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D E C I S I O N
of 26 June 2003

Case Number: T 0676/00 - 3.3.8

Application Number: 89910564.7

Publication Number: 0435911

IPC: C12N 5/00

Language of the proceedings: EN

Title of invention:
Cell culture medium for enhanced cell growth, culture longevity and product expression

Patentee:
CHIRON CORPORATION

Opponent:
Lonza AG

Headword:
Cell culture medium/CHIRON

Relevant legal provisions:
EPC Art. 54, 56, 84

Keyword:
"Admissibility of additional documents filed after summons to oral proceedings (no)"
"Main request: novelty (yes)"
"Inventive step (no)"
"Auxiliary request 1: novelty (yes)"
"Inventive step (no)"
"Auxiliary request 2: clarity (no)"
"Auxiliary request 3: inventive step (no)"

Decisions cited:
T 0606/89, T 0950/99

Catchword:
-

Case Number: T 0676/00 - 3.3.8

D E C I S I O N
of the Technical Board of Appeal 3.3.8
of 26 June 2003

Appellant: (Opponent)
Lonza AG
Münchensteinerstrasse 38
CH-4002 Basel (CH)

Representative:
Ritthaler, Wolfgang, Dr. rer. nat. Dipl.-Chem.
Winter, Brandl, Fürniss, Hübner,
Röss, Kaiser, Polte
Partnerschaft
Patent- und Rechtsanwaltskanzlei
Alois-Steinecker-Strasse 22
D-85354 Freising (DE)

Respondent: (Proprietor of the patent)
CHIRON CORPORATION
4560 Horton Street
Emeryville, CA 94608-2912 (US)

Representative:
Duckworth, Timothy, John, Dr.
J.A. KEMP & CO.
14 South Square
Gray's Inn
London WC1R 5JJ (GB)

Decision under appeal:
Interlocutory decision of the Opposition Division of the European Patent Office posted 9 March 2000 concerning maintenance of European patent No. 0435911 in amended form.

Composition of the Board:

Chairman: L. Galligani
Members: T. J. H. Mennessier
M. B. Günzel

Summary of Facts and Submissions

I. The opponent (appellant) lodged an appeal against the interlocutory decision of the opposition division dated 9 March 2000, whereby the European patent 0 435 911 was maintained on the basis of the first auxiliary request then on file, the main request not having been allowed for lack of novelty vis-à-vis documents (D1) and (D2) (cf section XIII, infra).

II. The set of claims allowed by the opposition division consisted of 22 claims.

Claim 1 read:

"1. A composition comprising

a Primary Supplement comprising:
(a) a first reagent selected from glutamine and glutamate;
(b) phospholipid precursors including at least choline and ethanolamine, the phospholipid precursors optionally being supplied in complex form; and
(c) amino acids;

and comprising the following Class I reagents: (i) a reducing agent, (ii) metal ions, (iii) a metal chelator, and (iv) vitamins,

wherein the Primary Supplement components and Class I reagents are supplied in amounts that effectively maintain cells in culture for a prolonged time in a pseudo-stationary phase growth phase and wherein:

(a) the amino acids comprise tryptophan at a concentration that when added to culture media is more than 20 mg/L, but less than about 200 mg/L; and
(b) the glutamine or glutamate is present at a concentration that when added to culture media is above about 5 mM, but below about 40 mM; and
(c) the choline is present at a concentration that when added to culture media is greater than about 4 mg/L; and
(d) the ethanolamine is present at a concentration greater than about 1 mg/L, but less than about 100 mg/L."

Claims 2 to 7 were dependent claims.

The remaining claims were directed to: i) a cell culture medium or a cell culture medium supplement comprising a composition as defined in claim 1 (see claims 3 to 18), ii) a method of growing cells using such a composition, or cell culture medium, or cell culture medium supplement (see claims 19 and 20), iii) the use to grow cells and produce biochemicals of such a composition, or medium, or cell culture medium supplement (see claim 21), and iv) a method of producing a biochemical based on the use of such a composition, or cell culture medium, or cell culture medium supplement (see claim 22).

III. The appellant, who had opposed the patent on the grounds on lack of novelty and lack of inventive step, lodged an appeal and set out the grounds of appeal in a statement filed on 12 July 2000.

IV. The patentee (respondent) filed a response to the appeal.

V. Together with the summons to oral proceedings, the board sent a communication pursuant to Article 11(2) of the Rules of Procedure of the Boards of Appeal presenting some preliminary and non-binding views on the matters of the case.

VI. In reply to the board's communication, the appellant filed two additional documents and submitted further arguments.

VII. In reply to the board's communication, and taking into account the latest appellant's submissions, the respondent filed a new main request and three auxiliary requests on 23 May 2003.

VIII. The new main request differed from the claims allowed by the opposition division only in that claims 19 and 21 were formally amended by correcting back-references to previous claims and renumbering a feature, respectively.

IX. Oral proceedings took place on 26 June 2003. They were attended by both parties. In the course of the proceedings, the appellant filed three new auxiliary requests (referred to thereafter as auxiliary requests 1, 2 and 3, respectively), to replace the previous ones.

