Biomedical Reviews 1996; 6: 111-119

ISSN 1310-392X

DANCE ROUND

WE DANCE ROUND *IN A RING AND SUPPOSE,*
BUT THE SECRET SITS IN THE MIDDLE AND KNOWS.
ROBERT FROST

CHEMICALLY DEFINED CULTURE MEDIA
Rational recipes or witches' brew?

Jeroen van Bergen and Egbert A.J.F, Lakke
Neuroregulation Group, Department of Physiology, Medical Faculty, University of Leiden, Leiden, The Netherlands

Scale of dragon, tooth of wolf,
Witches' mummy, maw and gulf
Of the ravin 'd salt-sea dark,
Liver of blaspheming Jew,
Gall of goat, and slips of yew
Sliver 'd in the moon's eclipse,
Nose of Turk, and Tartar's lips,
Finger of birth-strangled babe
Ditch-delivered by a drab,
Make the gruel thick and slab:

Add thereto a tiger's chaudron,
For the ingredients of our cauldron.

Double, double toil and trouble;
Fire burn and cauldron bubble.

Cool it with a baboon's blood,
Then the charm is firm and good.

William Shakespeare
Macbeth, Act IV, scene 1

• A rational approach to study cells, tissues or even organs is to isolate them from the body and bring them into a controlled, and therefore reproducible, environment. *In vivo,* cells are surrounded by the extracellular matrix, and the body fluids nourish them. *In vitro,* these fluids are replaced by culture media. In the early days of tissue culture, tissue was cultured in a drop of clotted lymph. The early-day natural nutrient media have gradually become replaced by media of a more defined composition, culminating in the advent of completely defined culture media.

The elimination of serum from culture media was motivated by the wish to study the role of growth factors and hormones *in vitro* (1). The composition of serum is unknown, and it may contain factors masking the effects of these agents. In addition, the composition of serum varies greatly from batch to batch, which makes it difficult to conduct reproducible, quantitative studies. Toxic and growth-inhibiting effects of serum have also been reported (2). *In vivo,* cells are surrounded by lymph or cerebrospinal fluid, and not by serum. Therefore, the elimination of serum may very well approximate the *in vitro* conditions to the conditions *in vivo.* For neuronal culture, the omission of serum is of special interest, since the presence of serum allows proliferation of non-neuronal cells, which might otherwise come to outnumber the neurons.

Chemically defined media (CDM) may be composed of more than 70 ingredients. This poses a methodological problem, since it is not possible to assay every possible combination of ingredients. This *Dance Round* describes the development of CDM for neural tissue in a historical perspective, emphasizing methodological considerations.

At present, many CDM are available, tuned for specific purposes. Most of these media are based on DMEM or Ham F12 and supplemented with hormones and growth factors, nutrients, binding and attachment factors. The recipes of twenty-nine CDM were compared with respect to their content of supplements, and their reported suitability. An attempt was made to trace the original introduction, and the reasoning be-

hind the introduction of each hormone and growth factor in the CDM recipes. Disappointingly, the rationale behind a novel ingredient was often not mentioned, and many hormonal additions seemed to be related to the availability of the pure substance. The various recipes were compiled in Table 1.

The group of compounds that differs most between the various media, is that of hormones and growth factors, suggesting that the requirements of these compounds are the most significant, with respect to the intended CDM specificity. We will argue that this significance reflects the developmental history of CDM, rather than specific virtues of these compounds. Finally, the question is addressed whether CDM are truly defined in the chemical sense.

HISTORY

• The history of CDM can be divided into several periods. A genealogical tree of chemically defined neural culture media was drawn to illustrate the description (Fig.l).

• Purely natural media

In 1907, Harrison succeeded in culturing the medullary tube of a frog embryo in a drop of clotted lymph (3). His observations of neurite outgrowth *in vitro* bear evidence to the viability of his preparation. This event marks the introduction of neural tissue culture as an experimental tool. Burrows (4), working in the same group, started using a plasma clot instead of a lymph clot. Shortly afterwards, it was discovered that embryo extracts possessed strong growth-promoting capacities (5). The use of plasma clots supplemented with embryo extracts soon became standard practice.

• Partially synthetic media: definition of essential low molecular weight compounds

From the early beginning, the factors in the medium necessary for growth and survival were under investigation. This work was started by Lewis and Lewis in 1911 (6). Harrison's coworkers also attempted to identify the essential ingredients in their media (7). It was found that dialyzed culture medium, which is deficient in low molecular weight components, was completely unable to maintain the life of cells (8). In contrast, dialyzed medium supplemented with salts, amino acids and substances that are important in the Kreb's cycle (vitamins, choline, creatine, C_4-acids) was able to maintain cells in culture (9). This finding provided the rationale for a systematic analysis of the low molecular weight substances.

