COMMISSIONER'S DECISION

OBVIOUSNESS: Human Liver Cell Line

The cell line of claims 1 and 2 being the product of a chance formation, were held unpatentable.

FINAL ACTION: Affirmed in part: claims 1 & 2 were rejected. Amendments were suggested for the other claims.

This decision deals with a request for review by the Commissioner of Patents of the Examiner's Final Action dated July 9, 1973, on application 086,556 (Class 195-35). The application was filed on June 25, 1970, in the name of Kostadin Apostolov and is entitled "Human Liver Cell Line." The Patent Appeal Board conducted a Hearing on March 26, 1975, at which Mr. K.P. Murphy represented the applicant.

The application relates to a novel human liver cell line, and cultures thereof. A cell line is defined as: "An established cell culture having infinite replication potential in vitro when passaged regularly in a suitable environment; such cells are usually nondiploid (e.g. aneuploid or heteroploid) and undergo altered morphology changes." This cell line can be reproduced by growing in a culture in vitro, and is useful for growing viruses for use in making viral vaccines.

The examiner in the Final Action refused all of the claims. The reasons for such rejection are that the claims do not distinguish inventively from the teachings of Canadian patent 630,490 to Westwood, and that the cell line, being the product of a chance transformation, is unpatentable.

In that action the examiner stated in part:

> The reference describes a process for transforming human liver cells by treating parent liver tissue with trypsin to obtain a cell dispersion which is then subjected to

prolonged incubation in a standard nutrient medium, such as Parker's no. 199 or Eagle's medium, supplemented with a mammaliam serum. During the course of the incubation the cells undergo a change in their characteristics. The transformed cells, according to Westwood et al, "grow as compact islands of flat polygonal epithelial cells". Other described features are a dense granular cytoplasm, nuclei exhibiting 2-8 dark nucleolia and a high rate of proliferation. These characteristics and the method of production are essentially the same as those of the applicant's claimed cell line. The reference also describes the further cultivation of the transformed cells under conditions similar to those used in the transformation process and the more or less conventional process of propagating viruses on the said cells in association with a standard medium. Claims 3 to 13 are not distinguishable from these teachings.

The applicant has argued that his cell line is morphologically distinctive in view of the statement, on page 4 of the patent, indicating a resemblance between that cell line and HeLa cells from human uterine carcinoma. However the subsequent qualifying clause in the statement: "but morphology is influenced by the nature of the serum used"- indicates that the apparent shape of the cells is not an absolute criterion.

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Lastly, the applicant indicates in the disclosure on page 4 lines 20 to 26 that the method of obtaining the cell line is a chance occurrence and this is affirmed in his letters of May 19, 1972 and April 4, 1973. In order to be patentable, a product must, inter alia, be consistently producable by a replicable process and, unless an operator can be certain of obtaining the product whenever that process is conducted, the said product may not be claimed regardless of whether or not there are also claims to the process itself. Thus, even if the cell line were new, since it cannot be predictably produced from normal liver tissue, it may not be patented.

The response dated January 9, 1974 to the Final Action reads (in part):

Considering first the Examiner's objection that "the cell line, being the product of a chance transformation, is therefore unpatentable".

This objection is presumably directed only against the claims directed to the cell line per se. It is respectfully pointed out that Applicant is not claiming the method of producing the cell line; rather Applicant is claiming the cell line itself and methods involving the use of the cell line; these latter methods are completely reproducible.

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The claims of the application are directed to a product and its use. A sample of the product is deposited and obtainable from a public depository, and the method claimed, which

utilizes the cell line is the method of culturing the cell line to produce an end product identical with the starting material but in larger quantity, is completely reproducible.

Consequently, it can be seen that the invention claimed is clearly reproducible and when the period of monopoly of a patent on the application has expired the public will be able to make successful use of the invention.

Having regard to the foregoing comments, reconsideration of this objection is requested.

The remaining objection on which the rejection of the claims is based is that the claims do not distinguish inventively from the teachings of Canadian Patent No. 630,490.

As has been indicated above and in the Specification of the Application, the cell line of the present invention resembles in morphology and in biochemical activity the functional liver cells in vivo from which it is derived. At the same time, the cell line enjoys the benefit of being heteroploid in contrast to the diploid nature of the parent liver cells.

At this stage it is important to emphasize that the resemblance of the cell line of the invention to the hepatocytes of the normal liver in morphology and biochemical activity is surprising in view of the manifold and fundamental differences existing between the parent liver cells and the cell line. Usually this is not the case and generally cells in a cell line revert to a state of relative non-entity and lose the structural and functional specialization that characterized the parent cells in vivo (reference is here made to "Principles of Cell Culture" by D.O. White of the Department of Bacteriology, University of Melbourne, particularly at page 173, copy enclosed).

With the development of the cell line of the present invention, Applicant believes that there has been produced for the first time a human epithelial cell line retaining the morphology and biochemical activity of the parent liver cells in vivo, while enjoying the benefit peculiar to heteroploid cell lines of increasingly rapid multiplication and hence increase in the rate of mass production of virus and vaccines.

