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D E C I S I O N
of 11 March 1998

Case Number: T 0780/95 - 3.3.4

Application Number: 85303302.5

Publication Number: 0162639

IPC: A61K 39/10

Language of the proceedings: EN

Title of invention:
Antigenic preparations and isolation of such preparations

Patentee:
Evans Medical Limited

Opponents:
SmithKline Beecham Biologicals SA
Connaught Laboratories Limited
Chiron Corporation

Intervening party:
Lederle Arzneimittel GmbH & Co.

Headword:
Antigenic preparations/EVANS MEDICAL

Relevant legal provisions:
EPC Art. 83

Keyword:
"Main request, first and second auxiliary requests: sufficiency of disclosure (no)"

Decisions cited:
T 0212/88, T 0301/87, T 0449/90, T 0027/92

Catchword:
-

Case Number: T 0780/95 - 3.3.4

D E C I S I O N
of the Technical Board of Appeal 3.3.4
of 11 March 1998

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Decision under appeal:

Interlocutory decision of the Opposition Division of the European Patent Office posted 26 June 1995 concerning maintenance of European patent No. 0 162 639 in amended form.

Composition of the Board:

Chairwoman: U. M. Kinkeldey
Members: R. E. Gramaglia
W. Moser

Summary of Facts and Submissions

I. European patent No. 0 162 639 with the title "Antigenic preparations and isolation of such preparations" was granted with 19 claims and for Austria with 12 claims, based on European patent application No. 85 303 302.5.

II. Oppositions were filed by the other party (opponent (O1)) and the appellants I and II (opponents (O2) and (O3)) on the grounds of Articles 100(a), 100(b) and 100(c) EPC, i.e. lack of novelty (Article 54 EPC), lack of inventive step (Article 56 EPC), insufficiency of disclosure (Article 83 EPC) and added subject-matter (Articles 123(2) and (3) EPC).

III. By its decision dated 26 June 1995 the Opposition Division maintained the patent in amended form.

IV. The appellants respectively filed a notice of appeal against this decision together with a statement of ground of appeal and paid the fee. On Monday, 5 February 1996 notice of intervention (Article 105 EPC) was filed by Lederle Arzneimittel GmbH & Co. (hereinafter: intervener) and the official fees for filing an opposition and lodging an appeal of DM 1200 and DM 2000, respectively, were paid. Attached thereto were a copy of the service letter of process by the Landgericht Hamburg along with the certificate of delivery by the post office (Postzustellungsurkunde) in proof of the delivery of the complaint of the respondent (proprietor of the patent in suit) to the intervener on 3 November 1995. The respondent filed counterarguments to the appeals and the intervention.

V. In a communication accompanying the summons to oral proceedings, the board stated some of the issues to be discussed.

VI. Oral proceedings were held on 10 and 11 March 1998, during which the respondent withdrew the preceding requests and filed a new main request and a first and second auxiliary request. Claim 1 of the main request read as follows:

"1. A purified, excluding immunopurified, Bordetella pertussis antigen which is extracted together with adenylate cyclase activity when extraction of the activity is performed using an aqueous, pH3 solution of 0.25M glycine; and which is characterized by the following features:

a relative molecular weight of 69,000 as determined by 12% (w/w) polyacrylamide gel electrophoresis; and

a ratio of proline to glutamic acid of substantially 1:1 as determined by amino acid analysis, said antigen being substantially free from intracellular B. pertussis material"

Claim 1 of the first and claim 1 of the second auxiliary request, while differing from that claim, also contain the feature "a ratio of proline to glutamic acid of substantially 1:1 as determined by amino acid analysis".

VII. The following documents are referred to in the present decision (the numeration suggested by the respondent in the submission of 18 October 1996 has been adopted):

(D1) Hewlett et al., J. Bacteriol., Vol. 127, pages 890 to 898 (1976)

(D2) Novotny et al., Dev. Biol. Stand., Vol. 61, pages 27 to 41 (1985)

(D4) Montaraz et al., Infection and Immunity, Vol. 47, pages 744 to 751 (1985)

(D5) Novotny et al., Infection and Immunity, Vol. 50, pages 199 to 206 (1985)

(D9) WO-A-91/15505

(D15) Brennan et al., Infection and Immunity, Vol. 56, pages 3189 to 3195 (1988)

(D17) Novotny et al., J. Inf. Diseases, Vol. 164, pages 114 to 122 (1991)

(D25) Prof Hewlett declaration dated April 1995

(D29) Dr Cozens Declaration dated 3 May 1995

(A6) Shahin et al., J. Exp. Med., Vol. 171, pages 63 to 73 (1990)

(A7) Declaration of Dr Rappuoli dated 24 October 1995

(A8) Capiau et al., Proceedings of the Sixth International Symposium on Pertussis, NIH, Bethesda, Maryland, USA, pages 75 to 86 (26 to 28 September 1990)

(A9) Gotto et al., Infection and Immunity, Vol. 61, pages 2211 to 2215 (1993)

(A11) Novotny et al., Dev. Biol. Stand., Vol. 73, pages 243 to 249 (1991)

(A12) WO-A-90/13313

(A30) Dr Cozens declaration dated 14 October 1996

(A39) EP-A-0 462 534

(A40) De Magistris et al., J. Exp. Med., Vol. 168, pages 1351 to 1362 (1988)

