



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(21) International Application Number: PCT/SE91/00558 (22) International Filing Date: 22 August 1991 (22.08.91) (30) Priority data: 9002732-7 24 August 1990 (24.08.90) SE (71) Applicant (for all designated States except US): KABI PHARMACIA AB [SE/SE]; S-751 82 Uppsala (SE). (72) Inventors; and (75) Inventors/Applicants (for US only) : KIHLEBERG, Reinhold [SE/SE]; Riddarväg 37 B, S-184 51 Österskär (SE). LINDGREN, Svante, Bengt [SE/SE]; Lättsta, Skepptuna, S-195 00 Märsta (SE). SANDBERG, Lars, Göran [SE/SE]; Björkskog 1213, S-762 00 Rimbo (SE).		(74) Agents: TANNERFELDT, Agneta; Kabi Pharmacia AB, S-112 87 Stockholm (SE) et al. (81) Designated States: AT (European patent), AU, BE (European patent), CA, CH (European patent), DE (European patent), DK (European patent), ES (European patent), FI, FR (European patent), GB (European patent), GR (European patent), IT (European patent), JP, LU (European patent), NL (European patent), NO, SE (European patent), US. Published <i>With international search report.</i>
(54) Title: PRODUCT CONTAINING GROWTH FACTOR AND GLUTAMINE AND USE OF GROWTH FACTOR FOR THE TREATMENT OF INTESTINAL MUCOSA (57) Abstract This invention relates to a product containing a) glutamine or derivative or analogue thereof, b) short- or medium-chain fatty acid and c) a growth factor or analogue thereof for simultaneous, separate or sequential use having anabolic effects and useful for the treatment or prevention of malfunction or disease of the intestinal mucosa. The growth factor is GH, GRF, IGF-1 or IGF-2 and preferably IGF-1. The invention also relates to the use of GH, GRF, IGF-1 or IGF-2 for the manufacture of a medicament for the treatment or prevention of malfunction or disease of the intestinal mucosa.		

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⁺ Any designation of "SU" has effect in the Russian Federation. It is not yet known whether any such designation has effect in other States of the former Soviet Union.

PRODUCT CONTAINING GROWTH FACTOR AND GLUTAMINE
AND USE OF GROWTH FACTOR FOR THE TREATMENT OF
INTESTINAL MUCOSA

The present invention relates to a product having anabolic effects and which also can be used for the treatment or prevention of malfunction or diseases of the intestinal mucosa. The product contains a growth factor together with glutamine and a fatty acid. The invention also comprises a procedure for the prevention or treatment of impaired function of the gut wall after, for example, trauma, sepsis, burn injuries, radiation therapy or cytotoxic chemotherapy, as well as different inflammatory conditions such as ulcerative colitis or morbus Chron. Thereby can growth factors optionally together with glutamine and/or a fatty acid be given by oral, intravenous, peritoneal, rectal, intramuscular or subcutaneous administration.

Background.

In trauma and sepsis, in addition to the conspicuous injuries or signs of infection, there are also metabolic changes. These changes can be divided into an early so-called ebb phase followed later by a so-called flow phase. In the ebb phase (shock phase), which can last for 1-3 days after the incidence of the injury, there exists a reduced energy turnover, increased lipolysis, hyperglycaemia, elevated catecholamine release and hormonal maladaptation, circulatory effects with decreasing blood pressure, reduced tissue perfusion and oxygen consumption, hypoxia, acidosis, increased body weight as a result of fluid and urine retention and a reduced body temperature. Protein synthesis is also reduced as well as blood albumin and amino acid concentrations.

After about 3 days, the flow phase appears which can last for 1-4 weeks. The flow phase is characterised by an increased energy turnover, increased release of substrates, negative nitrogen balance, rise in blood pressure, increased oxygen consumption and elevation

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of body temperature. Protein synthesis is reduced in mild trauma whilst both synthesis and breakdown increase in major trauma. Breakdown dominates resulting in a negative nitrogen balance which can reach 0.3-0.6g nitrogen per kg body weight and day. This is equivalent to approximately 1.2 - 2.4 kg muscle wastage per day in a normal individual.

