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(54) Title: HIGH PURITY ANIMAL INTERFERONS

(57) Abstract

Interferons from bovine, porcine, equine and ovine in high purity effective across species lines. The interferons are obtained by challenging viable leukocytes *in vitro* with an interferon inducer, preferably an inducer infectious to the host animal, then recovering the interferon from the culture medium. After purification to in excess of 106 International Reference Units per mg of protein the high purity animal interferons are non-antigenic in the human.

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Description

High Purity Animal Interferons

Background of the Invention

Since its discovery about twenty years prior to the date of this invention, Interferon has been the subject of such widespread intense activity that substantial bodies of art have developed to virtually every aspect of this anti-viral substance, for example: to its nature, to its mode of action, to its induction in vivo, to its production in vitro. The interferon art is progressing forward without, it seems, pause to cross-check initial conclusions and theories with the most recent laboratory results. Erroneous conclusions drawn from early work stand uncorrected and blindly repeated again and again in the literature and patent art. One such erroneous conclusion constituting now a most significant handicap facing the art is the widespread belief that interferon are species specific, c.f. Patent 3,970,749 (at Column 1) and Burke "The Status of Interferon", Scientific American, Vol. 236 (April 1977), pages 42-50, (on page 42).

The evidence that many forms of Interferon exist (fibroblast, leukocyte and "immune" interferons, to name but three) has been well explored by the art; c.f. Burke, supra. These different forms are readily distinguished by biological features as well as by antigenic properties, physio-chemical properties, etc., c.f. Burke, supra. However, the evidence that many forms of interferon exist and that one or more forms of interferon are effective across species lines appears to have had, heretofore, little if any effect on the direction followed by workers in the art; c.f. Burke, supra. Human leukocytes from blood bank sources are suggested for (large scale) production of inter-

ferons, despite the manifest absurdity of reliance upon such a high cost material of limited availability for the basic source of supply. The impossibility of the situation has even escaped the notice of those investigating the action of interferon across species lines, as witness "Pronounced Antiviral Activity of Human Interferon on Bovine and Porcine Cells" Nature, Vol 251, October 11, 1974, pp. 543-545, Gresser et al, wherein can be found the suggestion to prepare human leukocyte interferon for veterinary medicine purposes.

A far more feasible approach is preparation of animal interferon for human medicine purposes. The need to cross species lines is not as crucial for veterinary purposes, but even in veterinary medicine the capability of doing so is self-evidently desirable.

Rationale of this Invention

It has now been found that high purity leukocyte interferons from bovine, ovine, equine, and porcine, i.e., cattle, sheep, horses, and swine cross species lines, and are effective in human application. The bovine and porcine interferon have tested out as the most effective, and are the preferred embodiments.

Advantageously, the high purity interferons seem essentially to be non-antigenic in the human. Without being certain thereof, the belief offered for the non-antigenity is that existence of several distinct forms of interferon in the human with differing antigenic determinants has created a wide enough zone of antigenic tolerance for differences in interferon moleculr structures such that interferons from bovine and porcine leukocytes, in particular, are tolerated by the human immune system and are pharmacologically quite potent in the human.

A consequence flowing from the capability of high purity interferon to cross species lines is that one major concern of the art has been avoided. The concern

in question is often expressed as the desire to induce interferon production without employing a virus which will adversely affect the future patient. So long as patient and host were of the same species, and so long as human blood bank sources were contemplated, as the basic source material for interferon many infectious viruses were necessarily excluded from consideration as inducers. Now viruses most virulent in the host animal (but nonpathogenic in the human) can be considered for interferon inducing purposes.

The Invention

Thus, this invention relates to high purity interferons derived from porcine, equine, bovine and ovine. Since purity levels of proteins are difficult to establish, the term high purity as herein contemplated should be understood as the level of purity required to make the interferon essentially non-antigenic in humans. These high purity interferons are believed to be noval products. As a rule of thumb, high purity interferon comprises an interferon product with at least about 10^6 international reference units per mg of protein and preferably more than 10^7.

This invention also relates to the process of culturing leukocytes taken from the blood of porcine, bovine, ovine and equine in the presence of an interferon inducing agent, such as a virus for example, then recovering the interferon in high purity. In preferred modes of this invention the inducer is a virus virulent to the animal source of the leukocytes.

