DECISION
of 3 June 2004

Case Number: T 0285/01 - 3.3.8
Application Number: 88909905.7
Publication Number: 0394296
IPC: G01N 33/68
Language of the proceedings: EN

Title of invention:
Assay of a body fluid for measuring bone resorption

Patentee:
Washington Research Foundation

Opponents:
Osteometer Biotech A/S
F. HOFFMANN-LA ROCHE & CO. Aktiengesellschaft
Metra Biosystems Inc.

Headword:
Bone resorption/WASHINGTON RESEARCH FOUNDATION

Relevant legal provisions:
EPC Art. 56, 83, 84, 123(2)
EPC R. 29(6)

Keyword:
"Main request: allowable amendments (yes)"
"Clarity (yes)"
"Inventive step (no)"
"First auxiliary request: clarity (no)"
"Second auxiliary request: sufficiency of disclosure (yes)"
"Inventive step (yes)"

Decisions cited:
T 0190/99, T 0522/00

Catchword: -
Case Number: T 0285/01 – 3.3.8

DECISION
of the Technical Board of Appeal 3.3.8
of 3 June 2004

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Composition of the Board:

Chairman: L. Galligani
Members: T. J. H. Mennessier
          M. B. Günzel
Summary of Facts and Submissions

I. Opponent 1 (appellant I) and opponent 2 (appellant II) each lodged an appeal against the interlocutory decision of the opposition division dated 25 January 2001, whereby the European patent No. 0 394 296 was maintained on the basis of the main request (claims 1 to 12) filed at the oral proceedings on 21 January 1999. The patent had been granted on European application No. 88 909 905.7 which originated from an international application published as WO 89/04491 (to be referred to in the present decision as the application as filed).

II. The patent had been opposed by a third opponent (opponent 3) which has not appealed.

III. The grounds for opposition were that, as set forth in Article 100(a) EPC, the invention was neither novel (Article 54 EPC) nor inventive (Article 56 EPC), and, that, as set forth in Article 100(b) EPC, the invention was not sufficiently disclosed (Article 83 EPC).

IV. In reply to the statements of grounds of appeal filed by the appellants, the respondent filed a first auxiliary request (together with a letter dated 13 December 2001) and stated that the claims which had been accepted by the opposition division were still its main request.

VI. In reply to that communication, additional observations were received with letters dated 30 April 2004.

VII. At the oral proceedings which took place on 3 June 2004 in the absence of opponent 3, the respondent replaced its main claim request, ie the claims as accepted by the opposition division, by the first auxiliary request filed with letter dated 13 December 2001 which became its main claim request (referred to thereafter as the "main request") as well as two further claim requests, referred to thereafter as the "first auxiliary request" and the "second auxiliary request".

VIII. Claim 1 of the main request read:

"1. A method of determining the rate of bone resorption, the method comprising quantitating by immunological means the concentration of a peptide fragment of molecular weight less than 5000, in a body fluid, which is derived from bone collagen resorption and has a 3-hydroxypyridinium cross-link that is lysyl pyridinoline and/or hydroxylysyl pyridinoline."

(emphasis added by the board)

Claim 1 differed from claim 1 as granted in that it contained the additional expressions (shown in bold in the claim) "by immunological means" and "of molecular weight less than 5000".

IX. Claim 1 of the first auxiliary request read:

"1. A method of determining the rate of bone resorption, the method comprising quantitating by immunological means the concentration of a peptide
fragment of molecular weight less than 5000, in a body fluid, which is derived from bone collagen resorption and has a 3-hydroxypyridinium cross-link that is lysyl pyridinoline and/or hydroxylysyl pyridinoline, and which peptide appears in the elution profile of Figure 3a or Figure 3b."

Claim 1 differed from claim 1 of the main request (see section VIII, supra) in that it contained the additional expression (also in bold in the claim) "and which peptide appears in the elution profile of Figure 3a or Figure 3b".

X. The second auxiliary request consisted of 10 claims, claim 1 thereof being identical to claim 8 as granted and claims 2 to 10 being identical to claims 10 to 18 as granted.

Claim 1 read:

"1. A peptide fragment, derived from the aminoterminal telopeptide domain of type I collagen linked through a 3-hydroxypyridinium cross-link, comprising the amino acid sequence:

Asp-Glu-K-Ser-Thr-Gly-Gly

\|

Gln-Tyr-Asp-Gly-K-Gly-Val-Gly

\|

K
where $K$ represents hydroxylsyl pyridinoline or lysyl pyridinoline, and $K_{Gln}$ is glutamine or wholly cyclized pyrrolidone carboxylic acid."

Claim 2 was directed to a hybridoma capable of producing monoclonal antibodies specific for the peptide fragment of claim 1.

