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D E C I S I O N
of 30 November 1999

Case Number: T 0745/96 - 3.3.4

Application Number: 86904268.9

Publication Number: 0229809

IPC: C12N 5/02

Language of the proceedings: EN

Title of invention:
Animal cell culture

Patentee:
Alusuisse Holdings A.G.

Opponent:
Roche Diagnostics GmbH
Dr Karl Thomae GmbH
Chiron Corporation

Headword:
Animal cell culture/ALUSUISSE HOLDINGS A.G.

Relevant legal provisions:
EPC Art. 123(2)(3), 87 to 89, 84, 54, 56

Keyword:
"Lack of inventive step - all requests"

Decisions cited:
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Catchword:
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Boards of Appeal

Chambres de recours

Case Number: T 0745/96 - 3.3.4

D E C I S I O N
of the Technical Board of Appeal 3.3.4
of 30 November 1999

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Decision under appeal: Interlocutory decision of the Opposition Division
of the European Patent Office posted 2 July 1996
concerning maintenance of European patent
No. 0 229 809 in amended form.

Composition of the Board:

Chairman: U. M. Kinkeldey
Members: F. L. Davison-Brunel
W. Moser

Summary of Facts and Submissions

- I. The appeal lies against the decision of the Opposition Division maintaining the patent in suit in amended form on the basis of 16 claims filed on 28 November 1995 as second auxiliary request.

Claims 1 and 5 read as follows:

"1. A process for the fed batch culture of animal cells comprising culturing the cells in nutrient medium and continuing the culturing into the decline phase of the culture to provide the product(s) of the cells, wherein, during the exponential growth phase of the culture and for a prolonged period of time extending beyond the exponential growth phase of the culture, the medium is supplemented with a combined feed of glutamine and a sugar as an energy source, characterised in that the supplemental feed additionally comprises one or more of the other "essential" amino acids."

"5. The process of any one of claims 1 to 4 wherein the supplemental feed additionally comprises one or more of the "non essential" amino acids."

Dependent claims 2 to 4, 6 to 16 related to specific embodiments of the process of claim 1.

- II. The Appellants I, II and III (Opponents 01, 02 and 03) lodged an appeal against this decision, paid the fee and submitted a statement of grounds of appeal. The Respondents (Patentees) lodged an appeal but did not submit a statement of grounds of appeal.

III. In response to a communication by the Board, submissions were sent by the Appellants and by the Respondents; the latter further filed a first and a second auxiliary request on 29 October 1999.

Claim 1 of the second auxiliary request read as follows:

"1. A process for the fed batch culture of animal cells comprising culturing the cells in a nutrient medium and continuing the culturing into the decline phase of the culture to provide the product(s) of the cells, wherein, during the exponential growth phase of the culture and for a prolonged period of time extending beyond the exponential growth phase of the culture, the medium is supplemented with a combined feed of glutamine and a sugar as an energy source,

characterised in that

the supplemental feed additionally comprises one or more of the other "essential" amino acids, said additional amino acid components comprising from 50-90 per cent of the amounts of the amino acids present in the medium at the start of the culture."

IV. Oral proceedings were held on 30 November 1999. During these proceedings, the first auxiliary request then on file was replaced. Claim 1 of the new first auxiliary request read as follows:

"1. A process for the fed batch culture of animal cells comprising culturing the cells in a nutrient medium and continuing the culturing into the decline phase of the

culture to provide the product(s) of the cells, wherein, during the exponential growth phase of the culture and for a prolonged period of time extending beyond the exponential growth phase of the culture, the medium is supplemented with a combined feed of glutamine and a sugar as an energy source,

characterised in that

the supplemental feed additionally comprises one or more of the other "essential" amino acids, the amounts of said glutamine, said sugar and said "essential" amino acids in the feed being such as to restore and maintain the concentration of said glutamine, said sugar and said "essential" amino acids which are depleted by growth at the levels present in the medium at the start of the culture."