This invention also relates to a procedure for purifying crude interferon recovered from the culture medium to high purity levels, e.g. to more than 10^6 international reference units per mg of protein.

Detailed Practice of the Invention

As has already been pointed out, practice of this invention contemplates generation of interferon in a culture medium by appropriate challenge to porcine, bovine, equine and ovine leukocytes, followed by recovery and purification of the interferon. The techniques developed for generating human leukocyte interferon (e.g., as briefly described by Burke, supra) can be employed, and are contemplated herein.

The leukocyte source materials herein contemplated offer many advantages to the interferon arts. The output from a production facility is no longer restricted by the limited availability of human blood. In addition, the leukocytes can be made available immediately after the blood is removed from the animal. Moreover, the plasma associated with the animal source leukocytes constitutes a by-product of possible value, being available for culture medium nutrient purposes, for example.

Fresh whole blood obtained by bleeding or butchering of the animals is separated (promptly) into its components, by conventional techniques, e.g., centrifugation to remove the plasma from cellular constituents, then the white cells or leukocytes are recovered e.g., as a buffy coat. Commonly lysis is employed to eliminate a residual red cells contaminant from the recovered leukocytes. Lysis of red cells to separate them from leukocytes is also contemplated for practice of this invention. In any event the leukocytes from an animal source can be suspended in a nutrient medium and challenged by the interferon inducer, in as little as an hour after the whole blood is collected, and certainly while the leukocytes are still fresh and viable.

Conveniently, the nutrient medium in which the leukocytes are suspended and cultured may be derived in whole or in part from the plasma fraction of the whole blood. Such a use for the plasma has many advantages,

not the least of which is its availability almost without cost. However, the plasma contains a significant protein content of a nature which makes purification of the interferon product more difficult. (c.f. 3,800,035). Therefore the exact proportion of plasma employed in the nutrient medium is a matter of coice to be weighed against relative costs of purifying the interferon generated therein to high purity levels.

As has already been pointed out, the species divorcement between host animal and patient created by practice of this invention allows employment of a wider variety of viral inducers than heretofore possible, including notably the possibility offered for using a virus which attacks the host species. In general, however, the interferon inducer may be any of the many materials heretofore suggested to the art for inducing leukocytes to produce interferon, including viruses such as for example Newscastle disease virus, (NDV), Sendai virus, etc., and synthetic materials such as double stranded ribonucleic acids or single stranded ribonucleic acids, complexed or uncomplexed. Reference is made to Carter and DeClereq "Viral Infection and Host Defense", Science Vol 186, 1974 pp. 1173-1178.

Of particular interest to practice of this invention is use of viruses virulent to cows or pigs with, of course, those infectious only to the host animal especially notable, such as for example blue tongue virus (BTV). In such instance a special leukocyte/inducer interaction seems to take place. The minimum number of affective BTV virus particles per leukocyte cell necessary to induce a high level of interferon in the culture medium was found to be half or less of the number for NDV. Foot-and-mouth disease (FMDV) was also found to be an unusually efficient inducer of swine and bovine leukocyte interferon, requiring vis a vis NDV a lower multiplicity of infection (moi) or number of virus

particles per leukocyte during the inital interferon infection phase.

The actual mechanics of inducing production of interferon by viable leukocytes in vitro have been well explored by the art and, therefore, need not be described here. Suffice it to note here that the quantity of inducer and the character of inducer selected for practice of this invention are matters of choice being within the skill of the art and per se, do not bear upon practice of this invention except that (inducer purposes) use of a virus infectious to the animal host species, is considered to be within practice of this invention. Thus, for example, the suspended leukocytes are optimally primed with homologous interferon 10-50 units per 10^7 leukocyte cell count for about 2 hours, then are challenged with a virus 1 to 10 x 10^8 plaque forming units per 10^7 cell count. After 6-48 hours of incubation the cells are removed from the medium, e.g., by centrifugation, then the medium is dialyzed overnight against saline of pH-2 (to deactivate the virus). Thereafter the interferon is harvested after readjustment of the pH to 6.5-7.0. The interferon is then purified by affinity adsorption techniques consisting primarily of hydrophobic and lectin chromatography as Biochemistry 1976, pages 5182-5187, Vol 251 No. 15; Journal of Biological Chemistry, 1976, pages 5381-5385, Vol 251 No. 17; Journal of Virology, 1976, pages 425-434, 1976, Vol 19 No. 2.