Claim 3 was directed to an immunological binding partner for the peptide fragment of claim 1; claims 4 and 5 were dependent on claim 3. Claim 9 was directed to such an immunological binding partner for use as a diagnostic agent.

Claim 6 was directed to a test kit comprising such an immunological binding partner.

Claim 7 and claim 8 were each directed to a use of a peptide fragment of claim 1.

Claim 10 was directed to an immunogenic composition comprising a peptide fragment as defined in claim 1.

XI. The following documents are mentioned in the present decision:


(6) David R. Eyre et al., Anal. Biochem., Vol. 137, 1984, Pages 380 to 388;

(17) David Eyre, Meth. Enzymol., Vol. 144, 1987, Pages 115 to 139;


(27) Dennis A. Hanson et al., J. Bone Min. Res., Vol. 7, No. 11, 1992, Pages 1251 to 1258;

XII. The appellants' arguments may be summarised as follows:

Main request

Interpretation of claim 1

In view of the specific wording "quantitating [...] the concentration of a peptide fragment" used therein claim 1 was directed to a method comprising the step of quantitating the concentration of a single peptide fragment not that of a plurality of peptide fragments.

Article 123(2)EPC (Claim 1)

Due to the added feature "of molecular weight less than 5000" claim 1 contained added matter. When assessing that issue, the full context of the claim had to be taken into consideration. The two references to a figure of 5000 in the application as filed on pages 12 (lines 25 and 26) and 16 (lines 1 and 2) were not in connection with the determination of a particular peptide. The "less than 5000" feature on page 12 was cited in the context of preparing an antigen. There was no invitation to assess a particular peptide. There was no statement or suggestion that the concentration of a particular peptide had to be measured.

There was no teaching on page 12 that in general peptide fragments of less than 5,000 should be selected for measurement. The sentence there was simply the observation that the two peptides of Formula III and Formula IV and those equivalent to them had a molecular weight below 5,000, this having some impact on the best way to make antibodies to those particular peptides.
This observation did not amount to a teaching that peptides below 5,000 in general were a specific class of peptides that should be quantitated according to the invention. The figure of 5,000 in the cited passage of page 12 did not relate to the peptides to be measured. It indicated the peptide size below which conjugation to a carrier molecule was needed generally for antibody production.

On page 16 it was referred to a "1000-5000 Dalton" range of molecular weights not in the context of measuring the concentration of a particular peptide but in the only context of an electrochemical procedure.

**Article 84 EPC (Claim 1)**

The molecular weight of 5,000 was a relative molecular weight. As there was no indication in the claim of how it had been determined, it was an unclear feature.

**Article 56 EPC (Claim 1)**

Document (17) represented the closest prior art. The process described on page 135, based on the determination of total hydroxylysyl pyridinoline (HP) cross-links contained in a hydrolysate of urinary peptides, was concerned with the specific measurement of bone resorption. The observed increase of HP in the urine of a patient with Paget's disease was a reflection of the greatly increased bone turnover in this condition. The technical problem to be solved was regarded as the provision of a more convenient method avoiding the hydrolysation step of the process of document (17). The skilled person would have considered
that a readily appropriate alternative to said process was an immunoassay using antibodies specific for the urinary peptides. All the information necessary to isolate the peptide fragments was contained in document (17), in particular on page 136 which contained the explanation of how to separate cross-linked peptides and in Figure 9 on page 137 which showed an elution profile of peptide fragments in type III collagen of bovine aorta similar to an elution profile obtained with human bone type I collagen as represented in Figure 3a in the patent. Moreover, the use of immunoassays in the field was known from document (3).

First auxiliary request

Admissibility of the request

Whereas an unclear and complex feature had been introduced into claim 1, the appellants were taken by surprise at this late stage of the proceedings. They had not had the time necessary to evaluate the significance of the amendment. Therefore, the request should be regarded as inadmissible.

Article 84 EPC (claim 1)

Peptide fragments defined by means of elution profiles was a concept which did not exist in the application as filed. An elution profile such as the one of Figure 3a or of Figure 3b was not appropriate for a clear definition of the peptide fragments of interest. In particular, there was no indication that each and every peak corresponded to peptide fragments. Furthermore, the peptide of Formula IV as referred to in the elution
profile of Figure 3b did not exist. Moreover, as the elution profiles of Figures 3a and 3b had been obtained when performing the particular experiments reported on page 7 in the patent specification, they would not be re-obtained when performing other experiments. Therefore, claim 1 lacked clarity.

Second auxiliary request

Article 84 EPC (request as a whole)

Notwithstanding the fact that they corresponded (with same wording) to granted claims, appellant II submitted that the claims of the second auxiliary request lacked clarity.

