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[54] Title: TREATMENT OF DISEASES CAUSED BY RETROVIRUSES

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A method of treatment for a disease caused by a retrovirus, which comprises administering, to a patient in need of such treatment, a therapeutically effective amount of a compound having a saccharic carbon atom, said compound being selected from the group consisting of:

(a) a natural oligosaccharide,
(b) a synthetic oligosaccharide,
(c) a natural polysaccharide,
(d) a synthetic polysaccharide, and
(e) a pharmaceutically acceptable salt of (a), (b), (c), or (d);

wherein said compound has at least one 3-oxoacid group attached to said saccharic carbon atom through a linking group of lower molecular weight.

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TREATMENT OF DISEASES CAUSED BY RETROVIRUSES

FIELD OF THE INVENTION

The present invention relates to prevention, therapy, etc. of diseases caused by retroviruses. More particularly, the present invention provides a medicament (including veterinary medicament) containing as active ingredient a natural or synthetic oligo- or polysaccharide having at least one S-oxoacid group attached to the saccharic carbon atom through a linking group of lower molecular weight or a pharmaceutically acceptable salt thereof and a method of prevention and therapy etc. of the diseases caused by retroviruses, especially AIDS (acquired immune deficiency syndrome), ARC(AIDS-related complex), PGL (persistant generalized lymphadenopathy) and AIDS-virus carrier using such medicament.

BACKGROUND OF THE INVENTION

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Retroviruses refer to a family of virus which has RNA and reverse transcriptase (RNA-dependent DNA polymerase), which is essential to the first stage of its self-replication for synthesizing complementary DNA on the base of template RNA of the virus.

Retroviruses include various oncoviruses such as avian leukemia virus, avian sarcoma virus, avian reticuloendotheliosis virus, murine mammary cancer virus, murine leukemia virus, murine sarcoma virus, guinea pig type C virus, hamster type C virus, rat leukemia virus, feline leukemia virus, feline sarcoma virus, feline type C virus, ovine leukemia virus, bovine leukemia virus, swine type C virus, simian leukemia virus, Mason-Pfizer virus, simian sarcoma virus, simian T-lymphotropic virus, baboon type C virus, and the like. Among those infective to human, important ones are adult T-cell leukemia virus (ATLV), or human T-lymphotropic virus type I (HTLV-I), and type II (HTLV-II). The adult T-cell leukemia abounds in Japan, especially in the west part, but the effective treatment containing prevention and therapeutics of the disease has not been established.

On the other hand, retroviruses also include those having no oncogenecity, such as visna virus, ovine progressive pneumonia virus, ovine maedi virus, simian T-lymphotropic virus type III (STLV-III), equine infectious anemia virus, and the like. The viruses isolated from human

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as causative agents for AIDS or ARC etc. (HTLV-III, LAV1, LAV2, ARV, and other so-called AIDS-viruses.) belong to this subfamily. Recently, AIDS-causative viruses are called HIVs (human immune deficiency viruses).

Further, as the third subfamily, there is known a spumavirinae to which simian foaming virus belongs. Also, a retrovirus has been isolated recently as a causative virus for Kawasaki disease (mucocutaneous lymphonode syndrome).

World-wide interests have been focused on AIDS due to its unfavorable prognosis. It is a clinical syndrome characterized by recurrent oppotunistic infections, (e.g. preumocystis carinii preunonia, cryptococcal meningitis, disseminated toxoplasmosis), lymphadenopathy, and an aggressive Kaposi's sarcoma, and induces a high mortality more than 90 % by the dysregulation of immune system. It is also known that the helper-T cells are specifically destroyed by the infection of the virus.

In order to find out pharmaceutical agents effective on the treatment of AIDS, ARC, PGL, and AIDS-virus carrier, the present inventors, using a cell line of MT-4 established from T-cells of adult T-cell leukemia patient and HTLV-III which is a causative virus for AIDS, examined the effects of various substances on the infection and replication of HTLV-III.

The above MT-4 cell line is absolutely susceptible to the infection with HTLV-III followed by causing cell lysis

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(experimental HTLV-III infection). The present inventors found that when certain polysaccharides having sulfonate group ($-SO_3^-$) or mucopolysaccharides having sulfonate group or their additionally sulfuric acid esterified substances were added to the experimental HTLV-III infection system, the infection of HTLV-III on MT-4 cells and viral replication were strongly inhibited without accompanying any toxicity to the cells.

Further, the present inventors demonstrated that the above polymerized sugar inhibits the reverse transcriptase of the retrovirus in vitro, and thereby suppresses the replication of the virus.

RELATED DISCLOSURES

Among the sulfuric acid esters of polysaccharides, dextran sulfate with lower molecular weight has long been commercialized as an antilipemic or anti-arteriosclerosis agent. Also, dextran sulfate with relatively higher molecular weight is known to have an inhibitory action against herpes virus. (European Patent Laid-Open Publication No.0066379). However, since the herpes virus is a DNA virus, its replication is absolutely different from that of the retrovirus which depends entirely on reverse transcriptase for synthesis of DNA. Accordingly, the effectiveness on herpes virus does not necessarily mean the effectiveness on retrovirus alike. Furthermore, dextran

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sulfate with lower molecular weight less than 10,000 was found to be almost ineffective on herpes viruses.

Among the mucopolysaccharides or these sulfates, chondroitin sulfate is commercialized as a drug for sensorineural hearing impairment, neuralgia, lumbago and chronic nephritis, and also as a cornea-protective ophthalmic solution. Keratan sulfate is obtainable from the cartilage, teichronic acid from the cell walls of Bacillus subtilis, hyaluronic acid from shark skin, whale cartilage, or from human serum, heparan sulfate from bovine liver or lung, and chitin from arthropod or from fungus or yeast, respectively. The preparation process for the further sulfuric acid esterified compound of chondroitin sulfate is described in Japanese Patent Publication (JP, B2) No.9570/1971.

Heparin is known to inhibit various enzymes in vitro, e.g., DNA polymelase of phytohemagglutinin stimulated human lymphocytes and reverse transciptase of simian sarcoma virus (Cancer Research, 38, 2401 - 2407), but is not proved to inhibit the viral infection of cells.

SUMMARY OF THE INVENTION

In one aspect, the present invention provides a method of treatment of diseases caused by retroviruses which comprises administering an effective amount of a natural or synthetic oligo- or polysaccharide having at least one S-oxoacid group attached to the saccharic carbon atom

through a linking group of lower molecular weight or a pharmaceutically acceptable salt thereof to a subject in need of such treatment.

In another aspect, the present invention provides a use of the above oligo- or polysaccharide or a salt thereof for the manufacture of a medicament for treatment of diseases caused by retroviruses.

In a further aspect, the present invention provides a pharmaceutical composition comprising the above oligo- or polysaccharide or a salt thereof as an active ingredient in association with a pharmaceutically acceptable carrier, diluent or excipient.

BRIEF EXPLANATION OF THE DRAWINGS:

Figs. 1 - 7 show the reverse transcriptase inhibition activities of the test substances in Example 1.

Fig. 8 shows the reverse transcriptase inhibition activity of the test substance in Example 2.

Figs. 9 - 15, 16 - 22, and 23 - 29 show the effects of the test substances on cell growth, viability, and infected cell rate (%) of MT-4 cells infected with HTLV-III, respectively, in Example 3.

Figs. 30 - 33 show the reverse trascuptase inhibition activities of the test substance in Example 5.

Figs. 34 - 37, 38-41, 42 - 45 show the effects of the test substaces or cell growth, viability, and infected cell

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rate (%) of MT-4 cells infected with HTLV-III, respectively, in Example 6.

Figs. 46 - 48 show the effects of heparin on cell growth, viability and infected cell rate of MT-4 cells infected with HTLV-III, respectively, in Example 7.

DETAILED DESCRIPTION AND PREFERRED EMBODIMENT

The term "treatment" herein is intended to cover all controls of disease including prevention, sustention (i.e. prevention of aggravation), reducing (i.e. alleviation of conditions) and therapy.

The retroviruses includes all viruses having RNA and reverse transcriptase as the basic components including those exemplified above.

The diseases referred to herein cover all the diseases caused by retroviruses, including those bearing or not bearing the aforementioned virus name. Important diseases are the diseases caused by AIDS-viruses.

The oligo- or polysaccharide usable in the present invention are those having at least one S-oxoacid group attached to the saccharic carbon atom through a linking group of lower molecular weight. Such oligo- or polysaccharide may be natural or synthetic. The term "natural" is intended to mean that the oligo- or polysaccharide is obtainable from a natural source such as plant, microorganism or animal by extraction and other means. The term "synthetic" is intended to mean that the

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oligo- or polysaccharide is obtainable synthetically, for example, by introducing S-oxoacid group into other oligo- or polysaccharide which has or has not S-oxoacid group and which is nartural or unnatural (and synthetic).

The term "oligosaccharide" refers to a carbohydrate containing from two up to about nine monosaccharides linked together. For example, when a oligosaccharide contains three monosaccharides, one, two or three of the monosaccharides may have at least one S-oxoacid group.

The term "polysaccharide" refers to a carbohydrate containing about ten or more monosaccharides linked together. At least one and a minor or major part or the all of the monosaccharides may have at least one and normally up to four S-oxoacid groups.

The S-oxoacid group includes sulfo group ($-SO_3H$) and hydroxysulfinyl group (-SO.OH). Preferable S-oxoacid group is sulfo group.

The term "saccharic carbon atom" refers to a carbon atom which is a member of tetrahydrofuran or tetrahydropyran ring of monosaccharide contained in the oligo- or polysaccharide.

The linking group of lower molecular weight includes oxy (-O-), imino (-NH-), thio (-S-), methylene ($-CH_2-$), ethylidene ($-CH(CH_3)-$) groups and the like. The term "lower molecular weight" is intended to mean that the group has a

molecular weight from about 14 up to about 32. Preferable linking group is oxy and imino groups.

One class of the oligo- or polysaccharide is a natural polysaccharide having at least one hydrogen sulfate group ($-O-SO_3H$) and is obtained from a plant or a microorganism, or a synthetic polysaccharide having at least one hydrogen sulfate group ($-O-SO_3H$) and is formed by esterifying a polysaccharide obtained from a plant or a microorganism.

