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D E C I S I O N
of 25 April 1995

Case Number: T 0906/91 - 3.3.4

Application Number: 83303822.7

Publication Number: 0098179

IPC: C12P 1/00

Language of the proceedings: EN

Title of invention:
Hybridoma cell lines and monoclonal antibodies to theophylline

Patentee:
E.I. DU PONT DE NEMOURS AND COMPANY

Opponent:
Behringwerke Aktiengesellschaft
Boehringer Mannhein GmbH Patentabteilung

Headword:
Hybridoma/E.I. DU PONT

Relevant legal provisions:
EPC Art. 56

Keyword:
"Inventive step -no; obvious to replace antisera by monoclonal antibodies with reasonable expectation of improved specificity; no difficulties overcome in doing so."

Decisions cited:
T 0499/88

Catchword:
-

Case Number: T 0906/91 - 3.3.4

D E C I S I O N
of the Technical Board of Appeal 3.3.4
of 25 April 1995

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Decision under appeal: **Interlocutory decision of the Opposition Division of the European Patent Office dated 10 June 1991, posted 16 July 1991 concerning maintenance of European patent No. 0 098 179 in amended form.**

Composition of the Board:

Chairman: U. M. Kinkeldey
Members: C. Black
L. C. Mancini

Summary of Facts and Submissions

I. European patent No. 0 098 179 (application No. 83 303 822.7) was granted on the basis of a set of 9 claims of which Claims 1 to 3, 7 and 8 read as follows:

"1. Monoclonal antibody to theophylline having 5% or less cross-reactivity with caffeine.
2. A monoclonal antibody according to Claim 1 having 30% or less cross-reactivity with theobromine and 5% or less cross-reactivity with 3-methylxanthine.
3. A hybridoma cell line producing antibody as claimed in Claim 1, which cell line is a hybrid of a spleen cell from a mouse immunized with an 8-substituted theophylline-carrier conjugate and a mouse myeloma cell.
7. A cell line producing antibody as claimed in Claim 1, which hybridoma cell line is ATCC HB 8152, ATCC HB 8153 or ATCC HB 8154.
8. An immunoassay for theophylline which utilizes a monoclonal theophylline antibody as claimed in Claim 1 or 2."

Claims 4 to 6 and 9 relate to preferred embodiments of Claims 3 and 8 respectively.

II. Oppositions to the granted patent were filed by Behringwerke AG (01), specifying the grounds mentioned in Article 100(a),(b) and (c) EPC, and by Boehringer Mannheim GmbH (02), specifying the grounds mentioned in Article 100(a) and (b) EPC.

III. The Opposition Division maintained the patent in amended form, finding that the amended claims were novel and inventive over the cited prior art. The Division further confirmed that the late filing of a deposit number during the examination proceedings was allowable as a

correction under Rule 88 EPC, and dismissed the related objections by the opponents on the ground of insufficiency and added subject matter.

IV. The present appeal by Opponent 01 lies against this decision.

V. Documents referred to by the parties during the appeal proceedings are as follows, wherein the identification depends for the most part on the party which introduced the document:

D1: Cook et al., Research Communications in Chemical Pathology and Pharmacology 13 (1976), 497-505.
D2: Li et al., Clin. Chem. 27 (1981), 22-26.
D3: EP-A-O 044 441
D4: US-A-4 156 081 (Singh et al.)
D5: Singh et al., J. Immunoassay 1 (1980), 309-322.
D6: T 499/88, Immunoglobulins/Unilever
D7: Eshhar et al., Protides of Biological Fluids, Proceedings of Colloquium 29, XXVII, Pergammon Press (1982), 823-825.
D8: J.W. Goding, J. Immunol. Methods **39** (1980), 285-308.
D9: Sevier et al., Clin. Chem. **27** (1981), 1797-1806.
D10: McMichael and Bastin, Immunology Today (1980), 56-61.
D11: EP-A-0 077 896.

B1: D.E. Yelton and M.D. Scharff, "Monoclonal Antibodies", American Scientist, vol. 68, No. 5, Sept/Oct. 1980, 510-516.

B2: B.A. Diamond et al., "Monoclonal Antibodies, A New Technology for Producing Serologic Reagents". The New England Journal of Medicine, vol. 304 (1981) 1334-1349.

B3: C. Milstein, "Monoklonale Antikörper", Spektrum der Wissenschaft, Dezember 1980, 97-108.

B4: C. Milstein, "Monoclonal Antibodies", Scientific American, October 1980, 56-64.

B5: G.S. David et al., "The Hybridoma - An Immunochemical Laser", Clinical Chemistry, vol. 27/9 (1981), 1580-1585

B6: E.D. Sevier et al., "Monoclonal Antibodies in Clinical Immunology", Clinical Chemistry, vol. 27/11 (1981), 1797-1806.

