In the "Monoclonal Anti-Ep" section, the erythropoietin preparation, purified by HIC, DIAC and RIAC, is used as an immunogen to generate hybridomas secreting monoclonal antibodies including, specifically, the antibodies produced by clone "7A7". At page 38, lines 3-16, the immunization protocol is described. The required performance characteristics are set forth with the notation:

> "These performance characteristics are rather formidable, considering the weak immunogenic properties of Ep. Accordingly, the EP used for immunization should be the purest possible and the number of mice immunized should be relatively large. Generally, assuming careful selection and execution of the immunization protocol, about one mouse in six immunized will exhibit an acceptable immune response."

While the P.N.A.S. paper refers generally to the 1975 Kohler and Milstein procedures, page 38, lines 28-33 refer to a different reference for the fusion procedure and also refer to "modifications" of the procedure as actually applied by Lee-Huang. As noted at page 39, lines 14-17, the 7A7-producing clone was one of three stable clones isolated from a total of 6460 hybridomas generated from a total of 10 fusions.* At page 41, lines 11-13, the 7A7 clone was noted as a 10-fold higher producer than the other two clones. In the published patent application, the 7A7 antibody was distinguished from the others by type (IgG2a/k as versus IgG1/k) but the P.N.A.S. reference is wholly silent on this matter.

Support for the above conclusion that the skilled worker would not have been able to prepare the pure immuno-

* This appears to be the type of "discovery" which has been characterized as patentable in Ex parte Old, 229 U.S.P.Q. 196 (Bd. App. & Int., 1985).

215 223

gen employed to generate the 7A7 antibody without "inventive skill" is found in the recent issuance of U.S. Letters Patent 4,568,488 to Lee-Huang (Applicant's Reference A-22) based on the "Ep Purification Patent Application" Serial No. 570,075. Presumably, the U.S. Patent Office was convinced that extraordinary skill -- indeed a patentable invention -- was involved in the preparation of highly purified erythropoietin having a specific activity of 66,000 U/mg as described in column 15, lines 43-45. (This activity corresponds exactly to that ascribed to the starting material used in immunizations for monoclonal antibody 7A7 preparation set out in the Lee-Huang P.N.A.S. publication.) It is not known whether Lee-Huang's Application Serial No. 570,039 is still pending, and whether it claims an invention in the specific 7A7 antibody. The clear implication derived from the published PCT application, however, is that extraordinary skill in the art would be needed to produce the antibody.

Additional support for the conclusion that the 7A7 antibody was not publicly available to skilled workers at the time of the P.N.A.S. publication is provided by Applicant's attached Declaration (Exhibit No. 4 hereto) relating to attempts to secure a sample of the antibody. As set out in the Declaration, Applicant recently sought to obtain a sample of the 7A7 antibody from Dr. Lee-Huang and, after a long delay, his request was denied upon his refusal to assure that it would be used only for personal purposes, unrelated to work for his employer. As of this day, the high affinity, 7A7 antibody whose use was asserted to be critical to the practice of the isolation procedures of the Lee-Huang P.N.A.S. article remains unavailable to the public at large.

A 7091

Because the record clearly demonstrates that the cDNA isolation work described in the Lee-Huang reference could not be duplicated without exercise of extraordinary skill, the publication is not properly urged as a bar to patentability of the present invention and the rejection of the claims under 35 U.S.C. §102(a)/103 may properly be withdrawn.

7. The Rejection of Claims 14, 15, 17-20, 24, 33, 34, 36, 58, 61-66, 69, 70 and 71 Under 35 U.S.C. §§102(a)/103 Based on Publications by Applicant and His Co-Workers May Properly Be Withdrawn

It was the Examiner's position that the prohibitions of Sections 102(a) and/or 103 dictate rejection of essentially all pending claims based on a 1984 Abstract jointly authored by Applicant and his co-workers [J.Cell. Biochem., Suppl. 8B, p. 45 (1984)]. As noted by Applicant's counsel in the Interview Outline, this Abstract and another of substantially the same caliber [appearing in Exp.Hematol., 12, 357 (1984)] do not provide any basis for ignoring Applicant's Declaration as to prior sole inventorship of the claimed subject matter filed in this and his "parent" applications dating back to December 13, 1983. Nonetheless, Applicant has attached hereto as Exhibit Nos. 5 and 6 two separate Declarations, consistent with the decision of In re Katz, 215 U.S.P.Q. 14 (CCPA, 1982), establishing sole inventorship of the claimed subject matter. Applicant submits that no basis exists for the continued rejection of claims 14, 15, 17-20, 24, 33, 36, 58, 61-66, 69, 70 and 71 based on Applicant's publications and that the outstanding rejection may properly be withdrawn.