Within this class, a preferable subclass is polysaccharide composed of non-amino monosaccharide (including sugar acid) as the repeating unit. This polysaccharide, however, may contain a trace amount of nitrogen. Examples of the non-amino sugar repeating units include xylose, arabinose, rhamnose, fucose, glucose, galactose, glucuronic acid, galacturonic acid, mannuronic acid, etc. The natural polysaccharide includes carrageenan (galactan sulfate obtainable from Gigartina tenella, etc.) and fucoidin (polyfucose sulfate obtainable from Laminaria brown seaweed). Carrageenan includes κ-carragheenin, λ-carrageenan, ι-carrageenan, etc. which have the different content of hydrogen sulfate group. The synthetic polysaccharide includes those to be obtained by sulfuric acid esterification of polysaccharides, e.g., starch and partial hydrolyzate thereof, dextran which is produced by Leuconostoc sp. and partial hydrolyzate thereof (usually having the molecular weight of 500 - 2,000,000, ordinarily

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2,000 and 300,000, preferably 2,000 - 10,000, most suitably 3,000 - 8,000, e.g., 7,000 - 8,000), glycogen, pectin, cellulose, plant viscous liquids (gum arabic, tragacant gum, etc.), plant mucilage products (those obtainable from Hibiscus esculentus, Aloe, Brasenia schreberi, xylan, etc.), viscous liquids of marine and fresh water algae (alginic acid, laminarin, etc.) or polysaccharide derived from microorganism (lentinan, pluran, mannan, etc.). They include known ones (dextran sulfate, cf., European Patent Laid-open Publication No.0066379) and novel ones. The novel ones may be produced in the same manner as in the known ones. An example of the preparation process is shown, as follows:-

Chlorosulfonic acid is added dropwise to dry pyridin of 8 - 10 fold volume while cooling. To the mixture are added small amounts of formamide and dextran (about 1/4 weight of chlorosulfonic acid), and the mixture is heated to 55 - 65° C under stirring. After stirring the mixture for several hours, the solvent is distilled off, and the residue is purified for example by recrystalization, dialysis, etc. Within the synthetic polysaccharide, those obtained by further sulfuric acid esterification is represented by the term "polysulfate".

Another class of the oligo- or polysaccharide is a natural polysaccharide having at least one hydrogen sulfate group ($-O-SO_3H$) and is obtained from an animal, or a

synthetic polysaccharide having at least one hydrogen sulfate group ($-O-SO_3H$) and is formed by esterifying a polysaccharide obtained from an animal.

Within this class, a preferable subclass is mucopolysaccharides, which is composed of amino monosaccharide (including N-acyl or $NH-SO_3H$) as the repeating unit. This may further contain as another repeating unit non-amino sugar or an acid derivative thereof. The repeating amino-sugar unit or its N-acylated (preferably N-acetylated) derivatives include glucosamine, galactosamine, N-acetylated derivatives of them, and sulfuric acid ester or partial hydrolyzate of the above compound. Examples of monosaccharide or acid (preferably, hexulonic acid) includes glucose, galactose, glucuronic acid, iduronic acid, etc. The mucopolysaccharides containing such repeating unit include heparin, keratan sulfate, chondroitin-4-sulfate, chondroitin-6-sulfate, dermatan sulfate, teichuronic acid, hyaluronic acid, heparitin sulfate, chitin, and their partial hydrolyzates, modified derivatives (e.g., partial acylated products), and synthetic polysacchrides containing the repeating unit such as above.

The mucopolysaccharide polysulfates are defined as the products which are synthesized by additional sulfuric acid esterification of the above mucopolysaccharides having sulfate group. This esterification may be carried out, for example, according to the procedure described in Japanese

Patent Publication No.9570/1971. In general, the esterification is carried out by treatment of the mucopolysaccharides with one of sulfating reagents such as concentrated sulfuric acid or chlorosulfonic acid.

These reactions are usually carried out with or without a solvent at low temperature. The reaction product is separated by conventional procedure, e.g., neutralization, concentration, precipitation, recrystarization, chromatography, etc.

The term "pharmaceutically acceptable salt" is intended to mean that the salt has the biological activity of the parent compound and is not unusably toxic at the administration level. Such salt includes the salt of inorganic base such as sodium salt, potassium salt, ammonium salt, etc., and salt of organic base such as diethanolamine salt, cyclohexylamine salt, amino acid salt, etc. These salts are produced from the corresponding acids by the conventional procedures. The above oligo- or polysaccharides and their salts may be solely used or as a mixture with the metal salts such as zinc, aluminum, etc. The oligo- or polysaccharide should be administered at a dose sufficient to produce the effect for the desired treatment. For example, the dosage of the sulfates of the above polysaccharide or their salts sufficient to produce blood concentration for anti-virus activity is generally 0.2 - 200 mg/kg, preferably 0.5 - 100 mg/kg. In the case of

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human, an amount of about 10 mg - 10 g/day, preferably about 50 mg - 5 g/day, is administered in 1 - 4 divisions a day, or as a sustained release form. The administration route can be optional such as oral, rectal, nasal, local(including sublingual), injection(including subcutaneous, intracutaneous, intramuscular and intravenous), inunction etc. Preferable route is selected depending on various factors including kind of active ingredient, conditons and age of patient, severity of infection etc.

The dosage of the mucopolysaccharides or their polysulfate or the salts thereof sufficient to produce a concentration for anti-virus activity is generally 0.2 - 200 mg/kg, preferably 0.5 - 100 mg/kg. In the case of human, an amount of about 10 mg - 20 g/day, preferably about 50 mg - 10 g/day is administered in 1 - 4 divisions a day, or as sustained release form.

The administration route can be optional such as oral, local, injection, inunction, etc.

For administration, the effective ingredient may be mixed with a pharmaceutical carrier such as organic or inorganic solid or liquid excipient e.g. suitable for internal administration or injection, and administered in the form of the conventional pharmaceutical preparation. Such preparation includes solids (e.g., tablet, granule, powder, capsule, etc.) and liquids (e.g., liquid, emulsion, suspension, etc.), and ointment. The above carriers include

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starch, lactose, glucose, sucrose, dextrin, cellulose, paraffin, fatty acid glyceride, water, alcohol, gum arabic, etc. If necessary, auxiliary, stabilizer, emulsifier, lubricant, binder, pH regulating agent, isotonicity agent, and other additives in ordinary use may be added.

The toxicity of the above oligo- or polysaccharide is extremely low. For example, the acute toxicity (LD_{50}) of sodium dextran sulfate (molecular weight 7,000 - 8,000, S-content 17 - 20 %) is 21,000 mg/kg when orally, and 4,500 mg/kg when intravenously administered to mice.
The acute toxicity (LD_{50}) of sodium chondroitin sulfate is 4,000 mg/kg or more when intraperitoneally, and 7,500 mg/kg or more when orally administered to mice. The acute toxicitiy (LD_{50}) of sodium heparin is 1,500 - 2,000 mg/kg when intravenously injected to mice.

The following examples will illustrate the present invention in further detail.

Preparation 1

Preparation of chondroitin polysulfate from chondroitin sulfate

Chondroitin sulfate (5g) was added to 95 % sulfuric acid (10 ml) cooled at below -25°C with stirring. After addition, the reaction mixture was stirred at the same temperature for 90 minutes. After the end of the period, the reaction solution was gradually poured onto ice (120 g)

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with stirring. To the resulting solution was gradually added calcium carbonate with well stirring. The precipitates were filtered off, which, then were washed well with water. To the combined filtrates (240 ml) was added ethanol (60 ml), and the solution was kept to stand overnight at 5 °C to precipitate calcium sulfate. The precipitates were filtered off, and the filtrate was adjusted to pH 10 with sodium carbonate. After addition of acetic acid to make the solution weakly acidic, the solution was concentrated to about 20 ml, then diluted with ethanol (100 ml), and kept to stand overnight at 5 °C. The precipitates in the solution were isolated with centrifugation, washed with ethanol, and with ether, and dried under vaccum to give the white powder of the title compound.

Preparation 2

Preparation of keratan polysulfate from keratan sulfate.

Preparation 1 was repeated except that keratan sulfate (100 mg) is used as a starting material and 1 ml in place of 10 ml of 95% sulfuric acid is used, to give the title compound.

Formulation 1

Sodium dextran sulfate (molecular weight: 7,000 - 8,000, S-content: 17 - 20%)	150 mg

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Corn starch	45 mg
Lactose	300 mg
Magnesium stearate	5 mg

The above ingredients are mixed, granulated, and pressed according to the conventional procedure to make tablets, which were then enterically coated.

Formulation 2

Sodium dextran sulfate (molecular weight: 7,000 - 8,000, S-content: 17 - 20%)	600 mg
Physiological saline	q.s. to 10 ml.

Formulation 3

Sodium dextran sulfate (molecular weight: 5,000, S-content: 13 - 14%)	600 mg
Physiological saline	q.s. to 10 ml.

Formulation 4

Sodium salt of chondroitin sulfate	150 mg
Corn starch	45 mg
Lactose	300 mg
Magnesium stearate	5 mg

The above ingredients are mixed, granulated, and pressed according to conventional procedure to make tablets, which were then enterically coated.

Formulation 5

Sodium salt of keratan polysulfate	400 mg

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Lactose	195 mg
Magnesium stearate	5 mg

The above ingredients are mixed according to the conventional procedure and filled in hard gelatine capsules.

Formulation 6

Sodium salt of chondroitin polysulfate	300 mg
Physiological saline	q.s. to 10 ml.

Formulation 7

Sodium heparin	25,000 units
Physiological saline	q.s. to 10 ml.

Formulation 8

Calcium heparin	5000 units
Procain hydrochloride	10 mg
Water	q.s. to 10ml.

Example 1 (Inhibition of reverse transcriptase activity)

Test substances were assayed for inhibition against the enzyme activity of reverse transcriptase (authentic sample) derived from Avian Myeloblastosis Virus (abbrev. AMV), a kind of retrovirus.

Five microliters of $(\gamma A)_n$ (template RNA), 4 µl of $(dT)_{12-18}$ (primer DNA), and 1 µl of water were mixed with 5 µl of 0.5M Tris-HCl (pH 8.4) including 0.1 % triton X-100, 5 µl of 1nM-$MgCl_2$, 5 µl of 20 mM-DDT, 5 µl of water, and [^{3}H]-TTP (tritium labeled thymidine triphosphate). To this mixture, test substances in solutions (final concentrations:

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1, 0.1, and 0.01 μg/ml, 5 μl) or buffer solutions (control, 5 μl) at various doses were added. Then, 5 μl (one unit) of the authentic reverse transcriptase derived from AMV was added and the reaction mixture was incubated at 37°C for 30 minutes. The reaction was stopped by addition of trichloroacetic acid, and after filtering the reaction mixture, the radioactivity of the polymerized (^{3}H-T)n retained on the filter was measured using a liquid scintilation counter. As the test substances, sodium dextran sulfate (molecular weight: 5000), same (molecular weight: 8000), same (molecular weight: 500000), fucoidin, κ-carrageenan, λ-carrageenan, and ι-carrageenan were used. The results are shown in Figs. 1-7.