B7: G. Köhler, "Gewinnung und Vorteile monoklonaler Antikörper", Deutsche Gesellshaft für klinische Chemie, Mitteilungen 3/82 (1982), 106-108.

B8: C. Milstein, "Monoclonal Antibodies from Hybrid Myelomas: theoretical aspects and some general comments" in "Monoclonal Antibodies in Clinical Medicine" (A.J. McMichael and J.W. Fabre, eds.) Academic Press, London, 1982, 3-16.

B9: S.H. Sacks and E.S. Lennox, "Monoclonal Anti-B as a New Blood Typing Reagent", Vox Sang. 40 (1981), 99-104.

B10: C.J. Barnstable et al., "Production of Monoclonal Antibodies to Group A Erthrocytes, HLA and Other Human Cell Surface Antigens - New Tools for Genetic Analysis", Cell 14 (1978), 9-20.

B11: GB-A-2 083 836 (WO 81/02899)

B12: B.E. Bång et al., "Studies of monoclonal and polyclonal anti-digoxin antibodies for serum digoxin radioimmununoassay", Scand. J. clin. Lab. Invest., vol. 41 (1981), 75-78.

B13: T.W. Rall, "Central Nervous System Stimulants: The Xanthines" in Goodman and Gilman's "The Pharmacological Basis of Therapeutics", MacMillan Publishing Co. New York, 1980, 592.

P1: Pratt **et al**, Eur. J. Nuclear Med., **4**, 159-170 (1979)

P2: Berzofsky **et al**, Mol, Immunol., **18**, pages 751-763 (1981)

P3: Lane **et al**, Nature, **296**,pages 200-202, March 1982.

P4: Meredith Mudgett Hunter **et al**, J. Immunol., **129**(3), pages 1165 to 1172, September 1982.

P5: Samarajeewa **et al**, Steroids, **38**(6), pages 667-678, December 1981.

P6: Text and slides for oral presentation by Dr. Stephan G. Thompson at meeting of the American Association of Clinical Chemists on 22 July 1981.

VI. At oral proceedings attended by the Appellant, Opponent 02 (party as of right to the proceedings) and the Respondent (patent proprietor), the Appellant requested that the decision under appeal be set aside and the patent revoked. The Respondent requested that the patent be maintained in amended form on the basis of Claims 1 to 9 filed with the letter dated 18 September 1992 (main request) or on the basis of Claims 1 to 9 filed at the oral proceedings as first auxiliary request, or on the basis of Claims 1 to 4 filed at the oral proceedings as second auxiliary request.

VII. Claim 1 according to the main request reads as follows:

"Monoclonal antibody to theophylline having 5% or less cross-reactivity with caffeine when evaluated in a particle-enhanced turbidimetric inhibition immunoassay for theophylline, cross-reactivity being defined as the percentage error in measurement introduced when a potential cross reactant is present at a final concentration of 10 µg/ml in a sample containing 10 µg/ml theophylline, said monoclonal antibody having been raised against an 8-substituted theophylline-carrier conjugate."

Claims 2 to 9 according to the main request are identical to those of the granted patent (see paragraph I above).

Claims 1 and 2 according to the first auxiliary request reads as follows:

"1. Monoclonal antibody to theophylline having 5% or less cross-reactivity with caffeine having 30% or less cross-reactivity with theobromine and 5% or less cross-reactivity with 3-methylxanthine when evaluated in a particle-enhanced turbidimetric inhibition immunoassay for theophylline, cross-reactivity being defined as the percentage error in measurement introduced when a potential cross reactant is present at a final concentration of 10 µg/ml in a sample containing 10 µg/ml theophylline, said monoclonal antibody having been raised against an 8-substituted theophylline-carrier conjugate.

2. A monoclonal antibody according to Claim 1 having 5% or less cross-activity with theobromine."

Claims 3 to 9 are the same as those of the main request.

Claim 1 according to the second auxiliary request reads as follows:

"Monoclonal antibody produced by the cell line ATCC HB 8152, ATTCC HB 8153 or ATCC HB 8154."