If the catabolic component of the flow phase continues for too long, a life threatening situation can develop with substantial losses of fat and muscle tissue as well as impaired wound healing and defence against infection (Ljusk S Eriksson, Mossberg T, Wahren J; pp 99-117, Klin Nutr Almq & Wiks 1987).

The concentration of glycogenic amino acids (such as alanine, glycine, serine and glutamic acid) drops in the plasma whilst a simultaneous increase in the plasma levels of branched chain amino acids (such as leucine, isoleucine and valine), aromatic amino acids (such as phenylalanine, tyrosine and tryptophan) as well as methionine, is observed. An approximately 50% reduction of the intracellular glutamine concentration is also typical. The above mentioned changes can partly be explained as disturbances in transport over the cell membrane. Alanine and glutamine are taken up and metabolised by the gut mucosa and the liver. In the liver they are utilised for, amongst other things, gluconeogenesis. In burn patients the splanchnic (liver and gut) uptake of amino acids is increased approximately 2-6-fold compared to a healthy control. The protein catabolism, which is evident mainly as increased urea production, not only affects striated muscles and visceral proteins but also smooth muscles such as those found in the gut wall and in the respiratory pathways. This situation can negatively affect vital bodily functions as well as secretory transport in the bronchii, with secretory stagnation and development of pneumonia, a common post-traumatic complication.

The gut mucosa is important not only for the selective and active absorption of different nutrients, but also as a barrier against foreign organisms and toxins. In recent years, it has been shown

that the barrier function of the gut mucosa deteriorates in association with major trauma and sepsis. Furthermore, both cytotoxic chemotherapy and radiation therapy have a deleterious effect on gut mucosal function. Intravenous nutritional therapy, which is applied when the patient has difficulties to eat or assimilate orally administered food, has been shown to contribute to atrophy of the gut mucosa. Gut atrophy appears in laboratory animals and in man after intravenous feeding. This can increase the risk for translocation of bacteria over the gut mucosa with consequent risk for sepsis and increased mortality.

Atrophy of the gut combined with simultaneous translocation of bacteria over the gut mucosa is most likely in patients who have suffered major trauma and extensive clinical intervention, in sepsis, in major burn injuries and even after radiation therapy and/or cytotoxic chemotherapy in patients with abdominal or urogenital tumours. Patients with inflammatory diseases of the gut such as ulcerative colitis and morbus Chron also run the risk of being stricken by life threatening infections caused by atrophy of the villi and consequent development of ileus with toxic dilatation and release of bacteria into the peritoneum.

The observation of a marked decline in the intracellular concentration of glutamine in muscle following trauma and sepsis initiated interest in glutamine metabolism and its significance. It has been shown that glutamine is an important energy substrate in intestinal tissue and that glutamine utilization increases in the catabolic state. In studies on experimental animals it has been demonstrated that glutamine administration can prevent a fall in plasma and muscle glutamine concentrations following trauma. Further, an accelerated mucosa recovery after injury induced by 5-fluorouracil was observed in rats when glutamine was added to the intravenous nutrition solutions (Dwyer ST et al, Clin Res 367a, 1987).

During an international symposium "Glutamine metabolism in health and disease", January 26-27, 1990, in San Antonio, Texas,

USA, it was reported that in human subjects, post-operative glutamine supplementation of a total parenteral nutritional regimen improved nitrogen balance and counteracted the decline in protein synthesis usually seen post-operatively. Further it was reported that a number of positive effects had been observed in patients undergoing bone marrow transplantation who were given considerable amounts of glutamine.

In a patent for the treatment of catabolic dysfunction in an animal, a composition including 5-30 g of L-glutamine and/or 5-25 g of alpha-keto glutaric acid is claimed (WO 87/01589). Another patent comprises aqueous solutions for parenteral nutrition which preferentially contain glutamine together with other organic nitrogen-containing compounds (WO 87/03806). Furthermore, the presentation of glutamine in the form of dipeptides has been patented (see EP 87750 and DE 3108079).

The gut has an active endogenous microflora of which the majority of the organisms are facultative or obligatory anaerobes. This means that the major products of fermentation are the short-chain fatty acids (SCFA), mainly acetic acid, propionic acid and butyric acid. The substrates for this fermentation are the carbohydrates that reach the large intestine.