Figs. 1-7 show that the enzyme inhibition increases with the increasing doses of the above test substances.

Example 2 (Inhibition of reverse transcriptase activity)

The assay procedure of Example 1 was repeated using disrupted HTLV-III virions as a crude reverse transcriptase in order to evaluate the reverse transcriptase inhibitory effect of dextran sulfate (DS, molecular weight 7,000 - 8,000, S-content 17 - 20 %). The result is shown in Fig. 8.

Fig. 8 shows that DS has an inhibitory effect against the reverse transcriptase derived from AIDS-virus, HTLV-III.

Example 3 (Anti-AIDS virus activity)

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To MT-4 cells (30 x 10^4/ml) cultured in RPMI-1640 medium containing 10 % bovine serum, was inoculated HTLV-III, and the suspension was incubated at 37 °C for 1 hour to cause the adsorption of the virus. The cell : virus ratio was 500:1. The cells were then washed, and cultured with or without various doses of the test substances (same as those of Example 1) at 37 °C under 5 % CO_2 for 3 days, after which cell growth, viability, and percentage of infected cells were recorded. The infected cells were distinguished from the uninfected cells by indirect immuno-fluorescence method. Thus ; the cultured cells were fixed with cold methanol on a slide glass, reacted with antibody to the HTLV-III-specific antigens, and further with the secondary antibody (having fluororescent label). The results are shown in Figs. 9-29, wherein, ▽, △ and □ show the controls without virus, ▼, ▲ and ■ show the infection experiments with HTLV-III. The cell growth is indicated in number of cells, the viability (%) in number of viable cells x 100/number of total cells, and the infected cell rate (%) in number of fluorescent-positive cells x 100/number of total cells.

Figs. 9-22 demonstrate that when no test compound was added to the medium, the cells did not grow and were killed by viral infection, whereas depending on the increase in the dose of the test substance, the number of cells and viability came near to the values of the control without

virus. Also, it is shown from Figs. 23 - 29 that when the test substance is not added, almost all cells are infected (- 100%), whereas depending on the increase in the dose of the test substances, the infection of cells was strongly inhibited.

Accordingly, it is evident that the test substances have excellent inhibiting activities against infection of AIDS virus to host cells and viral proliferation.

Example 4 (Cytotoxicity)

As the anti-virus substances often show toxicity to the host cells, the following experiment was conducted to know whether or not the test substances (used in Example 1 and 3) would induce cytotoxicity.

MT-4 cells were cultured with or without 1 - 100 μg/ml of each test substance which is the same as in Example 1 and 3 and the proliferation and viability of cells were recorded. The results are shown in the following Table.

Substance (μg/ml)	Cell number ($\times 10^4$ cells/ml)	Viability (%)
Sodium dextran sulfate (molecular weight: 5000, S-content: 13%)		
100	121	93
10	127	92

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1	123	90
0	124	87

Sodium dextran sulfate (molecular weight: 7000 - 8000, S-content: 17 - 20%)

100	130	94
10	138	91
10	120	90
0	124	87

Sodium dextran sulfate (molecular weight: 500,000 S-content: 16%)

100	126	86
10	139	94
1	124	88
0	124	87

Fucoidin

100	71	93
10	112	99
1	141	92
0	124	87

κ-Carrageenan 80 % + λ-Carragheenin 20 %

100	111	92
10	149	93
1	147	93
0	124	87

λ -Carrageenan

100	83	94

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10	203	94
1	147	89
0	124	87
ι-Carrageenan		
100	144	80
10	128	93
1	135	94
0	124	87

The above results show that the test substances have little cytotoxicity.

Example 5 (Inhibition of reverse transcriptase activity)

The effects of the test substances on the reverse transcriptase activity of AMV were evaluated by the method described in Example 1. The test substances used are chondroitin sulfate (S-content: 6.2 - 6.9 %), chondroitin polysulfate (S-content: 11.6 - 12.1 %), keratan sulfate (S-content: 7.0 - 8.0 %), and keratan polysulfate (S-content: 9.7 %). The results are shown in Figs. 30 - 33.

Figures 30 - 33 indicate that the enzyme inhibition increases with the increasing doses of the above test substances. The above results also demonstrate that the reverse transcriptase inhibitory activity of the test substances is closely related to the number of sulfate group in the molecule, as evidenced by the fact that the synthetic

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substances (e.g. condroitin polysulfate and keratan polysulfate) have stronger activity than the natural substances (e.g. condroitin sulfate and keratan polysulfate).

Example 6 (Anti-AIDS virus activity)

Test substances were assayed for the anti-AIDS virus activity in the same manner as in Example 3 using cell culture. The test substances are the same as those used in Example 5. The results are shown in Figs. 34 - 45, wherein ▽, △ and □ show the controls without virus, ▼, ▲ and ■ show the infection tests with virus.

Figs. 34 - 41 demonstrate that, without the test substances, the cells did not grow and were killed by viral infection, whereas depending on the increase in the dose of the test substance, the decrease in number of cells and loss of viability were prevented. Also, Figs. 42 - 45 demonstrate that when the test substance was not present, almost all of the cells were infected ($\simeq$ 100%) with HTLV-III, whereas depending on the increase in the dose of the test substances, the infected cell rate (%) was significantly reduced.

The above results also indicate that the synthetic mucopolysaccharide polysulfates having higher S-content had stronger anti-AIDS virus activities than those of the natural products.

Example 7 (Anti-AIDS virus activity)

The anti-AIDS virus activity of heparin was evaluated in the same manner as in Example 3. The results are shown in Figs. 46 - 48, wherein ▽, △ and □ show the controls without virus, and ▼, ▲, and ■ show the infection tests with virus.

Figs. 46 and 47 show that without heparin, the cells did not grow and were killed by viral infection, whereas depending on the increase in the dose of heparin, the number of cells and viability were maintained to that of control. It was also shown from Fig. 48 that when heparin was not present, almost all of the cells are infected, whereas depending on the increase in the dose of heparin, they become less susceptible to the viral infection.

Example 8 (Cytotoxicity)

As the anti-virus substances often show toxicity to the host cells, experiment was conducted to know whether or not heparin would induce such cytotoxicity.

Without the virus, MT-4 cells were cultured in the same manner as in Example 4 except the test sample was heparin, and proliferation and viability of cells were recorded. The results are shown in the following Table.

Heparin (μg/ml)	Cell number ($\times 10^4$ cells/ml)	Viability (%)
100	133	94

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10	142	89
1	143	91
0	124	87

The above results demonstrate that heparin has little cytotoxicity.

Example 9 (Anti-AIDS activity)

In order to examine the correlation between the anti-AIDS virus activity and the molecular structure (especially, molecular weight and S-content or number of sulfate group in this case) of various test substances including those used in Examples 1 - 7, the anti-AIDS activities were evaluated for the various naturally occurring polysaccharides, polysaccharides having sulfate group, mucopolysaccharides, mucopolysaccharide sulfate, and mucopolysaccharide polysulfate. Further, similar experiments were carried out with various other sulfates which were synthetically obtained. The experimental procedures employed are identical to that in preceding experiments. The cultured MT-4 cells were infected with HTLV-III and the inhibitory effects of various test subsutances on the infected cell rate (number of fluorescent cell x 100/ total cell, %) were determined at 6th day. The results are shown in the following Table.

1) Dextrans, their synthetic sulfates, and monosaccharides having sulfate groups.

Test substance	Molecular weight	S-content (%)	Infected cell(%)* 10μg/ml	100μg/ml	1000μg/ml
Dextran	9,000	0	100	100	
"	300,000	0	100	100	
Dextran sulfate	5,000	≃13	18	0	
"	8,000	≃14	25	0	
"	500,000	≃16	20	0	
"	7,000-8,000	17 -20	1	0	
"	3,500	6	100	82	1
(Monosaccharides)					
Glucose-6-sulfate		12	100	100	100
Glucose-polysulfate		22	100	100	100
N-acetylglucosamine polysulfate		18	100	100	100

* The control without test substance shows the value of 100% as the infected cell rate under the same conditions.

2) Polysaccharides derived from algae and their sulfates.

Test substance	Molecular weight	S-content (%)	Infected cell(%) 10μg/ml	100μg/ml
κ-Carraheenan		≃ 7	95	1
γ- "		≃ 16	3	1
ι- "		≃ 12	100	31
Fucoidan		≃ 15	32	1
Agarose	60,000 - 180,000	2 - 3	100	100
Alginic acid	32,000 - 240,000	0	100	100
Alginic acid sulfate	50,000 - 300,000	14	7	4

3) Chitin and chitosan and their sulfates

Test substance	Molecular weight	S-content (%)	Infected cell(%) 10μg/ml	100μg/ml
Chitin		0	100	100
Chitin sulfate		9	100	81
Chitosan		0	100	100
Chitosan sulfate		18	1	1

4) Mucopolysaccharides derived from animals, and their sulfates and polysulfates

Test substance	Molecular weight	S-content (%)	Infected cell(%) 10μg/ml	100μg/ml
Chondroitin	25,000 - 30,000	0	100	100
Chondroitin polysulfate	5,000 - 8,000	13	3	1
Chondroitin-4-sulfate	30,000 - 50,000	6	90	80
Chondroitin-4-sulfate polysulfate		16	2	1
Dermatan sulfate	20,000 - 40,000	6	100	80
Chondroitin-6-sulfate	30,000 - 50,000	6	100	100

Chondroitin-6-sulfate polysulfate		15	2	1
Heparin	7,000 - 30,000	13	41	1
Heparitin sulfate	15,000	7	100	90
Keratan sulfate	4,000 - 20,000	7	100	60
Keratan polysulfate		10	40	20
Hyaluronic acid	10,000 - 100,000	0	100	100
Hyaluronic acid sulfate		8	100	70

5) Other polysaccharides

None of pectin, coromic acid, inulin, raffinose, and methylcellulose showed any anti-AIDS virus activities.

From the above results, it can be clearly seen that the anti-AIDS virus activity is closely related to the S-content or number of sulfate group in this case rather than to the molecular weight. Substances without sulfate group showed no anti-AIDS activity. Further, the anti-AIDS activity was intensified with increasing S-content (number of sulfate group) of the molecule. With respect to the relation with the molecular weight, there was no effect at all in the monosaccharides. However, in the substances having molecular weights of 5,000 and higher, the increase of the molecular weight did not affect the anti-AIDS virus activity as seen in e.g. dextran sulfate.

