Commonwealth of Australia
The Patents Act 1952
DECLARATION IN SUPPORT

In support of the ~~(Convention)~~ Convention Application made by: DAVID CULLIS-HILL

for a patent for an invention entitled: Preparation of hyaluronic acid

I (We) DAVID CULLIS-HILL

~~of and are the applicant company~~ do solemnly and sincerely declare as follows:

a) I am (~~We are~~) the applicant(~~s~~) for the patent

or

b) ~~I am (We are) authorised by the applicant(s) for the patent to make this declaration on its behalf.~~

Delete the following if not a Convention Application.

~~The basic application(s) as defined by section 141 (142) of the Act was (were) made~~

on in

on in

on in

by

The basic application(s) referred to in this paragraph is (are) the first application(s) made in ~~a Convention country in respect of the invention the subject of the application~~.

a) I am (~~We are~~) the actual inventor(~~s~~) of the invention.

or

b)

~~is (are) the actual inventor(s) of the invention and the facts upon which~~

~~is (are) entitled to make the application are as follows:~~

The basic application as defined by section 141 of the Act was made on 9 May 1985 in Australia by DAVID CULLIS-HILL

The basic application referred to in this paragraph is the first application made in a Convention country in respect of the invention the subject of the application

Declared at SYDNEY this 31st day of December 19 86

Signed [signature] Status Applicant/Inventor

Declarant's Name DAVID CULLIS-HILL

F. B. RICE & CO PATENT ATTORNEYS

This form is suitable for any type of Patent Application. No legalisation required.

(12) PATENT ABRIDGMENT (11) Document No. AU-B-58198/86
(19) AUSTRALIAN PATENT OFFICE (10) Acceptance No. 593556

(54) Title
HIGH PURITY HYALURONIC ACID FROM SYNOVIAL FLUID

International Patent Classification(s)
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(56) Prior Art Documents
GB 1088304

(57) Claim

1. A method of preparing high purity hyaluronic acid with a protein impurity of less than or equal to 0.5% w/w from synovial fluid comprising the steps of:-

(a) removing high molecular weight impurities, if present, in a sample of synovial fluid;

(b) dissolving a metallic salt and a free radical inhibitor in the synovial fluid;

(c) precipitating hyaluronic acid and protein by adding sufficient of a water miscible alcohol to said synovial fluid;

(d) separating the precipitate from step (c) and dissolving the hyaluronic acid contained therein in a solution in which the protein is insoluble;

(e) adding an effective amount of a free radical inhibitor to the step (d) solution;

(f) digesting the protein by treating said solution with an effective enzyme at a temperature of 37°C for a period of from 2 to 3 hours; and

(g) recovering hyaluronic acid.

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PCT

WORLD INTELLECTUAL PROPERTY ORGANIZ
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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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C08B 37/08	A1	(43) International Publication Date: 20 November 1986 (20.11.86)

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(81) Designated States: AT (European patent), AU, BE (European patent), CH (European patent), DE (European patent), FR (European patent), GB (European patent), HU, IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent), US.

Published
With international search report.

(54) Title: PREPARATION OF HYALURONIC ACID

(57) Abstract

A method for preparing high purity hyaluronic acid comprising treating a proteinaceous solution containing hyaluronic acid to precipitate protein and said hyaluronic acid from the solution, denaturing the protein, adding the hyaluronic acid and denatured protein to a solution in which the hyaluronic acid is soluble, digesting the protein and recovering hyaluronic acid.

PREPARATION OF HYALURONIC ACID

The present invention relates to a method for the preparation of hyaluronic acid and more particularly to a method for producing high purity hyaluronic acid derived from the synovial fluid of selected animal species.

Hyaluronic acid is known to occur in many animal species including man. In the majority of animals, it is widely distributed in tissues and extra cellular fluid. In particular, in man, it is found in synovial fluid, the vitreous humour of the eye, Wharton's jelly of the umbilical cord and articular-bone cartilage. It also occurs in small quantities in the connective tissue of animals and in some micro-organisms.

It is to be noted that hyaluronic acid is a mucopolysaccharide and as such, the molecular weight of the compound varies between animal species and also within a single animal species depending on its location in the tissues.