VIII. The gist of the written and oral argumentation of the Appellant and the other party (Opponent 02) is that antisera to the theophylline which had acceptably low cross-reactivity with other xanthines were known from documents D1 and D2. From the numerous other cited documents which establish what was common general knowledge in the art at the priority date of the patent in suit, the weight of evidence was that monoclonal antibodies showed advantages over polyclonal antibodies inter alia in respect of specificity and therefore cross-reactivity. It was therefore obvious to replace the polyclonal antibodies known from documents D1 or D2 by monoclonal antibodies with the reasonable expectation of improved cross-reactivity. The use of an 8-substituted theophylline-carrier conjugate as immunogen is also known from documents D1 and D2 and the protocol employed for preparing the hybridomas as well as the selection protocol had by the priority date of the patent become routine. The cross-reactivity required by Claim 1 (5% or less with caffeine) corresponds, according to the patent proprietor, to a 5 to 8 fold improvement compared with antisera but for the average skilled person such an improvement is not surprising. In any case it is not apparent from the patent in suit that the best antisera (in terms of cross-reactivity) were selected for the comparison.

The Respondent's counter-arguments may be summarised as follows: The extent to which monoclonal antibodies had been recognised at the priority date of the patent in suit as real, as opposed to potential, replacements for

polyclonal antibodies is open to question. The review articles reflecting common general knowledge in the art indeed contain optimistic predictions but these are qualified with cautionary statements. Moreover these review articles are concerned almost exclusively with macromolecules as immunogens, and the predictions do not necessarily apply in the case of small molecules (haptens) which require conjugation to a carrier before they can function as immunogens. This applies particularly to theophylline which is one of the smallest haptens known.

It could not have been predicted with any degree of certainty that monoclonal antibodies could have been prepared to theophylline that were capable of distinguishing, using the reliable assay method required by the patent in suit, between theophylline and the structurally very similar caffeine. The disclosure in the documents D7, B12 and P5, relating to monoclonal antibodies to haptens, demonstrated that monoclonal antibodies were not necessarily associated with lower cross-reactivity than the corresponding polyclonal antibodies. The comparison of the cross-reactivities associated with the monoclonal antibodies of the patent in suit with those of antisera prepared substantially according to document D1, when evaluated by the method according to the patent in suit rather than the unreliable 50% displacement method used in D1 showed a 5 to 8 fold improvement for the claimed antibodies which could not have been foreseen and is indicative of the presence of an inventive step.

Reasons for the Decision

1. The appeal is admissible.

2. The requirements of Article 123(2) and (3) are not at issue in this appeal and the Board can accept that no objection arises in this respect.

3. Novelty of the subject-matter of the claims according to the three requests is also not at issue, the Appellant having acknowledged novelty of the claims according to the present main request in point 1 of the letter dated 23 December 1994.

4. The background to subject-matter of the patent in suit is the following. Theophylline is an anti-asthmatic drug with a very narrow therapeutic range, and the concentration of the drug in the blood of the patient being treated has to be monitored to ensure that it remains within this narrow range. Assay of theophylline is complicated by the presence in blood of structurally closely related compounds, particularly caffeine and theobromine derived from coffee, tea and common soft drinks. (Theophylline is 1,3-dimethyl xanthine, caffeine 1,3,7-trimethyl xanthine and theobromine 3,7-dimethyl xanthine). A useful assay method for theophylline has therefore to distinguish it from the potentially interfering compounds.

5. It is undisputed that for the evaluation of inventive step, the most appropriate prior art is either document D1 or D2. Document D1 discloses a radioimmunoassay for theophylline which uses an antiserum obtained by immunising rabbits with a conjugate of 8-(3-carboxypropyl)-theophylline with bovine serum albumin. According to page 502, lines 4 and 5 of the section

"Discussion", the antigen was designed with a view to minimising cross-reaction with other xanthines. The hapten (theophylline) was attached to protein via an alkyl linkage at the 8-position, thus leaving positions 1,3,7 and 9 free to influence antibody selectivity. The antiserum is stated to show good selectivity for theophylline against other theophylline derivatives when measured by the 50% displacement method (see abstract) and the table on page 500 indicates inter alia a cross-reactivity of 4.2% with caffeine. A "more recent" antiserum (page 502, first paragraph, lines 9 to 12) exhibited a cross-reactivity of 1% with caffeine. On page 503, second paragraph, lines 5 to 8, it is concluded that although the gross cross-reactivity with caffeine and theobromine was low as predicted, further comparisons are needed to make sure that caffeine levels in a random patient population will not interfere significantly with the analytical results.

D2, published some five years after document D1 and about a year before the priority date of the patent in suit, contains a similar disclosure. Again a conjugate of 8-(3-carboxypropyl)-theophylline is used as immunogen and the specificity of the antiserum is such that cross-reactivity with other xanthines by the 50% displacement method is of no practical significance taking into account the usual amounts of these in blood serum (page 25).