This is quite different from the pattern of manifestation of heretofore known activities of polysaccharide sulfates against herpes virus.

In view of the fact that the polysaccharides with higher molecular weight and their sulfate are known to have the high toxicities to human being and animals, the experimental evidence obtained in the persent invention that the dextran sulfate with lower molecular weight show sufficient anti-AIDS activity, is extremely important in

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developing it as a medicament for prevention and therapy of the viral disease.

Among the above test substances, those which showed particularly strong anti-AIDS virus activities are dextran sulfate, λ-carrageenan, alginic acid sulfate, chitosan sulfate, chondroitin polysulfates, further sulfated chondroitin-4-sulfate and -6-sulfate, heparin, etc. having S-content more than 10%.

Example 10 (Anti-Friend leukemia virus (F-MuLV) activity)

(Procedure 1)

Anti-FMuLV activity of dextran sulfate (molecular weight: 7000 - 8000, S-content: 17 - 20 %) was determined by a XC-plaque assay method. BALB3T3 cells were cultured in adhesive form in a 35 mm-dish at 5×10^4 cells/dish (2 ml). After removing the culture medium, a fresh medium with or without indicated concentrations of the test substance (1 ml each) and 0.2 ml of the virus preparation were charged, and the cells were cultured overnight. On the following day, the culture media were replaced with those (2 ml) containing or not containing the above substances, the incubation was continued for three additional days to progress the infection and replication of the virus. After the removal of the medium, the further progression of viral replication was stopped by UV irradiation. To this dish, the suspension of XC-cells (2 ml) was added and cultured for three days and

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the plaque formation produced by the virus particle induced cell-fusion, was observed. The number of plaques was shown in the following Table.

Table

Anti-Freind leukemia virus activity by Procedure 1

DS (μg/ml)	Number of plaques per dish	Inhibition (%)
Control	168	(0)
1	14	92
5	12	93
10	11	93
50	13	92
100	6	96
1000	0	100

As observed from the above Table, DS inhibited 90 % or more the formation of plaque at the concentrations of 1 - 100 μg/l, indicating that the infection and replication of the virus was strongly inhibited. The plaque formation was not detected at 1,000 ug/ml of DS.

DS at 1 - 100 ug/ml did not show any cytotoxicity to BALB 3T3 cells.

(Procedure 2)

Procedure 1 was repeated except that after adsorption of the virus in the medium without DS, the non-adsorbed viruses were removed and the culture was carried out in the

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medium (2 ml) containing or not containing DS. The results are shown in the following table.

Table

Anti-Friend leukemia virus activity by Procedure 2:

DS (μg/ml)	Number of plaques per dish	Inhibition (%)
Control	35	(0)
0.01	33	6
0.1	19	45
1	14	61
10	17	52
100	16	54
500	0	100

The above results indicate that, also in Procedure 2, DS inhibited the infection and replication of the virus by about 60 % at the concentration of 1 μg, and almost completely at 500 μg/ml.

From the above results, it is evidenced that DS inhibits the infection and replication of the oncogenic virus (Oncovirinae) including F-MuLV, as well as the cytolytic virus (Lentivirinae) including AIDS-virus.

WHAT IS CLAIMED IS:

1. A method of treating disease caused by a retrovirus selected from the group consisting of avian leukemia virus, avian sarcoma virus, murine leukemia virus, murine sarcoma virus, guinea pig type C virus, hamster type C virus, rat leukemia virus, feline leukemia virus, feline sarcoma virus, feline type C virus, ovine leukemia virus, bonvine leukemia virus, swine type C virus, simian leukemia virus, Mason-Pfizer virus, simian sarcoma virus, simian T-lymphotropic virus, baboon type C virus, adult T-cell leukemia virus (HTLV), human T-lymphotropic virus type I (HTLV-I) and type II (HTLV-II), visna virus, ovine progressive pneumonia virus, ovine maedi virus, simian T-lymphotropic virus type III (STLV-III), equine infections anemia virus, human T-lymphotropic virus type III (HTLV-III), LAVI, LAV2, ARV and other HIVs, simian foaming virus, and Kawasaki disease-causative virus, which comprises administering, to a patient in need of such treatment, a therapeutically effective amount of a compound having a saccharic carbon atom, said compound being selected from the group consisting of:

(a) a natural oligosaccharic containing two to about nine monosaccharides linked together,

(b) a synthetic oligosaccharide having S-OXO-acid group attached to polysaccharides, and containing two to about nine monosaccharides linked together,

(c) a natural polysaccharide selected from the group consisting of plant, micro-organism and animal, and containing about ten or more monosaccharides linked together,

(d) a synthetic polysaccharide having S-OXO-acid group attached to polysaccharides, and containing about ten or more monosaccharides linked together, and

(e) a pharmaceutically acceptable salt of (a), (b), (c), or (d);

wherein said compound has at least one S-oxoacid group selected from the group consisting of sulfo group ($-SO_3H$) and hydroxysulfinyl group (-SO.OH), attached to said saccharic carbon atom through a linking group selected from the group consisting of oxy (-O-), imino (-NH-), thio (-S-), methylene ($-CH_2$) and ethylidene ($-CH(CH_3)-$) groups.

2. The method according to claim 1, wherein the said S-oxoacid group is a sulfo group ($-SO_3H$).

3. The method according to claim 1, wherein the said linking group is an oxy group (-O-) or an imino group (-NH-).

4. The method according to claim 1, wherein the said treatment is the prevention for a disease selected from the group consisting of: PGL, ARC, AIDS, ATL, and Kawasaki disease.

5. The method according to claim 1, wherein the said retroviruses are human retroviruses.

6. The method according to claim 5, wherein the said human retroviruses are HIVs, said HIVs selected from the group comprising HTLV-III, LAV, and ARV.

7. The method according to claim 5, wherein the said human retroviruses are selected from the group consisting of HTLV-I, HTLV-II, and Kawasaki disease causative retroviruses.

8. The method according to claim 1, wherein the said retroviruses are animal retroviruses.

9. The method according to claim 1, wherein the said retroviruses are selected from avian myeloblastosis virus and Friend murine leukemia virus.

10. The method according to claim 1, wherein the said treatment is prevention from infection by retroviruses.

11. The method according to claim 10, wherein the said retroviruses are HIVs.

12. The method according to claim 1, wherein the said compound has the ability to inhibit a reverse transcriptase of a retrovirus.

13. The method according to claim 1, wherein the said compound is further selected from the group consisting of:

(a) a natural polysaccharide sulfate ester containing about ten or more monosaccharides linked together,

(b) a synthetic polysaccharide sulfate ester having S-OXO-acid group attached to polysaccharides, and containing about ten or more monosaccharides linked together and

(c) a pharmaceutically acceptable salt of (a) or (b).

14. The method according to claim 13, wherein the said compound is selected from the group consisting of:

(a) a natural polysaccharide having at least one hydrogen sulfate group ($-O-SO_3H$) containing about ten or more monosaccharides linked together,

(b) a synthetic polysaccharide having at least one hydrogen sulfate group formed by esterifying a polysaccharide, having S-OXO-acid group attached to polysaccharides, and containing about ten or more monosaccharides linked together, with a sulfating agent, and

(c) a pharmaceutically acceptable salt of (a) or (b).

15. The method according to claim 14, wherein the said compound is a synthetic polysaccharide selected from the group consisting of: dextran sulfate, alginic acid sulfate, lentinan sulfate, pulluran sulfate and xylan polysulfate.

16. The method according to claim 15, wherein the said dextran sulfate has a molecular weight between 500 and 2,000,000.

17. The method of claim 16, wherein the said dextran sulfate has a molecular weight between 2,000 and 300,000.

18. The method of claim 17, wherein the said dextran sulfate has a molecular weight between 2,000 and 10,000.

19. The method of claim 18, wherein the said dextran sulfate has a molecular weight between 3,000 and 8,000.

20. The method according to claim 15, wherein the said dextran sulfate has a sulfur content of between approximately 5% and 22%.

21. The method of claim 20, wherein the said dextran sulfate has a sulfur content between 10% and 20%.

22. The method of claim 21, wherein the said dextran sulfate has a sulfur content between 15% and 20%.

23. The method according to claim 14, wherein the said compound is a natural polysaccharide selected from the group consisting of carrageenan and fucoidin.

24. The method according to claim 13, wherein the said compound is selected from the group consisting of:

(a) a natural polysaccharide having at least one sulfo group (-SO3H) containing about ten or more monosaccharide linked together,

(b) a synthetic polysaccharide having at least one sulfo group ($-SO_3H$) formed by esterifying a polysaccharide, having S-OXO-acid group attached to polysaccharide, and containing about ten or more monosaccharide linked together, with a sulfating agent, and

(c) a pharmaceutically acceptable salt of (a) or (b).

25. The method according to claim 24, wherein the said compound is a natural mucopolysaccharide selected from the group consisting of plant, microorganism and animal, and composed of aminomonosaccharide or acylated aminomonosaccharide, both containing sulfuric acid ester or $NH-SO_3H$ group, and optionally of non-amino sugar or an acid derivative thereof, as the repeating unit.

26. The method according to claim 24, wherein the said compound is a synthetic mucopolysaccharide having S-OXO-acid group attached to mucopolysaccharide, and composed of aminomonosaccharide or acylated aminomonosaccharide, both containing sulfuric acid ester or $NH-SO_3H$ group, and optionally of non-amino sugar or an acid derivative thereof, as the repeating unit.

27. The method according to claim 24, wherein the said polysaccharide is selected from the group consisting of heparin or a pharmaceutically acceptable salt thereof.

28. A method according to claim 24, wherein the said compound is a natural polysaccharide selected from the group consisting of: chondroitin sulfate, dermatan sulfate, heparitin sulfate, keratin sulfate, hyaluronic acid, teichronic acid, chitin and chitosan; or a pharmaceutically acceptable salt thereof.

29. The method according to claim 24, wherein the said polysaccharide is a synthetic polysaccharide selected from the group consisting of chondroitin polysulfate, dermatan polysufate, heparitin polysulfate, keratin polysulfate, hyaluronic acid sulfate; teichronic acid sulfate, chitin sulfate and chitosan sulfate, or a pharmaceutically acceptable salt thereof.

30. The method according to claim 1, wherein the said treatment is the sustention for a disease selected from the group consisting of: PGL, ARC, AIDS, ATL, and Kawasaki disease.

31. The method according to claim 1, wherein the said treatment is the reducing or the therapy for a disease selected from the group consisting of: PGL, ARC, AIDS, ATL, and Kawasaki disease.