Hyaluronic acid is known to perform a number of functions in man and other animals including the lubrication of the joints, the maintenance of the gel-like character of the vitreous humour of the eye and the contribution to the ground substance around cells where it functions as an inter-cellular lubricant and flexible cement.

In man, hyaluronic acid has been used to maintain the hydration and condition of the eye during various surgical procedures such as corneal grafts. More recently, because of its joint lubricant function, investigations have been directed in an attempt to elucidate its potential to alleviate the imflammatory joint conditions such as arthritis. In animals such as the horse, it is currently used as a method of treatment of imflammatory joint conditions.

In addition, because it is known to be a constituent

of the ground substance of cells, hyaluronic acid is being incorporated into various cosmetic preparations for the skin. In this role it is proposed that the addition of hyaluronic acid to the skin is able to raise the level of hyaluronic acid present in the cells coats in the dermal layers thereby improving the condition of the skin.

As used in this specification, "hyaluronic acid" refers to the acid and to its metallic salts.

In the prior art, the methods of preparation of the hyaluronic acid have been directed towards extraction from the tissue sources in which hyaluronic acid occurs in high concentration in man and other animal species. In particular, the coxcomb of the chicken and the human umbilical cord have been identified as suitable sources. However, the combination of these sources and the many steps required to isolate hyaluronic acid in the prior art have resulted in the cost of hyaluronic acid being exceptionally high. Further the present inventor believes that some of the prior art sources and preparation methods may result in a hyaluronic acid compound which has an unsatisfactory biological activity in man. It is postulated that the unsatisfactory activity is due to methods of preparation which disrupt the composition and structure of the molecule unduly, including producing a high level of protein impurity and a hyaluronic acid of unsatisfactory molecular weight.

Representative of the prior art is US 4,141,973 (Balazs). This discloses a long and complex procedure for the isolation of "ultrapure" hyaluronic acid using coxcombs and human umbilical cords as the hyaluronic acid containing starting material. The basic steps in the disclosed process for the production of hyaluronic acid are

(1) Cleaning and freezing starting material, slicing the frozen material and extracting the material with 95% ethanol

(2) Extracting the material with water and chloroform

(3) Addition of sodium chloride to the water/chloroform extracts, rejection of chloroform phase; acidification of aqueous phase to pH4-5 followed by extraction with chloroform; as an alternative to chloroform extraction, DNase or RNase enzymes may be used

(4) Adjust pH to 6.0-7.0 and extract aqueous phase with chloroform; reject chloroform; centrifugation at 70,000 to 110, 000g may also be used; and

(5) Filtration of aqueous phase through 0.2 micron filter, followed by a series of precipitations of hyaluronic acid using ethanol followed by solution into sodium chloride solution; acetone is also used as a precipitant and washing agent to obtain the desired purity of hyaluronic acid.

The present inventor has realised that the synovial fluid of some animal species represents a potentially economical source of hyaluronic acid. Such suitable animal species include sheep, cattle and pigs. By coupling such a source with a method of extraction that does not unduly damage the molecule, the present invention is able to produce a high purity hyaluronic acid having a molecular weight suitable for use in man. By high purity hyaluronic acid, it is meant hyaluronic acid having not more than five micrograms of protein per miligram of hyaluronic acid when determined by the Lowry method.

The present invention provides a method for preparing high purity hyaluronic acid comprising treating a proteinaceous solution containing hyaluronic acid to precipitate protein and said hyaluronic acid from the solution, denaturing the protein, adding the hyaluronic acid and denatured protein to a solution in which the hyaluronic acid is soluble, digesting the protein and recovering hyaluronic acid.

The present invention provides a method for preparing high purity hyaluronic acid with a protein impurity of less than or equal to 0.5% w/w from synovial fluid comprising the steps of:-

(a) removing high molecular weight impurities, if present, in a sample of synovial fluid;

(b) dissolving a metallic salt and a free radical inhibitor in the synovial fluid;

(c) precipitating hyaluronic acid and protein by adding sufficient of a water miscible alcohol to said synovial fluid;

(d) separating the precipitate from step (c) and dissolving the hyaluronic acid contained therein in a solution in which the protein is insoluble;

(e) adding an effective amount of a free radical inhibitor to the step (d) solution;

(f) digesting the protein by treating said solution with an effective enzyme at a temperature of 37°C for a period of from 2 to 3 hours; and

(g) recovering hyaluronic acid.