Article 83 EPC

A necessary (although not sufficient) requirement for an antibody for the immunological measurement of a bone resorption-derived peptide fragment containing a LP or HP cross-link would be an immunological specificity for the presence of the pyridinium cross-link combined with an immunological specificity for a type I collagen peptide sequence. Neither the respondent nor any other party had described any such antibody.

There was no disclosure in the patent of the existence of any antibody produced by the respondent, let alone a deposit thereof. The description of antibody production was entirely on a theoretical basis.

Furthermore, the lysyl pyridinoline (LP) and hydroxylysyl pyridinoline (HP) cross-links were not the
only cross-links formed in type I collagen of human bone. As described in document (24) there were also pyrrole cross-links which formed at the same sites in the collagen chains of the LP and HP cross-links and which were connected to the very same amino acid sequences in each collagen molecule so joined. Antibodies capable of discriminating between fragments differing in the nature of their cross-link were neither disclosed in the patent nor even in the post-published document (27).

Article 56 EPC (claim 1)

Either document (20) or document (19) in combination with document (37) had to be taken into consideration.

Document (20), page 21, right-hand column, reported that in the fibril the triple-helical molecule could be cleaved by the mammalian collagenase at a distinct position between glycine 775 and isoleucine 776 into two pieces, TC\(^A\) and TC\(^B\) which presumably would lead to a peptide fragment according to claim 1.

Document (37) described the preparation of type I collagen fragments from calf aorta. The fragments were C-terminal fragments comprising amino acid chains cross-linked by pyridinium. Claim 1 covered similar structures which, however, were derived from the N-terminal of the molecule.

Document (19) at page 8 (Section 3) described such N-terminal fragments isolated after cleavage of insoluble collagen with CNBr or trypsin. Figure 4 on page 8 showed the amino acid sequence of the nonhelical cross-
link region of the $\alpha_1$ (I), $\alpha_2$ (I), and $\alpha_1$ (III) chains at the N terminal end of calf skin collagens. Even though the exact sequence of the equivalent human sequence was not given, the means of obtaining the human fragment by protease digestion of human collagen and fragment purification by prior art techniques were made available to the skilled person.

XIII. The respondent's arguments may be summarised as follows:

Main request

Interpretation of claim 1

The application as originally filed related to the monitoring of bone resorption by reference to a multiplicity of cross-linked peptide fragments, as illustrated by various references throughout the specification to peptide fragments in the plural form, in particular on page 5, lines 35 to 37, and by the elution profiles represented in Figures 3a and 3b which showed multiple peaks comprising telopeptide cross-linked peptide fragments of interest.

Article 123(2) EPC (Claim 1)

Because the application as filed taught that a multiplicity of cross-linked peptide fragments could be studied, it was quite apparent that there was a relevant "group" within such groups. Indeed, the sentence on page 12 in the application as filed starting at line 25 referring to peptide fragments which are "less than 5,000" was a clear teaching that the group of fragments of interest had molecular
weights less than 5,000. In the absence of any other indications, the only sensible interpretation of this sentence was that each of the various peptide fragments was less than 5,000. Significant importance was also to be placed upon the teaching in the paragraph bridging pages 15 and 16 of the application as filed referring to the use of Bio-Gel P2 or Sephadex G-20 columns for fractionation of urine samples, which made clear that the population of peptide fragments selected for quantification eluted in the 1,000 to 5,000 dalton range.

Article 84 EPC (Claim 1)

There was a need to take an objective, logical and sensible interpretation of the claims as ruled in decision T 190/99 of 6 March 2001 or in decision T 522/00 of 24 January 2003. When this standard was applied to interpretation of the reference to 5000 in claim 1, bearing in mind the teaching of the patent specification, it was immediately apparent that the skilled person would have had no difficulty in interpreting that an absolute molecular weight as calculated from the atomic structure of the peptides to be measured was referred to. The skilled person wishing to put the invention into effect or to determine whether or not an activity fell within the scope of the claim needed merely to identify the nature of the peptides being detected and to calculate their molecular weight. There was no need for a reference in claim 1 to a means of measurement of molecular weight, especially in consideration of the fact that it was in relation to small molecules.
Article 56 EPC (Claim 1)

Document (17) merely reported that the urine of patients with active Paget's disease exhibited a great increase in the content of HP which presumably reflected the greatly increased turnover in this condition.

Document (17) simply confirmed what document (3) (cited therein as reference 30) had already reported: urine of patients with Paget's disease contained lots of HP. Now, whereas document (3) hydrolysed an entire urine sample (containing both free and peptide-linked pyridinolines), document (17) hydrolysed a crude fraction of urinary peptides. Thus, document (17) essentially eliminated the free pyridinoline component from the urine sample, by dialysis, and then eliminated other fluorescent contaminants, before hydrolysing the total peptide-linked pyridinoline component that remained.