Briefly stated purification of the interferon is a multi-step procedure of:

1. Removing glycoproteins from the interferon solution by adsorption, then

2. Removing the interferon from the interferon solution by adsorption of the interferon on a first substrate and eluting it back into a new interferon solution, thereafter

3. Removing the interferon from the new interferon solution by adsorption the interferon on a second substrate and eluting it into a second new interferon solution, and thereafter

4. Fractionating the second new interferon solution by molecular weight e.g., with a molecular sieve, to prepare a protein solution of 20,000-30,000 dalton protein.

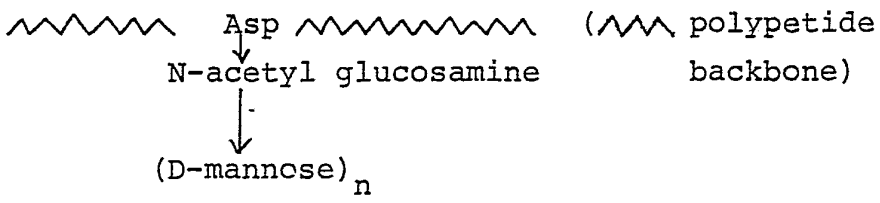
In total the purification recovery sequence recovers about 15% of the initial interferon activity in the form of high purity interferon with more than about 10^6 international reference units per mg of protein. The final solution contains more than one million biological units per ml of solution. The concentration purification of interferon (initially $5x10^2$-$5x10^3$ per mg of protein) is in excess of 1000 fold.

For further understanding of the purification sequence it is below described in exemplary detail, according to a preferred mode of practice of this invention.

Example A

After initial harvest, the crude interferon is of about 100-1000 biological units/ml of medium, specific activity of $5x10^2$ to $5x10^3$ international reference units/mg of protein (measured by Lowery or luorometric methods). The purification sequence now described purifies the (porcine) leukocyte interferon several thousand fold, resulting in a highly purified non-antigenic product suitable for human use as a parenteral drug.

In the first step of the purification, the crude product was passed through a column of concanavalin A, as described in Biochemistry, 1976, page 704 Vol 15. The majority of interferon passes through the column largely unretarded whereas contaminating glycoproteins having the prosthetic group:



were retained and required the competing sugar, methyl mannoside for their complete displacement. Substantial purification was achieved separating the interferon from contaminating glycoprotein in the normal porcine serum.

In the second step of purification, the leukocyte interferon solution was passed through a column of blue dextran (cibacron Blue F3GA-dextran) which had been immobilized on cyanogen bromide activated agarose as described, in Biochemistry, 1976, pages 5182-5187, Vol 15. The chromatography principle here employed is hydrophobic chromatography and the results indicate that the interferon is a hydrophobic protein which can be separated effectively from other contamination proteins in porcine and bovine sera. It is specifically observed that the serum albumin, a potential antigenic protein for man, is efficiently removed at this step as it passes through the column essentially unretarded, whereas the interferon requires a high concentration of sodium chloride for its efficient elution.

When a linear gradient of sodium chloride was applied to the column practically all of the leukocyte interferon was recovered (over 80%) with a resultant high level of purification.

In the third step of purification, the leukocyte interferon was bound to a straight chain hydrocarbon decyl (C_{10}) agarose column. The interferon was bound tightly whereas most of the remaining contaminating protein were bound less tightly and could be removed with a solution of sodium phosphate, the interferon requiring an elutant consisting of 1M NaCl and a polarity reducing agent, ethylene glycol for its full and efficient removal. The manner of purifying animal inter-

feron by hydrophobic chromatography is known to the art, e.g., see Journal of Biolgoical Chemistry, 1976, pages 7620-7625, Vol 251 no. 23.

In the fourth step of purification, the highly purified leukocyte interferon was further freed to high molecular weight and very low molecular weight contaminating proteins by the principle of molecular sieving using a Sephadex G-100 column precalibrated with known molecular weight markers such as bovine serum albumin, ovalbumin, soybean trypsin inhibitor, chymotrypsinogin and ribonuclease, etc. All interferon activity was eluted completely in the zone between ribonuclease and ovalbumin and particularly close to chymotrypsinogen, thus indicating its apparent molecular weight of about 20,000 to 30,000 daltons.