A 7092

- 25 -

217 225

8. The Rejections of All Claims Under 35 U.S.C. §103 Based Variously on Sugimoto, et al., Cohen, et al., Paddock, Farber, et al., Bennetzen, et al., Gouy, et al., and Lewin May Properly Be Withdrawn

It was the Examiner's position that the subject matter of claims 14, 15, 17, 18, 20-24, 34-36, 58 and 61-72 was statutorily obvious upon consideration of Sugimoto, et al. (PTO Reference A, U.S. 4,377,513; Applicant's Reference A-8), in view of the disclosures of Paddock (PTO Reference B, U.S. 4,563,151; Applicant's Reference A-18), and Cohen, et al. (PTO Reference C, U.S. 4,468,464; Applicant's Reference A-17). The Examiner specific position was as follows:

> "Sugimoto, et al. teach cells from which erythropoietin RNA can be isolated, as they have a high erythropoietin production. Paddock teaches making cDNA from RNA, and Cohen, et al. teach cloning of a desired strand of DNA. Further, Sugimoto, et al. suggest that the erythropoietin gene could be so cloned. Thus it would be obvious to one or ordinary skill in the art to isolate and clone the erythropoietin gene, as the techniques for doing so are well known in the art and the expected result is obtained."

Claim 19, specifically directed to monkey species erythropoietin DNA, was rejected on the same grounds as above, in further view of the Farber, et al. Abstract appearing in Exp.Hematol. VII, Suppl. 4, Abstract 101 (PTO Reference T; Applicant's Reference C-32).

Finally, claims "25-30" (sic, 33?) were rejected based on Sugimoto, et al., Paddock and Cohen, et al., in further view of Bennetzen, et al. [J.Biol.Chem., 257(6), 3026-31 (1982); PTO Reference R[1]; Applicant's Reference C-133], Gouy, et al., [Nucleic Acids Res., 10, 7055-7074 (1982); PTO Reference U] and Lewin, [page 307 in Genes, John Wiley & Sons (1983); PTO Reference S[1]].

24/226

A 7093

Applicant respectfully disagrees with the Examiner's conclusions of obviousness for the claimed subject matter and affirmatively submits that the Examiner's position is without factual support. The references relied upon neither disclose nor suggest the making of the invention claimed. As set forth with greater particularity below, it is Applicant's position that the "primary" Sugimoto, et al. reference provides no teaching of a source for erythropoietin RNA and the "secondary" references by Cohen, et al. and Paddock are wholly uninformative with respect to the correct methods and materials means for identifying erythropoietin encoding genetic material so as to allow the present invention to be attained. The remaining, "tertiary" references fail to bar patentability because the primary and secondary references do not suggest the invention. Finally, Applicant submits that the Lee-Huang P.N.A.S. article appears to describe a "failed experiment" which constitutes evidence of patentability of the present claims.

Turning first to the Sugimoto, et al. reference, at the outset of its text it is noted that the alleged invention resides in means for obtaining erythropoietin from human lymphoblastoid cells in culture. Beginning at column 1, line 55, the reference proposes culturing any erythropoietin-producing lymphoblastoid cells and goes on to note that such cells could include "human lymphoblastoid cells in which there has been introduced the human erythropoietin genetic sites" from a variety of normal and neoplastic cells. The reference then states that:

> "These erythropoietin production governing genetic sites may be introduced by means of cell fusion using polyethylene glycol or

Sendai virus, or by genetic recombination techniques using DNA ligase, nuclease and DNA polymerase." (Emphasis supplied.)

It is thus clear that the authors of the Sugimoto, et al. reference do not suggest that lymphoblastoid cells are a potential source of genetic material. Rather, the cells are proposed as a host for insertion of "genetic sites" borrowed (e.g., by cell fusion) from other cell sources. There is no mention at all of RNA or of the reverse transcriptase enzyme needed to secure cDNA from messenger RNA. Contrary to the Examiner's position, then there is no suggestion in Sugimoto, et al. that the erythropoietin gene could be cloned, nor any "teaching" of "cells from which erythropoietin RNA can be isolated, as they have a high erythropoietin production".* Even if there had been a direct disclosure in Sugimoto, et al. of a potential source of "high levels" of erythropoietin-encoding messenger RNA (such as might be attributed, for example, to the Farber, et al. disclosure of isolated kidney cells from phenylhydrazine-treated baboons), it still remains the case that neither Sugimoto, et al. nor any of the secondary references disclose any means whatever for ascertaining which message (from among the innumerable "messages" present in the cells) would encode erythropoietin or which reverse transcript (cDNA) of such a message would encode erythropoietin.

* The Examiner will also recall that the "effect" of being able to isolate high levels of a secreted polypeptide from a culture may result from a variety of "causes" other than high levels of mRNA in the cells. As examples, one may note the possibility of more efficient translation of the same quantity of RNA, or of more efficient secretion of an essentially fixed quantity of RNA translation product, or even less efficient activity of destructive proteolytic enzymes in the cells or medium.

Even a most casual analysis of the secondary references to Cohen, et al. and Paddock reveals that the skilled worker can obtain no help from them in solving the problem of erythropoietin gene identification and isolation from erythropoietin-producing cells. One must note, for example, that the methods of the Cohen, et al. reference presume the independent existence of a means for identifying and isolating the "second DNA segment foreign to said cell and having at least one intact gene". Likewise, Paddock or any other reference which might address the successful "reverse transcription" of RNA to generate cDNA, also presumes either a means for isolating only the desired messenger RNA (prior to reverse transcription) or a means for isolating the desired cDNA sequences (following transcription and amplification of multiple messages).