6. Starting from this prior art, the problem which is the basis of the patent in suit can be seen as improving the immunoassay for the theophylline in respect of the feature of cross-reactivity with other xanthines of closely-related structure, in particular caffeine. In this respect the Board observes that document D1 refers to caffeine, theobromine and 3-methylxanthine as potential sources of interference in a radioimmuno-assay

for theophylline (page 502 under "Discussion") and caffeine is singled out for specific mention, e.g. page 503, lines 14 to 17. This view of the problem is moreover wholly consistent with what is derivable from the patent in suit, page 2, lines 21 to 31, where again potential interferants are listed (lines 25 to 27) and again caffeine is singled out (lines 27 to 31).

7. *Main request*

This problem is solved according to Claim 1 of the main request by a monoclonal antibody having 5% or less cross-reactivity with caffeine when evaluated by a particular immunoassay for theophylline, cross-reactivity being particularly defined and the antibody having been raised against an 8-substituted theophylline-carrier conjugate (see paragraph VII above).

8. The subject-matter of Claim 1 differs from the disclosure in documents D1 or D2 in that the antiserum of documents D1 and D2 is replaced by a monoclonal antibody and the question to be answered is therefore whether an inventive step can be seen in this replacement and the improvement in cross-reactivity thereby achieved.

9. To answer this question it has first to be established what was, for the person of average skill in the art, common general knowledge. The pioneer work of Köhler and Milstein on monoclonal antibodies dates from 1975 and it has not been disputed in this appeal that the basic steps of their technique for producing hybridomas, involving choice of immunogen, cell fusion, screening and selection of the desired hybridomas, had by the priority date of the patent in suit become routine, although still time-consuming and laborious. Of the

documents referred to in paragraph V above, many are review articles, which can be seen as reflecting common general knowledge in the art. Their relevant content can be summarised as follows.

In the introduction to document D8, the author, discussing hybridoma technology, states that there is no best approach and that success will usually depend on adaptation of the methods to each individual problem. He goes on to say that while the euphoria about monoclonal antibodies is believed to be justified, they may be too specific, or lack the necessary affinity etc. He concludes, in the introduction, that when extreme specificity is paramount, or when very large quantities of antibody are needed, hybridomas can provide the ideal solution. Later (page 294) it is stated that it cannot be emphasised too strongly that the screening procedure is the key to success in hybridoma production.

Document D9, comparing polyclonal and monoclonal antibodies states that in contrast (to polyclonal antibodies) unwanted reactivity may be eliminated from consideration in the production of monoclonal antibodies by merely selecting against antibodies responsible for such cross-reactivity during the screening phase (page 1797, right column, last paragraph). The importance of the screening procedure is repeated on page 1798, right column, second paragraph, and in the summary on page 1802. The use of monoclonal antibodies as replacements for polyclonal sera is referred to on page 1800 under "Immunodiagnostics".

Document D10 also refers to the importance of selection of the clones of interest, and mentions such difficulties as instability and overgrowth by uninteresting clones.

Document B1 refers to the possibility of producing virtually unlimited supplies of homogeneous antibodies which overcome many of the problems inherent in classical serology (page 510). The problems of weak immunogen or poor response of the animal to immunisation are mentioned on page 513, right column, last paragraph, and on page 514, right column, second full paragraph, in respect of unacceptable cross-reactivity, of the need to generate another monoclonal antibody. However it is then stated that the amount of labour involved in producing hybrids and the technical difficulties are minor obstacles in view of the benefits of having a monoclonal antibody.

Much of this is repeated in document B2. Further, in the sentence bridging the columns on page 1347 it is stated that the titer of a monoclonal against a cross-reacting antigen could be higher than the titer against the immunising antigen but is usually lower. The question is posed whether the production of monoclonal antibodies is worth the effort and it is concluded that while sometimes it may be better to remain with antisera, it seems inevitable that most immunologic assays will eventually be conducted with hybridoma antibodies.

Documents B3 and B4 contain similar disclosures to each other and express guarded optimism, for example, in document B4, page 60 it is warned that there are no miracles and on page 63 that monoclonal antibodies are slowly beginning to replace conventional antiserums in standard kits. Document B3, page 105, middle column, indicates that the method (of producing monoclonal antibodies) seems to be of general applicability, including the use of haptens as immunogen.

Document B6 is the same as document D9, and document B5, sharing authors with documents B6/D9 contains much the same disclosure. In document B7, published in March 1982, Köhler and Milstein express less guarded optimism concerning monoclonal antibodies than did Milstein over a year earlier in documents B3 and B4.

10. What emerges from the foregoing is that at the priority date of the patent in suit, the advantageous properties of monoclonal antibodies over polyclonal antibodies (antisera) were common knowledge, these being the possibility of selection during their production for high specificity, a desired affinity and/or avidity, and the fact that an unlimited supply became available once they had been obtained. Accordingly, taking into account the various cautionary statements contained in the above-cited documents, the weight of their content is that at the priority date of the patent in suit it was an obvious step to replace polyclonal antibodies by monoclonal antibodies with a reasonable expectation of an improvement in properties, including that of specificity.