~~A preferred~~ The source of hyaluronic acid is synovial fluid obtained from the joints of suitable freshly slaughtered animals. Those animals that are suitable include cattle, sheep and pigs. Any method may be used to obtain the fluid but it is preferably accomplished using a syringe and a suitable cannula. Typically, one obtains approximately 20 mL of synovial fluid from the carpal joints of a beast the concentration of hyaluronic acid in the synovial fluid being of the order of from 1.5% to 4.0% w/v.

Depending on the source of hyaluronic acid, it may be advantageous to carry out a purification step prior to using the method of the invention. Thus, in the case of synovial fluid, following its collection, it is preferably stored at 5°C for a week prior to any further processing. This refrigeration step facilitates the removal of high molecular weight impurities.

Thus a proteinaceous hyaluronic acid solution may be obtained by centrifuging the previously refrigerated synovial fluid preferably at 2000 rpm wherein the high molecular weight impurities are spun down. This allows the proteinaceous hyaluronic acid solution to be poured off for further processing.

It is to be noted that alternative methods may be used to remove such impurities, the methods being tailored to suit the source of hyaluronic acid and the nature and level of the high molecular weight impurities.

In a preferred embodiment of the invention, synovial fluid after having been stored at 5°C for a week, is filtered using glass wool to remove the high molecular weight impurities.

The proteinaceous hyaluronic acid solution may be treated directly with a substance to cause precipitation of the protein and hyaluronic acid.However, ~~it is preferred that~~ prior to this treatment,the proteinaceous

hyaluronic acid solution has added to it a metallic salt soluble in said solution. The salt is added to the proteinaceous hyaluronic acid solution to produce preferably a concentration of salt in the range of from 2 to 3 molar. In a preferred embodiment of the invention, the metallic salt is sodium chloride.

A free radical inhibitor is added to the proteinaceous hyaluronic acid solution as well as a metallic salt. A preferred inhibitor is dimethylsulfoxide, preferably in a concentration of from 2 to 3% v/v.

Preferably, the denaturation of the proteinaceous hyaluronic acid solution, using the aforementioned acetic acid and absolute ethanol solutions, is carried out with boiling of the solutions at a temperature of about 85°C.

hyaluronic acid solution has added to it a metallic salt soluble in said solution. The salt is added to the proteinaceous hyaluronic acid solution to produce preferably a concentration of salt in the range of from 2 to 3 molar. In a preferred embodiment of the invention, the metallic salt is sodium chloride.

In another embodiment, a free radical inhibitor may be added to the proteinaceous hyaluronic acid solution as well as a metallic salt. A preferred inhibitor is dimethylsulfoxide, preferably in a concentration of from 2 to 3% v/v.

In another embodiment, where a free radical inhibitor has been included in the proteinaceous hyaluronic acid solution, the step of denaturation using the aforementioned acetic acid and absolute ethanol solutions is carried out with boiling of the solutions at a temperature of about 85°C.

The solution which includes the precipitate containing protein and hyaluronic acid is treated with a protein denaturing agent. Preferably, the denaturant includes a water miscible alcohol but other denaturing agents may be used, for example heat. Where alcohol is the denaturant, it is preferred that absolute ethanol is used. The denaturing agent may, however, include an acid together with an alcohol, at a concentration providing that the pH of the solution containing precipitated protein and hyaluronic acid, after the denaturing agent has been added, remains at not less than 5 and/or providing that the viscosity of the precipitated hyaluronic acid remains substantially unaltered after the denaturing agent has been added to the solution.

In a preferred embodiment, the denaturing agent includes a 2% solution of glacial acetic acid in absolute ethanol. In a particularly preferred embodiment, two volumes of a solution containing 2% glacial acetic acid in

absolute ethanol is mixed with one volume of the solution containing the protein to be denatured.

It is to be noted that the steps of precipitation and protein denaturation may be accomplished simultaneously. For example,in those embodiments of the invention where an alcohol is used as the denaturant, precipitation of the protein and hyaluronic acid will also occur.