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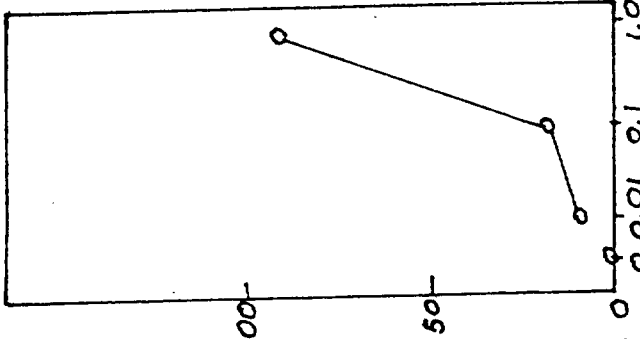
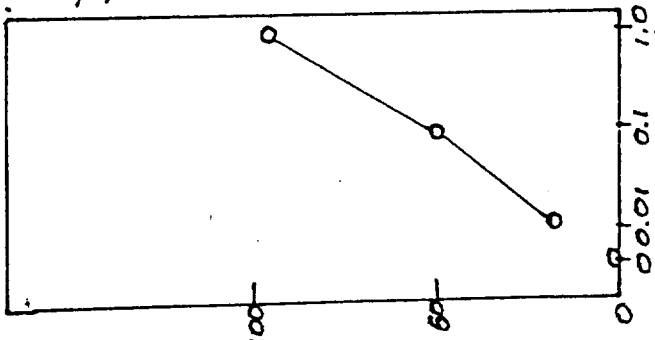
FIG. 1



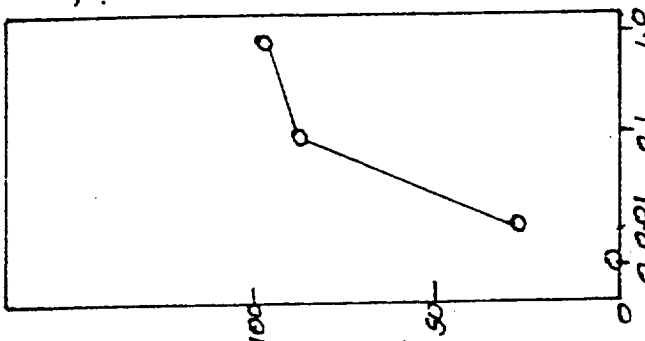
Na Dextran sulfate (M.W. 5000)

FIG. 2



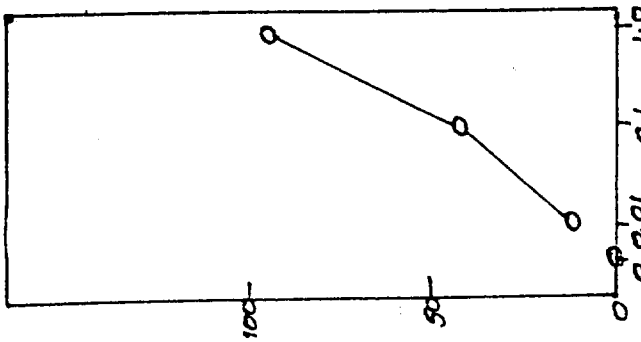
Na Dextran sulfate (M.W. 8000)

FIG. 3



Na Dextran sulfate (M.W. 500000)

FIG. 4



Fucoidin

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TREATMENT OF DISEASES CAUSED BY RETROVIRUSES

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FIG. 7

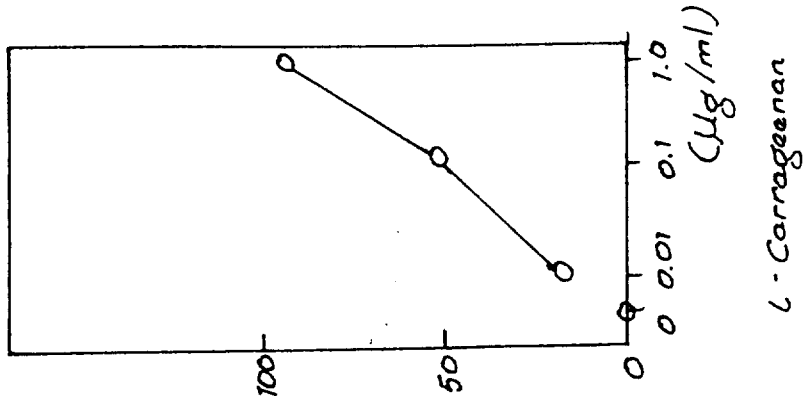

FIG. 6

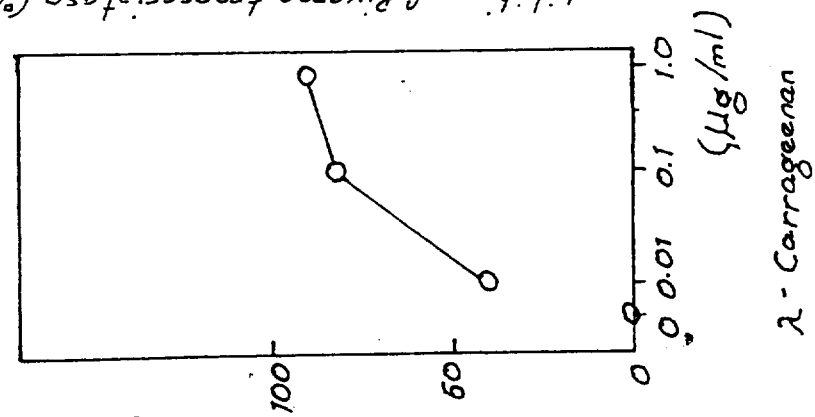

FIG. 5

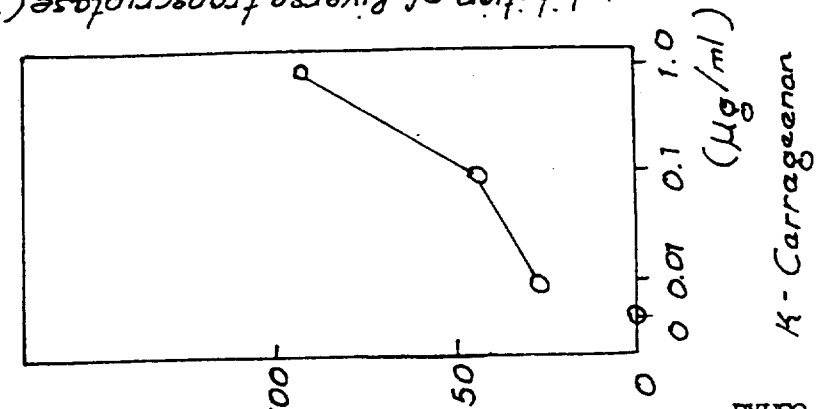

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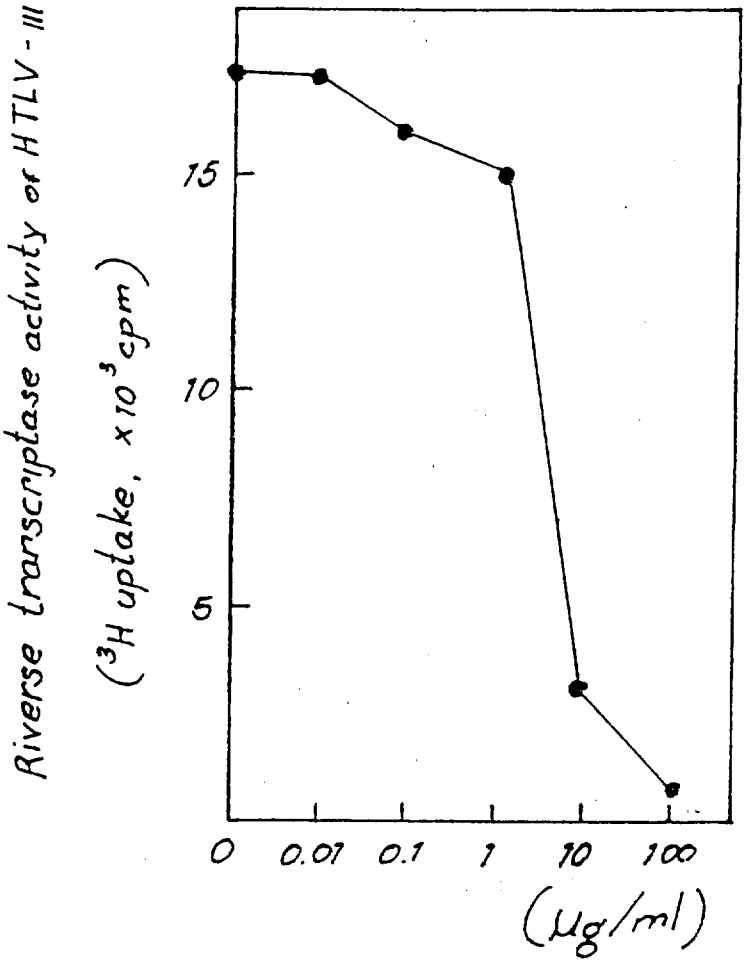
FIG.8