(A41) EP-A-0 437 687

(A49) Prof Barber Affidavit dated November 1995

(A59) Prof Ivanyi declaration dated 10 February 1998

(A62) Report from Dr Guiso for the English Patents Court dated 17 September 1997

(A64) Charles et al., Eur. J. Immunol., Vol. 21, pages 1147 to 1153 (1991)

(A67) Friedmann et al., Infection and Immunity, Vol. 55, pages 129 to 134 (1987)

(A70) Repetition of the Examples of the patent by Appellant (03)

(A72) Robinson et al., Infection and Immunity, Vol. 40, pages 523 to 528 (1983)

(A80) Report from Prof Higgins dated 25 September 1997

(A87) Annex CFH-6 to document (A80)

(A102) Pagliaccia et al., Arch. Microbiology, Vol. 68, pages 437 to 440 (1997)

(A105) Pages from the transcript of evidence of Dr Chubb at the UK trial regarding experiments performed in the Wellcome Laboratoires under Dr Novotny in the early 1980's

(A113) Dr Plainchamp declaration dated February 1998

VIII. As regarded insufficiency of disclosure (Article 83 EPC) the appellants and the intervener argued essentially as follows:

- The experiments described in the patent in suit exactly matched those of a later publication (document (D5)) by a team including the present inventor, Dr Novotny, and relating to Bordetella (hereinafter: B.) bronchiseptica, not to B. pertussis as the patent in suit. Therefore, by following the disclosure of the patent in suit, the skilled person would not arrive at the P69 antigen (pertactin) because the isoelectric point (pI) of 7.0 to 7.4 and the proline to glutamic acid ratio of 1:1 recited in the patent in suit related to the 68 kDa protein from B. bronchiseptica and not to pertactin from B. pertussis which in fact had a pI of 5.6 to 6.2 and a Pro/Glu ratio of 0.80:1.

- Repetition of the Examples by appellant II (see documents (A7) and (A70)) did not lead to the claimed pertactin. By applying the purification of Example 1 (acid glycine hydrolysis) and Example 2(a) (DEAE chromatography) no significant purification of pertactin could be obtained and pertactin could not be found in the 0.2 M NaCl fraction but rather in the 0.1 M NaCl fraction. By

repeating Example 2(b), the true pI of pertactin was found at a pI = 5.94, ie, outside the pI range of 7.0 to 7.4 recited in the patent in suit. The pI of 5.94 was consistent with the ones reported for pertactin by later literature.

- The presence of enzymatic activity (adenylate cyclase) and the positivity in the Kendrick test were the only handles for tracking pertactin during the purification steps in the patent in suit. However, purified pertactin turned out to exhibit neither adenylate cyclase activity (see document (A8), pages 77 to 78) nor protection in the Kendrick test (loc. cit., page 83).

- Wishing to purify pertactin to a degree of purity such as to exhibit the Pro/Glu ratio recited in claim 1 of the patent in suit, one needed a monoclonal antibody (hereinafter: MAb) against pertactin. However, there was insufficient information in the patent in suit for making and identifying the MAb specific for pertactin. Neither pure antigen nor a biochemical function on which one could base the screening were available. There was only one characteristic, of all the characteristics listed, which was right for pertactin, namely the molecular weight range. As for everything else, they were wrong. It was not adenylate cyclase. It did not have adenylate cyclase activity. It did not focus at the pI described, and it was not in a pure form active in the Kendrick test. It took almost one year to find monoclonal antibodies BB05 and BB07 used by Dr Novotny for immunopurifying pertactin. BB05 and BB07, however, had been raised against the 68kD protein of B. bronchiseptica, a membrane protein corresponding to pertactin from B. pertussis. The patent in suit however gave no indication that a

MAb to a protein from a different B. strain should have been used to immunopurify pertactin. It was impossible to confirm that a selected MAb was directed against pertactin: if the skilled person used the molecular weight as a selective feature for the antigen, there were a great many proteins in the 67-73 kDa range.

- Since there were no other possible biochemical tests for tracking the protein looked for, the skilled person would have turned to adenylate cyclase activity as the handle to be used for screening by a enzymatic activity quenching assay and arrive at a MAb binding to adenylate cyclase, thus discarding any hybridoma secreting monoclonal antibodies against pertactin.

IX. As regarded insufficiency of disclosure (Article 83 EPC) the respondent argued essentially as follows:

- The appellants' and intervener's arguments of insufficiency of disclosure of the patent in suit were contradicted by earlier statements made by them in document (A40)(see page 1352, lines 13 to 14 from the bottom), document (D9) (see page 3, lines 11 to 23) and document (A41) (see page 2, line 51 to page 3, line 7) that pertactin could be purified by using the procedures disclosed in the patent in suit.

- Where a claim in a patent was directed to a new product, the patent had not to disclose how to obtain the product in a pure form in order to satisfy the requirements for sufficiency. This principle had been pointed out in decision T 212/88 (OJ EPO 1992, 28).

- The reproduction of the Examples of the priority document performed by appellant II were not carried out properly so as to repeat the teachings of the priority document.

- In any case, there was an admission by the author of document (A7), who reproduced the Examples, that pertactin was present in the fractions specified in the priority document (flow-through and 0.2 M fraction).