X. **Auxiliary request 1** consisted of 20 claims.

Claim 1 read:

"1. A **method of growing cells comprising contacting the cells with cell culture medium wherein individually or in combination the components of a composition are added to the cell culture medium over the time of cell culture to maintain the cells in a pseudo-stationary growth phase, which** composition comprises:

Primary Supplement comprising:
(a) a first reagent selected from glutamine and glutamate;
(b) phospholipid precursors including at least choline and ethanolamine, the phospholipid precursors optionally being supplied in complex form; and
(c) amino acids;

and comprising the following Class I reagents: (i) a reducing agent, (ii) metal ions, (iii) a metal chelator, and (iv) vitamins,

wherein the Primary Supplement components and Class I reagents are supplied in amounts that effectively maintain cells in culture for a prolonged time in a pseudo-stationary phase growth phase and wherein:

(a) the amino acids comprise tryptophan at a concentration that when added to culture media is more than 20 mg/L, but less than about 200 mg/L; and
(b) the glutamine or glutamate is present at a concentration that when added to culture media is above about 5 mM, but below about 40 mM; and

(c) the choline is present at a concentration that when added to culture media is greater than about 4 mg/L; and
(d) the ethanolamine is present at a concentration greater than about 1 mg/L, but less than about 100 mg/L." (bold-type characters added by the board in order to emphasize the difference in respect of claim 1 allowed by the opposition division)

Claims 2 to 7 were dependent claims.

The remaining claims were directed to: (i) a method for growing cells comprising contacting the cells with a cell culture medium or a cell culture medium supplement comprising a composition as defined in claim 1 (see claims 8 to 18), (ii) the use of a method according to any one of the preceding claims to grow cells and produce biochemicals (see claim 19), and (iii) a method of producing a biochemical based on the use of such a composition, or cell culture medium, or cell culture medium supplement (see claim 20).

XI. **Auxiliary request 2** consisted of 20 claims.

Claim 1 read:

"1. A method of growing cells comprising contacting the cells with cell culture medium wherein individually or in combination the components of a composition are added to the cell culture medium over the time of cell culture to maintain the cells in a pseudo-stationary growth phase, which composition comprises:

Primary Supplement comprising:
(a) a first reagent selected from glutamine and glutamate;
(b) phospholipid precursors including at least choline and ethanolamine, the phospholipid precursors optionally being supplied in complex form; and
(c) amino acids;

and comprising the following Class I reagents: (i) a reducing agent, (ii) metal ions, (iii) a metal chelator, and (iv) vitamins,

wherein the Primary Supplement components and Class I reagents are supplied in amounts that **can be used to** effectively maintain cells in **batch** culture for a prolonged time in a pseudo-stationary phase growth phase and wherein:

(a) the amino acids comprise tryptophan at a concentration that when added to culture media is more than 20 mg/L, but less than about 200 mg/L; and
(b) the glutamine or glutamate is present at a concentration that when added to culture media is above about 5 mM, but below about 40 mM; and
(c) the choline is present at a concentration that when added to culture media is greater than about 4 mg/L; and
(d) the ethanolamine is present at a concentration greater than about 1 mg/L, but less than about 100 mg/L." (bold-type characters added by the board in order to emphasize the difference in respect of claim 1 of auxiliary request 1)

Claims 2 to 20 were identical in wording to claims 2 to 20 of auxiliary request 1 with the exception of claim 8 and 16 which had each been amended in the same way as claim 1 to contain the expression "that **can be used to** effectively maintain cells in **batch** culture".

XII. **Auxiliary request 3** consisted of 20 claims.

Claim 1 read:

"1. A method of growing **antibody secreting** cells comprising contacting the cells with cell culture medium wherein individually or in combination the components of a composition are added to the cell culture medium over the time of cell culture to maintain the cells in a pseudo-stationary growth phase, which composition comprises:

Primary Supplement comprising:
(a) a first reagent selected from glutamine and glutamate;
(b) phospholipid precursors including at least choline and ethanolamine, the phospholipid precursors optionally being supplied in complex form; and
(c) amino acids;

and comprising the following Class I reagents: (i) a reducing agent, (ii) metal ions, (iii) a metal chelator, and (iv) vitamins,

wherein the Primary Supplement components and Class I reagents are supplied in amounts that effectively maintain cells in culture for a prolonged time in a pseudo-stationary phase growth phase and wherein:

(a) the amino acids comprise tryptophan at a concentration that when added to culture media is more than 20 mg/L, but less than about 200 mg/L; and
(b) the glutamine or glutamate is present at a concentration that when added to culture media is above about 5 mM, but below about 40 mM; and
(c) the choline is present at a concentration that when added to culture media is greater than about 4 mg/L; and
(d) the ethanolamine is present at a concentration greater than about 1 mg/L, but less than about 100 mg/L." (bold-type characters added by the board in order to emphasize the difference in respect of claim 1 of auxiliary request 1)

Claims 2 to 20 were identical in wording to claims 2 to 20 of auxiliary request 1 with the exception of claim 16 which had been amended in the same way as claim 1 to contain the expression the term "**antibody secreting**" and claim 20 which had been amended to be directed to "A method of producing **antibodies**".