Fischer's supplement consisted of 47 components (9), far to many to allow the investigation of all possible combinations. Instead, he demonstrated the importance of amino acids by showing that cells died quickly when cultured with the supplement mixture lacking amino acids. He subsequently omitted single amino acids from the mixture, in order to investigate their importance. Although the omission of several amino acids greatly decreased growth, no single component was demonstrated to be absolutely essential. It was obvious that the composition of the final minimal medium greatly depended on the arbitrary choice of amino acids in the initial mixture.

Several researchers continued Fischer's work and developed synthetic media (10-12), but a major breakthrough was achieved by Eagle (13). Using the same approach as Fischer, he was able to establish the essential amino acid requirements for a mammalian cell line (strain L). Likewise, he defined the minimum vitamin requirements (14). Supplementation of the minimal essential medium (MEM) (15) with 0.25 to 2.0 % dialyzed serum was still necessary. At this point, it could not be excluded that the dialyzed serum supplied protein-bound trace elements, vitamins, or even amino acids. The amino acids and vitamins shown to be essential were obviously not present in the serum fraction. If they were present in concentrations sufficient for survival and growth, the omission of these ingredients would not lead to cell death.

Growth of mammalian cells in a completely synthetic medium was first reported in 1954 by Healey and colleagues (16). Ham (17) was the first to describe colony formation and growth of a clonal cell line in a completely synthetic medium. Chinese hamster ovary cell lines demonstrated clonal growth in the

Abbreviations: Age: E - embryonic, F -fetal, N - neonatal, A - adult. Animal: R - rat, C - chick, M - mouse, X - Xenopus laevis, Q - quail. Culture type: CL - cell line, RD1S - reaggregating dissociated cells, DIS - dissociated cells, EXP - explant, PC - primary culture, ON - optic nerve. LMWF - low molecular weight fraction.

To several media extra putrescin (already present in Ham's F12) was added. The medium of Wilson et al (52) further contained ceruloplasrnin, uridin, and collagen. Creatine andfumarase (not listed in this table) were used by Annis et al (33). Likewise several media contain extra linoleic acid or lipoic acid, which are also components of Ham's F12. Zinc is already present in Ham's F12, but nevertheless further zinc was added in some recipes (32, 49, 52, 99). Glutathion cooperates with selenium and Vitamins A andE to protect the cell against damage from oxygen-derived free-radicals. Vit B12 and H are constituents of Ham's F12, but were supplemented to several media.