Having regard to the process of document (17), the technical problem to be solved was more general than the mere provision of an alternative method for determining bone resorption in unhydrolysed urine.

There was no teaching that the urinary peptides referred to on page 135 of document (17) were of a particular size range, namely with a molecular weight of less than 5,000, nor that the production of such peptides was commensurate with the rate of bone resorption such that they could be used as an index of bone resorption. Document (17) did not, for example, teach a proportionate increase in the excretion of HP
compared to the rate of bone resorption, nor did it teach whether the amount of HP varied in Paget's disease under different conditions.

Thus, the patent taught for the first time that there was a correlation between such peptide fragments and the rate of bone resorption, and that the relevant fragments were not further metabolised upon their production by osteoclasts but stably appeared in body fluids such as urine in such a manner as to permit a reproducible measure of the rate of bone resorption.

In order to prepare immunogens the peptides should first be separated. Nevertheless, the information contained on page 136 et seq. in document (17) did not relate to bone collagen. This was illustrated in Figure 9 on page 137 where type III collagen of bovine aorta was referred to. The detailed conditions of the protocol of the patent were not contained in the relevant passage of page 135.

First auxiliary request

Admissibility

Respondent had repeatedly referred to the protocol for the isolation of urinary peptides on pages 9 and 10 of the application as filed and to the corresponding Figures 3a and 3b. Therefore, referring to said elution profiles in claim 1 was not something new and surprising for the appellants. Thus, the request should be considered admissible.

Article 84 EPC (claim 1)
Figures 3a and 3b represented the invention. Preparing antibodies which reacted with peptide fragments contained in the elution fractions corresponding to the peaks of the elution profiles was only routine experimentation. As emphasised in the description, in particular on page 6, lines 25 and 27, in the patent specification, these profiles were typical, ie they were not variable and could be re-obtained when testing urine samples of patients with Paget's disease. Even if Formula IV was erroneous, the peak corresponding thereto in the elution profile did mark the presence of the major C-telopeptide fragment identified by the inventor. Therefore, the reference to the elution profiles of Figures 3a and 3b was appropriate for a clear definition of the peptide fragments of interest.

Second auxiliary request

Article 84 EPC (Claim 1)

The claims exactly corresponded to claims as granted. Therefore, they were not at this stage of the proceedings open to any objection of lack of clarity.

Article 83 EPC

There was no need for a highly specific single fragment-responsive antibody. Antibodies with a broader range of specificity could quite satisfactorily be employed. There where nothing to prevent the skilled person from using the whole panoply of standard antibody techniques and approaches to pursue the invention. For example, a possibility was the use of
two antibodies, one being specific for an amino acid sequence in the region of the cross-link and the other being specific for a type I collagen amino acid sequence.

If similar peptide fragments having different cross-links, such as pyrrole cross-links, coexisted in the body fluid to be tested with the peptide fragments of the invention, it did not matter whether the antibody was not so specific as to discriminate between 3-hydroxypyridinium cross-links and the other cross-links, provided that all cross-links be generated with the same proportionality as a result of bone resorption.

*Article 56 EPC (Claim 1)*

On the basis of the mere passage on page 21 of document (20) on which the appellants had relied, it could not be established that the claimed peptide fragment existed. Therefore, the skilled person would not have been in a position to identify the peptide fragment of claim 1.

Document (37) concerned man-made C-terminal fragments of calf aorta, whereas claim 1 concerned an N-terminal structure isolatable from body fluids.

Document (19) gave some bovine sequence information, but did not disclose any particular fragment that could be regarded as relevant to the present invention.

XIV. Opponent 3 did not make any submissions in these appeal proceedings.
XV. The appellants (opponents) requested that the decision under appeal be set aside and that the European patent No. 0 394 296 be revoked.

XVI. As main request the respondent (patentee) requested that the decision under appeal be set aside and the patent be maintained on the basis of the claims of the first auxiliary filed with letter dated 13 December 2001. As first auxiliary request the respondent (patentee) requested that the decision under appeal be set aside and the patent be maintained on the basis of the first auxiliary request, filed during the oral proceedings. As second auxiliary request the respondent (patentee) requested that the patent be maintained on the basis of the claims and the amended description pages of the second auxiliary request, filed during the oral proceedings.