XIII. The following documents are referred to in the present decision:

(D1): WO-A-87/00195 (from which the post-published patent EP-B1-0 229 809 B1, the document actually cited by the appellant and relied upon by the opposition division, was derived);

(D2): Hiroki Murakami et al., Proc. Natl. Acad. Sci. USA, Vol. 79, February 1982, Pages 1158 to 1162;

(D3): Hiroki Murakami, "Serum-Free Media Used for Cultivation of Hybridomas", in "Monoclonal Antibodies: Production and Application", Alan R. Liss, Inc., 1989, Pages 107 to 141;

(D4): English translation (Pages 1 to 18) of "Hiroki Murakami et al., Nippon Nogeikagaku Kaishi, Vol. 58, No. 6, 1984, Pages 575 to 583";

(D5): H. Murakami et al.: "Serum-free stirred culture of human-human hybridoma lines", in Murakami et al. (eds.) "Growth and Differentiation of Cells in Defined Environment", Tokyo/Berlin, Kondansha/Springer-Verlag, 1985, Pages 111 to 116;

(D6): Patrick J. Farrell et al., Biotechnol. Bioeng., Vol. 64, No. 4, 20 August 1999, Pages 426 to 433;

(A): The front page of the IGN cell biology catalog 1995/96, together with page 24 thereof containing information with respect to the Dulbecco's Modification of Eagle's Medium (DMEM).

XIV. The submissions of the appellant as made in writing and at the oral proceedings, insofar as they are relevant to the present decision, may be summarized as follows:

Admissibility into the appeal proceedings of documents (D5) and (D6):

Documents (D5) and (D6) were filed in reply to the latest respondent's arguments. They could not have been filed earlier because the appellant had difficulties in being provided with the cells cited in those documents which had been ordered with the view of assessing the experiments described therein. Both documents should be admitted into the appeal proceedings.

Main request (claim 1): novelty and inventive step

Claim 1 lacked novelty over document (D1), more particularly in view of Example 4.6 thereof. The concentrations of the supplemented glutamine, tryptophan, tyrosine, cysteine, glucose, choline and soluble amino acids were those indicated in Table 1 on page 16. The concentration of ethanolamine was not explicitly referred to, but the person skilled in the art, aware of documents (D2) and (D4), would have used said component at a concentration comprised within the range of claim 1. Cystine and cysteine could be used intraconvertibly as a reducing agent in culture media. Therefore, regardless of whether the term "cysteine" on page 27 of document (D1) should read "cystine" or not, the composition of Example 4.6 contained a reducing agent. Moreover, cystine was regarded in the patent in suit itself (see page 7, lines 39 to 42 in the patent specification) as a preferred "reducing agent".

Document (D1) or document (D4) could be chosen as the closest prior art. Starting from document (D1), the person skilled in the art would have derived directly from document (D2) the ethanolamine concentration to be used when preparing the composition according to Example 4.6 of document (D1). Similarly, starting from

document (D4), the skilled person would have derived directly from Table 1 on page 16 of document (D1) the tryptophan concentration to be used when preparing the basal medium according to Example 4.6 of document (D1). Therefore, the subject-matter of claim 1 did not involve an inventive step in view of the combination of document (D1) with either document (D2) or document (D4).

Auxiliary request 1 (claim 1): novelty and inventive step

The method of claim 1 was identical to the process described in Example 4.6 of document (D1). The same composition was used. Therefore, for the reasons given with respect to the main request, the subject-matter of claim 1 was not new and did not involve an inventive step. Document D1 on its own was sufficient to establish lack of inventive step.

Auxiliary request 2: clarity and inventive step

The amendment according to which "the component/reagent amounts can be used to maintain cell in batch culture" as contained in claim 1 meant that a prolongation of the stationary phase was not necessary; this was in contradiction with the requirement, also contained in the claim, of maintaining the cells in a pseudo-stationary growth phase.

Notwithstanding said defective amendment, the reasoning made for the main request and auxiliary request 1 for novelty and inventive step also applied to claim 1 of the auxiliary request 2.

Auxiliary request 3 (claim 1): inventive step

The process of Example 4.6 in document (D1) was appropriate to the culture of animal cells in general and, more particularly, as illustrated in the examples, to the culture of hybridomas, whether they were antibody-secreting or polypeptide/protein-producing (as an effect of a transfection). Therefore, the person skilled in the art would have considered that the process of Example 4.6 was applicable to the culture of antibody-secreting hybridomas and would have been prompted to combine that document with document (D4), which dealt with the culture of antibody-secreting hybridomas, or document (D2), thereby arriving, without the exercise of inventive skill, to the method of claim 1. The person skilled in the art would have known that the use of supplemented serum-free media was advantageous because contaminants present in serum were avoided and the purification process when recovering the protein produced was simplified. Therefore, the subject-matter of claim 1 did not involve an inventive step.

XV. The submissions of the respondent as made in writing and at the oral proceedings, insofar as they are relevant to the present decision, may be summarized as follows:

Admissibility into the appeal proceedings of documents (D5) and (D6)

Documents (D5) and (D6), which were filed only with a letter sent in preparation of the oral proceedings before the board, were to be regarded as late-filed. Figure 3 of document (D5) corresponded to Figure 4 of document (D3). Document (D5) was a citation of document (D3). Document (D5) was therefore known to the appellant and could have been filed long before, presumably already during the opposition proceedings. Furthermore, document (D5) was not relevant to the invention because it dealt with a perfusion system and was not concerned with the development of culture media. Moreover, as the serum-free medium in the study referred to in document (D5) was continuously exchanged for the same volume of fresh medium every day, the tryptophan concentration in the cell culture medium could not increase compared to the concentration in the medium at the beginning of the cell culture. Therefore, document (D5) was not *prima facie* novelty-destroying. Document (D6) was a post-published document. Neither of documents (D5) and (D6) should be admitted into the appeal proceedings.