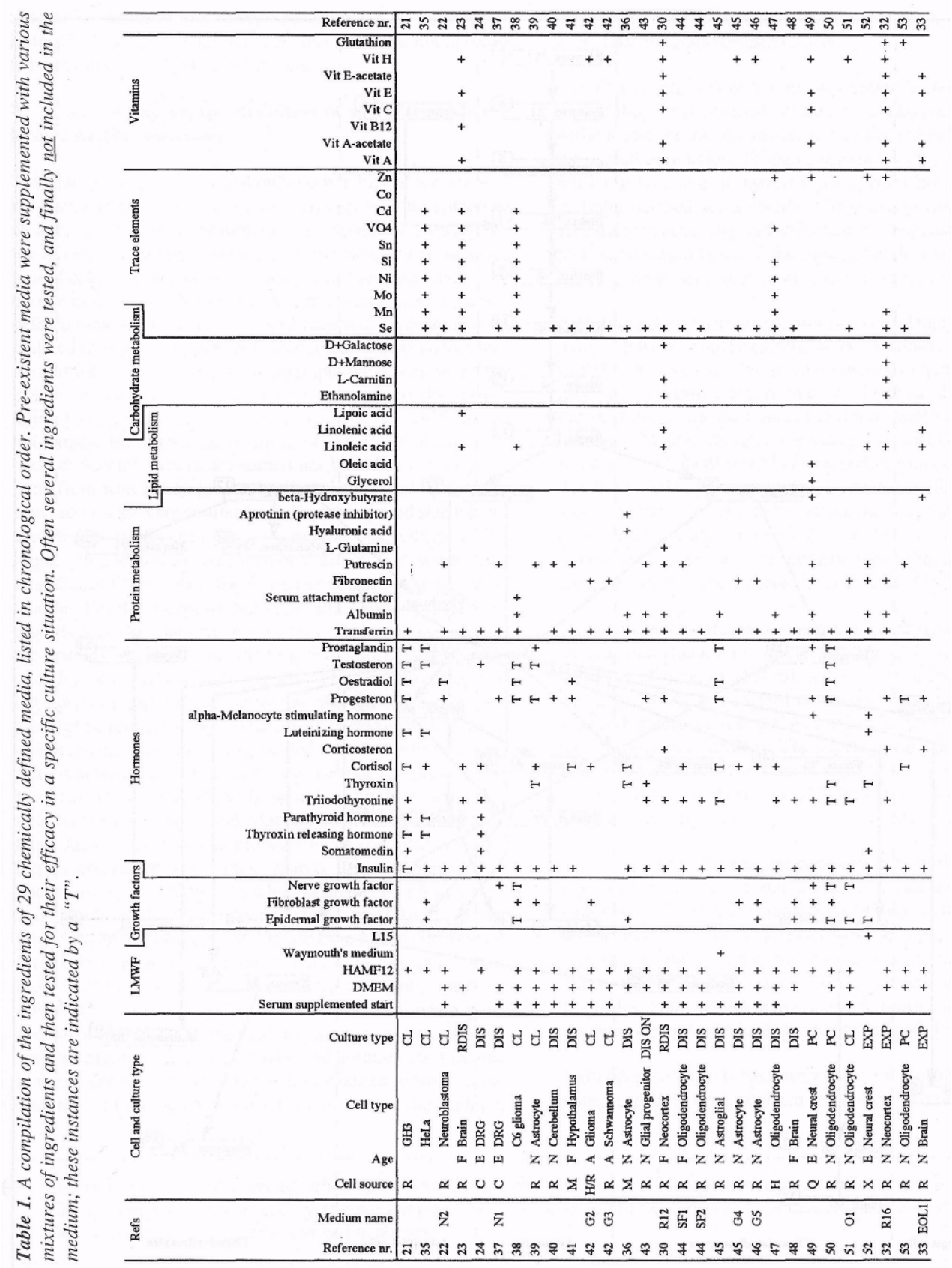
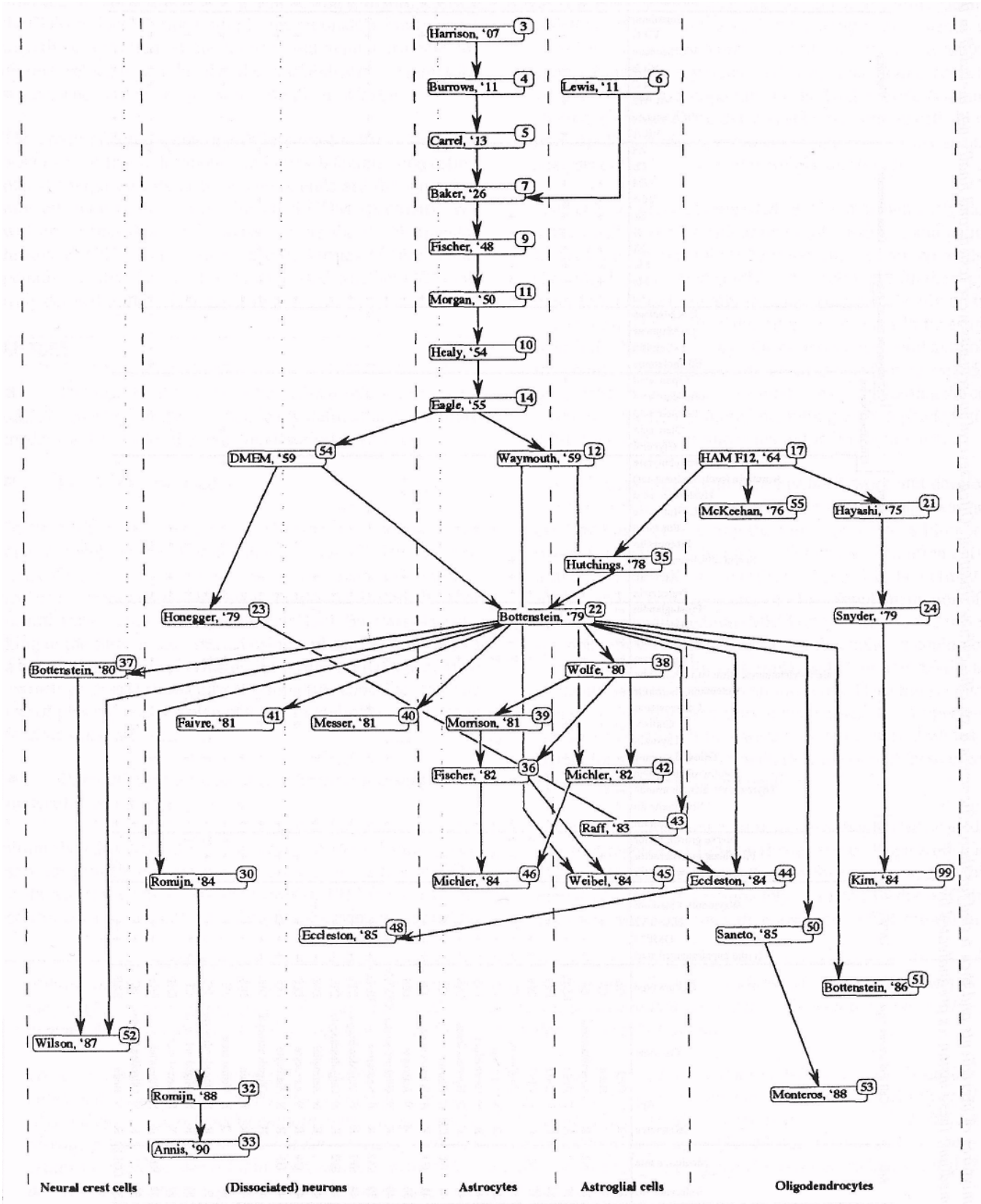