In so-called germ-free rats there is an atrophy of the gut mucosa. These rats also have a low endogenous production of SCFA which represents only about 1% of the production seen in normal rats (Hoverstedt and Midtvendt, J. Nutr. 116, 1772-76, 1986).

A fiber-free diet (one week) or total parenteral nutrition (two weeks) reduces the fecal loss of SCFA in man by 50% and 80%, respectively (Hoverstedt, Symposium, held at The Wennergren-Gren Center, Stockholm, Sweden, June 1th-4th, 1988).

Some experiments with SCFA-enriched nutritional solutions have been described in the literature. In all cases but one the experiments were carried out in animals that had been subjected to

small bowel resection and given intracolonal or intracaecal infusions.

In a study of Koruda et al. (Am. J. Clin. Nutr. 51,685-9, 1990) SCFA-enriched total parenteral nutrition was employed, but since there was no fat in the regimen, the diet was not balanced (lacking essential fatty acids). Furthermore, a mixture of SCFA (acetate, propionate and butyrate) was used in which acetate and propionate were in higher concentration than butyrate. These shorter acids have been shown to be more toxic than butyrate.

It has been suggested (WO 87/04074) that protein accretion or nitrogen retention can be promoted in the case of a hypocaloric diet by the administration of growth hormone.

In a study with intravenously fed rats, an increase in mucosal protein was observed in the small bowel and in the colon following the simultaneous administration of L-glutamine and epidermal growth factor (Jacobs D O et al. Surgery 104 (2), 358-364 (1988)). IGF-1 has been shown by O'Sullivan et al. (Endocrinology 125: 2793-95 81989) to prevent weight loss in starved mice, whereas GH (bovine) was ineffective in this situation. A relative increase in kidney and spleen weights has been reported for GH and IGF-1 treated rats (Skottner A et al. Endocrinology 124, 2519 (1989)).

It is quite clear, however, that several of the problems mentioned above have until now not been solved with existing nutritional therapy.

At present there is no clinical application of a growth factor alone or together with glutamine and/or a short- or medium-chain fatty acid for the improvement of gut function or for the combination having anabolic effects.

Description of the invention.

The present invention relates to a product containing glutamine or a derivative or analogue thereof, a short- or medium-chain fatty acid and a growth factor or analogue thereof for simultaneous,

separate or sequential use having anabolic effects and/or for treatment or prevention of malfunction or disease of the intestinal mucosa as well as the use of these components for the manufacture of a medicament or a kit for the treatment or prevention of malfunction or disease of the intestinal mucosa.

The invention also discloses a method for the treatment or the prevention of malfunction or disease of the intestinal mucosa characterised in that a pharmacologically effective dose of a growth factor or analogue thereof is administered to a patient optionally simultaneously, separately or sequential with either or both of glutamine or a derivative or analogue thereof and/or a short- or medium-chain fatty acid.

The components should be in a pharmaceutically acceptable form. By product is meant here not only a product or kit containing these three components, but also when the components are admixed with other components in a TPN, such as amino acids, carbohydrates and fat, preferably as an emulsion.

The components can also be administered separately, e.g. a short -or medium- chain fatty acid, glutamine and the growth factor in an aqueous medium. The fatty acid and glutamine can both be mixed with the nutrient and the growth factor administered separately. Glutamine can be administered e.g. as the L-amino acid, as N-acetyl-L-glutamine or as a glutamine-containing peptide.

By short-or medium-chain fatty acids are meant here saturated fatty acids of C2-C12 carbon chain length, preferably short chain of 2-4 carbons, such as butyric acid which is preferably administered intravenously.

The short- or medium-chain fatty acids can be administered as, for example, sodium or potassium salts and a medium fatty acid can be administered as a triglyceride.

Growth factors include here especially growth hormone (GH), Insulin-like growth factors IGF-1 and IGF-2, GRF (growth hormone releasing factor) or analogue thereof etc. By analogue is meant substances which have the same physiological effect as the Growth factors, mainly IGF-1, can be injected or can be administered intravenously either as an aqueous solution or

incorporated into a fat emulsion or as a component in micelles or liposomes.