Main request (claim 1): novelty and inventive step

The composition of claim 1 was novel. It differed from the composition referred to in Example 4.6 of document (D1) in that (i) the ethanolamine concentration was not indicated, (ii) there was no clear disclosure of a reducing agent therein (cystine and not cysteine had been actually used, cystine not being a reducing agent), and (iii), in view of Figure 4 of document

(D1), it was doubtful whether the cells used in Example 4.6 had been maintained for a 'prolonged' time in a pseudo-stationary phase.

It was doubtful whether the person skilled in the art assessing document (D1) would have looked at Example 4.6 rather than at the other examples which, while dealing with compositions containing no ethanolamine, as shown in Figure 1, gave a substantially longer stationary phase. Furthermore, if, nevertheless, that person had considered Example 4.6 of document (D1), he/she would have found no incentive to further look at document (D2), a document concerned primarily with stimulation of the cell growth rather than with the development of a composition which would allow to obtain a prolonged stationary growth phase. As the medium of document (D2) was very different from the medium of Example 4.6 of document (D1), the person skilled in the art would have had no incentive to adjust the ethanolamine concentration of the medium of Example 4.6 of document (D1) in such a way that it would have corresponded to the concentration referred to in document (D2). Compared to the invention, document (D4) addressed a quite different technical problem, the emphasis therein being put on obtaining cells in higher density and, therefore, on optimizing the compound concentrations in the culture medium. As a result, the person skilled in the art would have found no incentive in a further increase of the tryptophan concentration of the e-RDF medium of document (D4). Therefore, the composition of claim 1 involved an inventive step.

Auxiliary request 1 (claim 1): novelty and inventive step

The composition referred to in claim 1 was identical with the composition of claim 1 of the main request and differed in the same way from the composition of Example 4.6 of document (D1). Therefore, the method of claim 1 was novel. That method contained the active functional feature of maintaining the cells in a pseudo-stationary growth phase. Document (D1) was also the closest prior art. As documents (D2) and (D4) addressed two different technical problems, namely and respectively, improving the growth of the cells and increasing cell densities, there would have been no incentive for the person skilled in the art to combine document (D1) with either of document (D2) or document (D4). Therefore, the composition of claim 1 involved an inventive step.

Auxiliary request 2 (claim 1): clarity and inventive step

Claim 1 was clearly formulated. While document (D1) related specifically to a process for culturing cells where the medium was supplemented during the culture, ie a continuous feeding was involved, claim 1 implied that the composition could be used in a simple batch culture, a feature which could not be derived from the state of the art. Therefore, the method of claim 1 involved an inventive step.

Auxiliary request 3 (claim 1): inventive step

The technical problem solved by the method of claim 1 was the provision of means to grow cells which are in a stationary phase. Example 4.6 of document (D1) was concerned with the culture of transfected hybridomas producing not antibodies but tPA. Therefore, the person skilled in the art would have looked primarily at the other examples which were concerned with the production of antibodies, and which, moreover, showed a more prolonged stationary phase than Example 4.6 (as shown in Figure 1 compared with Figure 4). In addition, it was believed that transfected hybridomas behaved differently in culture than antibody-secreting hybridomas. Therefore, the method of claim 1 involved an inventive step.

XVI. The appellant requested that the decision under appeal be set aside and that the European patent No. 0 435 911 be revoked.

XVII. As main request the respondent requested that the decision be set aside and the patent be maintained on the basis of the main request filed on 23 May 2003. As auxiliary requests 1 to 3 the respondent requested that the patent be maintained with any of auxiliary requests 1 to 3 filed during the oral proceedings, taken in their numerical order.

Reasons for the Decision

Admissibility into the appeal proceedings of documents (D5) and (D6)

1. Two additional documents, namely (D5) and (D6), were filed within the one month time limit fixed by the board, for making written submissions in preparation of the oral proceedings. The respondent considers that they were filed at a very late stage of the proceedings and, therefore, objects to their admission into the proceedings. With respect to document (D5) the respondent submits that (i) it is used against novelty which was not a ground on which the appeal was based in the statement setting out the grounds of appeal and (ii) it is not *prima facie* relevant.

2. Although, in principle, an appeal should be essentially based on facts and evidence which were already available to the department of the first instance, parties in their effort to make a full statement of the grounds why the revision of the contested decision is requested often rely on additional evidence. Such evidence is not necessarily defined as being "late-filed". Much depends on its *prima facie* relevance, the board being empowered essentially either i) to disregard it under Article 114(2) EPC or ii), having admitted it, either to remit the case to the department of first instance under Article 111(1) EPC for further prosecution, or to decide on the case (see decision T 0950/99 of 11 November 2002, point 4 of the reasons).

3. In the present case, the board, exercising its discretion, decides not to admit documents (D5) and (D6) into the appeal proceedings for the following reasons:

Document (D5)

4. Document (D5) was published before the priority date and, therefore, is part of the state of the art as defined in Article 54(2) EPC. It is citation No. 61 of document (D3) (Figure 3 of document (D5) being Figure 4 of document (D3)), a document cited by the appellant in the notice of opposition filed in 1996. Consequently, document (D5) was well-known to the appellant as from the beginning of the opposition and could have been submitted earlier. The fact that the appellant may have had difficulties in being provided with the cells mentioned in this document cannot be accepted as a justification for withholding the document as such.