11. Nevertheless it remains to be considered whether this general conclusion applies in the particular circumstances of the present case.

The Respondent has argued that the above documents reflecting common general knowledge are concerned almost exclusively with monoclonal antibodies raised against macromolecules, for which the optimistic predictions could be said to be justified. The reasoning behind this is that macromolecules possess a vast repertoire of epitopic sites, so that there is a reasonable chance that, of the antibodies elicited against a macromolecule, one or more monoclonal antibodies can be obtained having a desired specificity. Haptens on the

other hand are relatively small molecules with a limited number of functional groups for providing epitopic sites, and in the Respondent's view, the odds against producing a monoclonal antibody which will distinguish between structurally closely related haptens are very high. This applies particularly in the case of theophylline which is one of the smallest haptens known (molecular weight 180) and this differs from the potential cross-reactant caffeine only in the absence of a methyl group at the 7-position.

The Board does not follow this line of argument. As regards haptens generally, four of the cited documents (D3, D7, P5 and B12) relate to the production of monoclonal antibodies to haptens, and three of these (D3, D7 and B12) contain certain statements to the effect that this was done with the aim of achieving increased specificity - see document D3, sentence bridging pages 3 and 4: "The use of monoclonal antibodies would maximize the ability to discriminate between the drug under assay and potential cross-reacting interference"; document D7, Introduction: "The monoclonal antibodies of defined immunochemical specificities can serve this purpose (high specificity) and replace conventional antibodies", document B12, page 75, final paragraph: "We decided to study whether selected hybridoma lines would produce antibodies without disturbing cross-reactions". Therefore, before the priority date of the patent in suit, others were investigating the production of monoclonal antibodies to haptens in the expectation of achieving improved specificity. That is to say, the optimistic predictions contained in the documents referred to in paragraph 9 above were believed to be valid in the case of haptens also. The Board would agree that theophylline is a somewhat smaller molecule than those mentioned in documents D3, D7, P5 and B12 (the disclosure in

document D3 relating to theophylline is minimal) but does not see anything in this which would deter the average skilled person from attempting to raise monoclonal antibodies to it. It is known to be immunogenic (see e.g. documents D1, D2). Further, while it is true that because of the structural similarity between the theophylline and caffeine molecules, monoclonal antibodies raised against theophylline may also recognise caffeine, the molecules are nevertheless different and this difference will be reflected in a different affinity in the antibody-antigen reaction. This difference is one factor which is exploited in selecting the hybridomas which produce antibodies having the desired specificity.

Further, in document B1, page 514, right column, second complete paragraph, it is stated, in respect of macromolecules as immunogens, that since monoclonals are, by definition, monospecific, if a monoclonal raised against a particular immunogen is found to cross-react with another macromolecule, the two molecules must share an antigenic determinant. For the average skilled person, the logical conclusion is that haptens, comprising a single or few epitopic sites, will show less cross-reactivity than macromolecules, providing a relatively large number of epitopic sites.

12. The Respondent has also argued that a contribution to inventive steps should be seen in the feature that the claimed antibody is raised against an 8-substituted theophylline-carrier conjugate. According to the Respondent it was conventional wisdom at the priority date of the patent in suit that the further the site of attachment of a carrier to a hapten from the antigenic determinant, the better would be the specificity of a monoclonal antibody raised against that carrier-hapten conjugate. Cited in support of this contention were

document D5, sentences bridging pages 317 and 319: "Since the only common substitute of these compounds is the methyl at position 3 of the xanthine nucleus precise molecular recognition at this site should be of limited importance. Accordingly, we concluded that an immunogen prepared by attaching the drug to carrier proteins at the 3 position might elicit the most specific immune response", and document P5, page 673, section "Discussion": "It has been proposed that the carrier protein should be attached to a site on the steroid molecule remote from the distinguishing functional groups on the molecule". The Respondent also referred to documents D11 and P6 in this respect. D11 is state of the art under Article 54(3) EPC and is not therefore of direct interest in evaluating inventive step. However it does disclose the use as an immunogen of the theophylline derivatives at the 9-position and states on page 4 that antibodies obtained possess an unexpectedly extremely low cross-reactivity for caffeine compared with antibodies obtained using 7- and 8-substituted theophylline. This may well be typical patent specification language; nevertheless it does not support the Respondent's above-stated contention regarding conventional wisdom, because if this were valid it should not have been surprising that the 9-position derivatives showed an improvement over the 7- and 8-position derivatives. Further, document D11 relates almost exclusively to antisera, only a short paragraph on page 10 referring to monoclonal antibodies without any information concerning cross-reactivity.
Document P6, discussing haptens lacking suitable functional groups for coupling to the carrier, states in connection with slide 3 that in such cases it is necessary to derivatise the drug with a functional group at a position that is removed from the more interesting portions of the drug. (The Board can accept the Respondent's information that the disclosure in