V. The following documents are referred to in this decision:

(5): Eagle, H., Science, vol.30, pages 432 to 437, 1959,

(6): Griffiths, J.B. and S.J. Pirt, Proc.Roy. Soc.B. 168, pages 421 to 438, 1967,

(8): Stoner, G.D. and D.J. Merchant, In Vitro, vol. 7, No. 5, pages 330 to 343, 1972,

(12): Clark J.M. and M.D. Hirtenstein, Annals New York Acad.of Sciences, vol. 369, pages 34 to 46, 1981,

- (14): Adamson S.R, et al., Biotechnology Letters, vol. 5, No. 9, pages 573 to 578, 1983,
- (16): Glacken, M.W. et al., Ann. New York Acad.Sci., vol. 413, pages 355 to 372, 1983,
- (23): Reuveny, S. et al., Develop.biol. Standard., vol. 60, pages 185 to 197, 1985,
- (25): Reuveny, S. et al., Abstract No. 032 of the 85th Annual meeting of the American Society for Microbiology, 3 to 7 March 1985,
- (26): Reuveny, S. et al., J.Imm.Methods, vol. 86, pages 53 to 59, 1986,
- (28): Reuveny, S. et al., Abstract of the Joint ESACT/IABS Meeting, 21 to 25 May 1984,
- (29): Declaration by Dr. Reuveny dated 14 May 1995.

Exhibit(D): slide accompanying document (28),

Exhibit(G): slide accompanying document (25).

VI. The submissions in writing and during oral proceedings by the Appellants can be summarized as follows:

Main request: claim request accepted by the Opposition Division (cf.par.I supra):

- Claim 1 was not clear as it related to a supplemental feed comprising "one or more of the **other** essential amino acids" (emphasis added)

whereas the only amino acid mentioned earlier in the claim was not an essential amino acid.

- The process described in the priority application on page 2, lines 17 to 21 was different from the process claimed in claim 1. Furthermore, the priority application did not disclose that the supplemental feed had to contain "one or more essential amino acids" (claim 1) or "one or more non essential amino acids" (claim 5). Accordingly, neither claim 1 nor claim 5 enjoyed priority rights from 28 June 1985 which implied that documents (23) and (26) were part of the state of the art.

- The subject-matter of claim 1 lacked novelty over the teachings of document (14) or (6) as well as over Exhibit (D). Furthermore, claim 1 lacked novelty over the teachings of document (25), an abstract accompanied by a slide submitted into the proceedings as Exhibit (G), as this document described a process for the in vitro production of monoclonal antibodies (Mabs) which presented all of the features of the claimed process.

- Document (25), Exhibit (G), was the closest prior art to the subject-matter of claim 1. Starting from this document, the problem to be solved could be defined as devising an improved process for the production of desired products in fed-batch culture. The solution provided was to grow the cells into the decline phase while adding to the culture essential amino acids (rather than fresh medium) together with glutamine and sugar. This

solution was rendered obvious by the combination of the teachings of document (25) with, for example, those of document (5) or (16).

First auxiliary request: claim request filed at oral proceedings:

- The requirements of Article 123(2) EPC were not fulfilled because the application as filed did not disclose that glutamine and the energy source should be replaced at levels present in the medium at the start of the culture. This disclosure was also missing in the priority document which meant that priority rights were not valid.
- The feature that the amounts of glutamine, sugar and essential amino acids to be added were such that their concentration was maintained at the original level did not impart inventive step to the claimed process because the skilled person would know that the concentration of nutrients in a cell culture medium was so devised as to ensure optimal growth conditions. And, besides, no unexpected effect attached to this concentration had been demonstrated with regard to the production of the desired product.

Second auxiliary request: claim request filed as second auxiliary request with the submission dated 29 October 1999:

- Claim 1 covered embodiments which were also comprised in the subject-matter of claim 1 of the first auxiliary request. Accordingly, and for the

same reasons as presented in relation to this earlier claim, it did not involve an inventive step.

VII. The Respondents' submissions were essentially as follows:

Main request:

- As early as 1959, the skilled person knew that glutamine was an essential amino acid insofar as cell cultures were concerned. Considering that claim 1 was about growing animal cells and that glutamine was mentioned in the first part of the claim, the skilled person would have no problems in understanding the expression "one or more **of the other** essential amino acids" found in the second part of the claim (emphasis added).

- The claimed process was described in the priority application on page 2, lines 22 to 32.

A basis could be found on page 2, lines 31 to 32, page 3, lines 24 to 28 and page 5, lines 4 to 8 of said application for a supplementary feeding with "one or more essential" or "one or more non essential" amino acids. The priority rights were, thus, valid and documents (23) and (26) did not belong to the state of the art.

- Document (6) disclosed a batch culture, document (14) related to a dialysis process. Exhibit (D) showed the production of Mabs in a fed-batch process where culturing was not carried out into

the decline phase of growth. None of them was relevant to novelty.

Document (25) was a short abstract corresponding to an oral presentation. It did not disclose that a solution of glutamine and sugar **in growth medium** was added to the culture. There was no reason to believe that in the short time of the oral presentation, such detail would have been described. Document (29) which purportedly reported what was said during the oral presentation was not credible as it had been written some ten years after the meeting took place. Document (25) was not relevant to novelty.