5. Novelty was a ground of opposition. It has been taken into consideration by the opposition division in its decision. Therefore, it is comprised within the legal frame of the present appeal. As a result, the late submission of document (D5) could not be regarded as inadmissible for the reason that the document has been cited against novelty.

6. The key question to be answered is whether document (D5) is *prima facie* relevant.

7. Document (D5) describes a serum-free cell culture system which grows human-human hybridomas cells in high density and produces monoclonal antibodies in a large quantity. A medium referred to as "eRDF" is used which

is supplemented with insulin, transferrin, ethanolamine and selenite (these four compounds being all together referred to as "ITES"). Neither the precise composition of the medium nor the concentration of the supplemented compounds are indicated. A mere reference to document (D4) on page 113 lets assume that the eRDF medium corresponds to the e-RDF medium that is described in detail on page 11 of that document. On the premise that the medium is continuously exchanged for the same volume of fresh medium every day, the appellant infers, without providing any technical evidence such as tryptophan concentrations measured during the course of the culture, that in document (D5), at least after two days of culture and due to the repeated supply of the ITES supplemented eRDF medium, a composition is used which has the technical features of the composition of claim 1, with in particular a tryptophan concentration of more than 20 mg/L. The board cannot *prima facie* adhere to this reasoning because concentrations as such are not cumulative: if 1 litre of used medium containing tryptophan at a concentration of 4 mg/l is replaced by 1 litre of fresh medium containing tryptophan at a concentration of 16 mg/L, one cannot conclude that a medium with a tryptophan concentration of at least 20 mg/L has been used. Therefore, document (D5) is not *prima facie* relevant to the present decision.

Document (D6)

8. Document (D6) investigates the capability of transformed lepidopteran insect cells to overexpress human tissue plasminogen activator (tPA). The emphasis is not put on the composition/medium used to grow the

cells. Moreover, it is a post-published document, ie a document which does not belong to the state of the art to be taken into consideration for the assessment of novelty and inventive step. Therefore, also document (D6) is not *prima facie* relevant to the present decision.

Main request

Formal requirements; Articles 123(2) and (3) and 84 EPC

9. The appellant has no objection as regards the compliance of the amended claims with the requirements of Articles 123(2) and (3) and 84 EPC. Also in the board's judgment these requirements are met.

Novelty (claim 1); Article 54 EPC

10. The appellant submits that document (D1) deprives the claimed invention of novelty for the reason that a composition as defined in claim 1 is described in Example 4.6 of that document.

11. Document (D1) describes a process for the culture of animal cells for producing a polypeptide/protein. That process relies on the finding that continued feeding serves to prolong viability of the culture giving rise to enhancement of overall product yield (see page 5, lines 10 to 17). It preferably applies to hybridomas, whether they are antibody-secreting hybridomas or protein-producing transfected hydridomas. Example 1 describes a fed batch culture of an antibody-secreting mouse-mouse hybridoma cell line. The cells are grown in a 5-litre airlift fermenter in a medium consisting of

Dulbecco's modification of Eagle's medium (DMEM) supplemented with foetal calf serum. Shot additions of three supplements are made. The composition and mode of addition of the supplements are indicated in Table 1 (see page 16). The supplemented nutrients consist of a) a shot addition of glutamine, b) a shot addition of cystine, tyrosine and tryptophan, and c) a pumped feed of glucose, choline chloride and of soluble amino acids. Example 4.6 describes a fed batch culture of a tPA-producing transfected hybridoma. The cells are grown in a 5-litre airlift fermenter (ie as in Example 1) in a serum-free formulation consisting of a DMEM base supplemented *inter alia* with albumin, insulin, **transferrin**, **ethanolamine**, **choline**, **vitamins**, and **trace metals** (see page 27). During the culture, additional nutrients are added which consist of a) a shot addition of **glutamine**, b) a shot addition of the "insoluble" **amino acids** tryptophan, tyrosine and **cysteine**, and c) a pumped feed consisting of glucose, **choline** and the "soluble" **amino acids**. Figures 1 (with respect to Example 1) and 4 (with respect to Example 4) show, with different scales, **a prolonged stationary growth phase** (see the second sentence at the bottom of page 16).

12. Although in Example 4.6 the concentrations of the nutrients added during the culture are not directly indicated, it is evident that, since the same type of culture (fed batch culture, same fermenter, and same added nutrients) is carried out in Examples 1 and 4.6, the added nutrient concentrations in Example 4.6 are those of Table 1 reported in Example 1, where the same headings a), b) and c) with identical compounds (except for cystine/cysteine, see point 13, infra) are given for the supplements.

13. The respondent denies that the nutrients added during the culture in Example 4.6 are the same as those added during the culture in Example 1, because the term "cysteine" is referred to in Example 4.6 only, the term being replaced in Example 1 by the term "cystine". As cysteine in Example 4.6 is referred to as an insoluble amino acid (together with tryptophan and tyrosine) and as it is common knowledge that cystine but not cysteine is insoluble, the board is convinced that in Example 4.6 the term "cysteine" has been mistakenly substituted for the term "cystine" (see also the statement on page 10, lines 1 and 2 in document (D1), where cystine is explicitly referred to as an insoluble amino acid).