Reasons for the Decision

Main request

Claim 1

1. Claim 1 concerns a method of determining the rate of bone resorption wherein the concentration in a body fluid of a bone collagen-derived peptide fragment of molecular weight less than 5,000 is determined by immunological means, said fragment having a 3-hydroxypyridinium cross-link that is lysyl pyridinoline (LP) and/or hydroxylysyl pyridinoline (HP). Contrary to the view of the opposition division (cf decision under
appeal, page 10, fourth paragraph), the board cannot see why claim 1 as worded (cf Section VIII, supra) should exclude from its ambit an assay wherein quantification of more than one fragment fulfilling the structural conditions given is performed. The wording "a peptide fragment" is not to be interpreted narrowly as meaning only "one peptide fragment", but rather as meaning "any peptide fragment", which implies the quantification of one or more fragments, i.e. at least one fragment. This is supported by the description of the patent specification which repeatedly refers in the text and in the figures to peptide fragments.

**Article 123(2) EPC**

2. While no objection was raised by the appellants to the introduction in claim 1 of the feature "by immunological means" (the board also has no objections), the feature "of molecular weight less than 5000" qualifying the peptide fragment to be determined was objected to under Article 123(2) EPC. It was submitted that the figure "5000" is found in the application as filed either in connection with the general observation that peptides which are less than 5,000 should be conjugated to an hapten for making antibodies (cf page 12, lines 25 to 27) or in the context of a specific procedure for isolating bone-collagen peptide fragments (cf passage bridging pages 15 and 16), both offering no fair basis for the amendment.

3. The board cannot follow these objections. The whole of the application as filed makes abundantly clear that the method proposed relies on the determination in a body fluid of bone collagen-derived peptide fragments...
having 3-hydroxypyridinium cross-links. Within this framework, some specific peptide fragments with Formulæ III and IV which have molecular weights, respectively of about 2000 and 4000 as well as equivalents thereof with amino acids additions or deletions, are referred to on pages 11 and 12. On page 12, when describing the immunological procedure for quantitating such peptide fragments, reference is made to the preparation of monoclonal and polyclonal antibodies specific for the peptides of Formulæ III and IV and their equivalents and it is stated "because the molecular weights of these peptides fragments is less than 5,000, it is preferred that the hapten be conjugated to a carrier molecule" (emphasis added). When describing the alternative electrochemical procedure for quantitating such peptide fragments, the population of peptide fragments selected for quantification eluted in the 1,000 to 5,000 dalton range (cf passage bridging pages 15 and 16). Thus, in the board's judgement, the content of the original application as a whole unambiguously indicates that the bone collagen-derived peptide fragments to be quantitated are those "of molecular weight less than 5000". Therefore, no objection under Article 123(2) EPC arises.

Article 84 EPC

4. It was submitted that the feature "of molecular weight less that 5000" per se is unclear if the method for its determination is not stated. It is generally true that whenever molecular weight data are provided for large molecules (eg proteins) the method of determination has to be indicated because the data can vary in relation
to the method used. However, in the present case the peptide fragments are small, and thus the method of determination is not critical as the molecular weights can already be calculated on the basis of their formula. Thus, no clarity objection is seen by the board.

Article 56 EPC (Claim 1)

The closest prior art and the background art

5. Document (17) is considered to represent the most appropriate starting point for the discussion of inventive step.

6. Document (17) is a general review about collagen cross-linking amino acids. The structures of the mature 3-hydroxypyridinium HP and LP cross-links are given (see Figure 1c on page 117). The section entitled "Special Applications" of the Chapter which from page 127 onwards deals more generally with the direct quantitation of hydroxypyridinium cross-links in tissue hydrolysates by high-performance liquid chromatography (HPLC) and fluorescence detection, is of particular relevance. It is reported on page 135 therein that the HP and LP content of urinary peptides can be measured after dialysis of urine in reduced porosity tubing (nominal 3500 mw cut-off) resulting in free HP-cross-links being discarded and hydrolysing the freeze-dried non-diffusate. As described under the heading "Resolution and Detection of Cross-Linked Peptides Containing HP and LP", the HP and LP content was measured by fluorescence detection in a procedure involving chromatography and HPLC assay. By reference to document (3) (cf reference 30 in the document), it
is indicated that, when the method was applied to the urine of patients with active Paget's disease, a great increase in the excretion of HP was found, confirming the previous results. It is stated that this presumably reflects the greatly increased bone turnover in the condition.

7. Document (3) (cf reference 30 in document (17)) describes an enzyme-linked immunoassay for the determination of HP, and reports an increased urinary excretion in patients with bone and joint disorders. The assay was based on the use of anti-pyridinoline sera (polyclonal antibodies) prepared against an antigen consisting of pyridinoline linked to bovine serum albumin (BSA). It is stated (see page 617, left-hand column, second paragraph) that the procedure was meant to provide "a unique index of the degradation of certain forms of mature collagen by analysis of physiological fluids". In the immunoassay, hydrolysed human urine was contacted with the antibodies.