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The formation of the cell line of the invention is described in the disclosure at page 5 and comprises the steps of:

a) trypsinising human embryo liver tissue and

b) retaining the trypsinised tissue in Eagle's Minimum Essential Medium mixed with 10% bovine serum at 37°C for a few months.

> Considering now the cited Canadian Patent to Westwood et al, this is concerned with improvements in culture systems for the cultivation of viruses. The stated object of the invention is to provide a method of making a culture system from animal tissue for the population of viruses, especially from animal tissue from non-primate origin. However, the patent also describes the formation of culture systems from animal tissue of primate origin.
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> The Canadian Patent broadly describes the transformation of the parent tissue material by first forming a cell dispersion of the tissue by treatment with trypsin and/or versene followed by dispersion of the cell dispersion in a suitable nutrient medium. The pH of the medium is substantially neutral being from 7 to 7.8 and the cells are cultured.
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> The Canadian Patent indicates that the time of first appearance of transformed cells varies but that generally a minimum time of 25 days is required whereas the upper limit appears to be "as much as 70 days".
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> ...
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> In summary, it is emphasized that there is no evidence of deposition or existence of a human liver cell line derived by Westwood et al utilizing bovine serum and all attempts to obtain further data concerning such a cell line have met with failure. It cannot be accepted that the vague disclosure of the cell lines of Westwood et al which are almost certainly different from that of the present invention can be sufficient to anticipate the present invention.

The first question which the Board will consider is whether "the cell line is the product of a chance transformation and is therefore unpatentable."

Claim 1 and 2 relate to:

> A human epithelial heteroploid liver cell line, which forms individually separated islands or discrete clumps when cultured in a growth medium, has a morphology closely resembling that of hepatocytes of the human liver and a generation time not more than 24 hours, manifests increased production of glycogen in the presence of 1% glucose in the media, and is capable of supporting viruses.
>
> A human epithelial heteroploid liver cell line, as deposited with the American Type Culture Collection under accession number CL48.

It is noted that the applicant has not claimed the process of producing the products in claim 1 and 2.

It may well be that the applicant has developed a human epithelial heteroploid liver cell line which resembles in morphology and in biochemical activity the functional liver cells in vivo from which it is derived, while at the same time enjoying the benefit of being heteroploid in contrast to the diploid nature of the parent liver cells; and that the morphology and biochemical activity is "extremely surprising" in view of the manifold and fundamental differences existing between the parent liver cells and the cell line.

The development of a cell line may give rise to patentable subject matter satisfying the requirement of a new and useful invention under Section 2 of the Patent Act, provided the applicant can also satisfy the requirements of Section 36 of the Patent Act, which read in part:

> The applicant shall in the specification correctly and fully describe the invention and its operation or use as contemplated by the inventor, and set forth clearly the various steps in a process, or the method of constructing, making, compounding or using a machine, manufacture or composition of matter, in such full, clear, concise and exact terms as to enable any person skilled in the art or science to which it appertains, or with which it is most closely connected, to make, construct, compound or use it .... (emphasis added)

It is clear from the section that every specification must set forth the invention in "such full clear concise and exact terms" as to enable any person skilled in the art "to make, construct, compound or use it."

The applicant argues that he should be permitted to claim the "cell line." However, as shown from the following quote from page 3 of the disclosure at line 20, the cell line is the product of a chance transformation: "Although the indicated public availability (recognized culture collections) is the simplest method for obtaining a cell line according to the present invention, it is not altogether impossible or improbable that similar and functionally substantially identical human epithelial heteroploid liver cell lines might be produced by other methods or similar unexpected chance occurrences."

The applicant also argues that he has taught how to use the cell line (with which we agree), and how to make it (by culturing the newly developed cell line). In our view this is not enough. He must also teach persons skilled in the art how to make it from the original source by mutation of human cells. That he cannot do, since the mutation was admittedly an unexpected chance occurrence. The only method he can teach to make the cell line (reproductive culturing) presupposes and pre-requires the existence of the cell line. In other words the cell line was already in existence through a fortuitous circumstance before the applicant did anything which could be considered an invention. There is no inventive step in the chance occurance of the product of claims 1 and 2.

The examiner did not raise an additional objection to claim 1 which we would like to have seen explored. That is whether it is proper and possible to patent a living organism. Since that objection was not made, and since there are other reasons to refuse claim 1, we will not pursue it further.

The applicant points out (and we agree) that "the ability to obtain patent protection is a prerequisite for the encouragement of continuing research in this new technology from which the benefits to mankind may be almost unlimited." This incentive, however, can be provided by claims other than 1 and 2.

We are satisfied therefore that the applicant is not entitled to per se patent protection for the alleged invention as defined by claims 1 and 2. In our view they do not relate to patentable subject matter. They fail to comply with Section 36 of the Patent Act.