14. In view of document (A), the composition referred to in Example 4.6, made of the culture medium as such, ie the supplemented DMEM base, and the nutrients added during the culture, contains:

- **glutamine** at a concentration of about 6 mM (584 mg/L already contained in the DMEM base (see document (A)), ie about 4 mM, plus 2 mM supplied during the culture (see Table 1 on page 16));

- **choline** at a concentration of at least 9 to 20 mg/L (4 mg/L already contained in the form of choline chloride in the DMEM base (see document (A), plus an unspecified amount added thereto as a supplement, plus 5 to 15 mg/L supplied during the culture, also in the form of choline chloride);

- **ethanolamine** (the concentration of which is not specified at all in Example 4.6 or elsewhere in document (D1)),

- **amino acids** (contained in the DMEM base (see document (A)) and supplied during the culture), including **cystine** and **tryptophan** at a concentration of 26 to 36 mg/L (16 mg/L (see document (A)) plus 10 to 20 mg/L (see Table 1 on page 16 of document D1))

- **metal ions** (contained in the DMEM base (see document (A)) and also added thereto as a supplement),

- **a metal chelator** (according to the definition given on page 8, lines 8 and 9 of the patent specification two metal chelators are referred to in Example 4.6, namely, albumin and **transferrin**) added to the DMEM base as a supplement, and

- **vitamins** (contained in the DMEM base (see document (A)) and also added thereto as a supplement).

In view of Figure 4 of document (D1), said components (including cystine) are supplied in amounts that effectively maintain cells in culture for prolonged time in a stationary growth phase.

15. The respondent argues also that there is no clear disclosure of the presence of a reducing agent in the composition of Example 4.6 of document (D1), cystine being used instead of cysteine (see point 13, supra). However, it is noted that in the patent in suit, **cystine** but not cysteine is referred to as a preferred reducing agent (see page 7, line 42 in the patent specification) which is an admission by the respondent

that cystine (which is recognised as the reduced form of cysteine) may be used in the composition of the invention as a source of cysteine or derivatives thereof. This, in fact, corresponds to the common knowledge that in culture media cystine is used as the stable form of cysteine (cf composition of DMEM medium, document (A)).

16. Therefore, the composition referred to in Example 4.6 of document D1, consisting of the supplemented DMEM base and all the nutrients added during the culture, contains **glutamine** at a concentration that is above 20 mg/L, but less than about 200 mg/L, **choline** at a concentration that is greater than about 4 mg/L, **ethanolamine, amino acids** including **tryptophan** at a concentration that is more than 20 mg/L, but less than about 200 mg/L, **a reducing agent** (cystine), **metal ions**, **a metal chelator** (such as transferrin or albumin), and **vitamins**, all said compounds being supplied in amounts that effectively maintain cells in culture for prolonged time in a stationary growth phase. At this point, the board considers that the term "stationary" used in document (D1) (see the last but one sentence at the bottom of page 16) has the same meaning as the term "pseudo-stationary" (see page 4, lines 21 to 23) as used in the patent specification: it marks a period of culture growth occurring after the exponential phase and before the decline phase.

17. The board notes that the said composition differs from the one of claim 1 only in that the ethanolamine concentration has not been specified. Therefore, in view of this sole difference, document (D1) does not disclose the composition of claim 1 and the

requirements of Article 54 EPC are met by the main request, in which all remaining claims refer to this composition.

Inventive step (claim 1); Article 56 EPC

State of the art

18. In addition to document (D1) (see point 11, supra), documents (D2) and (D4) are taken into consideration.

19. Document (D2) reports results of a study based on the premise that cultivation of hybridomas that continue to secrete splenic antibody in a defined medium would be facilitated by eliminating serum. It essentially describes the use of a serum-free medium which is a 1:1 mixture of DMEM with Ham's F-12 medium (globally referred to as "SFFD") supplemented with one or more of four compounds, insulin, transferrin, **ethanolamine**, and selenite, to grow a variety of antibody-secreting hybridomas and plasmacytoma-B-lymphoma hybrid cells. Ethanolamine was found to be a necessary growth-promoting material for all hybridoma lines tested (see bottom of the left-hand column on page 1158), no growth of the cells being obtained in SFFD without ethanolamine, even if any of the factors listed in Table 1 (see page 1160) is supplemented in addition to insulin, transferrin, and selenite (see the second sentence in the left-hand column on page 1162).

20. Document (D4) describes a basal medium (referred to as e-RDF) which upon supplementation with insulin, transferrin, **ethanolamine** and selenite (those four compounds being all together referred to as "ITES") allows for growth of cells such as hybridomas with an

increase of cell densities while enabling accumulation of immunoglobulin at high concentrations in the medium. The composition of the medium is given in Table III (see page 11). It contains glutamine, choline, amino acids including tryptophan and cystine, this latter amino acid representing a reducing agent within the meaning of the patent in suit, metal ions, vitamins, and, upon supplementation with ITES, a metal chelator (transferrin) as well as ethanolamine.

Closest prior art

21. In accordance with the case law (see, for example, decision T 606/89 of 18 September 1990, point 2 of the reasons), the closest prior art for the purpose of objectively assessing inventive step is generally that which corresponds to a similar use requiring the minimum of structural and functional modifications.

22. No stationary growth phase is identifiable on Figure 5A or any other figures of document (D4). No prolongation of the cell viability is referred to in the document. What is expected when using the e-RDF medium supplemented with ITES is an increase of the growth density and a corresponding increase of the antibody secretion.