8. The background art had already indicated that hydroxypyridinium cross-links were excreted in urine in peptide form, and that their measure could be a useful index of collagen degradation (cf eg documents (1) and (5)). Document (6) noted that a sensitive, enzyme-linked immunoassay of HP had been developed in document (3) (cf reference 14 in document (6)) which however could not distinguish the LP form of the crosslink (cf page 381, left-hand column).
Subject-matter encompassed by claim 1

9. Claim 1 contains the alternative "and/or" (cf Section VIII, supra). Thus, in its simplest form, one of the embodiments covered is a method comprising the quantification by immunological means in urine (e.g. of Page's disease patients) of the concentration of HP cross-link-containing peptide fragments of molecular weight less than 5,000.

Analysis of inventive step

10. The essential difference between this subject-matter and that of document (17) lies in the fact that, while in document (17) HP cross-links are determined by fluorescence after hydrolysis of non-diffused urinary peptides, claim 1 demands the use of immunological means in quantitating HP cross-link-containing peptide fragments of molecular weight less than 5,000.

11. No relevant difference is seen in the intended use of the method of quantification: claim 1 refers in general terms to "determining the rate of bone resorption", while document (17) refers to "bone turnover". In view of the generality of the terms, the skilled person would not see an essential difference between the two expressions as it was well-known that the elevated bone turnover characteristic of Paget's disease was associated with an excessive loss of bone material as a result of activity of osteoclasts. In fact, in this disease more bone than normal being resorbed by abnormal osteoclasts, bone has to be made faster by the body with the result of badly structured areas of bone. Thus, the person skilled in the art would have
understood that the measurement in document (17) of peptide-associated HP cross-links in urine, while generally reflecting bone turnover did reflect bone resorption. This view is confirmed also by the fact that other prior art documents have put the presence of hydroxypyridinium residues in urine in relation to collagen degradation (cf eg document (5), in particular page 725).

12. In view of the above considerations, the technical problem to be solved may be regarded as the provision of an alternative method for quantitating peptide-associated HP cross-links in urine, one solution being that outlined in point 9, supra.

13. As document (17) already indicated that fluorescent contaminants co-eluting with the HP cross-links rendered the chromatograms noisy, the option of changing from a fluorescent method to an immunological method was readily open to the skilled person, especially in view of the fact that the immunological approach applied to HP cross-links had already proved to be a valid alternative (cf documents (3) and (6)). Document (3) had based the immunological approach on the preparation of antisera against an antigen in which pyridinoline had been linked to BSA (cf point 7, supra). In the board's judgment, the skilled person, knowing that HP cross-links were present in urine in peptide form, would have readily come to the idea of using similarly peptide fragments isolated from urine as haptens in the preparation of sera to be used in immunoassays for the quantitation of HP cross-links in urine. This approach presented no technical difficulties of any kind and constituted an alternative
way which would have been regarded by the skilled person as offering a reasonable expectation of success. In this, the skilled person would have also expected the peptide fragments present in urine to be relatively small, being degradation products. Already document (1), for example, had given some indications in this respect (cf page 761, right-hand column). Thus, the feature "molecular weight less than 5000" cannot be seen as contributing in any way to an inventive step.

14. It is thus concluded that claim 1 encompasses at least one embodiment (cf point 9, supra) which lacks an inventive step. For this reason, the main request is not allowable under Article 56 EPC.

First auxiliary request

15. In comparison with claim 1 of the main request, claim 1 of this request contains the added wording "and which peptide appears in the elution profile of Figure 3a or Figure 3b". The appellants' view is that the request should not be admitted into the proceedings because it was late-filed and, moreover, it contained an unclear additional feature.

16. The board used its discretion and admitted the claim request into the proceedings in spite of it having been filed at a later stage. This was because the possibility had been given to the respondent during oral proceedings to try to overcome the objection of lack of inventive step which lead to the rejection of the main request.
17. A first question which arises is whether the feature introduced in the claim satisfies the clarity requirements of Article 84 EPC.

18. As stated in Rule 29(6) EPC, "claims shall not, except where absolutely necessary, rely, in respect of the technical features of the invention, on references to the description or drawings. In particular, they shall not rely on such references as: "as described in part ... of the description", or "as illustrated in figure ... of the drawings". Exceptions are occasionally made when - eg in the case of protein or DNA sequences - reference to the description or figures avoids the recitation in the claim of long sequences. These exceptions are however only possible when the feature which is meant is clear as required under Article 84 EPC.