The amount of the components given to humans could be varied depending on the patient and disease. Normally they are within the doses normally given, such as 0.01 - 1 g/kg, day for glutamine, preferably 0,2-0,7g/kg, day and 0,005-0,3 g/kg per day for fatty acid, preferably 0,02-0,15 g/kg per day. The amount of IGF-1 could be 0.02-20 mg/kg per day and preferably 0.05-2 mg/kg per day, and for GH up to 1 IU/kg per day, preferably 0.05-0.7 IU/kg per day. (4 IU GH is equal to 1.3 mg GH).

As can be seen from the background and description of the problem, the function of the gut mucosa can be of critical importance in the outcome of different illnesses. In a series of experiments, mainly in the rat, we have studied the effect on the gut mucosa of intravenous administration of commercially available solutions (for example, amino acid solutions, glucose solutions and fat emulsions) both with and without supplementation with glutamine, short-chain or medium-chain fatty acids and growth factors,

The measured variables have been primarily growth, gut mucosa morphology, villus height, crypt depth and cell proliferation in the gut mucosa measured with a radiolabelled thymidine incorporation technique.

In the above mentioned studies we observed a pronounced atrophy of the gut mucosa after total parenteral nutrition. As expected we noted a 30% reduction in the villus height and crypt depth compared to an orally fed control group. Addition of glutamine improved the gut mucosa; villus height and crypt depth increased by about 20 % and a similar improvement was seen after the administration of either a short-chain fatty acid (mainly butyric acid) or IGF-1.

When glutamine, butyric acid and IGF-1 were administered together, an unexpected synergistic effect was observed on both growth and the gut mucosa. Growth rate was three-fold higher than

that of rats infused with fat, amino acids and glucose, which was totally unexpected and an anabolic effect was thus clearly shown. Moreover, for the first time we could reach a villus height in the TPN-fed rat which approached 400 μm (compared to slightly over 300 μm after non-supplemented infusion). This finding was confirmed by the results of the mucosal cell proliferation studies.

Example 1

Male Sprague Dawley rats (180-190 g body weight, BW) were surgically fitted with a permanent central catheter which was protected by a harness. Total parenteral nutrition was given to the rats in the form of a standard regimen:

Fat	7.6 g/kg BW and day
Nitrogen	1.35 g/kg BW and day
Total energy intake	270 kcal/kg BW and day
Total volume intake	300 ml/kg BW and day

The ratio between glucose and fat in this experiment was 30/70 expressed as a % of the non-protein energy intake. The TPN (total parenteral nutrition) mixtures were obtained by mixing Vamin® 14 EF (a solution of essential and non-essential amino acids for parenteral nutrition), a 50% glucose solution and Intralipid® 20% in relevant proportions after which was followed by addition of the necessary amounts of electrolytes, trace elements and vitamins required by the rat.

On the morning of the first experimental day the rats were randomised into three groups: oral controls, intravenous controls and the glutamine group. The oral control rats received a standard diet (R3, Ewos AB, Södertälje, Sweden) ad libitum during the experimental period and the intravenous control rats received the above-described standard TPN regimen.

The glutamine group received glutamine in the form of the dipeptide glycyl-glutamine (which was administered intravenously in isonitrogenous and isocaloric amounts at the expense of the amino acid solution) as well as the other nutrients supplied according to the regimen given to the intravenous control group.

The infusions were given for 7 days and growth was recorded daily. The total weight gain was 41.4 ± 4.2 , 14.9 ± 3.2 and 8.6 ± 3.5 for the oral controls, intravenous controls and glutamine groups, respectively.

After 3 hours' fasting, blood samples were taken under pentobarbital anaesthesia for analysis of the clinical chemistry. The results are shown in Table 1.

Table 1. Clinical chemistry analyses in the serum.

Group	ASAT	ALAT	ALP	LD	Bilirub	Chol	betaHB
Oral control.	1.4	0.87	8.2	3.6	0.7	2.0	1.3
iv. control...	1.3	0.37	4.4	5.8	1.7	2.8	1.1
Glutamine..	1.3	0.44	5.0	3.5	1.6	2.5	1.1

Abbreviations: ASAT=aspartate aminotransferase(microkat/l); ALAT = alanine aminotransferase (microkat/l); ALP=alkaline phosphatase microkat/l); LD=lactate dehydrogenase (microkat/l); Bilirub= Bilirubin (micromol/l); Chol= cholesterol (mmol/l); β -HB= β -hydroxy butyrate (mmol/l).