document P6 was publicly available before the priority date of the patent in suit.) There is however no escaping the fact that document D1 discloses the use of 8-substituted theophylline as immunogen and that document D2, in spite of the intervening teaching of document D5 and the corresponding document D4, continued to employ the same immunogen. It is true that document D5, in the sentence bridging pages 319 and 320 refers to significant cross-reactivity observed in assays using antisera based on the 8-substituted derivatives. However cross-reactivity between 1-methyl xanthine and theophylline, although the highest observed, is still relatively low in spite of the fact that the only difference between these, a methyl group in the 3-position, is removed by derivatisation, so that 100% cross-reactivity would have been expected. In fact the immunogens used in documents D4 and D5 are as much based on derivatives of 1-methyl xanthine as of theophylline, because 1-methyl xanthine is the starting material for their preparation. It would seem therefore that the links between carrier protein and hapten need not be remote from the distinguishing functional groups. It is noted that in the above-quoted passage in P6, the word "removed" is used and not "remote" so that the 8-position is not excluded. P6 does not state in words where theophylline is derivativised (see the text relating to slide 12). However the text relating to slide 3 indicates that it is necessary to derivatise the drug and goes on to say that the drugs are derivatives containing a short carbon chain terminated with a reactive amine or carboxyl group, and that the derivative is coupled via a variety of methods to the carrier. Slide 5 on the other hand describes the drug labelled with ß-galactosyl umbelliferone (ß-GU) for use in a competitive immunoassay. Document P6 is therefore distinguishing between the derivatised drug and the labelled drug. Slide 14, illustrating theophylline

structures, shows 8-(3-carboxypropyl)-theophyline and ß-GU-theophylline, and in the text relating to slide 16, 8-(3-carboxylpropyl)-theophylline is referred to as the derivative and again distinguished from the labelled drug (final sentence). It would appear to follow, therefore, that document P6 is disclosing the use of 8-substituted theophylline, bound to a carrier, as the immunogen for preparing anti-theophylline antibodies. The Board considers this to be a valid interpretation but has not relied on this interpretation in coming to the present decision, seeing it rather as confirming, or at least being entirely consistent with, its findings based on the other cited documents. On the other hand the Board cannot agree with the assertion of the Respondent (top of page 41 of the letter dated 18 September 1992) that 8-(3-carboxypropyl)-theophylline was used to prepare the labelled drug. This is nowhere stated in document P6 and from the structural formulae given in slide 14 it appears unlikely.

As noted by the Respondent (pages 39 to 42 of the said letter), documents D2,D3, D11 and P6 are of substantially the same provenance, and (page 42) in respect of documents D2 and D11, relating to antisera, it is stated that fundamentally different conclusions are reached as to the design of a theophylline-carrier conjugate. The Board does not see it this way, but is rather of the opinion that when all four documents are taken into consideration, it is apparent that several avenues were being explored at the same time. The Board would add that the disclosure of monoclonal antibodies to theophylline in document D3 may be seen in a different light when document P6 is taken into account, the first acknowledgement in document P6 referring to the work of the inventors in document D3 in developing the monoclonal antibodies discussed there.

All in all, there is in the Board's opinion no compelling reason why the person of average skill in the art faced with the problem set out in paragraph 6 above would be deterred from using the 8-substituted theophylline-carrier conjugate, particularly in view of the promising cross-reactivities reported in documents D1 and D2, of which more will be said later. Accordingly, no contribution to inventive steps of the subject matter of Claim 1 (main request) is seen in the choice of conjugate.

13. A further point of the Respondent's argumentation is that it could not have been predicted from the prior art that a monoclonal antibody to theophylline could be prepared having the very high specificity of the claimed antibody, corresponding to a cross-reactivity of 5% or less with caffeine when evaluated by the method defined in Claim 1. In support of the contention that monoclonal antibodies are not necessarily more specific the corresponding antisera let alone that they would show the 5 to 8 times improvement of the claimed antibodies, the Respondent cites documents D7, B12 and B5. It is true that in each of these documents the results of cross-reactivity experiments would appear to bear out the Respondent's contention. Nevertheless there are sufficient positive results to be consistent with the conclusion expressed in paragraph 10 above that there is reasonable expectation of improved specificity when polyclonal antibodies are replaced by monoclonal antibodies. Further in document B12, Table II, monoclonal antibodies are being compared with four antisera which were specially selected on the basis of low cross-reactivities. On the other hand, while in each of the documents the production of the hybridomas secreting antibodies to the immunogen is described in some detail, in no case is there any indication of measures taken during selection to minimise cross-