Interferon was assayed in monolayers of several cell lines including porcine kidney, bovine skin and human fibroblasts. The colorimetric method of Finter was used, see Journal of General Virology, 1969, pages 419-427, Vol 5, and Journal of Molecular Biology, 1972, pages 567-587, Vol 70. Vesicular stomatitis virus, at a multiplicity of infection of about 0.15 plaque forming unit/cell, was the challenge virus. An international reference standard of human interferon was used also.

A typical example of the assay illustrating that porcine (and bovine) leukocyte interferon are suitable for human use is as follows: aliquots of a solution containing a known quantity of 100 units of porcine leukocyte interferon was added to tissue culture wells (cell monolayers) of porcine, human and bovine cells. Forty to fifty percent of the activity observed in the homologous cell was observed in the human cells. That is, the 100 units of porcine leukocyte interferon assayed as 40 to 50 units in the heterologous cells of human fibroblasts. In normal (by karyology) human cells highly sensitive to the interferon effect the porcine leukocyte interferon was often 100 percent active in the

human cells tested.

To confirm that the interferon crosses species lines well, a consequence of vaue to human medicine, a human cell line (G258) which has a trisomy of the 21st chromosome was used for the test cell. The 21st chromosome, c.f. Burke, supra, is responsible for formation of specific receptors on the surface of the human cell for interferon. With the G258 cell line, the 100 units of porcine leukocyte interferon (as assayed on porcine cells) actually measured 300-500 units illustrating the high activity of this purified porcine leukocyte interferon for human tissue and its strong ability to protect these cells against cytolytic viral infections. The activity indicated the existence of an extensive region of homology, or similarity, in amino acid sequence between porcine leukocyte interferon and human leukocyte interferon. A precedence for such similarity is known elsewhere in the arts as in the example of the close similarity between porcine and bovine insulins, and human insulin.

For further understanding of this invention reference is made to the following examples:

Example 1

Porcine Leukocyte Cell Separation and Inteferon Production

A sample of 125 ml of whole blood and 30 ml ACD (a commercially available anticoagulant) was concentrated at 400 rpm for 10 minutes in 125 ml conical bottles. Then after differential centrifugation the fractions containing the plasma and white cells were added to a 50 ml syringe pre-loaded with 5.0 ml of plasmogel and 7.5 ml ACD, total volume being 50 ml. The syringe contents were mixed well, then the syringe was inverted and left to stand for 45 minutes.

The upper fraction of the syringe contents was expressed into a 40 ml centrifugal tube and spun 500 rpm for 10 minutes. Then the supernatant was aspirated and the pellet returned to the remaining plasma in the syringe, and 5.0 ml of 199 media + 5% Fetal Calf Serum was added, total volume being 7.5 ml with a Coulter cell count of 1.6×10^7 leukocytes/ml (1.2×10^8 total). (Depending upon the efficiency of recovery, $1\text{-}10 \times 10^8$ viable leukocytes are recovered from a starting sample of 125 ml of porcine or bovine whole blood).

The white cells were resuspended to 12.0 ml (of medium 199 or RPMI 1640) induced with 0.6 ml of NDV (Newcastle disease virus) at 5×10^8 plaque forming units /ml then incubated under a CO_2 atmosphere at 37° for 48 hours with gentle stirring to prevent the leukocytes from settling to the bottom of the vessel. It is observed that the pH must remain above 7.0 for the optimal interferon production to occur.

Thereafter the suspension was clarified at 10,000 rpm for 10 minutes. The supernatant, pH 7.12, was acidified to pH 2.0 (with 1N HCL), and left overnight, then dialyzed for 60 hours at 4°C against 0.15-M Na Cl, pH 2. (If desired the dialysis step may be omitted). The dialysis solution was then changed to a solution of phosphate buffered saline adjusted to pH 7.4 and the leukocyte interferon was dialyzed for an additional 24 hours.

Thereafter the interferon purified according to the procedure of Example A.

Example 2

Bovine and Porcine Leukocyte Separation

Two 15 ml samples each of both calf and porcine whole blood were isolated and treated, each with 120 ml of cold 0.83% ammonium chloride and stood for 10 minutes on ice. The resulting lysates were centrifuged for 10 minutes at 400 rpm. This method, utilizing ammonium

chloride lysis, greatly reduces the residual number of red blood cells.