- The process described in document (25) had no relevance to inventive step because it comprised the continuous addition of fresh medium to the culture which ensured that the cells always had the opportunity to grow, whereas in the claimed process, the synthesis of the desired product took place while the number of cells decreased. Furthermore, the skilled person would not have thought of combining the teaching of document (25) with that of document (5) or (16) to arrive at a process such as claimed where essential amino acids were continuously added to the culture medium with the aim of furthering the synthesis of the desired product because these latter documents advised adding amino acids to the culture medium to increase cellular growth.

It was just by chance that both the claimed process and the protocol in document (25) happened

to make use of glucose and glutamine and of a solution containing essential amino acids, or of essential amino acids.

It was surprising that the claimed process led to an increase in the product yield even as the cell count decreased.

First auxiliary request:

- A basis for claim 1 could be found in the passage bridging pages 8 and 9 of the application as filed.

The same information could be derived from the priority document, page 2 and example 2.

The claim was clear in the light of the description which disclosed which method to use to determine which nutrients had been depleted by growth.

The requirements of Article 123(2), 87 to 89 and 84 EPC were fulfilled.

- The experimental findings that the increase in product yield could be directly achieved by adding the relevant supplemental feed rather than indirectly achieved by having more cells synthesizing the desired product was non obvious. Document (16) did not suggest that the concentrations of the depleted glutamine, sugar and essential amino acids should be brought back to the initial concentrations. On page 363, it was

stated that sugar and glutamine should be continuously fed to keep their concentrations at a consistently low level. Document (5) was concerned with cellular growth rather than product formation.

Second auxiliary request

- The request was different from the first auxiliary request in that, in claim 1, the supplemental feed was defined in terms of the essential amino acids only. It was this supplemental feed which was used in the examples of the patent in suit. Its addition to the growth medium in the claimed conditions led to an unexpected increase in product yield. The subject-matter of claim 1 and of all claims dependent thereof was thus inventive.

VIII. The Appellants requested that the decision under appeal be set aside and that the European patent No. 0 229 809 be revoked.

The Respondents requested that the appeals be dismissed (main request); or that the decision under appeal be set aside and that the patent be maintained on the basis of the following documents:

- (i) claims 1 to 16 submitted during oral proceedings as first auxiliary request; or
- (ii) claims 1 to 16 filed on 29 October 1999 as second auxiliary request

Reasons for the Decision

1. The appeals of Appellants I, II and III are admissible whereas the appeal of the Respondents is not admissible under Article 108 EPC because they failed to submit any statement of grounds for their appeal. Nevertheless, the Respondents are a party to the proceedings as of right under Article 107, second sentence, EPC.

Main request

Article 84 EPC: clarity; claim 1

2. In the Board's judgment, the reference to "one or more **of the other** essential amino acids" (emphasis added) in the second part of claim 1 is not ambiguous considering that this expression is preceded in the first part of the claim by the mention of the amino acid glutamine which the skilled person would know as an amino acid essential for the growth of animal cells in a culture (document (5), page 432). There is, thus, no problem of clarity.

Article 87 to 89 EPC: right of priority; claims 1 and 5

3. The priority application, page 2, lines 22 to 32 discloses a process with the same steps as those of the process of claims 1 or 5, the feed supplement being described as containing beside glutamine "one or more of glucose, choline and an amino acid". Furthermore, it is specified on page 3 that the feed supplement is designed to restore the concentration of **each** amino

acid. The skilled person would, thus, understand that the culture medium could be supplemented with more than one amino acid (irrespective of whether it is an essential amino acid or not). Accordingly, the right of priority claimed in respect of claims 1 and 5 is acknowledged; thus, documents (23) and (26) do not belong to the state of the art.

Article 54 EPC: novelty; claims 1 and 5

4. Neither document (14) nor document (6) deal with a fed batch process but rather with a dialysis culture or with batch and chemostat cultures, respectively. They are not relevant when considering novelty.
5. Exhibit (D) is a slide which accompanied an oral presentation, the corresponding abstract of which is on file as document (28). It shows the growth curve of antibody-producing cells propagated according to the fed batch method and the corresponding curve of antibody production. It provides no information as to the nature of the supplemental feed involved in the fed-batch method nor about the way, the feeding was carried out. The same applies to document (28).
6. On the basis of document (29), ie a declaration by the scientist who made the oral presentation, Appellants III argued that the characteristics of the supplemental feed were described during said presentation and are those disclosed in the post-published document (23) (Figure 6): namely the addition of 5% to 10% of growth medium (ie of a medium containing glutamine, glucose and essential amino-acids), for 10^6 cells, every 24 hours. Appellants III further submitted that it could