19. This is not the case here. Figures 3a and 3b relate to a typical reverse phase HPLC natural elution profile of the aminoterminal telopeptides or carboxyterminal telopeptides, respectively, showing the location of the major peptide fragment containing 3-hydroxypyridinium cross-links. The volume of elution of the fraction containing the peptides fragments with "molecular weight less than 5000" can only be roughly guessed on the basis of the main peaks showing specific structures, no exact boundaries being given. Such elution profiles cannot be equated with an actual description of peptides present in a urine sample. They merely offer a presumption that peptides having fluorescence properties are present therein without providing any means to carry out a precise and reliable identification of said peptides. Such profiles, in
spite of their qualification as "typical" are the result of given experiments in a complex procedure which begins from urine collected from a pool of patients with Paget's disease and involves a series of chromatography steps. Said procedure is not described in detail. In particular, regarding the patients, no data are given which, such as the age, the sex, the stage of the disease, the diet, the moment in the day at which the urine samples were collected, all factors which might influence the concentration and nature of the urinary peptides to be determined. Also the detailed procedure of the chromatography steps is not given. The exact reproduction of an identical elution profile would hardly be possible.

20. For these reasons, the feature introduced in claim 1 is not clear. Consequently, the request is not allowable under Article 84 EPC.

Second auxiliary request

Preliminary remarks

21. Claims 1 to 10 of this request correspond exactly to claims 8 and 10 to 18 as granted. Added matter was not a ground on which any of the oppositions had been based. Thus, assessment of whether the requirements of Articles 123(2) and 84 EPC are met is not at issue. Novelty is not contested by the opponents. Nor does the board consider that any of the cited prior art documents affect novelty of the claim request (Article 54 EPC). Therefore, it remains to assess whether the claimed invention is sufficiently disclosed
(Article 83 EPC) and involves an inventive step (Article 56 EPC).

**Article 83 EPC**

22. The appellants consider that the immunological aspects of the claimed invention (see claims 2 to 10) are not sufficiently disclosed. Their reasoning is essentially based on the observation that the description lacks the necessary teaching for producing antibodies specific for a peptide fragment according to claim 1.

23. Claim 1 is directed to a peptide fragment comprising a given specific structure which is that of Formula III. Whereas it is true that no particular antibody is disclosed in the patent specification and that no reference is given to a deposit of any antibody producing-biological material, such as a monoclonal antibody-secreting hybridoma, the description provides the skilled person with useful information for the preparation of such material.

24. The relevant part of the description is the Chapter entitled "IMMUNOLOGICAL PROCEDURE FOR INDEXING BONE RESORPTION" (see from line 56 at page 8 to line 1 at page 10 in the patent specification). It contains the indication that both monoclonal and polyclonal antibodies specifically binding the peptide fragments are prepared by methods known in the art (see page 9, lines 2 and 3). It explains that, to serve as an immunogen, a peptide fragment is preferably conjugated to a carrier molecule, a preferred protocol involving a step of coupling the peptide fragment to keyhole limpet hemocyanin with carbodiimide (see page 9, lines 13 to
15). This "would ensure that most of the peptide fragment would be conjugated through the Gly carboxyterminus, thereby presented the preferred epitope, namely Tyr and 3-hydroxy pyridinium cross-link, to the primed vertebrate antibody producing cells (e.g., B-lymphocytes)" (see page 9, lines 15 to 18). To produce monoclonal antibodies, it is preferred that immunisation be carried out in the mouse. Suitable protocols of the prior art are specifically referred to (see page 9, lines 29 to 39). Immunometric assays in which the so produced immunological binding partners (monoclonal and polyclonal antibodies) are employed to quantitate the concentration of peptide fragments having 3-hydroxy pyridinium (HP and LP) cross-links derived from bone collagen resorption in body fluids are described (see page 9, lines 43 to 55). Suitable detectable markers are mentioned. Examples of standard immunometric methods are referred to. There is also the indication that, while it is preferred that the immunometric methods be conducted directly on untreated body fluids, occasionally, however, contaminating substances may interfere with the assay, necessitating partial purification of the body fluid. Suitable purification procedures are recommended.

25. In the board's judgement, such information is sufficient for the skilled person to prepare without undue burden by methods known in the art antibodies against a peptide according to claim 1 and hybridoma cells producing them, and thus to perform also the embodiments of all the claims.
26. None of the appellants, who bear the burden of proof, have put forward any evidence demonstrating that this is not possible or that there are such difficulties that undue burden is put on the skilled person.

27. As a matter of fact, document (27), a post-published document relied upon by the appellants, which describes a specific immunoassay for monitoring human bone resorption by quantitating type I collagen cross-linked N-telopeptides in urine, confirms that a monoclonal antibody can be prepared which specifically recognises a range of peptide fragments encompassed by claim 1 (see Figure 3 of the document). The specificity of the antibody was considered by the authors of document (27) to be sufficient to provide a convenient index of the rate of human bone resorption.