reaction,so that valid comparisons cannot be made. The Board accepts that the 50% displacement method used in documents D1 and D2 and in many of the other cited documents for measuring cross-reactivity is unreliable and can grossly underestimate cross-reactivity, as evidenced by document P1, page 162, left column. Table II on page 6 of the patent in suit compares cross-reactivities in assays using the claimed monoclonal antibodies with conventional antisera using the percentage error method required by Claim 1 and defined in Example IV. The monoclonal antibodies are those derived from cell lines 30/15, 17/14 and 61/7, deposited as ATCC HB 8152, 8153 and 8154 respectively. The antisera were raised against theophylline-8-BSA substantially as in documents D1 or D2. The table shows cross-reactivities of 5% for the three monoclonal antibodies and 25% and 40% for the 2 antisera, hence the 5 to 8 fold improvement quoted above. As to the question of whether such an improvement goes beyond what would be predictable having regard to common general knowledge (see paragraph 9 above), in the Board's view it is no more than what would be expected when a comparison is made between monoclonal antibodies derived from hybridomas which have been screened for lack of cross-reactivity with caffeine (see the patent in suit, page 4, lines 38 to 41) and antisera which are merely typical of more than ten pools of antisera tested (page 5, lines 49, 50). The Board has noted that in document P6, text relating to slide 17, it is stated that the lowest cross-reactivity obtained by the experiments so far with theophylline subclone supernatants is about 6.0% (by the 50% displacement method - see slide 15) and therefore inferior to that required by Claim 1. However it is further stated that results were very preliminary and that it was hoped to improve cross-reactivity by a more rigorous selection process prior to cell fusion.

14. The Respondent cited documents P2 and P3 to demonstrate that at the priority date of the patent in suit there were two types of cross-reactivity, of which the type of specificity which corresponds to type 1 or true cross-reactivity can be applied to either homogeneous or heterogeneous antibody populations, but is the only type which applies to truly homogenous antibodies such as monoclonal antibodies (see document P2, page 753, beginning of final paragraph). The Respondent argued that going from polyclonal to monoclonal would eliminate type 2 cross-reactivity but it could not have been foreseen that type 1 might also be minimised. In the Board's view since type 1 cross-reactivity is the only one encountered with monoclonal antibodies, it is the only one which can be reduced or eliminated by an appropriate screening protocol, as referred to for example in document D9, page 1797, last complete sentence. The Board therefore cannot follow the Respondent's argument.

15. The Respondent further contends that just because anti-theophylline antibodies can be raised in **rabbits** as described in D1 and D2 it does not follow that theophylline will necessarily be a sufficiently strong immunogen in **mice**. However at the priority date of the patent in suit, rabbits were one of the animals of choice for producing antisera and mice the animals of choice for producing monoclonal antibodies. To go from rabbit to mouse was the normal route and was the route followed for example in B12, P5 and D7.

16. An indication of inventive step in the subject-matter of Claim 1 could have been seen if there had been any evidence of difficulties overcome in producing monoclonal antibodies, for example in the all-important screening protocol. However as argued by the Appellant in point 2.1.9 of the letter dated 23 December 1994, the

Respondent followed a prescribed route to the claimed subject-matter from choice of immunogen to the selection procedure. Any suitable assay can be used for detecting antibody production by the immunised mouse (page 3,lines 3 to 5 of the patent in suit) and also for presence of the desired antibody in the growing fused cells (page 3,lines 19, 20). Further, obtaining three antibodies having the desired specificity from 56 positive wells appears to the Board to be the sort of yield one would reasonably expect. The Board notes that in Example 1, step C, particle-enhanced turbidimetric inhibition immunoassay (PETINIA) was used to screen the mouse serum. PETINIA is stated to be described in EP-A-73 611 published after the priority date of the patent in suit. This was not the first disclosure of such an assay as is apparent from the relevant prior art recorded in the file history of EP-A-73 611 and also from DE-A-2 749 956 cited during the opposition proceedings of the patent in suit as an example thereof.There is therefore no question of a novel immunoassay being used here to aid in the selection procedure.

It is true that as argued by the Respondent the person of average skill in the art could have investigated other routes in seeking to solve the problem set out in paragraph 6 above. Examples are removing cross-reactants from the serum or "spiking" the assay sample with a potential cross-reactant as is disclosed in documents D4 and D5 (see column 11 of D4). Nevertheless in the Board's opinion, the obvious advantages of monoclonal antibodies, in particular the possibility of obtaining unlimited or at least very large quantities of a homogeneous reactant in addition to the expected high specificity would lead the skilled person to the route of the patent in suit.