Table 1. *A compilation of the ingredients of 29 chemically defined media, listed in chronological order. Pre-existent media were supplemented with various mixtures of ingredients and then tested for their efficacy in a specific culture situation. Often several ingredients were tested, and finally not included in the medium; these instances are indicated by a "T".*


Harrison, '07 3
Burrows, '11 4
Lewis, '11 6
Carrel, '13 5
Baker, '26 7
Fischer, '48 9
Morgan, '50 11
Healy, '54 10
Eagle, '55 14
DMEM, '59 54
Waymouth, '59 12
HAM F12, '64 17
McKeehan, '76 55
Hayashi, '75 21
Hutchings, '78 35
Honegger, '79 23
Bottenstein, '79 22
Snyder, '79 24
Bottenstein, '80 37
Wolfe, '80 38
Faivre, '81 41
Messer, '81 40
Morrison, '81 39
Fischer, '82 36
Michler, '82 42
Raff, '83 43
Romijn, '84 30
Michler, '84 46
Weibel, '84 45
Eccleston, '84 44
Kim, '84 99
Eccleston, '85 48
Saneto, '85 50
Bottenstein, '86 51
Wilson, '87 52
Romijn, '88 32
Monteros, '88 53
Annis, '90 33
Neural crest cells
(Dissociated) neurons
Astrocytes
Astroglial cells
Oligodendrocytes

absence of serum or serum-derived components. Moreover, Ham's F12 medium contained substances that did not appear to be essential in limited experiments.

• Serum-free media: definition of specific high molecular weight compounds

Thus far, growth in CDM had only been achieved for a number of clonal cell lines. These cases may very well be examples of selection of a subpopulation of cells capable of growing in the mixture of factors. Another conceivable event is adaptation of cells to defined culture media. For the maintenance of normal function of differentiated neurons, the addition of poorly defined biological extracts was still required. Because it was realized that these supplements contained critical factors for normal function *in vitro,* many attempts have been made to isolate and characterize them. Purification of growth stimulating factors from serum proved to be extremely difficult, if not impossible. First, many liters of starting material are needed. Second, factors are sometimes bound to carrier proteins from which they gradually dissociate. Third, many serum proteins are very similar regarding charge and size which complicates purification by conventional biochemical techniques. In addition to these technical difficulties, serum factors often act synergistically, further confusing separate purification. For these reasons, Sato (18) attacked the problem in a synthetic rather than an analytic way. He hypothesized that the primary role of serum was to provide hormones. Consequently, different cell lines required different serum-replacing hormone cocktails. Experiments showed that serum depleted of certain hormones no longer supported growth of cells, unless the medium was supplemented with the hormones that were removed (19, 20). This notion was not complete, since serum also contained nutrients, as well as binding factors for nutrients and hormones, and attachment factors. Binding proteins, such as albumin and transferrin, act like buffers, keeping the concentrations of their ligands at physiological level under various conditions. Attachment factors like fibronectin and globulin replace the supporting function of stroma in culture. Sato and coworkers (20-22) used the following strategy for the development of a serum-free nutrient medium: (/) initially, cultures were maintained with a defined medium (DMEM, Ham F12) supplemented with serum; when good growth of the cells was obtained, (/'/) the serum concentration was decreased in a stepwise manner, and hormones were added to the medium to compensate for the decreased growth; in the end the serum could be completely omitted from the medium, and (iii) by omission of single additives, the essentiality of every additive could be established.