- The pI range of 7.0 to 7.4 referred to in the patent in suit was consistent with peak I of Figure 1 of document (A9). The isoelectric focusing (IEF) technique was known to be affected by a considerable variability of the pI's. Therefore, should the skilled person carrying out preparative IEF on the flow-through material according to Example 2(b) not find pertactin in the pI range recited by the patent in suit, he/she would track pertactin by means of its molecular weight in the neighbouring pI ranges.

- Document (A39) contained an amino acid analysis of pertactin (see Table VII on page 12) showing that the Pro/Glu ratio was substantially 1:1 (0.86). Experimental errors of more than 10% were tolerable.

- The 69 kDa molecular weight for pertactin given in the patent in suit was an unique identifier which would have led the skilled person to make a MAb and then to use the latter for immunopurifying pertactin. There was no evidence that there were other proteins of this molecular weight in the preparations likely to be used by the skilled person for screening the hybridomas by Western blotting.

- Pertactin was highly immunodominant (see document (A64), paragraph 47). Thus, there was no difficulty in applying standard MAb techniques to obtain hybridomas secreting MAb's against pertactin.

- For screening the hybridomas use could have been made of the eluate from the DEAE column or the IEF fractions. Also the following property could have been used for screening: binding to outer membrane fractions of 67 to 73 kDa (Dr Cozen, see document (D29)). Also for selecting the MAb, the MAb that once solid phased, could yield a protein which resulted positively in the Kendrick test. The final test for the skilled person to know whether success had been obtained was to perform the Kendrick test because the protocol described in the patent in suit gave purified P69 antigen contaminated by a small quantity of pertussis toxin (PT) (see document (A113)). The Pro/Glu ratio recited in claim 1 could also have been taken as a test for selecting the MAb. Other characteristics of pertactin recited in the patent in suit could be used to confirm that a MAb recognizing pertactin had been obtained. These were: a molecular weight of 69 kDa, extraction together with adenylate cyclase when the acid-glycine hydrolysis technique is used, non-retardation and elution by 0.2 M in ion exchange chromatography, location in the outer membrane of B. pertussis and the pI's.

- The skilled person would not have been led to consider the enzymatic property of adenylate cyclase as a handle instead of the molecular weight of 69kDa since it was well known in 1984 that this activity was labile.

- In the event the skilled person encountered difficulties in finding the MAb or with the IEF technique, the claimed P69 antigen could then be obtained using the other methods recited on page 4, lines 31 to 37, such as chromatofocusing.

X. The appellants and the intervener requested that the decision under appeal be set aside and that European patent No. 0 162 639 be revoked. The intervener further requested that one of the fees paid (opposition or appeal fee) be refunded.

XI. The respondent requested that the decision under appeal be set aside and that the patent be maintained on the basis of

(a) main request:
claims 1 to 8 presented during oral proceedings as main request; or

(b) first auxiliary request:
claims 1 to 8 presented during oral proceedings as first auxiliary request; or

(c) second auxiliary request:
claims 1 to 8 presented during oral proceedings as second auxiliary request.

Reasons for the Decision

Admissibility

1. The appeals are admissible. Furthermore, the notice of intervention complies with Article 105 EPC. Consequently the intervener is a party to the appeal proceedings.

Main request

Sufficiency of disclosure (Article 83 EPC)

2. Claim 1 of the main request covers a B. pertussis outer membrane antigen defined by various technical features, inter alia, by a ratio of proline to glutamic acid of substantially 1:1 as determined by amino acid analysis (see Section VI supra). The respondent holds this Pro/Glu ratio as a critical feature since it enables the skilled person to establish whether he/she has obtained the claimed pertactin, and to discard other B. pertussis proteins not having said ratio. The patent in suit indeed states on page 3, lines 16 to 18 that "ACAP contains an unusually high proportion of proline, such that the Pro/Glu ratio is about 1:1 and this feature serves to distinguish ACAP from other B. pertussis proteins". The board also considers the Pro/Glu ratio in claim 1 to be a critical distinguishing feature. An expert of the respondent, Dr Hewlett, (see document (D25), page 5, paragraph (c)) actually used the Pro/Glu ratio for distinguishing two B. pertussis outer membrane proteins having substantially the same molecular weight around 69,000, namely "ACAP" (Pro/Glu ratio = 1:1) from adenylate cyclase (Pro/Glu = 0.32:1).

3. For the purposes of evaluating sufficiency of disclosure of the patent in suit, the board has to establish whether or not a product having the technical features recited in claim 1, inter alia a Pro/Glu ratio of substantially 1:1, can be achieved by the skilled person without undue difficulty when following the instruction of the patent in suit, optionally supplemented by the common general knowledge of that person.

4. In the present case, the board observes that characterizing a protein by an amino acid ratio such as the Pro/Glu ratio is only meaningful in the context of a pure protein preparation; otherwise, an "averaging" takes place, i.e. owing to the presence of contaminant proteins having a "normal" (and hence lower) proline content, the overall Pro/Glu ratio of the mixture will be considerably lower than 1:1. As a consequence, the distinguishing character of this technical feature vanishes. That amino acid analysis is only meaningful when performed on highly purified proteins is shown eg, by post-published document (A39) (see Table VIII and page 11, line 40: "the 69 Kd protein exhibit a purity of 99%"). Nevertheless, the parties agreed that amino acid analysis may be affected by an experimental error of ± 10%. Therefore, it is reasonable to assume that the material being subjected to amino acid analysis has to exhibit a purity of no less than about 90%, in order to render said analysis meaningful.

