURE, and G.H. SATO	310

Immune (γ) Interferon Produced by Murine T-Cell Lymphomas S. LANDOLFO and B. ARNOLD	314
TCGF-Dependent Growth of Naturally Autoreactive Cells: Regulatory Role of the Thymus C. RICCARDI, G. MIGLIORATI, and L. FRATI	317
Serum Replacement in Culture of Human Erythroid and Megakaryocytic Precursors G. VINCI, N. CASADEVALL, C. LACOMBE, J. CHAPMAN, W. VAINCHENKER, B. VARET, and J. BRETON-GORIUS	320
Participation of Pterins and of a Pteridine Binding Variant of Alpha ₁ -Acid Glycoprotein in the Control of Lymphocyte Transformation and of Lymphoblast Proliferation I. ZIEGLER, U. HAMM, and J. BERNDT	324

LIVER- AND KIDNEY-DERIVED CELLS

Conversion of Heterogeneous Liver Parenchyma Cells into a Homogeneous Cell Population During Cell Culture with Respect to PEPCK B. ANDERSEN and K. JUNGERMANN	330
Preservation of the Adult Functionality of Hepatocytes in Serum-Free Cultures J.V. CASTELL, M.J. GÓMEZ-LECHÓN, J. COLOMA, and P. LOPEZ	333
Isolation and Characterization of MDCK-Subcultures E.M. GIESEN, C. WELSCH, M. SCHMIDT, J.L. IMBS, A. STEFFAN, and J. SCHWARTZ ..	337
The Role of Fetal Calf Serum (FCS) During the First Stages of Hepatocytes Cultures M.J. GÓMEZ-LECHÓN and J.V. CASTELL	340
Modulation of Specific Functions in Adult and Fetal Hepatocytes Maintained in a Co-Cultured System C. GUGUEN-GUILLOUZO, G. LESCOAT, G. BAFFET, E. LE RUMEUR, B. CLÉMENT, D. GLAISE, and A. GUILLOUZO	344
Cell Culture and Differentiated Properties of Nephron Epithelial Cells in Defined Medium M. HORSTER, P.D. WILSON, M. SCHMOLKE, and D. KÜHNER	347
Multiplication of Adult Rat Hepatocytes in Monolayer Cultures D. PAUL and A. PIASECKI	351
Short-Term Modulation of Glycogenolysis, Glycolysis and Gluconeogenesis in Cultured Hepatocytes by Arterial and Venous Oxygen Concentrations D. WÖLFLE, H. SCHMIDT, and K. JUNGERMANN	354

MUSCLE CELLS, EPITHELIAL CELLS, FIBROADIPOGENIC CELLS, MELANOCYTES AND CHONDROCYTES

Differentiation of Primary Muscle Cells Cultured in a Serum-Free Chemically Defined Medium P. DOLLENMEIER and H.M. EPPENBERGER	358
--	-----