It is preferred that following the steps of precipitation and denaturation, the precipitate, which is in a stringy form, is separated from the solution by decanting off the solution, teasing the precipitate and allowing any excess solution to drain from the precipitate. At the end of this step, the level of alcohol, if used as the denaturant, should be present only as a trace. In some circumstances, the precipitate may include some metallic salt if it has been used to treat the proteinaceous hyaluronic acid solution prior to the step of precipitation. If this is present, at this stage it is preferred that it is removed by washing the precipitate, preferably with a solution that includes by volume 2% glacial acetic acid, 65% of an alcohol and 33% of a buffer having a pH of 7. The precipitate is than treated as described above.

Following the separation of the precipitated protein and hyaluronic acid, it is preferred that it is added to a solution in which hyaluronic acid is soluble and substantially stable. Preferably the solution is a buffer having a pH of 7. The hyaluronic acid is caused to dissolve in the solution whilst the protein being insoluble will be present in the solution as a milky precipitate.

Once the hyaluronic acid is in solution, it is preferred that the protein is degraded using the technique of protein digestion. The protein digestion step preferably comprises treating the hyaluronic acid solution

containing the precipitated denatured protein with an enzyme capable of causing the breakdown of the denatured protein. Preferred enzymes include trypsin and pronase. ~~It is also preferred that a~~ A free radical inhibitor is added to the solution to ensure that no degradation of the hyaluronic acid molecule occurs. A preferred free radical inhibitor is sodium azide in a concentration of 0.05 molar.

Generally the protein digestion is carried at a temperature of about 37°C for a period of from 2 to 3 hours or until the milkiness in the solution due to the protein has been removed, the solution being substantially clear.

Following the protein digestion step it is preferred that the steps of precipitation of protein and hyaluronic acid, protein denaturation and dissolution of hyaluronic acid as hereinbefore described are repeated. At this stage, it is estimated by the present inventor that approximately 5%w/w of protein remains associated with the hyaluronic acid.

In a preferred embodiment, so obtained hyaluronic acid is further treated by centrifugation at 5,000 to 10,000g, followed by filtration of the supernatant preferably through a filter having a pure size of 0.22 microns. The filtrate is then treated as outlined below.

To reduce the level of protein further, it is preferred that the steps of precipitation of protein and hyaluronic acid, protein denaturation and dissolution of hyaluronic acid as hereinbefore described are repeated. At this stage, it is estimated that the level of protein associated with the hyaluronic acid is less than 0.5%w/w.

It will be appreciated that the aforementioned steps may be repeated as required to obtain hyaluronic acid of even greater purity.

Once hyaluronic acid is obtained of desired purity, its concentration may be conveniently adjusted in solution

by taking precipitated hyaluronic acid and dissolving it in the desired solvent to produce the required concentration. It is important to note that during all stages of the process of the invention, the hyaluronic acid must remain adequately hydrated, otherwise it may degrade to produce material having an unacceptable biological activity.

The present process in its preferred embodiments may be distinguished over the prior art methods through its use of a ready source of hyaluronic acid namely synovial fluid, and the recognition that protein may be effectively removed using the steps of precipitating hyaluronic acid and protein, denaturing and digesting the protein in the presence of a free radical inhibitor without damaging the hyaluronic acid molecule and repeating precipitation and denaturation until hyaluronic acid of the desired purity is achieved.

The relatively few steps used in the process of the invention together with the relatively rapid production time, with attestant cost advantages, are in marked contrast to the method disclosed in the aforementioned US 4,141,973 (Balazs).

Hereinafter by way of example only is a preferred embodiment of the present invention:-

Hyaluronic acid of high purity was prepared as follows:

One litre of synovial fluid was obtained from the joints of cattle. It was stored at $5^{o}C$ for one week and following the storage period, the synovial fluid was filtered through glass wool. To the filtrate was added sufficient sodium chloride to produce a concentration of 2 molar.The protein and hyaluronic acid present were then precipitated and the protein denatured in a single step.

This was accomplished by adding to the solution absolute ethanol containing 2% acetic acid, in a ratio of

2 volumes of 2% acetic acid ethanol solution to every volume of solution. The solution then contained precipitated hyaluronic acid and precipitated denatured protein.