23. Therefore, not document (D4) but document (D1), which discloses a composition the components of which are supplied in amounts that effectively maintain cells in culture for a prolonged time in a stationary growth phase, represents the closest state of the art, the only difference between the composition of Example 4.6 of document (D1) and the composition of claim 1 being

that in the composition of document (D1) the ethanolamine concentration has not been specified (cf point 17, *supra*). The respondent argues that the person skilled in the art would have had no incentive to look at Example 4.6 of document (D1) because no "prolonged" stationary growth phase is observed. The argument cannot be accepted: as the term "prolonged" is an ambiguous term which has not been defined in the patent, there is no reason not to consider a stationary growth phase of about 40 hours, as recognisable in Figure 4 of document (D1), as a prolonged one.

Assessment of inventive step

24. Starting from document (D1), the technical problem to be solved by the invention may be defined as the provision of a composition which is appropriate for the culture of cells in the absence of serum (note that it was a major object of the invention to provide a composition, including a protein-free supplement, being particularly effective in production of antibodies using hybridoma cells in **serum-free culture**; see page 2, lines 54 to 57 in the patent specification), the solution to that problem being the provision of a composition which, as defined in claim 1, has *inter alia* an ethanolamine concentration greater than about 1 mg/L, but less than about 100 mg/L.

25. As document (D1) does not specify the ethanolamine concentration, the skilled person would have had to look in a prior art document in order to find a suitable one. As a matter of fact, as ethanolamine is referred to already in the title as an essential component for the growth of hybridoma cells in serum-

free medium, he/she would have been prompted to look in document (D2) and would have been convinced that a concentration of 20 µM, ie about 3,7 mg/L, in the culture medium (as indicated in the left-hand column of page 1160), was an appropriate one.

26. While looking in document (D2), the person skilled in the art would have paid no particular attention to the details of the experiments referred to therein, except the ethanolamine concentration. Notwithstanding this remark, the argument, made by the respondent, that the ethanolamine concentration was determined in document (D2) in relation with a very special medium and cannot be extrapolated to other media, cannot be accepted, in view of the fact that the SFFD medium is a 1:1 mixture of DMEM medium and Ham's F12 medium, two well-known commonly used basal media (see page 2, line 10 and page 4, line 4 in the patent specification), and in the absence in the document of any statement which would discourage the skilled practitioner to repeat the experimentation with another serum-free medium.

27. Therefore, the person skilled in the art, starting from the composition of document (D1), prompted by document (D2) to use an ethanolamine concentration of 20 µM, ie about 3,7 mg/L, would have come, without the exercise of inventive skill, to a composition having all the technical structural and functional features of the composition of claim 1. Therefore, claim 1 does not involve an inventive step and, as the requirements of Article 56 EPC are not complied with, the main request is not allowable.

Auxiliary request 1

Formal requirements; Articles 123(2) and (3) and 84 EPC

28. The appellant has no objections as regards the compliance of the amended claims with the requirements of Articles 123(2) and (3) and 84 EPC. Also in the board's judgment these requirements are met.

Novelty (claim 1); Article 54 EPC

29. The appellant submits that document (D1) deprives the claimed invention of novelty for the reason that a method as defined in claim 1 is described in Example 4.6 of that document.

30. Claim 1 is directed to a method of growing cells comprising contacting the cells with cell culture medium wherein, individually or in combination, the components of a composition as defined in claim 1 of the main request are added over the time of cell culture medium to maintain the cells in a pseudo-stationary growth phase. The cells are not limited as to their nature. According to the description, they are animal cells (see page 2, line 3 and page 4, lines 31 and 32, in the patent specification).

31. As explained at points 10 to 17 (see supra), the composition referred to in Example 4.6 of document D1 differs from the composition of claim 1 only in that, in the composition of document (D1), the ethanolamine concentration has not been specified. In Example 4.6 a method of growing cells is described which involves continuous feeding. Transfected hybridomas are inoculated in the supplemented DMEM base contained in

the fermenter and during the culture further nutrients are added. Therefore, the claimed method differs from the method of Example 4.6 only in that a different composition is employed. This difference is sufficient to establish novelty of the claimed method. As a result, the method of claim 1 is new, and auxiliary request 1 meets the requirements of Article 54 EPC.

Inventive step (claim 1); Article 56 EPC

32. The state of the art to be taken in consideration is the same as for the main request. Document (D1), being the only document which describes a process for prolonging the longevity of animal cells while giving rise to en enhancement of overall product yield, represents the closest state of the art.

33. Starting from that closest prior art, the technical problem to be solved by the invention may be regarded as the provision of a method of growing animal cells based on a continuous feeding which allows the cells to be cultured in the absence of serum with the view of maintaining the cells in a pseudo-stationary growth phase, the solution to that problem being the provision of a method as defined in claim 1 which uses a composition containing ethanolamine at a concentration greater than about 1 mg/L, but less than about 100 mg/L.

34. Document D2 would have provided the person skilled in the art with the teaching that ethanolamine used at a concentration of 20 µM, ie 3,7 mg/L, promotes the growth of cells such as hybridomas. Therefore, the skilled person would have found in document (D2) an incentive to perform the process of Example 4.6 of document (D1) while using ethanolamine at that concentration, and, thereby, would have come, without the exercise of inventive skill, to a method of growing cells having all the technical structural and functional features of the method of claim 1. Therefore, claim 1 does not involve an inventive step and, as the requirements of Article 56 EPC are not complied with, auxiliary request 1 is not allowable.