The first report (21) of serum substitution by hormones described the growth of an established rat pituitary cell line (GH,) without altering the characteristics of the individual cells or the overall population. Triiodo-L-thyronine (T_3), thyrotropin-releasing hormone, transferrin, parathyroid hormone, and a partially purified somatomedin (5000-fold purification from serum) were necessary and sufficient to compensate for complete serum elimination. T, became one of the most frequently used hormones in neural tissue culture (Table 1).

After this report, serum-free culturing evolved rapidly and soon many cell types could be cultured under serum-free conditions. In 1979, three groups almost simultaneously reported successful long-term culture of various types of nerve cells (22-24). In the following years, these three CDM were adapted for specific purposes. Most of these custom-made neural CDM were derived from N2 medium (22) for neuroblastoma cells (Fig.l). The hormonal requirements of cells *in vitro* are likely to be the similar to those *in vivo,* so that during the design process many of the specific requirements of different cells were revealed. Research into the regulation of neural ontogeny thus profited from the refinement of the various defined media (25-28).

Interesting from a methodological viewpoint is an extensively documented elaboration of a serum-free CDM for long-term culturing of reaggregated rat cerebrocortical tissue (29-32). These studies used the CDM mentioned above (22), and subsequently improved it stepwise, and in adherence to a cumulative improvement strategy. The effects of various single components or cocktails (Bottenstein and Sato-mix) were consecutively tested, and every time the optimal concentration was used in following tests.

The condition of the reaggregated cells was evaluated on the basis of morphological and sometimes electrophysiological parameters. The mean mammalian cerebrospinal fluid level (or, if unavailable, the blood serum level) could provide a guideline for the final concentration of a component. Other considerations were its biochemical properties and possible functions in the development and maintenance of brain cells, as well as the concentration profile of all other compounds in the medium.

Most adapted CDM recipes were designed for culturing established cell lines or dissociated cells of various origins. It was

Figure 1. *Genealogical tree of publications pertaining to the elaboration of chemically defined media for nervous tissue. The reference numbers specific to the present paper are indicated. Relations between the various papers are indicated by the respective arrrows, and explained in the text.*

Romijn who first succeeded in culturing neocortex explants instead of separate cells in a serum-free medium (32). Only recently, a CDM for organotypic slice culture in a roller tube was developed (33), which indicates the youthful stage of neural tissue culture in CDM.

Summarizing, early this century neural tissue was cultured in a drop of clotted lymph, a "natural" medium. The low molecular weight part of natural medium was then replaced by a mixture of ammo acids, vitamins and salts. Upon application of the "artificial" low molecular weight fraction of the natural medium, it was discovered that the remnant fraction of the natural medium could now be replaced by a tissue-specific mixture of high molecular weight molecules, such as hormones and transport proteins. The advent of these chemically defined high molecular weight supplements in return allowed further refinement of the composition of the low molecular weight fraction. This process is strikingly similar to Baron von Munchhausen escaping the swamp by pulling himself up by his own hairs.

By first establishing the low molecular weight requirements of tissue, using these for defining the high molecular weight compounds, and subsequently readjusting the low molecular weight portion, the reliability of the resulting media critically depends on the initial choice of compounds in the low molecular weight fraction. Statements concerning the *in vivo* requirements based on the composition of these media should be distrusted, since the composition of these media was based in the first instance on *a priori* suppositions about these requirements (another von Mtinchhausen's manoeuvre).

Fortunately, we may, at least partially, escape from the swamp. In the first place, *in vitro* observations may be corroborated by *in vivo* observations. The fact that cells *in vitro* generally looked like their *in vivo* counterparts morphologically and electrophysiologically, and expressed plasma membrane specific proteins, convincingly supports statements about the *in vivo* requirements. Here von Munchhausen pops up again, since for the generation of the antibodies that recognize these membrane proteins, cell specific antigens purified from cultured cells were used. In the second place is it not entirely correct to state that the suppositions about low molecular weight necessities were completely *a priori.* Biochemical research had, by the time Fischer performed his experiments (9), already established many of the basal metabolic requirements (34).