also be derived from the growth curve in Exhibit (D) that the cells were still capable of producing significant amounts of Mabs while in the stationary phase which, in their view, "can in principle be regarded as already corresponding to a decline phase". Thus, they conclude that the process which led to the results shown in Exhibit (D) must have been the same as the claimed process. In the Board's judgment, Exhibit (D) does not constitute evidence of a process involving a culturing in the decline phase because a stationary phase is a phase during which the number of living cells remains constant whereas a decline phase is a phase during which the number of living cells decreases as can be inferred from the patent in suit itself, page 7, lines 51 to 55. Thus, even if one were to accept that the characteristics of the supplemental feed were disclosed at the oral presentation, Exhibit (D) would not affect the novelty of claim 1 or 5 which requires that the desired product is produced while the cells are in the decline phase.

7. Document (25) describes a process for the production of Mabs in a fed batch culture whereby the yield of Mabs is increased by continuous feeding of glutamine and glucose. The cells are kept alive for up to two months by continuous medium feeding. This feeding is apparently carried out independently from the addition of the first two components. In addition, it is not disclosed that glutamine and glucose were dissolved in growth medium prior to their addition to the culture medium. Thus, document (25) does not relate to a process comprising the step of adding together glutamine, glucose and amino acids (even as constituents of the growth medium). Accordingly, it is

not novelty destroying for the subject-matter of claims 1 or 5.

8. Novelty is, thus, acknowledged.

Article 56 EPC: inventive step; claim 1

9. The closest prior art is document (25) together with its accompanying slide, ie Exhibit (G). As already stated above, this document shows that the production of Mabs in a fed batch culture where a continuous feeding of glucose and glutamine was initiated during the exponential phase is twice that obtained from a control culture. It also discloses that the cells may be kept alive for up to two months by adding growth medium, ie essential amino acids amongst other constituents.

10. Starting from this document, the technical problem to be solved can be defined as devising an improved process for the production of desired products in a fed batch culture. The solution provided is to culture the cells into the decline phase while adding essential amino acids together with glutamine and sugar for a prolonged period of time extending beyond the exponential growth phase.

11. The differences between the teaching of document (25) and the claimed process are thus, found

- firstly, in the process steps, since in the claimed process, amino acids are added at the same time as glutamine and glucose, and,

- secondly, in the fact that, in the claimed process, specific constituents of the growth medium (essential amino acids) are added rather than the growth medium itself.

12. Insofar as the first difference is concerned, it must be pointed out that the culture medium remains continuously replenished with glutamine, glucose and essential amino acids quite irrespective of whether or not these nutrients are added to the culture together (claimed process) or separately (document (25); as part of the growth medium). In other words, the qualitative composition of the medium (in terms of the three components) over the period of time when the process is run is identical in both cases. Therefore the difference is not significant in terms of assessment of inventive step.

13. In relation to the second of these differences, reference is made to document (16), in which the conditions for large scale production of mammalian cells and products thereof are discussed. On page 359 it is stated: "Cell growth and/or product formation can be prematurely inhibited due to nutrient limitation" (emphasis added). The effectiveness of adding fresh medium is viewed critically on page 360 where the following suggestion is made: "A better mode of operation would be a **fed-batch system** that feeds vital components **only as needed** to the culture". (emphasis added).

14. Prima facie, it would seem obvious to combine the teachings of documents (25) and (16) to arrive at a method such as claimed where the cells are cultured in

a fed batch process into the decline phase (ie over a long period of time), the culture medium being supplemented with glutamine, glucose and the specific nutrients only as needed for product formation.

The Respondents argue to the contrary that the skilled person would not have thought of combining both these documents to arrive at a process where the cells are cultured in the decline phase since there had been a general belief at that time that continuously producing a desired product was automatically linked to the fact of continuously increasing the number of cells. In this respect, reference was made to documents (5), (8), (12) and (16).

15. It is noticed that documents (5), (8) and (12) are publications solely concerned with the amino acid metabolism of mammalian cells, and not with the production of a desired product by these cells.