5. The appellants maintain that there is insufficient information in the patent in suit for enabling a skilled person to purify pertactin to a degree such as to exhibit the Pro/Glu ratio recited in claim 1 of the patent in suit. The respondent's answers to this objection are: (i) the Pro/Glu ratio is an intrinsic feature of pertactin within any mixture, independently of the proteins accompanying it, and (ii) if a claim in

a patent is directed to a new product, the patent need not disclose how to obtain the product in a pure form in order to satisfy the requirements for sufficiency (decision T 212/88 (OJ EPO 1992, 28)).

6. The board's position to the respondent's proposition (i) (see point 5 supra) is that even if one assumes the ratio Pro/Glu acid to be an intrinsic feature of pertactin within a mixture, this does not exempt the respondent from the obligation of rendering a material having this feature accessible to the public. This is only possible if the disclosure of the patent in suit provides the technical information necessary for a skilled person, optionally making use of his/her common general knowledge, to isolate from this mixture a material having a degree of purity such that the Pro/Glu ratio can meaningfully be determined (see point 4 supra). This is because a component within a mixture defined by a series of technical features cannot be said to have been made available to the public if it is impossible for the skilled person to isolate this component from the mixture and to check whether it has the technical features it is supposed to have. The principle according to which a molecule within a mixture cannot be considered to have been made available to the public if no means for isolating and identifying the molecule is available to the skilled person, has been emphasized in decision T 301/87 (OJ EPO 1990, 335, see point 5 of the reasons), wherein it was decided that in the absence of a probe or any other means enabling a well defined DNA sequence to be identified and isolated from a Lawn gene bank (i.e. a huge collection of DNA sequences), this DNA sequence was considered not to have been made accessible to the public in spite of the fact that this DNA sequence would have been present in the Lawn gene bank. Thus, in order that the composition as claimed, be it a rough pertactin mixture or pure pertactin, fulfils the

requirements of Article 83 EPC, the molecule together with all its technical features recited in claim 1, in particular the Pro/Glu ratio in question, should not only be in the hands of the respondent but has to be made accessible to the public and also has to be verifiable. This is because if the skilled person is not able to perform a meaningful measurement, inter alia, of said Pro/Glu ratio, he/she will be prevented from knowing whether the protein looked for has been obtained or is present in a mixture. This is equivalent to being unable to carry out the invention. The principle according to which problems under Article 83 EPC may arise from unreliable or non verifiable parameters in a claim emerges eg, from decision T 449/90 of 5 December 1991, point 3.3, where it was held that the technical feature of "virus inactivation" recited in the claims to a factor VIII composition was essential for distinguishing over the factor VIII compositions of the prior art. The competent board came to the conclusion that the "virus inactivation" feature had to be testable in order that the composition claimed to have said feature fulfilled the requirements of Article 83 EPC. It was argued that the reproduction of the claimed composition within the requirements of Article 83 EPC was only possible if the skilled person, after having taken the claimed process steps of lyophilizing, purifying and heating the composition, could be sure of the claimed effect to be achieved by the mentioned steps.

7. As to the respondent's arguments (ii) (see point 5 supra) based on decision T 212/88, the board is satisfied that the facts underlying this decision differ decisively from the present ones. In this earlier decision the competent board held that a product having the features (in particular the X ray diffraction pattern) recited in claim 1 directed to Theta-1 both in a pure form or admixed with impurities,

could be achieved by the skilled person when following the instruction of the patent, optionally supplemented by his/her common general knowledge, whereas in the present case, the distinguishing character of the Pro/Glu ratio vanishes since some of the impure mixtures of pertactin under consideration in the patent in suit comprise more than 90% extraneous proteins (cf. the DEAE-Trisacryl eluate of Example 2(a)), i.e. more than the tolerable 10% extraneous proteins (see point 4 supra). The two situations are thus in no way comparable.

8. The board now turns to the various methods disclosed in the patent in suit in order to evaluate whether or not they are susceptible, alone or in combination, to enable the skilled person making use of the common knowledge to obtain an antigen with the parameters defined in claim 1. These methods are acid glycine hydrolysis (Example 1), DEAE-Trisacryl chromatography (Example 2(a)), preparative isoelectric focusing (IEF)(Example 2(b) and chromatofocusing (page 4, line 37). The board does not have to take analytical isoelectric focusing of Example 2(c) into consideration because it is an analytical tool rather than a preparative one. A successful immunopurification of pertactin as mentioned in Example 3 is dependent upon the finding of a suitable MAb specific for pertactin. Thus, the fate of this issue is intimately linked to that of the MAb (see point 9 below).

9. The issue of whether or not the skilled person is able to arrive at a MAb specific for pertactin remains relevant since, as explained in details under points 12 and 15 infra, availability of such a MAb to the skilled person would have turned unsuccessful IEF and chromatofocusing methods into successful ones, i.e., under these circumstances, sufficiency of disclosure by the patent in suit would have to be acknowledged by the

board. The question whether or not the scant information to be found in the patent in suit (page 6, line 31: "a monoclonal immunoglobulin specific for ACAP") is sufficient to lead the skilled person to prepare a MAb specific for pertactin, is dealt with under points 18 to 33 infra.