28. A further argument of the appellants is that, whereas as shown in document (24) pyrrole cross-links are also present in type I collagen of human bone, the description fails to describe an antibody which is capable of discriminating between peptide fragments containing a pyridinoline (HP or LP) cross-link and otherwise similar peptide fragments containing a pyrrole cross-link. The board notes that in document (24) only assays were reported which were carried out on human cortical bone excised from the femur and then experimentally digested with bacterial collagenase. Therefore, it cannot be deduced from that document that peptide fragments derived from bone collagen resorption are excreted in body fluids such as urine without being further metabolised which, while having the amino acid sequences of the formula represented in claim 1,
contain a pyrrole cross-link. Therefore, the appellants' argument is not accepted.

29. Thus, the board concludes that the immunological aspects of the claimed invention (see claims 2 to 10) are sufficiently disclosed and for this reason the second auxiliary request as a whole complies with the requirements of Article 83 EPC.

Article 56 EPC

30. In respect of the inventive step involved in the particular peptide fragment which constitutes the subject-matter of this request, three documents have been relied upon the appellants, namely documents (20), (19) and (37).

31. Document (20) is a general review discussing the structure and function of collagen types. Under the heading "The Cross-link Sites", a schematic representation of the sites is given (cf Figure 5 on page 20) which are stated to be characterised inter alia by the presence of the sequence Hyl-Gly-His-Arg (with Hyl standing for hydroxylysine). This sequence is not present in the peptide of claim 1. Moreover, none of the cross-links represented in Figure 5 shows the amino acid sequences of the peptide of claim 1. Thus, the said document does not represent anything which might be considered structurally close to the claimed peptide. Furthermore, the submission by the appellants that the collagen cleavage into two parts as reported on page 21 might result in a peptide fragment according to claim 1 is a mere allegation devoid of any technical support.
32. Document (19) is part of a general review about the chemical properties of collagen, and in Figure 3 on page 8, which was relied upon, provides a schematic representation of two cross-link sites of an $\alpha_1$(I) chain of collagen. No peptide fragments of any sequence are reported therein. Figure 4 on the same page provides a representation of the amino acid sequence of the nonhelical cross-link region of the $\alpha_1$(I), $\alpha_2$(I) and $\alpha_1$(III) chains at the N terminal end of calf skin collagens. Here also no peptide fragments are described containing a cross-link sequence which might be considered to be structurally close to the HP or LP cross-link sequence as depicted in claim 1.

33. Document (37) describes the isolation and characterisation of a collageneous trimeric cross-linked peptide from the insoluble matrix of bovine aorta. The primary structure of the peptide is represented on Figure 8 (see page 434). The peptide fragment, which is a C-terminal fragment, does not appear to have any structural similarity or proximity with the peptide fragment of claim 1.

34. The above analysis of the documents relied upon by the appellants shows that none of them is considered to be relevant for a discussion of inventive step.

35. In the board's judgement, the most appropriate starting point for an analysis of inventive step is document (17) (cf point 6, supra). In the light of this document, the technical problem to be solved can be seen as the identification of peptide fragments in a body fluid such as urine useful for assaying bone resorption rates,
the solution being the particular peptide fragment referred to in claim 1.

36. Although - as explained above in relation to the main request - the general idea of using peptide-associated HP cross-links of small molecular weight from urine in an immunoassay for the determination of the rate of bone resorption would have readily occurred to the skilled person, nothing in the art pointed to any particular structure which could be regarded as the objective or to any particular amino acid sequence(s) which would be present. Thus, neither document (17) alone nor in combination with any of the three documents relied upon by the appellants suggested to the skilled person the particular structure of the peptide fragment of claim 1.

37. For these reasons, the board concludes that the peptide fragment of claim 1 involves an inventive step. As all remaining claims are directed to aspects of the invention which is defined with reference to that peptide fragment, their subject-matter also involves an inventive step.

Amendments to the description

38. The respondent has proposed amendments to the description pages 2, 4 to 11 which have not been objected to by the appellants. The board considers that said amendments result in an appropriate adaptation of the description to the claims of the second auxiliary request and are in compliance with the requirements of Article 123(2) EPC.
Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The case is remitted to the opposition division with the order to maintain the patent with the following documents:

   - Claims 1 to 10 of the 2nd auxiliary request filed during the oral proceedings;

   - Amended description pages 2, 4 to 11, filed during the oral proceedings; page 3 of the description as granted;

   - Figures 1 to 4B as granted.

The Registrar: W. Wolinski

The Chairman: L. Galligani