17. Accordingly the subject-matter of Claim 1 does not involve an inventive step having regard to the disclosure in D1 or D2 and what was common general knowledge in the art at the priority date of the patent in suit.

18. *First auxiliary request*

Claim 1 according to this request contains the additional feature that the antibody also has 30% or less cross-reactivity with theobromine and 5% or less with 3-methylxanthine. These additional features are derived from Claim 2 of the granted patent, which was substantially identical to Claim 2 as originally filed. It is true that the data in Table II (page 6 of the description), apparently constituting the basis for the claim, do not reflect the feature "or less". In fact the only reference in the description to cross-reactivities of less than 5% occurs on page 2, line 50 in respect of caffeine. However no doubt this could have been clarified had the need arisen, and the Board has examined the claim as it stands.

The claimed subject-matter is embodied in the antibodies derived from cell lines 30/15, 17/14 and 61/7 (see the Examples, in particular Table II and also page 2, lines 47, 48 in conjunction with page 2, lines 56 to 58). These were obtained using a screening protocol which selected for low cross-reactivity with caffeine (page 4, lines 38 to 41) and accordingly show the expected low-cross-reactivity. The fact that they also showed equally low cross-reactivity with theobromine and 3-methyl xanthine, or, in the case of the antibodies derived from cell lines 17/14 and 61/7, a somewhat higher but nevertheless tolerable cross-reactivity with theobromine could be considered as a bonus and therefore not contributing inventivity to the subject-matter of

the claim as compared with that of Claim 1 according to the main request. Indeed the additional features may be considered to be a further characterisation of the antibodies and in the Board's view the claimed subject-matter is providing the same solution to the same problem as that of Claim 1 according to the main request.

Nevertheless the Board has considered whether an inventive step can be seen in the particular pattern of cross-reactivities required by the claim. The Table on page 500 of document D1 lists the cross-reactivities of various xanthine derivatives with the theophylline antisera, but measured by the 50% displacement method so that a direct comparison cannot be made. Table II of the patent in suit discloses the cross-reactivity of two antisera with caffeine, but not with any of the other xanthines, so that there is no indication of a pattern here. Finally the Table on page 11 of the Respondent's submissions dated 18 september 1992 repeats the data in Table II for the monoclonal antibody derived from cell line 30/15 and gives the corresponding data obtained using the 50% displacement method. From a comparison of the results it is clear that results obtained using the percentage error method cannot be translated into results using 50% displacement method, because the differences are not proportional. Accordingly in the absence of any evidence as to what kind of pattern was to be expected using the percentage error method, the Board has to form its own conclusions.

It is true that the pattern derivable from the Table on page 500 of document D1 shows a relatively high cross-reactivity with caffeine as compared with theobromine and 3-methylxanthine (4.2%, 0.09% and 2% respectively). However, once the step, shown in paragraphs 11 to 17 above to be obvious, of replacing antisera by monoclonal

antibodies is taken, and when these antibodies are selected for low cross-reactivity with caffeine, the difference between the cross-reactivity with caffeine and that with the other xanthines will be minimised and, in view of the expected reduction in cross-reactivity in going from antisera to monoclonal antibodies rasied against an 8-substituted theophylline, the pattern of cross-reactivities will come closer to that required by the claim. In this respect it is noted that the range of cross-reactivities required by the claim is fairly wide and that the claim does not require that of theobromine to be higher than that of caffeine and 3-methylxanthine. Accordingly the Board sees nothing surprising in the particular pattern of cross-reactivities requested by the claim. The subject-matter of Claim 1 according to the first auxiliary request is therefore not seen as involving an inventive step.

19. *Second auxiliary request*

Claim 1 according to this request is restricted to the antibodies produced by the cell lines ATCC HB 8152, ATCC HB 8153 and ATCC HB 8154. However these antibodies have precisely the features which are claimed in Claim 1 of the first auxiliary request. In the absence of any non-obvious properties of these antibodies and since no difficulties had to be surmounted in producing them (see paragraph 16 above) the subject-matter of this claim also does not involve an inventive step.

20. Of the documents listed in paragraph V above, documents D6, D8, B9, B10, B11 and B13 have not so far been mentioned in this decision. Document D6 is a decision of Board of Appeal 3.3.2 wherein the issues under consideration corresponded closely to those in the present case. The present decision is wholly consistent therewith. The remaining documents contain nothing which in the Board's view contributes anything positive to the argumentation of any of the parties, the more so in respect of documents D8 and P4 which are not prepublished.

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:

L. P. McGarry U. Kinkeldey