The rats were sacrificed by exsanguination after heart puncture under pentobarbital anaesthesia after which obduction and macroscopic examination was performed. After sacrifice the liver, spleen, lungs, kidneys, heart and thymus were dissected out and

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weighed. The relative organ weights showed no differences of biological relevance between the groups. Similarly, there were no marked differences in the blood chemistry.

Sections of the gut were fixed in a Milla-Pott fixation medium and were evaluated with respect to villus height and crypt depth (Table 2).

Table 2. Morphometrical evaluation of the jejunum.

Group	jejunum	
	crypt depth (μm)	villus height (μm)
Oral control.....	165	460
iv. control.....	121	309
Glutamine.....	145	378

The morphometric analysis of the jejunum demonstrates an atrophy of the gut after parenteral nutrition of the rats for 7 days. This condition could be markedly improved by the administration of glycyl-glutamine.

In summary, the results show that intravenous administration of glutamine for 7 days was well tolerated and that a significant improvement of gut morphology, with approximately a 20% increase in both villus height and crypt depth.

Example 2

In an experiment performed similar to example 1, the effect of butyric acid on body weight, relative organ weight, blood chemistry, serum and urine β -hydroxy butyric acid levels as well as morphometrical evaluation of the jejunum was studied in rats. TPN was given to the rats in the form of the standard regimen described in example 1 and butyric acid was given intravenously as Na-salt.

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Infusions were given for 7 days and the growth of the animals was studied during the entire experimental period (Table 3).

Table 3. Growth after 7 days of TPN in the rat

Group	Weight increase (g)
iv. control.....	14.9±3.2
Butyric acid.....	15.5±2.5

Blood sampling, sacrifice and dissection were performed as described in example 1.

Total weight increase was not significantly different.

There were also no differences in the relative organ weights between the groups. Similarly, there were no differences in the blood chemistry.

Morphometric analysis of the jejunum and ileum demonstrated an atrophy of the gut after 7 days of parenteral nutrition in the rat. This condition was improved by the administration of 3 g butyric acid per kg body weight and day (Table 4).

Table 4. Morphometric evaluation of the jejunum after 7 days of TPN in the rat.

Group	crypt depth (µm)	villus height (µm)
iv. control.....	121	309
Butyric acid.....	150	361

In summary, the results show that 7 days of TPN containing 3g/kg butyric acid was well tolerated and that an improvement of gut morphology as a consequence of butyric acid could be seen.

Example 3

The effect of the simultaneous administration of glutamine, butyric acid and IGF-1 was studied in the rat. The experimental design as well as the parameters under study were identical to those described in examples 1 and 2. Besides a morphometric evaluation of the small intestine, a cytokinetic study using methyl-3H thymidine was performed on the jejunum. The test group received parenteral fat, amino acids, glucose, butyric acid and glutamine. Further, the rats received IGF-1 in the form of subcutaneous injections of 1 mg/day divided into two doses each day. This was repeated for 8 days. At the same time an intravenous control group of TPN rats was studied.

The experiment was terminated by anaesthetising the rats with pentobarbital and infusing them with radiolabelled methyl-3H-thymidine (1 μ C/kg). The rats were sacrificed by cervical dislocation and samples were taken from the jejunum for morphometric and cytokinetic analyses. See Table 5. Blood samples were taken for IGF-1 determination.

Table 5. Weight gain and morphometric evaluation of the jejunum after 7 days of TPN in the rat.

Group	Weight gain	jejunum	
		crypt depth (μ m)	villus height (μ m)
iv. control.....	8.8 $\pm$ 1.8	121	309
combination therapy.....	27.1 $\pm$ 4.6	144	395

In summary, after the simultaneous administration of a short-chain fatty acid, glutamine and a growth factor IGF1, a surprising and significant synergistic effect was observed both on growth and on the gut mucosa.

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The effect on weight increase when the three components were given together was surprisingly high when compared to the weight increase for a component given alone (Example 1 and 2). Thus an anabolic effect was obtained.

The effect on the gut mucosa was also clear and could even be seen at dissection. The results of the cytokinetic studies confirm these observations.