10. The eluate from the DEAE-Trisacryl column of Example 2(a) is a very crude mixture comprising only a few percent by weight pertactin. This has not been disputed by the respondent and is also confirmed by document (D2), page 31, end of first paragraph "Without the preparative step, the "purified material" would not be much different from the starting material". The preparative step alluded to is preparative IEF and the "purified material" is the one from the DEAE-Trisacryl column. Thus, this mixture per se does not make pure pertactin with inter alia a Pro/Glu ratio of substantially 1:1 accessible to the public (see point 6 supra), unless some other way for further processing the mixture is disclosed in the patent in suit. Of course, since the acid glycine hydrolysate of Example 1 is even cruder than the DEAE-Trisacryl column eluate, any negative consideration relating to the DEAE-Trisacryl column eluate also applies a fortiori to the acid glycine hydrolysate.

11. As regards the IEF of Example 2(b), the respondent provided a table showing that the pI of pertactin varies according the experimental conditions (see page 20 of the submission of February 1998 corresponding to page 52 of the submission of October 1996). The board agrees that the IEF technique is not as "robust" a technique as is SDS-PAGE from the point of view reproducibility of the results. However, the pI's reported for pertactin in later documents (see document (A102): 5.3-6.4; document (A105) page 876, line 15: 5.7-6.85; document (A70), page 34: 5.63-6.18;

document (D17), page 116: 5.6 and 6.2) are reasonably consistent and are much lower than the pI's of 7.0-7.4 of the patent in suit. The respondent argues that the pI's recited in the patent in suit are consistent with the peak at pI = 7.2 appearing in Figure 1 of document (A9). Yet, this peak, obtained by chromatofocusing, does not turn up on IEF which yields bands at pI's consistent with the pI's found in the above cited literature (see Figure 3). Thus, it must be concluded that by performing preparative IEF according to Example 2(b), the pertactin looked for will not be found in the pI fraction of 7.0-7.4 as recited by the patent in suit (page 6, line 10).

12. The respondent argued that if the skilled person does not find pertactin in the pI fraction he/she is told by the patent in suit, he/she would not blindly follow the teaching of the patent in suit but would check by SDS-PAGE the eluates from the IEF gel and take those fractions containing the 69 kDa protein, regardless of the pI thereof, because there were **only** three IEF fractions comprising the right molecular weight protein of 69 kDa, and these three bands were pertactin (see document (A70), compare the Coomassie stained gel at the centre of page 34 with the Western blot on the bottom of page 35). The patent in suit does not urge the skilled person to proceed as the respondent asserts. It merely teaches that the IEF fraction of 7.2-7.6 should be taken (page 3, line 19). No mention is made that the IEF fractions should be cut into strips and the eluted strips subjected to SDS-PAGE to see whether there are 69 kDa bands. Thus, in the board's view, the skilled person is not told in the patent in suit to proceed the way the respondent argues. This is even more true if one bears in mind that, without the help of a MAb specific for pertactin for tracking the antigen looked for, it would be difficult to establish **where** something went wrong.

Whether or not such MAb was available to the skilled person, or could be prepared in the light of the scant disclosure provided by the patent in suit will be discussed under points 18 to 33 infra. The skilled person could reasonably assume that failure to recover pertactin in the pI range recited by the patent in suit followed from eg, an error in the DEAE column step or in the acetone precipitation step. Furthermore, it can neither a priori be excluded that the skilled person would think that the molecular weight was wrong while the pI was correct: once the skilled person is faced with a failure, there is no reason for considering the molecular weight as sacrosanct, but not the pI, and vice-versa.

13. Even when assuming, as asserted by the respondent, that the skilled person decided to track the antigen looked for by SDS-PAGE, it should be noted that there are other bands which could also deserve the skilled person's attention in the molecular weight range of 67 to 73 kDa in the Western blot of the IEF fractions. These bands, shown by Figure 2A and B of document (D5) and by page 34 of document (A70), correspond to more acidic bands, known from document (D5) (see paragraph bridging l-h and r-h column of page 202) to comprise pertactin from B. bronchiseptica tightly complexed with outer membrane proteins such as lipopolysaccharides, which complex the skilled person is not able to dissociate to yield pure pertactin with a Pro/Glu ratio of 1:1. This is also true for pI-acidic pertactin from B. pertussis (see document (A87), "Program 320" page 7, first full paragraph). Moreover, the bands in the same molecular weight range at pI = 3.8-4.5 are also "good candidates", but these are unrelated to pertactin (see Figures 2A and B of document (D5)). There are also "alternative candidates" in the right pI range recited

by the patent in suit but having a molecular weight outside the 67 to 73 kDa range. In conclusion, even if the above proposition made by the respondent were true (what the Board denies), the likelihood of a second failure remains high.

14. Finally, it is stated in document (A87) (see "Program 320", page 4) that even after IEF made by taking the correct fractions (monoclonal anti-pertactin antibody BB05 was already available), there were several peptides and amino acid sequencing yielded two different overlapping amino acids: thus the board is not convinced that IEF-purified pertactin can be subjected to a meaningful Pro/Glu determination, the more so as the elution of bands from an IEF gel does not yield pure protein, since there are a lot of strong bands (see Figure 2A of document (D5)).