Study of a Teratoma-Derived Adipogenic Cell Line 1246 and Isolation of an Insulin-Independent Variant in Serum-Free Medium G. SERRERO-DAVÉ	366
Primary and Secondary Cultures of Rabbit Articular Chondrocytes in a Serum-Free Medium M. ADOLPHE, X. RONOT, B. FROGER, M.T. CORVOL, and N. FOREST	377
Vanadate Antagonizes Detrimental Effects of Serum Deprivation in Cultured Rat Heart Muscle Cells G. BAURIEDEL, K. WERDAN, W. KRAWIETZ, and E. ERDMANN	380
Characterization of Cardiomyocytes Cultured in Serum-Free Medium G. KESSLER-ICEKSON, L. WASSERMAN, E. YOLES, and S.R. SAMPSON	383
Serum-Free Cultures of Mouse LM Fibroblasts as a Tool for Biochemical and Genetic Studies in the Cellular Responses to Interferon P. MILHAUD, T. FAURE, J. CHAINTREUIL, and B. LEBLEU	387
Separation and Cultivation of Normal Human Melanocytes H.I. NIELSEN and P. DON	390
The Use of Cytodex 3 Microcarriers and Serum-Free Media for the Production of a Nerve Growth Promoter from Chicken Heart Cells G. NORRGREN, T. EBENDAL, H. WIKSTRÖM, and Ch. GEBB	393
Control of Cell Proliferation and Differentiation in the Myogenic Cell Line L6 by Manipulation of Culture Conditions Ch. PINSET and R.G. WHALEN	396
Serum-Free Culture of Adult Rat Cardiac Myocytes H.M. PIPER, I. PROBST, P. SCHWARTZ, J.F. HÜTTER, and P.G. SPIECKERMANN	400
Secretion of α -MSH-Like Molecules in a Human Melanoma Cell Line N. VAN TIEGHEM, M. FOOLIJ, P.-E. HENRY, F. LEGROS, and J.-M. PREVOST	403
Growth and Differentiation of Human Muscle Cells in Defined Medium R. YASIN and G. VAN BEERS	407
 TUMOR CELLS	
Tissue Culture of Human Colon Carcinomas. Therapy Experiments in Vitro J. VAN DER BOSCH	412
Human Prostatic Cells in Serum-Free Medium M.E. KAIGHN	418
Culture of Human Lung Carcinoma Cells in Serum-Free Media H. MASUI, K. MIYAZAKI, and G.H. SATO	430
Continuous Growth of a Human Breast Cancer Cell Line (MCF-7) in Serum-Free Medium P. BRIAND and A.E. LYKKESFELDT	436
Regulation of Oestrogen Responsiveness of MCF-7 Human Breast Cancer Cell Growth by Serum Concentration in the Culture Medium T.C. DEMBINSKI and C.D. GREEN	439

Induction of Differentiation in Human Solid Tumor Cells by
Anti-Tumor Agents in Serum Depleted Medium
A. REY, D. CUISSOL, E. URSULE, and B. SERROU 443

Hormonal Requirements of in Vitro Growth in Human Mammary and
Ovarian Carcinoma Cell Cultures
W.E. SIMON, F. HÖLZEL, and M. ALBRECHT 446

SUBJECT INDEX 449

Contributors

You will find the addresses at the beginning of the respective contribution

Adolphe, M. 377
Albrecht, M. 446
Ambesi-Impionbato, F.S. 285
Andersen, B. 330
Antoniou, M. 234
Arnold, B. 314
Artur, M. 127
Avola, R. 215
Baffet, G. 344
Banerjee, M.R. 234
Barritault, D. 123,132
Basset, P. 219
Baulieu, E.E. 114
Bauriedel, G. 380
Belleville, F. 127
Berndt, J. 324
Betsholtz, C. 103
Borel, J. 127
Boss, B. 120
Brenner, M.K. 300
Breton-Gorius, J. 320
Briand, P. 436
Brunet, N. 132
Brunner, G. 162,222,250,294
Bürk, R.R. 57
Bunge, R.P. 178
Carey, D.J. 178
Casadevall, N. 320
Castell, J.V. 333,340
Chaintreuil, J. 387
Chambard, J.C. 88
Chapman, J. 320
Clark, J. 6
Clément, B. 344
Coloma, J. 333
Corvol, M.T. 377
Courtois, Y. 123
Courty, J. 123
Cupissol, D. 443
Curti, D. 215
Darmon, M. 143
Daune, G. 229
De Lavergne, E. 127
Dembinski, T.C. 439
Dollenmeier, P. 358
Don, P. 390
Ebendal, T. 393
Eldridge, C. 178
Eppenberger, H.M. 358
Erdmann, E. 380
Eschenbruch, M. 107
Farkash, Y. 274
Farrant, J. 300
Faure, T. 387
Ferber, E. 304
Fischer, G. 189
Flesch, I. 304
Fooij, M. 403
Forest, N. 377
Franchi, A. 88
Frati, L. 317
Froger, B. 377
Gebb, Ch. 393
Genetet, N. 127
Geschier, C. 127
Giesen, E.M. 337
Gillery, P. 127
Glaise, D. 344
Gómez-Lechón, M.J. 333,340
Gourdji, D. 132
Green, C.D. 439
Güntert, B. 203
Guguen-Guillouzo, C. 344
Guillouzo, A. 344
Ham, R.G. 16
Hamm, U. 324
Hatzfeld, A. 307
Hatzfeld, J.A. 307
Heath, J.K. 111
Heldin, C.-H. 103
Henry, P.-E. 403
Herman, A. 127
Heulin, M.H. 127
Higgins, D. 178
Hölzel, F. 446
Honegger, P. 203
Horster, M. 347
Hütter, J.F. 400
Imbs, J.L. 337
Isacke, C.M. 111
Jimenez de Asua, L. 46,107,120
Joshi, J. 234
Jungermann, K. 330,354
Jung-Testas, I. 114
Kaighn, M.E. 418
Kawamoto, T. 310
Kessler-Icekson, G. 383
Ketelsen, U.-P. 304
Krause, U. 250
Krawietz, W. 380