Focussing on the development of serum-free media from serum-supplemented media, it should first be mentioned that from the majority of the reports reviewed here, very little information about the methodology of the specific medium elaboration could be extracted.

By omission of single additives, the essentiality of every additive could be established. In several experiments the result of the addition of single components was compared with the results obtained with the unsupplemented medium and the medium containing all supplements. Was the effect of single supplements negligible, the effect of complete supplementation optimal and the effect of single omission catastrophic, synergy of supplements had been demonstrated (22). In most reports (21, 35) only the results of single omission of the ingredients were mentioned. However, in some of these the single-addition experiments probably had been performed, since the Materials and Methods mentioned ingredients that were not a part of the unabridged supplement mixture (23, 24).

The "single omission strategy" has at least one drawback. If several compounds can be omitted individually without any effect, this does not necessarily mean that they can be omitted as group. Several compounds of the medium could be left out without influencing the quality of the cultures, but nevertheless continued their addition to the medium (24). It might be suggested that they tried the medium without these compounds with little success, and decided not to continue with a "single addition strategy" with the medium without these compounds as a basis.

For later adjustments to existing serum-free media for specific purposes not only experimental, but also theoretical arguments were considered. This approach could very well lead to an overestimation of the necessities, and therefore to unnecessarily expensive media. Glutamine was not omitted from R12 medium, despite the fact that its omission did not visibly affect the cultures, because "it certainly could not be excluded that glutamine supplementation had a beneficial effect" (30).

Not only the ingredients contribute to the contents of the medium, but also the products of the cultured cells. In a study on oligodendrocytes, for example, astrocytes may perturb the experiments by secreting factors that influence oligodendrocytes. To obtain pure cultures, selective culture media were designed, that select for a specific cell type (36). Another variable is often the number of cultured cells, which also influences the medium composition.

CONCLUSION

• The development of "chemically defined" media was a rational process, resulting in media of increasingly, but never completely, defined composition. The process was rational in that the methodology (single omission, single addition) was optimal, given the impossibility of trying every possible combination of putative ingredients. A disadvantage of the methodology is that the final composition of the media is critically dependent on which ingredients were tested, and on the order in which they were tested. Since the choice of ingredients to

be tested depended on previous reports, especially those describing the development of similar media, the ingredients of the first CDM for neural tissue culture were crucial. The term "completely defined medium" is misleading and incorrect, since all media contain undefined substances. The amount of undefined components, however, has been minimized. Given the variability of experimental conditions, complete definition of the media might not be necessary. Thus, chemically defined culture media can be viewed as *rationally designed witches' brews.*

REFERENCES

1. Barnes D, Sato G. Serum-free cell culture: a unifying approach. *Cell* 1980; 22: 649-655

2. Loo DT, Fuquay JI, Rawson CL, Barnes DW. Extended culture of mouse embryo cells without senescence: inhibitionby serum. *Science* 1987; 236: 200-202

3. Harrison RG. Observations on the living developing nerve fiber. *AnatRec* 1907; 1: 116-118

4. Burrows MT. The growth of tissues of the chick embryo outside the animal body, with special reference to the nervous system. *J Exp Zoo!* 1911; 10: 63-73

5. Carrel A. Artificial activation of the growth *in vitro* of connective tissue. *JExpMed* 1913; 17: 14-17

6. Lewis MR, Lewis WH. The growth of embryonic chicken tissues in artificial media, agar and bouillon. *Bull J HopkinsHosp* 1911; 22: 126-135

7. Baker LE, Carrel A. Effects of the amino acids and dialyzable constituents of embryonic tissue juice on the growth of fibroblasts. *J Exp Med'1926;* 44: 397-407

8. Fischer A. Die Bedeutung der Aminosauren fur die Gewebezellen *in vitro. Acta PhysiolScand* 1941; 2: 143-188

9. Fischer A. Amino acid metabolism of tissue cells *in vitro. Biochem J* 1948; 43: 491-497

10. Healey GM, Fisher DC, Parker RC. Nutrition of animal cells in tissue culture; synthetic medium no. 703. *Canad J Biochem Physiol* 1954; 32: 327-337

11. Morgan JF, Morton HJ, Parker RC. Nutrition of animal cells in tissue culture; initial studies on synthetic medium. *Proc Soc Exp Biol Med* 1950; 73: 1-8