15. The respondent argues that other methods recited in the patent in suit can be used in order to obtain pertactin with a Pro/Glu ratio of substantially 1:1. For instance, chromatofocusing is specifically mentioned on page 4, line 37. However, even when assuming that the skilled person would be able, merely on the basis of the word "chromatofocusing" found in the patent, to derive a technique based on chromatofocusing such as the one disclosed in document (A9), which assumption the Board cannot take for proven, the correct peaks would also need to be identified by means of a MAb specific for pertactin (see loc. cit., page 2211, right hand column, lines 16 to 17 from the bottom: "were recognized by an anti-69K MAb").

16. Further, the board observes that according to the later published document (A11) (see page 245, second full paragraph), addition of phenantroline as a proteolysis inhibitor turned out to be fundamental in order to avoid the formation of more than 10 isolelectric forms.

However, the skilled person cannot derive this important information from the patent in suit and, thus, he/she would not be able to cope with this unexpected further complication when designing a chromatofocusing technique for purifying pertactin.

17. For these reasons, the board must conclude that the methods discussed so far for producing the antigen of claim 1 according to the disclosure in the patent in suit, optionally supplemented by the common general knowledge, do not enable the skilled person to arrive at a product having the technical features recited in claim 1, inter alia the feature a Pro/Glu ratio of substantially 1:1. It remains to be examined whether or not it is possible to produce a MAb susceptible of healing these deficiencies.

Enablement of the patent in suit for the MAb

18. The appellants' experts conceded that there were no difficulties in producing hybridomas secreting MAb's against pertactin (see document (A49), point 9: "there is little doubt that monoclonal antibodies which recognize the 69 kDa antigen would be produced"). However, it is common general knowledge that obtaining hybridomas secreting a MAb looked for is to a large extent a matter of luck, especially when complex mixtures are used as immunogens. This view is supported by paragraph 8 of Prof Ivanyi's declaration (document (A59)) according to which Montaraz in his PhD thesis work used cell surface antigen (CSA) from B. bronchiseptica but merely found monoclonals which cross-reacted with other bacteria. He was only successful when using killed B. bronchiseptica whole cells as an immunogen.

19. Thus, fusion of spleen cells with tumour cells in the course of the cumbersome way of getting a desired hybridoma yields hundreds of hybridomas. Then, the screening of hybridomas secreting the MAbs looked for represents the difficult problem and is always a tedious work, and success is uncertain. The assay required for that screening must be highly specific, **fast**, and sensitive enough to allow an extremely selective screening of several hundred clones growing simultaneously. None of the screening techniques mentioned by the respondent fulfils these requirements.

20. As a first screening technique, the respondent suggested the selection of the hybridomas secreting monoclonal antibodies binding to an outer membrane fraction of 67 to 73 kDa, or binding to a band in the same molecular weight range on Western blots of crude preparations of B. pertussis protein (see eg document (D29), paragraph 19). This screening technique is likely to fail owing to the huge amount of extraneous proteins in the range 67 to 73 kDa. In fact, document (D5) relates to the identification of the protective antigen P68 from B. bronchiseptica (Figure 1b, lanes 4, 5,6 and 8) and shows that there are fractions comprising proteins of the same 68 kDa range which do not react with MAb BB05. The same pattern should be expected for the 69 kDa protein of the closely related strain of B. pertussis. Document (D5)(see Figure 1, lane S) shows a continuum of proteins in this range. On page 202, left hand column, lines 4 to 8, it is stated: "Several proteins of similar molecular weight (i.e., near 70,000) are present in the starting material but were quite well separated into several pools (e.g., in lanes 3, 4 and 5, Figures 1b) and did not react with MAbs.". Document (D4) (see Figures 3A and B, lane 6) also shows a band in the range 67 to 73 kDa not recognized by MAb BB05. Figure 3, lane 2 shows some of these bands below 68

kDa. Document (A6) (see page 71, second full paragraph) shows that filamentous haemagglutinin also has a molecular weight of 69 kDa. Finally, document (A80) (see paragraph 49) also cites a series of B. pertussis outer membrane proteins having molecular weights in the range 67 to 73 kDa. In conclusion, if one also takes into account the fact that each single protein contains a great many epitopes, a plethora of MAbs would bind to irrelevant material in the molecular weight range in question. It would mean undue burden for the skilled person to isolate among them the right hybridoma(s).

21. The respondent maintains that the MAb against pertactin could easily be screened by establishing which MAb binds to a band at 69 kDa by Western blotting against crude preparation of B. pertussis (see Declaration of Prof Yvanyi, document (A59), paragraph 15). The board first notes that, while the priority document teaches expressis verbis to raise a MAb against the 69,000 m.w. protein (see page 3, last paragraph; page 9, first paragraph and heading of Example 3), the patent in suit does not lay the same emphasis on the 69 kDa protein but recites that "ACAP has a molecular weight of 67,000 to 73,000, particularly 69,000" (page 3, lines 27 to 28). In conclusion, it would be unlikely that the skilled person would disregard the range 67 to 73 kDa and discard clones secreting MAbs directed eg, to proteins having molecular weights of eg., 70 kDa or 67,5 kDa. Secondly, even if the skilled person decided to disregard the range 67,000 to 73,000 and to rely on the 69 kDa band only, it should be borne in mind that it is impossible to get the 69 kDa band precisely in the absence of a side-by-side comparison with pure pertactin. Thus, an imprecision spanning the range of about 67 to 71 kDa is unavoidable. In conclusion, focusing upon a 69 kDa protein only would not reduce

the number of irrelevant clones arising from the width of the molecular weight range. Further, it can also not be said that there is only one protein with a molecular weight of 69 kDa or close to it (see points 23 and 24 infra).