The precipitate was separated from the solution by decanting and draining, the precipitate being teased apart to facilitate dissolution of the hyaluronic acid. Dissolution of the hyaluronic acid was effected in pH7 phosphate buffer. However, the protein remained present in solution as a milky precipitate.

Once the hyaluronic acid was in solution, in order to remove protein, 5 mg. of trypsin and sufficient sodium azide to produce a concentration of 0.05 molar in the solution was added to the buffer solution containing the dissolved hyaluronic acid. The digestion was carried out at 37°C for two hours when the solution became clear indicating the completion of the reaction.

Once the protein digestion was complete, sufficient sodium chloride was added to produce a concentration of 2 molar followed by two volumes of 2% glacial acetic acid in ethanol for every volume of hyaluronic acid solution when the hyaluronic acid and some of the low molecular weight protein components were precipitated.

The precipitate was removed from solution by decanting and draining, the precipitate being teased apart to facilitate dissolution in pH7 phosphate buffer.

The above steps of precipitation with salt and 2% glacial acetic acid in ethanol following by dissolution and pH7 buffer were repeated.

The resultant hyaluronic acid solution was then evaluated by the Lowry method (Lowry et al., J. Biol. Chem. 193, 265-275 (1951) and found to contain less than 5 micrograms of protein per milligram of hyaluronic acid.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:-

1. A method of preparing high purity hyaluronic acid with a protein impurity of less than or equal to 0.5% w/w from synovial fluid comprising the steps of:-

(a) removing high molecular weight impurities, if present, in a sample of synovial fluid;

(b) dissolving a metallic salt and a free radical inhibitor in the synovial fluid;

(c) precipitating hyaluronic acid and protein by adding sufficient of a water miscible alcohol to said synovial fluid;

(d) separating the precipitate from step (c) and dissolving the hyaluronic acid contained therein in a solution in which the protein is insoluble;

(e) adding an effective amount of a free radical inhibitor to the step (d) solution;

(f) digesting the protein by treating said solution with an effective enzyme at a temperature of 37°C for a period of from 2 to 3 hours; and

(g) recovering hyaluronic acid.

2. A method as claimed in claim 1, wherein the synovial fluid has been stored for a week at 5°C.

3. A method as claimed in claim 2, wherein after storage the synovial fluid is centrifuged to remove high molecular weight impurities.

4. A method as claimed in claim 2, wherein after storage the synovial fluid is filtered through glass wool to remove high molecular weight impurities.

5. A method as claimed in any one of claims 1 to 4, in which sufficient metallic salt is added to the synovial fluid to produce a concentration in the synovial fluid of from 2 to 3 molar.

6. A method as claimed in claim 5, the metallic salt being sodium chloride.

7. A method as claimed in any one of claims 1 to 6, the free radical inhibitor used in step (b) being dimethylsulfoxide in a concentration of from 2 to 3% v/v.

8. A method as claimed in any one of claims 1 to 7, wherein the water miscible alcohol is absolute ethanol.

9. A method as claimed in claim 8, wherein an acid is included with the ethanol in a concentration such that the pH of the solution after the addition of the ethanol and acid is not less than 5.

10. A method as claimed in claim 9, wherein the acid is glacial acetic acid in a concentration of 2% in absolute ethanol.

11. A method as claimed in claim 10, wherein two volumes of said acetic acid solution is mixed with one volume of synovial fluid.

12. A method as claimed in claim 11, wherein the solution is boiled following the addition of acid and ethanol.

13. A method as claimed in any one of claims 1 to 12, wherein the precipitate is separated from the solution by decanting off the solution, teasing the precipitate and allowing excess solution to drain therefrom.

14. A method as claimed in claim 13, wherein the separated precipitate is washed with a solution comprising by volume, 2% glacial acetic acid, 65% of an alcohol, and 33% of a buffer having a pH of 7.

15. A method as claimed in claim 13, wherein the separated denatured protein and hyaluronic acid is soluble.

16. A method as claimed in any one of claims 1 to 15, the enzyme being selected from the group consisting of

trypsin and pronase.