12. Waymouth C. Rapid proliferation of sublines of NCTC clone 929 (strain L) mouse cells in a simple chemically defined medium (MB 752/1). *J Nat Cancer Inst* 1959; 22: 1003-1017

13. Eagle H. The specific amino acid requirements of a mammalian cell (strain L) in tissue culture. *JBiol Chem* 1954; 214: 839-853

14. Eagle H. Nutrition needs of mammalian cells in tissue culture. *Science* 1955; 122: 501-504

15. Eagle H. Specific amino acid requirements of human carcinoma cell (strain HeLa) in tissue culture. *J Exp Med* 1955; 102: 37-48

16. Healey GM, Fisher DC, Parker RC. Nutrition of animal cells in tissue culture; synthetic medium no. 858. *Proc Soc Exp Biol Med* 1955; 89: 71 -77

17. Ham RG. Clonal growth of mammalian cells in a chemically defined, synthetic medium. *Proc Natl Acad Sci USA* 1964; 53: 288-293 .

18. Sato GH. The role of serum in cell culture. In: *Biochemical Action of Hormones.* Ed G Litwick, Academic Press, New York, 1975; pp 391-396

19. Armelin HA. Pituitary extracts and steroid hormones in the control of 3T3 cell growth. *Proc Natl Acad Sci USA* 1973; 70: 2702-2706

20. Nishikawa K, Armelin HA, Sato G. Control of ovarian cell growth in culture by serum and pituitary factors. *Proc Natl Acad Sci USA* 1975; 72: 483-487

21. Hayashi I, Sato GH. Replacement of serum by hormones permits growth of cells in a defined medium. *Nature* 1975; 259: 132-134

22. Bottenstein J, Sato GH. Growth of a neuroblastoma cell line in serum-free supplemented medium. *Proc Natl Acad Sci USA* 1979; 76: 514-517

23. Honegger P , Lenoir D, Favrod P. Growth and differentiation of aggregating fetal brain cells in a serum-free defined medium. Atom? 1979; 282: 305-307

24. Snyder EY, Kim SU. Hormonal requirements for neuronal survival in culture. *Neurosci Lett* 1979; 13: 225-230

25. Bruinink A, Reiser P. Ontogeny of MAP 2 and GFAP

antigens in primary cultures of embryonic chick brain. Effect of substratum, oxygen tension, serum and Ara-C. *Int JDev Neurosci* 1991; 9: 269-279

26. Gensburger C, Capo M, Deloulme JC, Sensenbrenner M. Influence of basic fibroblast growth factor on the development of cholinoceptive neurons from fetal rat cerebrum in culture. *Dev Neurosci* 1992; 14: 278-281

27. Heidet V, Faivre-Bauman A, Kordon C, Loudes C, Rasolonjanahary S, Epelbaum J. Functional maturation of somatostatin neurons and somatostatin receptors during development of mouse hypothalamus *in vivo* and *in vitro. Dev Brain Res* 1990; 57: 85-92

28. Nakajima M, Furukawa S, Hayashi K, Yamada A, Kawashima T, Hayashi Y. Age-dependent survival-promoting activity of vitamin K on cultured CNS neurons. *Dev Brain Res* 1993; 73: 17-23

29. Romijn HJ, Habets AMMC, Mud MT, Welters PS. Nerve outgrowth, synaptogenesis and bioelectrical activity in fetal rat cerebral cortex tissue cultured in serum-free, chemically deined medium. *Dev Brain Res* 1982; 2: 583-589

30. Romijn HJ, van Huizen F, Wolters PS. Towards an improved serum-free chemically defined medium for long-term culturing of cerebral cortex tissue. *Neurosci BiobehavRev* 1984; 8: 301-334

31. Romijn HJ. Development and advantages of serum-free, chemically defined nutrient media for culturing of nerve tissue. *Biol Cell* 1988; 63: 263-268

32 Romijn HJ, de Jong BM, Ruijter JM. A procedure for culturing rat neocortex explants in a serum-free nutrient medium. /*Neurosci Meth* 1988; 23: 75-83

33. Annis CM, Edmond J, Robertson RT. A chemically defined medium for organotypic slice cultures. *J Neurosci Meth* 1990; 32:63-70

34. Krebs HA, Johnson WA. The role of citric acid in intermediate metabolism in animal tissues. *Emymologia* 1937; 4: 148-156

3 5. Hutchings SE, Sato GH. Growth and maintenance of HeLa cells in s erum-free medium supplemented with hormones. *Proc Natl AcadSci USA* 1978; 75: 901-904