22. Prof Ivanyi (loc. cit.) states that non-specific antibodies would be rejected by this technique because they would bind to multiple bands in the Western blot. The board disagrees because it is impossible for the skilled person to get this information unless he/she knows in advance the behaviour of pertactin. Moreover, it cannot be taken for proven that any other protein which is not pertactin would automatically degrade yielding multiple bands on Western blots, while pertactin would not form fragments. Rather, the contrary appears to be true (see document (D15), page 3194, end of left hand column and also document (A87), "Programme 320", bottom of page 6: "the yield of the 69 Kd protein was very low and most of the protein decomposed into the 40 Kd form").

23. The respondent argued that by screening against the Western blots, there was no danger to pick up an hybridoma against a fragment of adenylate cyclase because the molecular weight of 70 kDa referred to in document (D1) had been carried out using disc gel electrophoresis. The molecular weight of adenylate cyclase was in fact 60 kDa by SDS-PAGE, and not 70 kDa according to document (A67). However, the board observes that document (A67)(see page 132, r-h column, third full paragraph) emphasizes that the molecular weight of 60 kDa relates to adenylate cyclase from crude urea extracts of B. pertussis purified on a calmodulin affinity chromatography column and that the molecular weight of adenylate cyclase depends on the biochemical method that has been used for the purification thereof. Thus the adenylate cyclase

fragment of 60 kDa by SDS-PAGE referred to in document (A67) is not the same as the one mentioned in document (D1), exhibiting a molecular weight of 70 kDa and having been purified from culture supernatant. Kessin and Franke in document (A67) (loc. cit.) also obtained an adenylate cyclase exhibiting a molecular weight of 70,000 from culture supernatant. Further, an expert of the respondent agreed that the 69 kD band contained a minor amount of adenylate cyclase (see document (A30), paragraph 23).

24. The respondent maintains that, since the examples of the patent in suit are carried out on a mixture comprising only the outer membrane proteins of B. pertussis, a single band on SDS-PAGE would correspond to one protein and not to a multiplicity of proteins. However, document (A6) (see page 71, first full paragraph shows overlapping on acrylamide gels of two outer membrane proteins at 69 kDa.

25. In Prof Ivany's declaration (loc. cit., paragraph 8), it is stated that Montaraz, in his thesis work, was able to raise only two viable hybridomas (BB05 and BB07) and that both were directed to the 68 kDa antigen. This, however, seems to be contradicted by document (D2) (see page 29, under the heading "Monoclonal antibodies") citing "Of seven clones investigated in detail". This suggests that these seven clones are a sub-set of the hybridomas found positive in the radioimmunoassay (RIA) using CSA (cell surface antigen) as antigen (see document (D4), page 745, under the heading "Production of MABs"). Of course, since CSA comprises a plethora of different proteins such as "very fine curled fibres and fimbriae, lipopolisaccharide and several proteins" (see

document (D2), top of page 28), the population of hybridomas which behave positively in the RIA using CSA as antigen should be expected to be very large. Thus it is unlikely that only anti-pertactin clones will be obtained.

26. In Dr Guiso's declaration (document (A62), page 62, paragraph 47.4) it is stated that Charles et al. (see document (A64), page 1148, left-hand column, first paragraph) easily obtained four hybridomas secreting monoclonal antibodies against pertactin by injecting mice with whole cell B. pertussis vaccine and screening against B. pertussis whole-cell sonicates. Though, the screening against B. pertussis whole cell sonicates referred to in this passage was a preliminary screening for excluding hybridomas unrelated to B. pertussis. It is not likely that this screening against a whole cell sonicate alone, thus comprising **all** the B. pertussis epitopes (both extracellular and intracellular), would lead only to the four hybridomas D5E9, E4D7, F6E5 and E4A8. It is more likely that confirmation of the right clones was made with pure pertactin since they indeed had pure P69 antigen (see loc. cit.: "purified pertactin"). Therefore, no comparison can be made between the Charle's team and a skilled person who had no purified pertactin at hand.

27. The respondent submits that the Examples of the patent in suit, up to Example 3, but not including it, do not require a MAb to be carried out. A material is obtained through these Examples which is much purer than the crude mixture of cell surface antigen. Use of this much purer material for screening inevitably will produce antibodies which recognize pertactin. The screening against the flow-through eluate from the DEAE column or the product of the ion exchange chromatography could be further purified before use in screening by SDS-PAGE and further elution of the band at 69 kDa. As regards

screening against the flow through eluate from the DEAE-Trisacryl column, the board notes that this is a very crude mixture comprising only a few percent by weight pertactin (see point 10 supra). Thus there are to be expected even more unrelated clones than in the case of screening against the 67 to 73 kDa range in Western blots. In connection with screening against the 69 kDa band eluted from the SDS-PAGE gel, this technique would imply the same drawbacks or prejudice by the skilled person already emphasized by the board under point 21 supra for the screening against the 69 kDa band on Western blotting.

28. If a screen were carried out against the IEF fraction recited by the patent in suit, no MAb of the kind discussed would be found, since these fractions do not comprise pertactin (see point 11 supra). In order that the skilled person, turning to this technique, be successful, he/she has first to use a MAb to identify the right bands since the MAb is crucial for tracking the kDa protein in both the eluate and the IEF gel. But this is a "catch 22".