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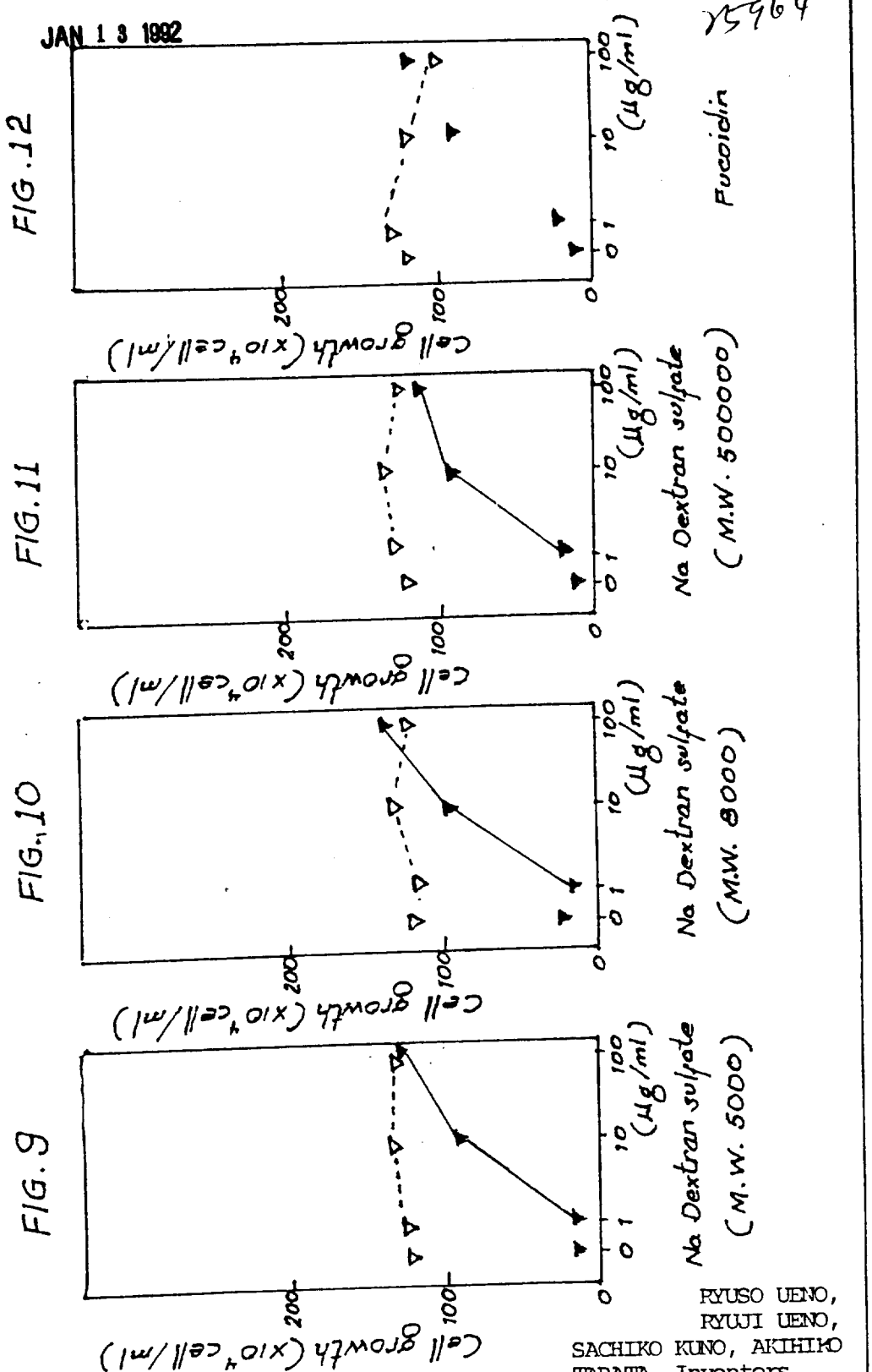

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FIG. 15

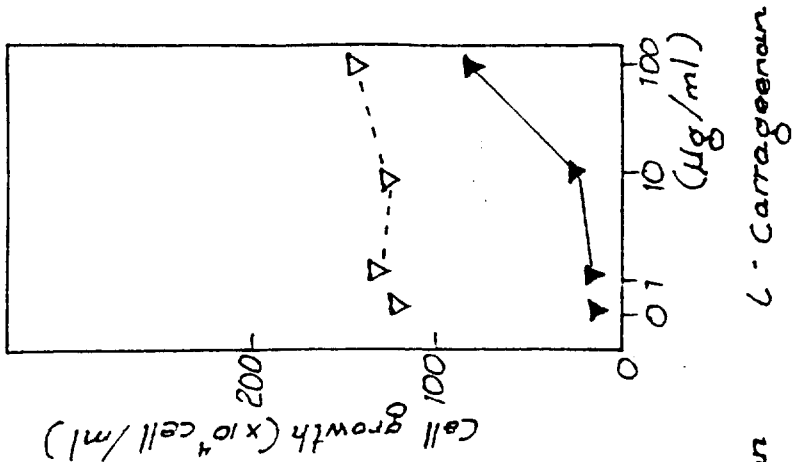


FIG. 14

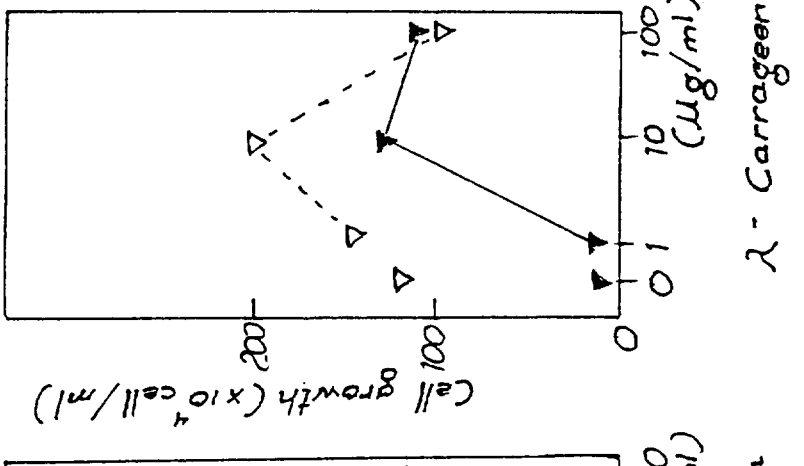
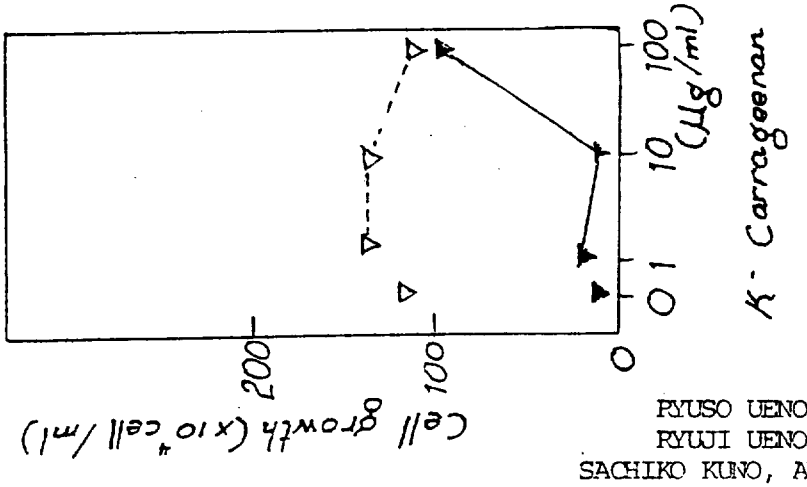


FIG. 13



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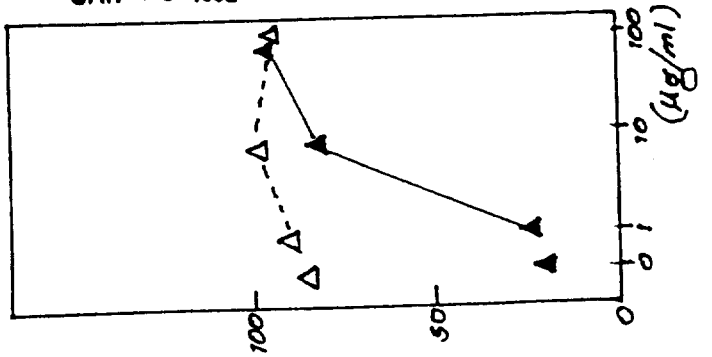