Thus, our studies show a surprisingly good effect on the gut mucosa when the three components were administered simultaneously, as compared to any of the components given alone.

CLAIMS

1. Product containing
 - a) glutamine or derivative or analogue thereof
 - b) short- or medium-chain fatty acid and
 - c) a growth factor or analogue thereoffor simultaneous, separate or sequential use having anabolic effects.
2. Product containing
 - a) glutamine or a derivative or analogue thereof
 - b) short- or medium-chain fatty acid and
 - c) a growth factor or analogue thereoffor simultaneous, separate or sequential use for the treatment or prevention of malfunction or disease of the intestinal mucosa.
3. Product according to any of claims 1 or 2, in which the growth factor is GH, GRF, IGF-1 or IGF-2 and preferably IGF-1.
4. Product according to any of claims 1 or 2, in which the short- or medium-chain fatty acid has 2-12, preferably 2-4 carbon atoms and is more preferably butyric acid.
5. Product according to any of claims 1 or 2, in which the short- or medium-chain fatty acid is butyric acid and the growth factor is IGF-1 or an analogue thereof.
6. Use of GH, GRF, IGF-1 or IGF-2 for the manufacture of a medicament for the treatment or prevention of malfunction or disease of the intestinal mucosa.

7. Use of
 - a) glutamine or a derivative or analogue thereof
 - b) short- or medium-chain fatty acid and
 - c) a growth factor or analogue thereoffor the manufacture of a medicament or a kit having anabolic effects and for the treatment or prevention of malfunction or disease of the intestinal mucosa.
8. Method for the treatment or the prevention of malfunction or disease of the intestinal mucosa characterised in that a pharmacologically effective dose of a growth factor or analogue thereof is administered to a patient optionally simultaneously, separately or sequential with either or both of:
 - a) glutamine or a derivative or analogue thereof
 - b) short- or medium-chain fatty acid
9. Method according to claim 8, in which the growth factor is IGF-1.
10. A method for preparing a medicament for the treatment or the prevention of malfunction or disease of the intestinal mucosa, comprising mixing the components:
 - a) glutamine or a derivative or analogue thereof
 - b) short- or medium-chain fatty acid and
 - c) a growth factor or analogue thereofwith a carrier or with different carriers.

INTERNATIONAL SEARCH REPORT

International Application No **PCT/SE 91/00558**

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 IPC5: A 61 K 37/36		
II. FIELDS SEARCHED		
Minimum Documentation Searched ⁷		
Classification System	Classification Symbols	
IPC5	A 61 K	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in Fields Searched ⁸		
SE,DK,FI,NO classes as above		
III. DOCUMENTS CONSIDERED TO BE RELEVANT⁹		
Category *	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
X	Dialog Information Services, File 155, Medline 66-91/MAY, Dialog accession no. 07118481, Jiang ZM et al: "Low-dose growth hormone and hypocaloric nutrition attenuate the protein-catabolic response after major operation", Ann Surg Oct 1989, 210 (4) p 513-24 --	1-7, 10
A	WO, A1, 8909268 (GENENTECH, INC.) 5 October 1989, see page 45, line 15 - page 47; claims 41-46 --	1-7, 10
A	EP, A2, 0177342 (GENENTECH, INC.) 9 April 1986, see the whole document -- -----	1-7, 10
* Special categories of cited documents:¹⁰ "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	
6th December 1991	1991-12-10	
International Searching Authority	Signature of Authorized Officer	
SWEDISH PATENT OFFICE	 Anneli Jönsson	

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. ☒ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE¹

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...8-9, because they relate to subject matter not required to be searched by this Authority, namely:

See PCT Rule 39.1 (iv).

Methods for treatment of the human or animal body by surgery or therapy as well as diagnostic methods.

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 LACKING²

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 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 PATENT APPLICATION NO.PCT/SE 91/00558**

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the Swedish Patent Office EDP file on 31/10/91
The Swedish 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	Publication date	Patent family member(s)	Publication date
WO-A1- 8909268	89-10-05	AU-D- 3217889 EP-A- 0406272	89-10-16 91-01-09
EP-A2- 0177342	86-04-09	JP-A- 61093129	86-05-12