- Kühner, D. 347
 Labourdette, G. 229
 Lacombe, C. 320
 Landolfo, S. 314
 Lang, E. 250
 Lang, K. 222,250
 Lasbennes, A. 127
 Le, Anh 310
 L'Allemain, G. 88
 Lebeurre, M.D. 127
 Lebleu, B. 387
 Legros, F. 403
 Le Rumeur, E. 344
 Lescoat, G. 344
 Lombardo, B. 215
 Lopez, P. 333
 Lykkesfeldt, A.E. 436
 Maciag, T. 68
 Majumder, P.K. 234
 Masui, H. 430
 Maquart, F.X. 127
 McClure, D.B. 310
 Migliorati, G. 317
 Milhaud, P. 387
 Miltenburger, H.G. 31
 Miyazaki, K. 430
 Nabet, P. 127
 Newton, Ch.A. 300
 Nielsen, H.I. 390
 Nielsen, J.H. 264
 Nilsen-Hamilton, M. 120
 Nitzgen, B. 250
 Norrgren, G. 393
 North, M. 300
 Olivié, M. 123
 Orly, J. 274
 Otto, A.M. 46,107,120
 Paris, S. 88
 Paul, D. 79,351
 Peterson, A.L. 225
 Pettmann, B. 229
 Piasecki, A. 351
 Picone, R. 285
 Pinset, Ch. 396
 Piper, H.M. 400
 Plouët, J. 123
 Pouysségur, J. 88
 Prevost, J.-M. 403
 Probst, I. 400
 Racké, K. 250
 Ragonese, P. 215
 Renis, M. 215
 Revel, M.O. 219
 Rey, A. 443
 Riccardi, C. 317
 Ricceri, G. 215
 Ronot, X. 377
 Roufa, D. 178
 Sampson, S.R. 383
 Sarem, F. 127
 Sato, G.H. 2,310,430
 Sato, J.D. 31C
 Schirmacher, V. 151
 Schmidt, H. 354
 Schmidt, M. 337
 Schmolke, M. 347
 Schwartz, J. 337
 Schwartz, P. 400
 Sensenbrenner, M. 229
 Serrero-Davé, G. 366
 Serrou, B. 443
 Simon, W.E. 446
 Spieckermann, P.G. 400
 Steffan, A. 337
 Stoltz, J.F. 127
 Straczek, J. 127
 Tixier-Vidal, A. 132
 Tramontano, D. 285
 Tschank, G. 294
 Ulrich, G. 219
 Ursule, E. 443
 Vainchenker, W. 320
 van Beers, G. 407
 van der Bosch, J. 412
 Van Obberghen-Schilling, E. 88
 van Tieghem, N. 403
 Varet, B. 320
 Vinci, G. 320
 Vlodavsky, I. 151
 Walum, E. 225
 Wasserman, L. 383
 Wasteson, Å. 103
 Weibel, M. 229
 Weinberger-Ohana, P. 274
 Weinstein, R. 68
 Welsch, C. 337
 Werdan, K. 380
 Westermarck, B. 103
 Weyman, Ch. 300
 Whalen, R.G. 396
 Wieser, R. 162
 Wikström, H. 393
 Wilson, P.D. 347
 Wölflé, D. 354
 Yasin, R. 407
 Yoles, E. 383
 Ziegler, I. 324
 Zwiller, J. 219