Applicant submits that two separate pieces of evidence further support the conclusion that the Sugimoto, et al., Cohen, et al. and Paddock references do not "combine" to render the presently claimed invention obvious. The first source of evidentiary support is provided by consideration of the specific examples of the specification which describe how Applicant actually made his discovery. The second piece of evidence is provided upon analysis of the Lee-Huang reference previously distinguished.

It is highly pertinent to the issue of whether the cited references render Applicant's invention obvious that his isolation of DNA encoding human erythropoietin did not proceed by cDNA techniques attributed to the references and that his isolation of monkey cDNA encoding erythropoietin employed DNA/DNA hybridization methods and materials nowhere described or suggested by the references. As conspicuously

noted in the present specification, Applicant's isolation of human EPO-encoding DNA was by screening of a human genomic library of 1,5000,000 viral plaques in order to isolate what is now generally recognized as a single copy gene in the human genome. The improved methodology employed by Applicant is itself the subject of non-elected claim 60 which describes use of multiple mixed probes, specific substrates, protease enzyme treatment, specific probe concentrations and specific hybridization conditions. The success achieved by Applicant through practice of these procedures must be viewed in the context of the essentially concurrent pronouncement of the Anderson, et al. reference (Applicant's Reference C-2) that such screening methods are "...impractical for isolation of mammalian protein genes when corresponding RNA's are unavailable". It is also noteworthy that PCT Application 85/01961, published May 9, 1985 (Applicant's Reference B-15) relates the opinion of its authors that, as of its 1984 filing date (and perhaps as of the earlier priority dates listed), a patentable invention resided in mixed probe genomic library screening to isolate the human gene for Factor VIII:C. See, e.g., claims 21, through 31 of the application. The genomic library screening process employed by Applicant is nowhere "taught" in the cited reference.

As further conspicuously noted in the specification, Applicant's isolation of monkey cDNA involved hybridization screening as described above and the use of DNA probes whose sequences were determined by Applicant based on information unavailable from any published source, much less from the cited references to Sugimoto, et al., Cohen, et al. and Paddock.

A 7097

- 28 -

952 230

outside the scope of the disclosure of the cited references is thus believed to support the conclusion of non-obviousness of the claimed invention.

Applicant next submits that non-obviousness is still further established by analysis of the Lee-Huang P.N.A.S. publication. A substantial basis exists for concluding that none of the cDNA sequences whose isolation is reported in the Lee-Huang publication actually encoded the polypeptide sequence of erythropoietin and, therefore, that the work of the publication constitutes a "failed attempt" to clone and express DNA encoding human erythropoietin. The principal indicators of failure are found in the publication's reports concerning the size and restriction enzyme digestion characteristics of the cDNA inserts alleged to encode erythropoietin, and in its reports concerning the molecular weights of the products of in vitro translation of messenger RNA used to generate the cDNA inserts.

It will be recalled at the outset that the publication's author concluded that erythropoietin cDNA had been cloned and expressed on the basis of following types of experimental evidence:

(1) polypeptide products of in vitro translation of a particular (sized) fraction of mRNA isolated from human renal carcinoma cells were immunologically reactive with a monoclonal antibody ("7A7") against human erythropoietin;

(2) the size (M_r) of these polypeptide translation products was about 29,000 and 15,000, and the larger of these appeared to correspond in size to the molecular weight

A 7098

253 207

of "aglycosylated" erythropoietin as projected by the author*; and

(3) cDNA derived from the same fraction of mRNA provided for three "positive" clones which (a) upon "expression" provided β-lactamase fusion products immunoreactive with the 7A7 antibody, and (b) hybridized "back" to the mRNA fraction which had yielded the immunoreactive in vitro expression products.

While the publication purports to address the "cloning and expression" of human erythropoietin cDNA, no expression product of the cDNA was ever isolated and sequenced, nor were any of the cloned cDNAs analyzed for their nucleic acid sequence constitution. Restriction enzyme digestion of the three separate "positive" clones (designated by Lee-Huang as pEp1, pEp2 and pEp3) revealed that the three cDNA inserts had approximate sizes of 1,400 base pairs, 600 base pairs and 200 base pairs, respectively. It was Lee-Huang's conclusion (at page 2712) that:

> "Judging from the M_r of the native urinary Ep, the cDNA insert of pEp1 is probably close to the coding size, while those of clones pEp2 and pEp3 are too short to encode the complete sequence of Ep." (Emphasis supplied.)

Applicant respectfully submits that knowledge of the nucleic acid sequence of the human erythropoietin gene as provided by the present application and as substantiated by later analytical work (including the independent work of

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* Please refer to the publication text beginning with the second paragraph of the "Results" section on page 2709. Immunoreactive polypeptide product of in vitro mRNA expression in a system incapable of glycosylation were sized at M_r 29,000 and 15,000. A series of comparative tests using crude and purified natural EPO were said to confirm that the M_r 29,000 polypeptide "may represent the aglycosylated form of Ep."