17. A method as claimed in claim 16, in which a free radical inhibitor is included in the solution to be digested.

18. A method as claimed in claim 17, the free radical inhibitor being sodium azide in a concentration of 0.05 molar.

19. A method as claimed in any one of claims 1 to 18, wherein after protein digestion is complete, steps (b)-(f) are repeated on the digested solution.

20. A method as claimed in any one of claims 1 to 19, wherein after completion of step (f) the digested solution is centrifuged at 5,000 to 10,000 g, and the supernatant obtained therefrom is filtered through a 0.22 micron filter.

21. A method as claimed in claim 20, wherein the steps (b), (c), (d), (e) and (f) are repeated on the filtered solution.

22. A method as claimed in any one of claims 1 to 21, wherein the hyaluronic acid in step (g) is recovered by precipitation.

23. A method as hereinbefore described with reference to the example.

24. High purity hyaluronic acid having a protein impurity of less than or equal to 0.5% w/w when prepared by a method as claimed in any one of claims 1 to 23.

DATED this 21 day of November 1989

DAVID CULLIS-HILL
Patent Attorneys for the
Applicant:

F.B. RICE & CO.

INTERNATIONAL SEARCH REPORT

International Application No PCT/AU86/00129

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int. Cl.[4] C08B 37/08

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC	C08B 37/08, 19/14

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

AU: IPC as above

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No [13]
X	GB, A, 1088304 (ETAPHARM CHEM. PHARM. LABORATORIUM GmbH) 25 October 1967 (25.10.67)	(1,2,20)
Y	US, A, 3862003 (OKUYAMA et al) 21 January 1975 (21.01.75) See Column 6	(1,6,11)
Y	US, A, 2583096 (HADIDIAN et al) 22 January 1952 (22.01.52)	(20-22,24)
A	JP, A, 58-037001 (GREEN CROSS CORP) 4 March 1983 (04.03.83)(Derwent English Language Abstract B04 35542 K/15)	(1)
A	US, A, 2585546 (HADIDIAN et al) 12 February 1952 (12.02.52)	(1)
A	US, A, 4141973 (BALAZS) 27 February 1979 (27.02.79)	(1,2)
A	AU, A, 35806/84 (MILES LABORATORIES INC) 30 May 1985 (30.05.85)	(1,2,20)
A	AU, A, 34148/84 (FIDIA S.p.A) 18 April 1985 (18.04.85)	(1,2,20)
A	CH, A, 518718 (L'OREAL) 30 March 1972 (30.03.72)	(1,20)

* Special categories of cited documents: [15]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
8 Aug 1986 (08.08.86)	26 AUGUST 1986 (26.08.86
International Searching Authority	**Signature of Authorized Officer**
AUSTRALIAN PATENT OFFICE	R.M.F. BOYS

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

A	FR, A, 1358465 (MALGOUZOU) 9 March 1964 (09.03.64)	(1)
A	JP, A, 58-084801 (GREEN CROSS CORP) 21 May 1983 (21.05.83)(Derwent English Language Abstract B04 62371K/26)	(1,6,11)
A	SU, A, 950935 (SARAT UNIV) 15 August 1982 (15.08.82) (Derwent English Language Abstract B04 63185K/26)	(1,11)

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers ....., because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claim numbers....., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim numbers....., because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON
INTERNATIONAL APPLICATION NO. PCT/AU 86/00129

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report	Patent Family Members		
US 3862003	CA 998634	DE 2333184	ES 416573
	FR 2190834	IT 1048127	JP 49026234
	US 3862003		
AU 34148/84	IT 49143	BE 900810	CA 1205031
	DK 4853/84	EP 138572	
	ES 536675	FI 843990	FR 2553099
	HU 36834	IL 73217	JP 61028503
	LU 85582	NO 844054	PT 79339
	ZA 8407942		
AU 35806/84	US 555224	DK 5588/84	EP 144019
	ES 537938	FI 844597	HU 36180
	JP 60133894	NO 844493	ZA 8409025
JP 58-084801		JP 59075140	JP 59084989
	JP 61066510		
JP 58-037001		JP 59026365	JP 60029198
	JP 61013414		

END OF ANNEX