29. It is also not feasible to use the Kendrick or Pro/Glu ratio as a screening test as above. The board agrees to the submissions by the appellants that the screening should be made quickly because, otherwise, the hybridomas will outgrow before they are cloned, with amounts of culture supernatants that are also very small. If columns are involved, the need arises to clone out and amplify the cells, make the ascites in order to obtain sufficient MAb to be immobilized onto the columns. All these steps would require a few months; thus, this kind of screening involving affinity columns cannot be defined as being fast. At most, these methods can be used for trying to confirm that a selected antibody recognizes pertactin.

30. But even by doing so, a series of difficulties arise. The first problem which arises is that MAb's which work in Western blots, i.e., which bind to denatured proteins, are not always effective in immunopurification, where the protein is in a native form, and vice-versa. Once a screen with Western blots is carried out, hundreds of hybridomas form, and many of the secreted MAbs are at low titre or low affinity and will not work in immunopurification. This is shown by document (D15), page 3191, left hand column: "BPD8 and BPE8 also reacted with the 69-kDa band by Western blotting, but **much greater amount of antibody** were required for detection" (emphasis added). Had MAb's BPD8 and BPE8 been screened by means of a Western blot instead of a bacterial agglutination (ibidem, page 3190, left hand column), it is likely that they would have been lost. Secondly, it is already difficult, departing from a panel of MAb's against the same antigen, to select the one suitable for affinity chromatography. In order that a immunoaffinity column be prepared, one has to ensure that the correct immobilization procedure is used and that the immobilized antibody retains its antigen binding capacity. A series of different eluants have to be checked until the one which gives the best degree of purification is found. It is, in the Board's judgement, unrealistic that anyone would turn to screening methods involving columns. It would represent an undue burden to do this. Finally, it should be borne in mind that pertactin alone does not pass the Kendrick test (see document (A12) page 11, table 11), and that even if the immunopurified antigen were protective in the Kendrick test, it would remain to be established whether it is the same antigen as the one recognized by MAb BB05.

This test is, moreover, not specific for pertactin since adenylate cyclase also pass the Kendrick test. As to the Pro/Glu ratio, there exist other proline rich B. pertussis outer membrane proteins, such as BrkA and Tcf (see document (A80), paragraph 49).

31. While it is admitted by the respondent that pertactin alone does not pass the Kendrick test, it is argued that the protocol described in the patent in suit leads to immunopurified pertactin contaminated by a small quantity of pertussis toxin (PT) (see document (A113)), which ensured that the preparation passes the Kendrick test. The board disagrees with this proposition. Before the priority date of the patent in suit, it was known (see document (A72), abstract) that tiny quantities of LPF (also named PT, see document (A11), abstract) could enhance protective activities in the Kendrick test of a series of outer membrane proteins of B. pertussis. Thus it should be expected that the skilled person would refrain from subjecting to the Kendrick test preparations contaminated with FHA and/or LPF; otherwise, it would be difficult to establish what induces protection. It would be indeed meaningless to put in the Kendrick test something which may be contaminated with something known to be active in the Kendrick test, with a view to proving that the antigen is active. The board's view is supported by the following evidence: According to document (A87), programme 320, page 11, the preparation used in the Kendrick test did not contain FHA and LPF because Dr Novotny specifically put it through columns to make sure it did not have PT and FHA in it, which were the two antigens which were known to give activity in the Kendrick test. This is in line with document (D4): three runs on the affinity column (see legend to Figure 3). Thus contaminants should be avoided. Document (D2)(see page 38, paragraph 1) further illustrates Dr Novotny's concern that the presence of

impurities could false the Kendrick test results. The presence of PT was excluded from B. pertussis immunopurified material by a test on rabbits which did not induce antibodies against PT once hyperimmunized with the same preparation.

32. The submission by the respondent that pertactin might have been highly "immunodominant" and that blood of patients immunized with B. pertussis comprised high titres of antibodies against pertactin are not convincing because, even if accepted as a fact, this has nothing to do with the screening procedure (see points 19 to 29 supra).

33. In conclusion, it is the Board's finding that it is not possible to arrive at a MAb specific for pertactin on the basis of the scant information provided by the patent in suit, which MAb would allow a purification of the B. pertussis 69 KDa antigen as claimed such that the feature of the ratio of proline to glutamic acid of substantially 1:1 contained in the claim can be determined to identify the antigen. Consequently, the patent in suit according to the main request does not disclose the invention in a manner sufficiently clear and complete for it to be carried out by a person skilled in the art. That request has thus to be rejected.

First and Second auxiliary requests

Sufficiency of disclosure (Article 83 EPC)

34. Claim 1 of these requests also covers pertactin with a Pro/Glu ratio of substantially 1:1 (see Section VI supra). Therefore, the conclusion arrived at by the board in relation to the main request also applies to these requests.

Reimbursement of a fee

35. The effectiveness of a notice of intervention filed during appeal proceedings depends on the payment of one fee only, namely the opposition fee (cf. decision T 27/92 of 25 July 1994). Thus, the reimbursement for the benefit of the intervener of an amount equivalent to the appeal fee, ie. DM 2000, has to be ordered.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The patent is revoked.

3. The reimbursement for the benefit of the intervener of an amount equivalent to the appeal fee, i.e. DM 2000, is ordered.

The Registrar: The Chairwoman:

D. Spigarelli U. Kinkeldey