The pooled pellets (2.0 ml) had a Coulter count of 3-5 x 10^7/ml (porcine leukocytes) and 1-2 x 10^7/ml (bovine leukocytes). They were diluted to 3.0 ml (of Medium 199 or RPMI 1640) then induced by either 0.3 ml of the NDV 5 x 10^8 units per ml (same virus stock as in Example 1) or 0.3 ml of FMDV (1 x 10^8 units per ml). Thus, one sample of each type of leukocytes was induced with each of the viral agents. The FMDV was by far the most effective inducer in that it required 2 to 5 fold less plaque forming units per ml to induce a resultant level of 80-100 interferon units per ml in both types of leukocytes. The four samples were incubated 18 hours at 37° in a CO_2 environment with gentle stirring to prevent the leukocytes from settling and in all ways handled identically.

The subsequent clarification of the leukocyte supernatants, inactivation of residual virus, dialysis and isolation of the leukocyte interferon fractions was then carried out in the manner described in Example 1.

In all four samples, 80-100 units per ml of interferon was induced. The two porcine leukocyte interferons demonstrated strong activity in the human cell system, being 40-50 percent as active as in the homologous cells. The porcine leukocyte interferons were 100% active in bovine cells. Similarly the two bovine leukocyte interferons demonstrated pronounced activity in human cells (as in Example 1).

Example 2B

Purification of Bovine and Porcine Leukocyte Interferon of Example 2

All 4 preparations were purified in the manner of example 1 with four affinity columns run consecutively on each preparation:

Column 1: concanavalin A. agarose
Column 2: blue dextran (cibacron Blue F3GA-dextran) agarose
Column 3: decyl (C_{10}) agarose
Column 4: Sephadex G 100

(inducer)	Overall Recovery	Purification Factor	RATIOS Biological Activity Normal Human v. Homologous cell*	Human Trisomy C21 v. Homo Cell
bovine leukocyte interferon (NDV)	20%	2500	0.40	1.0
porcine leukocyte interferon (NDV)	15%	3000	0.45	1.2
bovine leukocyte interferon (FMDV)	25%	2000	0.30	0.8
porcine leukocyte interferon (FMDV)	25%	2200	0.25	1.2

* homologous cell = porcine or bovine cell as indicated by original source of leukocyte interferon.

In a 5th and optional purification step, samples of the PLIF and BLIF from column 4 were chromatographed on phenyl Sepharose CL-4B. Samples were first dialyzed against 0.15M NaCl in 0.02 M sodium phosphate pH 7.4 (PBS) at 4°. The samples were then applied by means of a peristaltic pump on a column (0.9 x 8 cm) equilibrated with PBS. The column was washed with 10 ml of PBS and then 75% ethylene glycol was applied and both interferons were eluted. The porcine interferon was about 10^7 international reference units per mg. The bovine interferon was about 5×10^7 international reference units per mg. The additional purification of step 5 was 5-10 fold resulting in an overall purification of 10,000 to 30,000 fold. There were full retention of the heterologous protection seen in human cells.

Example 3

Equine and Ovine Leukocyte Cell Separation and Interferon Production

The same separating procedure as in Example 1 was applied to horse and ovine whole blood. In the instance of the horse blood the separation was quite good and the buffy coat could be clearly picked off, which made the total volume in the syringe only 40 ml.

The upper fraction of the contents was removed, centrifuged (as in Example 1) and the pellet returned to the plasma left in the syringe, along with 3 ml of medium 199 + 5% fetal calf serum. Total volume was 6.5 ml, Coulter cell count 7.64×10^7/ml, Total 5.0×10^8. The total ovine cell count was 2.5×10^8.

The white cells were resuspended to 50 ml (in RPMI 1640) and induced by a complex of polyriboinosinic, polyribocytidylic acids, (rI_nrC_n) c.f. Carter and Declercq, supra, then incubated for 48 hours at 37° under a CO_2 atmosphere.

Thereafter the suspension was clarified, dialyzed, etc. as in Example 1.

Recovery and Purification

These two interferons were purified using the procedure of Example 2 with an overall excellent recovery of 45% in both cases.

inducer	overall recovery	purification factor	RATIO biological activity normal diploid over homo.cell
equine leukocyte interferon (rI_nrC_n)	45%	300 fold	0.05
ovine leukocyte interferon (rI_nrC_n)	45%	450 fold	0.08

The purification sequence does not appear as good for equine and ovine as for bovine and porcine interferons.