FIG. 19

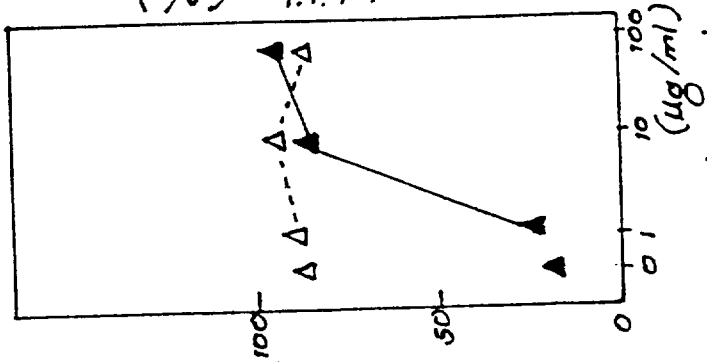

FIG. 18

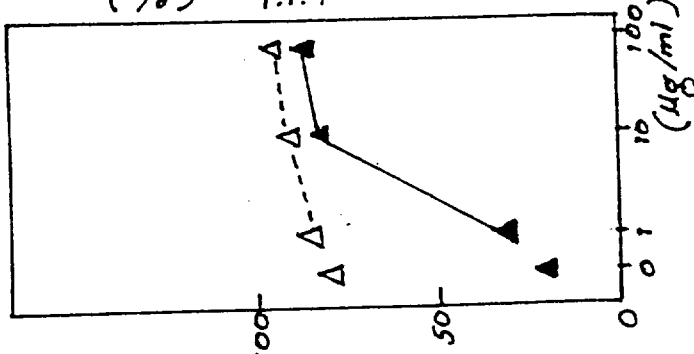

FIG. 17

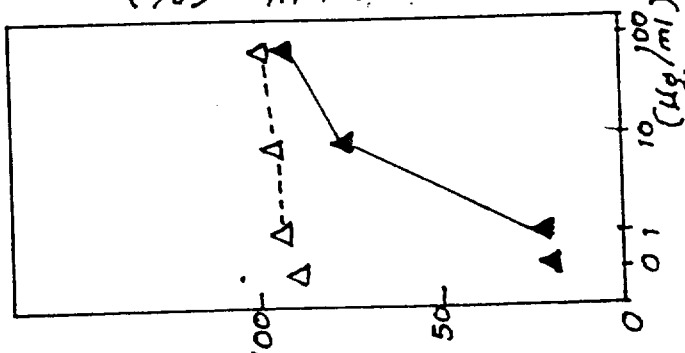

FIG. 16

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FIG. 22

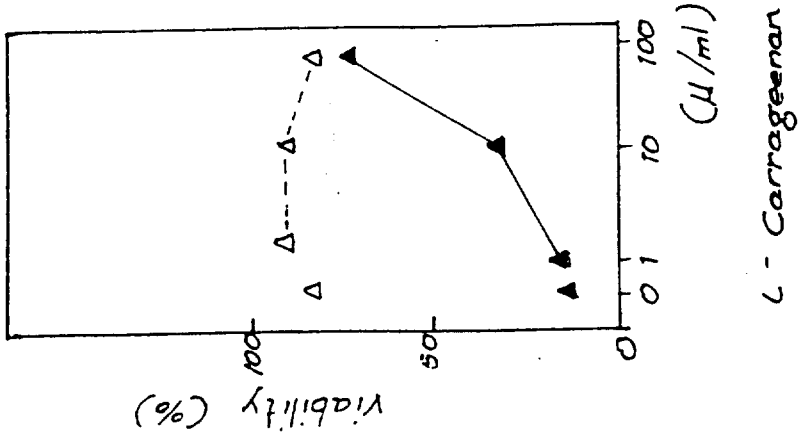

FIG. 21

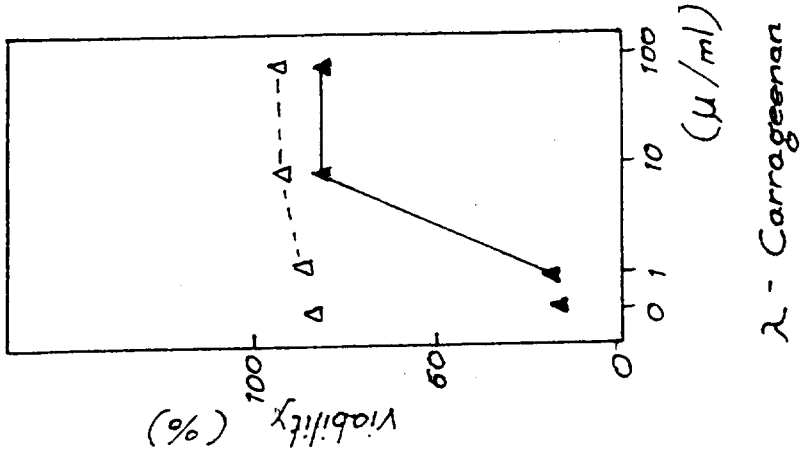

FIG. 20

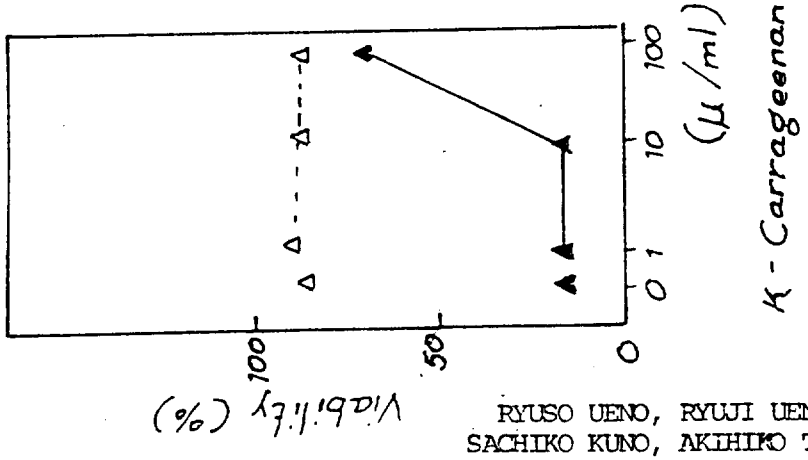

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FIG. 26

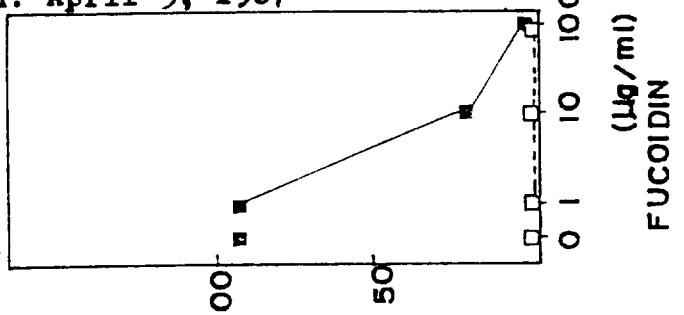

FIG. 25

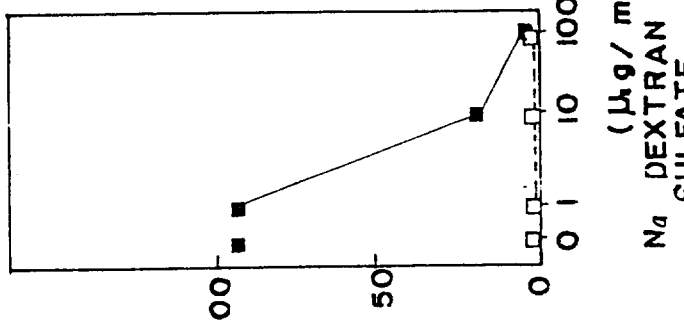

FIG. 24

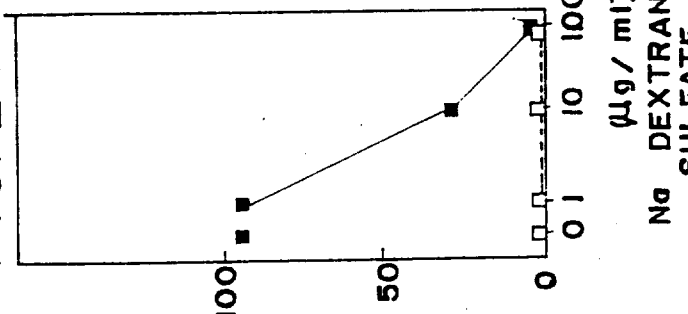

FIG. 23

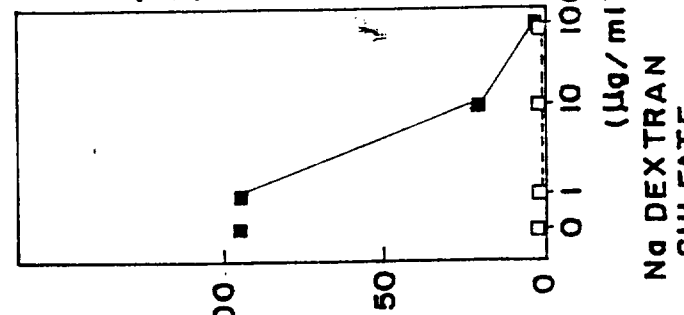

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FIG. 29

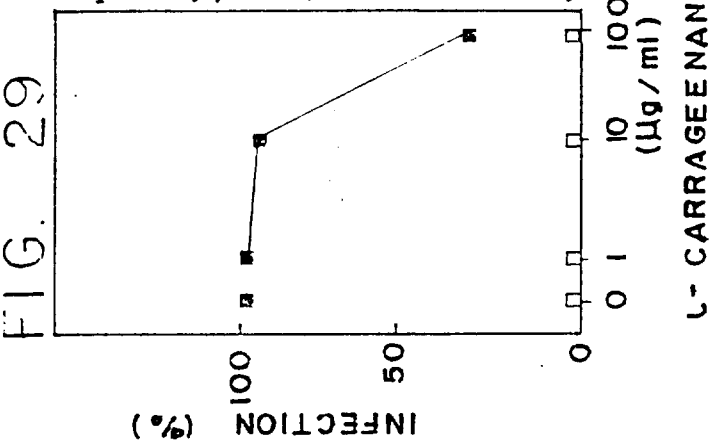

FIG. 28

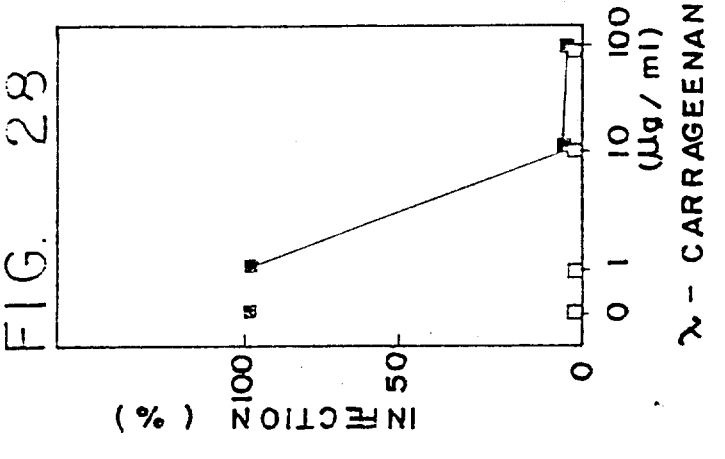

FIG. 27

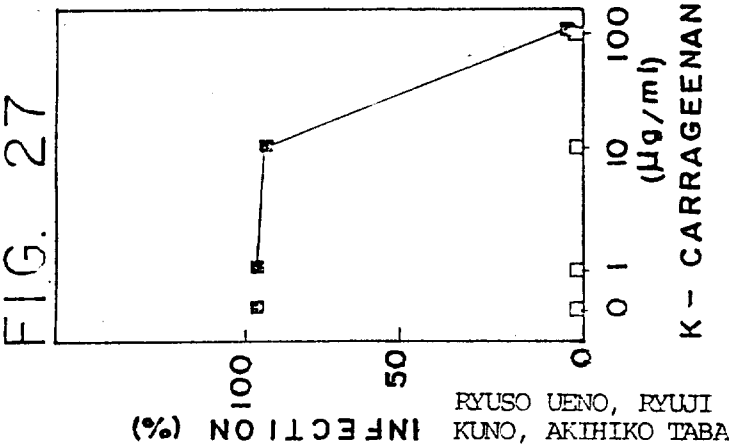

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FIG. 33

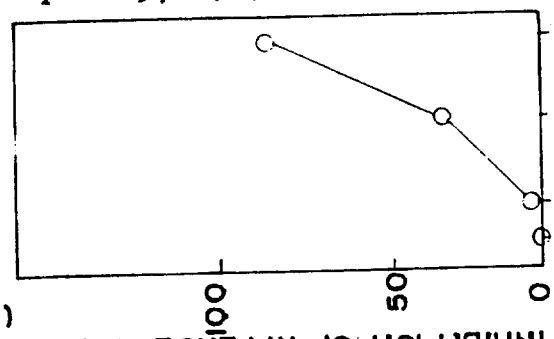

FIG. 32

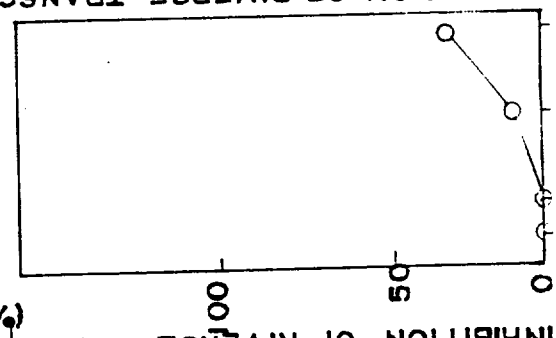