The next issue to decide is whether the subject matter of claims 3 to 13 distinguish inventively from the Westwood reference. There is no question

but that the subject matter of these claims complies with the requirements of Section 36. The starting materials have been made readily available (the cell line from a culture collection), and the process claimed, when worked, will produce the desired result in a man-made and controllable manner. Claims 3, 11 and 13 are representative:

Claim 3 — A method of culturing a heteroploid human epithelial liver cell line, as defined in claim 2, which comprises maintaining the cells in a nutrient culture medium.

Claim 11 — A method for cultivating viruses, which comprises inoculating a cell line as defined in claim 1, with a virus to which the cells are susceptible, and culturing the cell line in a nutrient culture medium.

Claim 13 — A virus culture, comprising a cell line or culture, as defined in claim 1 or 8, infected with viruses to which the cells are susceptible, in association with a nutrient culture medium.

The Westwood citation relates to the treatment of liver from a human foetus (as a specific example), treating the liver with trypsin, and cultivating for 33 days in standard medium containing 10% of human serum. Claim 1 of the Westwood patent defines:

> A culture system for the cultivation of viruses comprising viable transformed cells derived by culturing cells derived from mammalian tissue in a nutrient medium, said transformed cells being characterized by being generally small and polygonal in shape, possessing phase contrast illumination dense granular cytoplasm with generally circular nuclei of granular appearance, generally exhibiting 2-8 dark nucleioli, said cells being poorly phagocytic for carbon particles and producing generally unorientated growth.

It is noted that the Hlil cell line of Westwood resembles the HeLa cells. The Westwood disclosure at line 19, column 7, reads: "In appropriate media the cells closely resemble HeLa cells but morphology is influenced by the nature of the serum used."

The Declaration and Affidavit of Dr. Bauer, an expert in this field, which affidavit was presented at the hearing, establish that the

Apostolov cell line of the present application is quite different from that of the HeLa line. The following points of distinction can be made (as quoted from Dr. Bauer):

i) Colonial morphology - the Apostolov line forms individually separated islands or discrete clumps, typical of the liver lobules of the liver, whereas the HeLa line forms sheets of cells as in all typical tissue culture systems. (This is further borne out by P 707 line 12 of J. Exp. Med. op. cit which mentions cellular sheets;).

ii) Peroxisomes - these are large and numerous in the Apostolov line indicating a metabolic state close to the normal liver, whilst they are absent in the HeLa line;

iii) Mitochondria - in the Apostolov line these are thick-walled and have numerous cristae indicating a state of intense functional activity as in the patent cells; a low or restricted activity is indicated for the HeLa line in view of the thin walled mitochondria with few cristae;

iv) Pinosomes - these are not present in the patent cells nor in the Apostolov line but they are in the HeLa line, where they move from the periphery of the cytoplasm towards the nuclear area.

v) Endoplasmic reticulum - this is abundant in the Apostolov line, as in the parent cells, but only scanty in the HeLa line;

vi) Membranous extensions e.g. microvilli - these are present in great numbers on the surface of the cells in the HeLa line, but are not present in either the Apostolov line or the parent cells.

vii) Stored Glycogen - this is present in the Apostolov line and in the liver although not in the HeLa line (see later).

viii) Generation time - this is, by definition, no more than 24 hours with the Apostolov line, whilst the HeLa line is known to multiply only 15-fold in 7 days, (see ATCC Handbook) giving a considerable longer generation time for each doubling of the cell population.

In view of the fact that the HeLa line, by admission, is comparable to Westwood's Hlil cell line we are persuaded that the Westwood citation is not relevant to the Apostolov cell line. The examiner, after reviewing the evidence presented at the Hearing, was also persuaded that the Westwood citation should be withdrawn.

However we are not satisfied that Claims 3 and 8 are allowable in their present form. Claim 3 presently covers a broad method of culturing the cell line of claim 2. The cell line culture has no inherent utility other than to reproduce said cell line in greater quantities or to grow viruses therein or isolate metabolic products therefrom. The applicant did however discover an unexpected result. The morphology and biochemical activity of the new cell line is "extremely surprising". We would, therefore, allow the applicant a method of use claim on the same basis as if he discovered an unexpected utility of a known compound. Claim 3 would therefore be accepted if amended to read: "... in a nutrient culture medium <u>for the production of glycogen or enzymes</u>." Claim 8 would also be accepted if amended in the same manner. These claims will represent the novel and practical application of a new discovery.

We note that the applicant was successful in obtaining claims to the cell line in the United Kingdom, Switzerland and some other countries, but we are satisfied that claims 1 and 2 do not relate to subject matter patentable in Canada, and should be refused. We also recommend that the Westwood citation be withdrawn.

J.F. Hughes,
Assistant Chairman,
Patent Appeal Board.

I concur with the findings of the Patent Appeal Board and refuse to grant a patent for claims 1 and 2. The Westwood citation is withdrawn. The applicant has six months within which to delete claim 1 and 2, and make an appropriate amendment to claim 3 and 8 along the guide lines suggested, or to appeal this decision under the provisions of Section 44 of the Patent Act.

Decision accordingly,

A.M. Laidlaw,
Commissioner of Patents

Dated at Hull, Quebec
this 15 day of August, 1975.