Further Discussion of the Invention

By way of further explanation and discussion of the present invention, it is believed that what takes place during the sequence employed for purification of the animal leukocyte interferon is concentration and recovery of leukocyte interferon component or components most effective across species lines, from bovine, equine, ovine, or porcine to human, and removal of those interferon components that cross species lines poorly or not at all.

It should be appreciated that the unit activity of interferons are very high, at least 10^8 International Reference Units per mg of protein, and moreover that more than one interferon component is elaborated by human leukocytes in initially a very low concentration in the fluid. The very high degree of concentration desired for clinical products e.g., from 10^3 units activity to about 10^6 unit activity, requires that the purification technique for human interferon products be extremely selective for what is recovered. Even minor chemical and/or conformal differences between the protein molecule of the interferon sought and impurity proteins, even if the impurity constituted a different interferon molecule will cause the impurity to be rejected by the purification sequence. Accordingly past efforts to concentrate human interferon a thousand-fold are likely to have involved complete loss of interferon components that differ chemically or conformally from the interferon component successfully concentrated.

Considered in this light, it is significant that the purification sequence described above and other purification sequences developed by the art (e.g., the Cantell human leukocyte 1F procedure) have been developed to

recover high unit activity human source leukocyte interferon and according to practice of this invention now is applied to the animal leukocyte interferons. Suggested then is a facile explanation for the good results obtained by practice of this invention namely that the purification sequence has become so specific to a particular (human) leukocyte interferon component or set of human interferon components, that any equally high (to human cells) unit activity leukocyte interferon product of the same recovery and concentration sequence from animal sources leukocytes should be virtually identical to the human source product, and, therefore, should not be antigenic in the human.

If the above line of reasoning is correct, confirmation of sorts will be available from yield data . Evaluation of the unit activity data on tests of crude interferon and purified products against homologous cells, e.g., porcine v. porcine, bovine v. bovine, human v. human does, in fact, indicate consistently higher recovery losses during work up of animal leukocyte interferon (when using methodology explicitly slanted towards recovery of interferon components most homologous to human 1F). In other words, more interferon values are lost from the crude animal product than from the like crude human product; consistently product yield is distinctly lower.

The yield differences are 10-20%, sometimes even more, and occur both from the purification procedure described above and from adaptation of the Cantell procedure to the crude animal leukocyte interferon. The yield differences are believed to indicate selectively complete loss of some interferon component or components, which components are believed to have lower or perhaps non-existent species cross-over potential.

The prior art is not contradictory of a likelihood for selective complete loss of some interferon components during purification. Some researchers have reported heterogeneity in human leukocyte interferon and even that

one human leukocyte interferon component exhibits little cross species activity. See, for example, Virology 68, pg. 68-73 (1975) and 70 pages 451-458 (1976).

By the same line of reasoning, an interferon component that is species specific should exhibit such specificity all the more when purified to high unit activity levels. Some data confirmatory of this concept are available. A human fibroblast interferon was purified to about 10^7 ref. units per mg of protein by the procedures of the above examples, then tested against various cells. The results are tabulated below.