36. Fischer G, Leutz A, Schachner M. Cultivation of immature astrocytes of mouse cerebellum in a serum-free, hormonally defined medium, appearance of the mature astrocytic phenotype after addition of serum. *Neurosci Lett* 1982; 29: 297-302

37. Bottenstein JE, Skaper SD, Varon SS, Sato GH. Selective survival of neurons from chick embryo sensory ganglionic dissociates utilizing serum-free supplemented medium. *Exp Cell Res* 1980; 125: 183-190

38. Wolfe RA, Sato GH, McClure DB. Continuous culture of rat C6 glioma in serum-free medium. / *Cell Biol* 1980; 87: 434-441

39. Morrison RS, de Vellis J. Growth of purified astrocytes in a chemically defined medium. *Proc Natl A cad Sci USA* 1981; 78: 7205-7209

40. Messer A, Mazurkiewicz JE, Maskin P. Growth of dissociated rat cerebellar cells using serum-free supplemented media and varied transferrin concentrations. *Cell Mol Neurobiol* 1981; 1: 99-115

41. Faivre-Bauman A, Rosenbaum E, Puymirat J, Grouselle D, Tixier-Vidal A. Differentiation of fetal mouse hypothalamic cells in serum-free medium. *Dev Neurosci* 1981; 4:118-129

42. Michler-Stuke A, Bottenstein JE. Proliferation of glial-derived cells in defined media. *J Neurosci Res* 1982; 7: 215-228

43. Raff MC, Miller RH, Noble M. A glial progenitor cell that develops *in vitro* into an astrocyte or an oligodendrocyte depending on culture medium. *Nature* 1983; 303: 390-396

44 Eccleston PA, Silberberg DH. The differentiation of oligodendrocytes in a serum-free hormone-supplemented medium. *Dev Brain Res* 1984; 16: 1-9

45. Weibel M, Pettmann B, Daum G, Labourdeth G, Sensenbrenner M. Chemically defined medium for rat astroglial cells in primary culture. *Int J Dev Neurosci* 1984; 2: 355-366

46. Michler-Stuke A, Wolff JR, Bottenstein JE. Factors influencing astrocyte growth and development in defined media. *Int J Dev Neurosci* 1984; 2: 575-584

47. Kirn SU, Shin DH, Paty DW. Long term culture of human oligodendrocytes in serum-free chemically defined medium. In: *Experimental Allergic Encephalomyelitis, a useful model for multiple sclerosis.* Alan R. Liss, New York, 1984; 207-214

Eccleston PA, Gunton DJ, Silberberg DH. Requirements for brain cell attachment, survival and growth in serum-free medium, effects of extracellular matrix, epidermal growth factor. *DevNeurosci* 1985; 7: 308-322

Sieber-Blum M, Chokshi HR. *In vitro* proliferation and terminal differentiation of quail neural crest cells in a defined culture medium. *Exp CellRes* 1985; 158: 267-272

Saneto RP, de Vellis J. Characterization of cultured rat oligodendrocytes proliferating in a serum-free, chemically defined medium. *ProcNatlAcadSci USA* 1985; 82: 3509-3513 .

Bottenstein JE. Growth requirements *in vitro* of oligodendrocyte cell lines and neonatal rat brain oligodendrocytes. *ProcNatlAcadSci USA* 1986; 83: 1955-1959

Wilson HC, Milos NC. The effects of various nutritional supplements on the growth, migration and differentiation of Xenopus laevis neural crest cells *in vitro. In Vitro Cell DevBiol* 1987; 23: 323-331

Espinosa de los Monteros A, Roussel G, Neskovic, Nussbaum JL. A chemically defined medium for the culture of mature oligodendrocytes. *JNeurosci Res* 1988; 19: 202-211

Dulbecco R, Freeman G. Plaque production by the polyoma virus. *Virology* 1959; 8: 396-397

McKeehan WL, Hamilton WG, Ham RG. Selenium is an essential trace nutrient for growth of WI-3 8 diploid human fibroblasts. *ProcNatlAcadSci USA* 1976; 73: 2023-2028

Received 7 September 1996
Accepted 11 October 1996

For correspondence:
DrEgbertA.J.F. Lakke
Neuroregulation Group
Department Physiology
Medical Faculty
Leiden University
PO Box 9604
NL-2300 RC Leiden
The Netherlands

Tel: 31 (71)5276759
Fax: 31 (71)5276782
E-mail: e.a.j.f.lakke@physiology.medfac.leidenuniv.nl