Auxiliary request 2

Clarity; Article 84 EPC

35. Whereas claim 1 is directed to a method of growing cells with the explicitly expressed view of maintaining the cells in a pseudo-stationary growth phase, the claim contains the wording "*the Primary Supplement components and Class I reagents are supplied in amounts that* ***can be used to*** *effectively maintain cells in batch culture for a prolonged time in a pseudo-stationary phase growth phase*" (emphasis added by the board). This wording implies that amounts may be chosen which do not effectively maintain cells in batch culture for a prolonged time in a pseudo-stationary phase growth phase. This is in contradiction with the afore-mentioned aim of maintaining the cells in a pseudo-stationary growth phase. Therefore, the emphasized amendment contained in claim 1 renders it unclear.

36. As the requirements of Article 84 EPC are not complied with, auxiliary request 2 is not allowable.

Auxiliary request 3

Formal requirements; Articles 123(2) and (3) and 84 EPC

37. The appellant has no objections as regards the compliance of the amended claims with the requirements of Articles 123(2) and (3) and 84 EPC. Also in the board's judgment these requirements are met.

Novelty (claim 1); Article 54 EPC

38. The appellant does not have any novelty objection under Article 54 EPC.

39. Claim 1 of auxiliary request 3 differs from claim 1 of auxiliary request 1 only in that the terms "antibody-secreting" have been added to specify the cells to be grown. The cells grown in the experiment of Example 4.6 of document (D1) are not antibody-producing hybridoma cells but tPA-producing transfected hybridoma cells. Indeed, this represents, in addition to the ethanolamine concentration difference, a further difference between the claimed method and the method described in that example.

40. In the board's judgment, there are no documents on file which destroy the novelty of the subject-matter of claim 1. Novelty is thus acknowledged.

Inventive step (claim 1); Article 56 EPC

41. The state of the art to be taken in consideration is the same as for the other requests. Document (D1) still represents the closest prior art.

42. Starting from that state of the art, the technical problem to be solved by the invention may be regarded as the provision of a method of growing antibody-secreting cells based on a continuous feeding and which allows the cells to be cultured in the absence of serum with the view of maintaining the cells in a pseudo-stationary growth phase, the solution to that problem being the provision of a method as defined in claim 1 which uses a composition containing ethanolamine at a concentration greater than about 1 mg/L, but less than about 100 mg/L.

43. It has already been decided in relation to the previous requests that neither the composition *per se* (main request) nor a method of using it for growing cells in general (auxiliary request 1) are inventive. Thus, in respect of the present request, the question to be answered is whether the skilled person would have been prompted to use the method of Example 4.6 of document (D1) to grow an antibody-secreting hybridoma cell line instead of the transfected hybridoma cell line referred to therein.

44. Document (D1) describes a method of growing cells which generally applies to any animal cells, no particular distinction being made in particular between antibody-secreting hybridomas and transfected hybridomas. Of interest in this respect is the statement, found in the second paragraph of page 7, according to which

"[S]imilar supplemental feeding regimes may be used with hybridoma cells in general, and also transfected hybridoma and myeloma cells". Both Example 1, which describes the culture of an antibody-secreting hybridoma cell line, and Example 4.6, which describes the culture of a tPA producing transfected hybridoma cell line, rely on a fed batch culture carried out in a 5-litre airlift fermenter and using the same feeding regime. They differ in that the cells are grown in the fermenter in a medium consisting of a DMEM base supplemented in Example 1, with 3% v/v foetal calf serum, and in Example 4.6, with albumin, insulin, transferrin, ethanolamine, choline, vitamins and trace metals. This means that the foetal calf **serum** of Example 1 has been replaced in Example 4.6 by a supplement comprised of **albumin**, **insulin**, **transferrin**, **ethanolamine**, **choline**, **vitamins**, and **trace metals.**

45. In his/her assessment of whether the nutrient supplement of Example 4.6 would have been appropriate for the culture of antibody-secreting hybridomas, the person skilled in the art would have found a supplement of choline, vitamins, and trace metals to be convenient, as those components are already present in the DMEM base (see document (A)) used in Example 1. He/she would also not have been deterred from using albumin therein as albumin is an essential component of the serum used in Example 1. As regards insulin, transferrin and ethanolamine, he/she would have known from document (D2) that not only ethanolamine but also insulin and transferrin represented major growth factors for murine antibody secreting hybridomas cells. Therefore, the person skilled in the art would have tested the hybridoma cells of Example 1 in the method of growing

cells of Example 4.6 without any apprehension of failure.

46. Also informed by document (D2) that an ethanolamine concentration of 20 µM, ie about 3,7 mg/L, was appropriate, the person skilled in the art would have been prompted to apply the method described in Example 4.6 to the culture of antibody-secreting hybridomas with ethanolamine used at that concentration and, thereby, without the exercise of inventive skill, would have arrived at the method of claim 1. Therefore, claim 1 does not involve an inventive step, and, as the requirements of Article 56 EPC are not complied with, also auxiliary request 3 is not allowable.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The patent is revoked.

The Registrar: The Chairman:

A. Wolinski L. Galligani