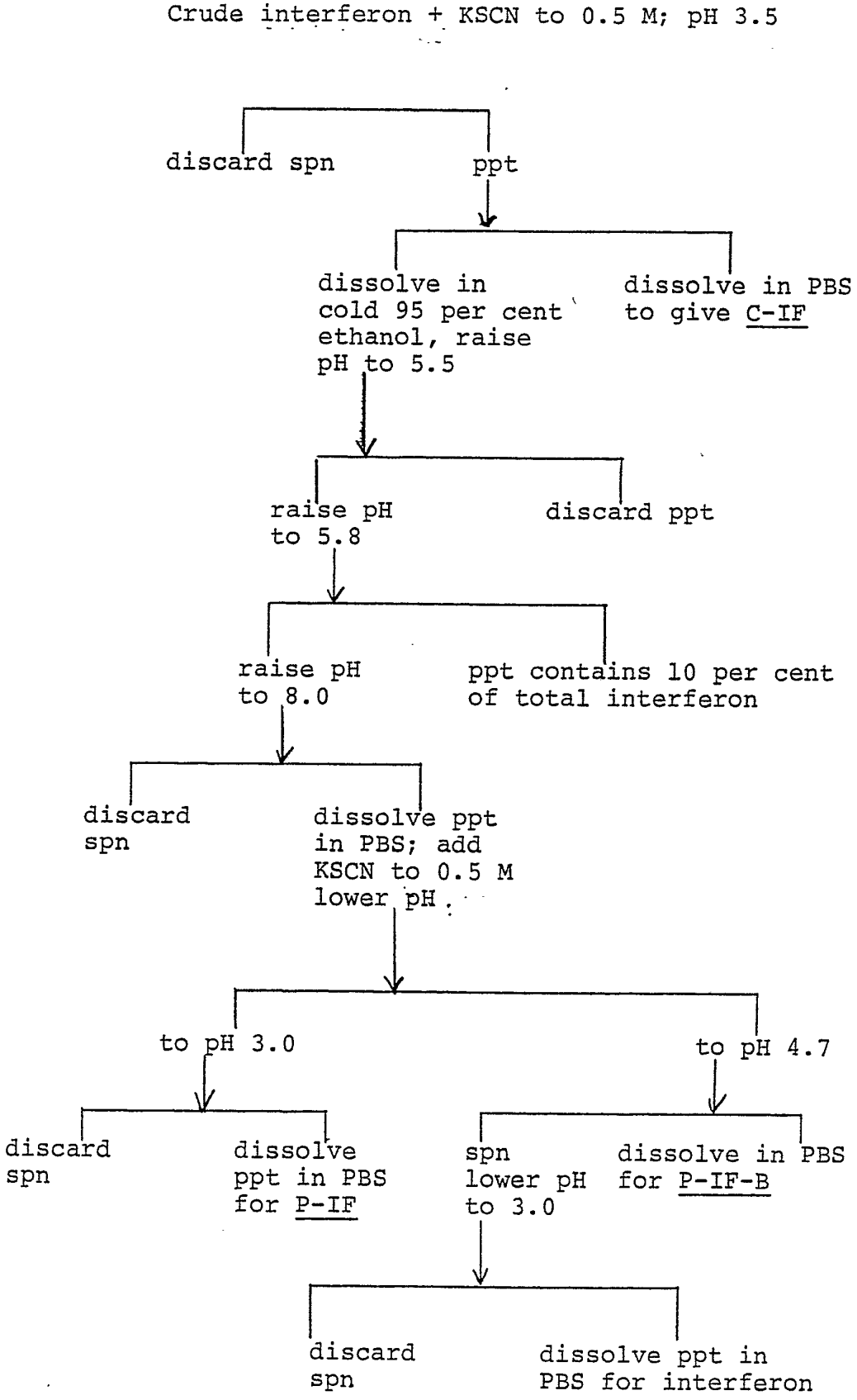
Test Cell	Species	Human Fibroblast IF	Percent Activity
Human amnion cells	Human	X	100%
Human cells (trisomy 21)	Human	X	300%
Bovine Embryonic trachea	Bovine	X	1%
MDBK	Bovine	X	1%
PK 15	Porcine	X	1%
FEA	Cat	X	1%

No comparative yield data is available, since efforts have not been made to subject animal fibroblast interferon to purification by the same techniques. However, the yield, (human) fibroblast interferon v. (human) leukocyte interferon is lower. In passing it may be noted that the data alluded to above also emphasizes how much more sensitive are the biologic tests to differences between various interferons than is the chemistry of the purification procedure. The human fibroblast and human leukocyte interferons were purified by the same procedure, a procedure manifestly not sensitive enough to differentiate between whatever chemical differences create the species specific nature of human fibroblast interferon.

Thus, the available data and the results reported by the art regarding human and animal leukocyte interferon are not inconsistent with the above expressed belief that the high activity and absence of antigenicity in humans exhibited by the animal leukocyte interferons herein described, are explainable in terms of selective complete removal of antigenic impurities present in the crude interferon product. Some of the removed impurities are probably interferon components of little or no cross-species activity.

Returning now to the above mentioned concept of deliberately employing procedures adapted to recovery of human leukocyte interferon, it may be appreciated that the procedures described above in detail are only exemplary, although preferred. Thus, the Cantell method for preparation of human leukocyte interferon for clinical use may be employed for production of the leukocyte bovine, ovine, porcine and equine inteferons. For description of the Cantell procedure reference is made to Mogensen and Cantell, "Production and Preparation of Human Leukocyte Interferon" in Pharm. Thera. C, Vol. 1, pp. 369-381, 1977, Pergamen Press, Great Brtain. For convenience the table outlining the purification scheme is reproduced below.