16. It is only in document (16) that the animal cells are used to synthesize a desired product. In this document, cellular growth and product formation are not considered to be necessarily linked as is clear from the above cited sentence found on page 359. It should also be noted that document (25) does not mention that the cells should grow, but only that the cells should be kept alive, which implies that their number remains constant rather than increases. As none of these documents links cellular growth to product production, it cannot not have been so surprising for the skilled person that the desired product is synthesized while the number of cells decreases, providing that the nutrients necessary for the synthesis of said product

were introduced into the medium as already disclosed in documents (25) and (16). In other words, the skilled person wanting to produce a desired product would have combined the teachings of documents (25) and (16) to arrive at the claimed process in an obvious manner.

17. The main request is rejected for lack of inventive step.

First auxiliary request:

Article 123(2)(3) EPC, Article 84 EPC

18. In the passage bridging pages 8 and 9 of the application as filed, it is stated that the feed "may comprise those components...which are depleted...and the amounts of these components...are preferably such as to restore and maintain the concentrations...at the levels present in the medium at the start of the culture". Taking into account that glutamine, glucose and essential amino acids are identified on page 3, line 2 and page 9, line 16 as nutrients which are depleted by growth of the cells, the Board considers that there is a basis in the original application for the subject-matter of claim 1.
19. The added technical feature results in a limitation of the scope of the claims.
20. The claim is clear in the light of the description (page 8, last paragraph), which advises that the amount of nutrients to be put in the supplemental feed may be determined by the analysis of the culture medium.

21. The requirements of Article 123(2)(3) EPC and Article 84 EPC are thus fulfilled.

Articles 87 to 89 EPC; Article 54 EPC: right of priority, novelty.

22. The priority document discloses using a supplemental feed devised to restore the concentration of each amino-acid to its original concentration in the fresh culture medium (page 3). In the Board's judgment, it would be implicit for the skilled person that the concentrations of all nutrients in the supplemental feed, including that of the energy source, should also be restored to their original concentration. The right of priority in respect of claim 1 is acknowledged; thus documents (23) and (26) do not belong to the state of the art.

23. In view of these findings the Board is of the opinion that none of the documents belonging to the state of the art is relevant in terms of assessment of novelty.

Article 56 EPC: inventive step

24. The process of claim 1 differs from the process disclosed in claim 1 of the main request in that the content of the supplemental feed has been defined further in quantitative terms: it should be such as to restore the concentrations of the depleted glutamine, glucose and essential amino acids to their original concentrations.

25. The Respondents pointed out that this limitation was nowhere suggested in the state of the art. They also

emphasized once again that it was surprising that the added nutrients would be used by the cells to produce the desired product while the number of cells decreased. However, they provided no evidence of a surprising effect linked to the specific limitation added to the claim. Neither was the Board able to find any evidence in the patent specification for such an effect. In fact, it is specified on page 4 that "preferably, the feed is such as to maintain the media components which are used either as energy substrates or as biosynthesis precursors **in excess** for the duration of the culture" (emphasis added) which puts no specific limits to their concentrations.

26. It must, thus, be concluded that the added technical feature does not change the reasoning on inventive step developed with regard to claim 1 of the main request. Accordingly, the previous conclusion of lack of inventive step applies here as well, and the first auxiliary request is refused.

Second auxiliary request

Articles 123(2)(3) and 84 EPC

27. The application as filed, page 10, provides a basis for the subject-matter of claim 1 as it is stated there as follows: "Generally, the amino acids components comprise from 50 to 90% of the amounts of the amino acids present in the medium at the start of the culture". This technical feature results in the limitation of the scope of the claim. The wording of the claim is clear. The requirements of Articles 123(2)(3) and 84 EPC are fulfilled.

Article 54 EPC: novelty

28. The Board agrees to the view shared by all parties that the subject-matter of claim 1 does not enjoy priority rights, and, that nevertheless none of the documents of the state of the art prior to the filing date of the patent in suit are relevant in terms of assessment of novelty. The requirements of Article 54 EPC are fulfilled.

Article 56 EPC: inventive step

29. The process of claim 1 differs from the process of claim 1 of the main request in that the supplemental feed is defined in quantitative terms insofar as amino acids are concerned: the additional amino acids components comprise from 50 to 90% of the amounts of amino acids present in the medium at the start of the culture.
30. This feature, however, is meaningless in terms of what the concentration in the culture medium might be because this concentration will be dependent on when, how often and how much of the supplementing feed is added to said medium. In other words, the added feature actually fails to provide any further true technical characterisation of the culture conditions. Accordingly, the reasoning on inventive step developed with regard to claim 1 of the main request equally applies. The second auxiliary request is thus refused.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.
2. The patent is revoked.

The Registrar:

The Chairwoman:

A. Townend

U. Kinkeldey