I. Purification Scheme



Note: C-IF, crude concentrated interferon; P-IF, partially-purified interferon, fraction B; PBS, 0.01 M phosphate-buffered saline, pH 7.2; SDS, sodium dodecyl sulphate (SDS-interferon, refers to interferon supposedly saturated with SDS); KSCN, potassium thiocyanate; ppt, precipitate; spn, supernatant.

II. Yields and Purity

	Volume	titer x 10^6 units/ml	specific activity x 10^6 units/mg protein	percent recovery
Crude	30 l	0.035*	0.01-0.05	
C-IF	1000 ml	0.5-2.0	0.01-0.05	50-100
P-IF	30 ml	5.0-20.0	0.10-0.50	50
P-IF-B	15 ml	10.0-20.0	0.50-1.00	25

* mean titer

When the Cantell procedure was applied to the animal leukocyte interferons of interest herein, the recovery varied in the 15%-20% range batch to batch. The specific activity of the animal leukocyte interferon to human cells was about 10^6 International Reference Units per mg of protein.

What is claimed is:

Claim 1 - A leukocyte interferon selected from the group consisting of the ovine, porcine, equine, and bovine species exceeding about 10^6 International Reference Units per mg of protein assayed against human cells.

Claim 2 - A bovine leukocyte interferon exceeding 10^6 International Reference Units per mg of protein assayed against human cells.

Claim 3 - A porcine leukocyte interferon exceeding 10^6 International Reference Units per mg of protein assayed against human cells.

Claim 4 - An interferon of claim 1 exceeding 10^7 International Reference Units per mg of protein.

Claim 5 - A process for manufacture of an interferon which protects human species cells against viral infections which comprises incubating live leukocytes from an animal of the species selected from bovine, equine, ovine and porcine with an interferon inducing agent, separating the interferon containing supernatant from said leukocytes then recovering the interferon, and thereafter purifying the recovered interferon to an activity level exceeding 10^6 International Reference Units per mg of protein assayed against human cells by a procedure adapted to purify human leukocyte interferon to a like activity level.

Claim 6 - The process of claim 5 wherein the interferon inducing agent is a virus infectious to the animal species source of the leukocytes.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US79/00268

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [3]

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

Int. Cl. A61K45/02; A61K 39/42
U.S. Cl. 424/85; 424/86

II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System	Classification Symbols
U.S.	424/85,86

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [5]

Chemical Abstracts, 1967-1978 under heading; Interferons

III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No. [18]
X	N, Chemical Abstracts, Vol. 77 Issued 1972, Vengris et al, Swine Interferon I. Induction in Porcine cell Cultures with Viral and Synthetic Inducers, Can. J. Comp. Med 1972, 36 (3), 282-7, See Abstract No. 111830e	1-4
X	N, Intervirology, Vol. 8 Issued 1977, Babiuk et al, Bovine Type II Interferon: Activity in Heterologous Cells, See pages 250-256	1-4
X	N, J. Gen, Virol, Vol. 36 Issued 1977, Tovey et al, Antiviral Activity of Bovine Interferons on Primate Cells, See pages 341-344	1-4
X	N, Proc. Soc. Exp. Biol. Med., Vol. 124(1), Issued 1967, Kono, Rapid Production of Interferon in Bovine Leucocyte Cultures, See pages 155-159	1-4

* Special categories of cited documents: [15]

"A" document defining the general state of the art

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

"L" document cited for special reason other than those referred to in the other categories

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

"P" document published prior to the international filing date but on or after the priority date claimed

"T" later document published on or 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

IV. CERTIFICATION

Date of the Actual Completion of the International Search [2]	Date of Mailing of this International Search Report [2]
24 October 1979	21 NOV 1979
International Searching Authority [1]	**Signature of Authorized Officer [20]**
ISA/US	Vincent D. Turner

Form PCT/ISA/210 (second sheet) (October 1977)

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

A	N, Biochemistry, Vol. 15, No. 3 Issued 1976, Davey et al, "Binding of Human Fibroblast Interferon To concanavalin A-Agarose. Involvement of Carbohydrate Recognition and Hydrophobic Interaction, See pages 704-713	5-6

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [10]

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 [12] 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 [13], specifically:

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [11]

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:

Remark on Protest

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

☐ No protest accompanied the payment of additional search fees.