FIG. 31

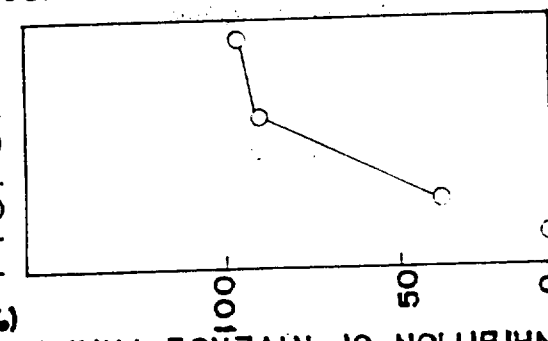

FIG. 30

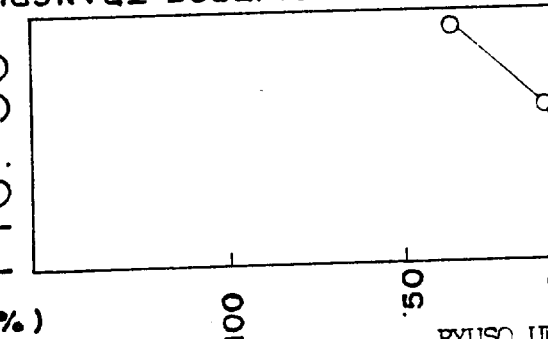

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FIG. 37

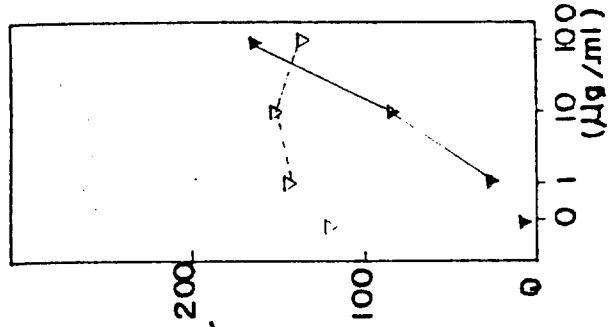

FIG. 36

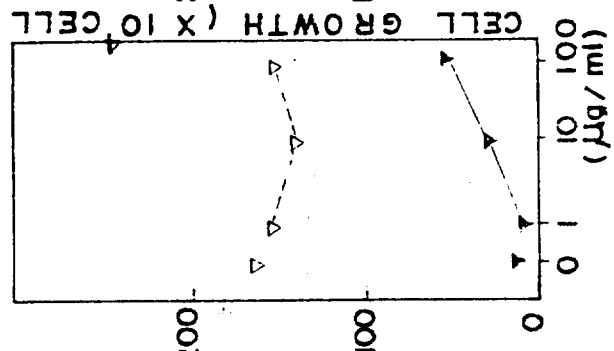

FIG. 35

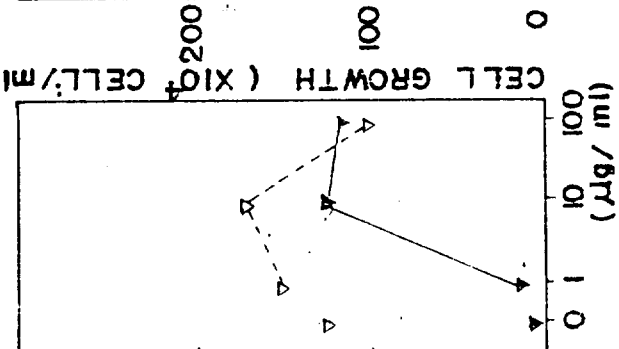

FIG. 34

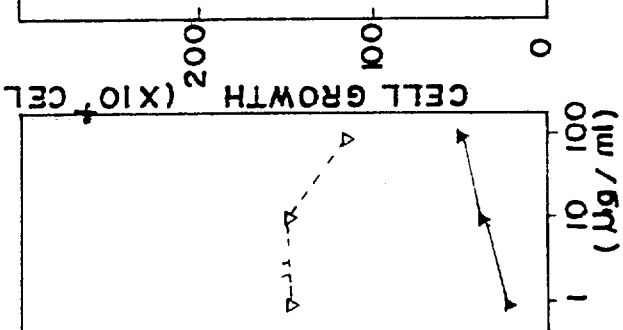

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FIG. 41

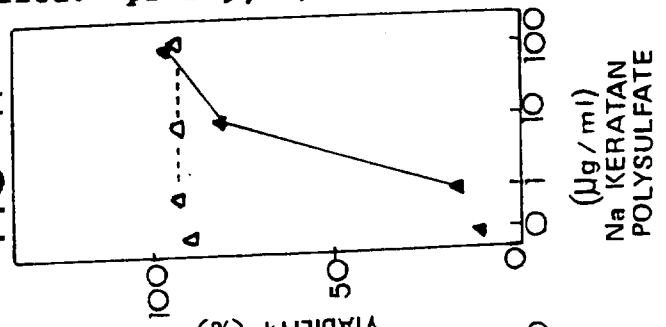

FIG. 40

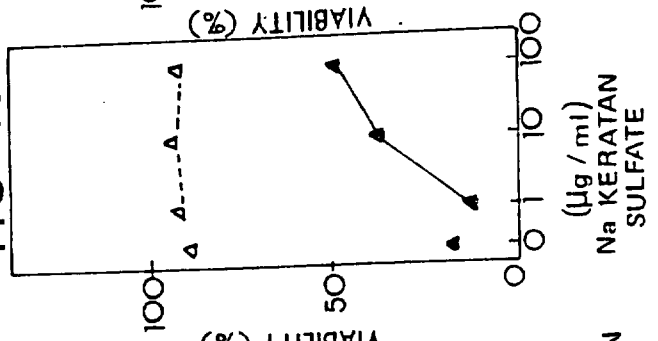

FIG. 39

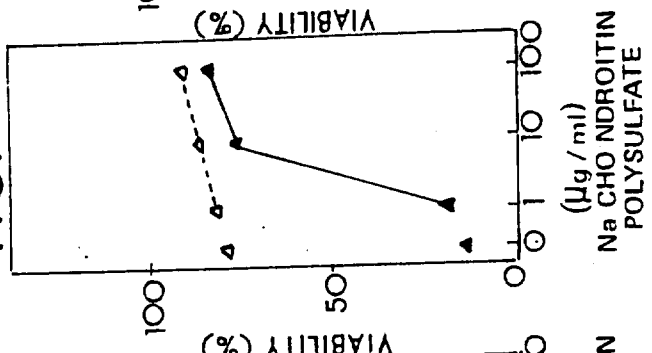

FIG. 38

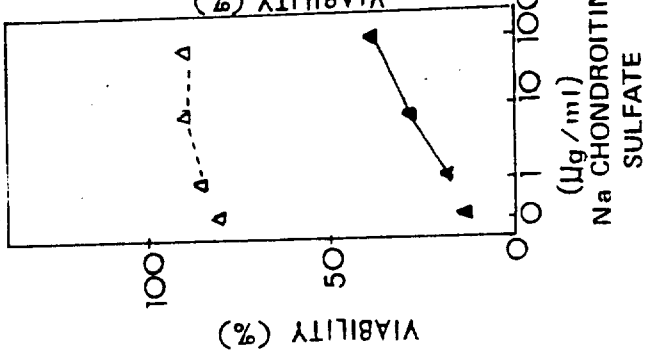

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FIG. 45

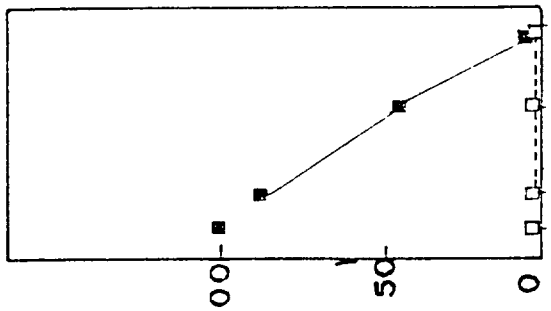

FIG. 44

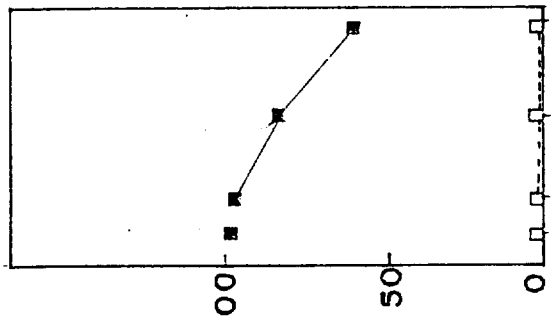

FIG. 43

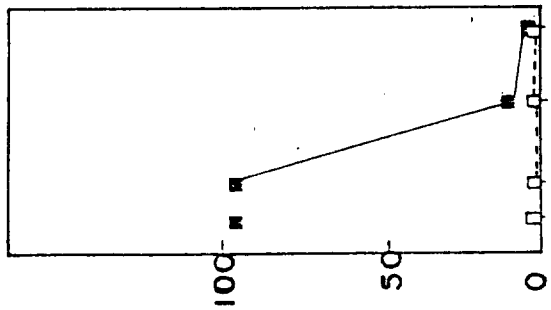

FIG. 42

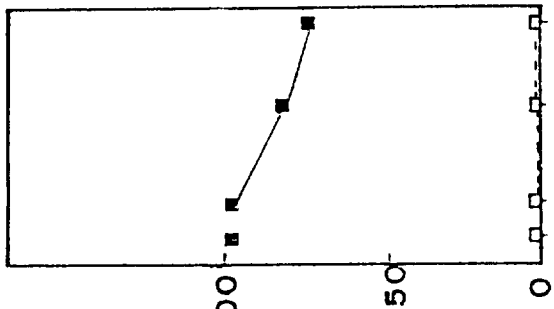

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FIG. 48

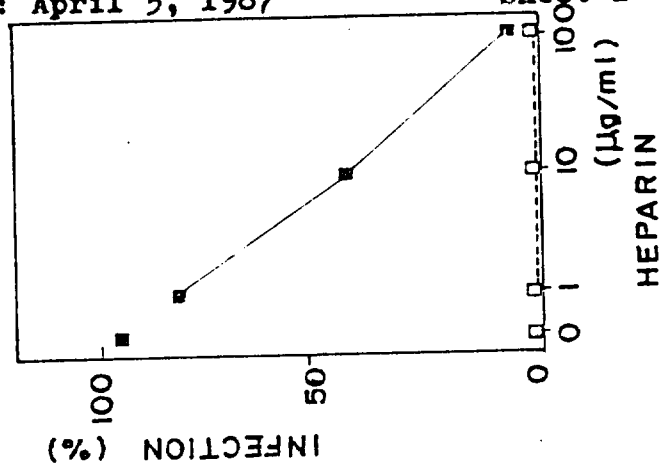


FIG. 47

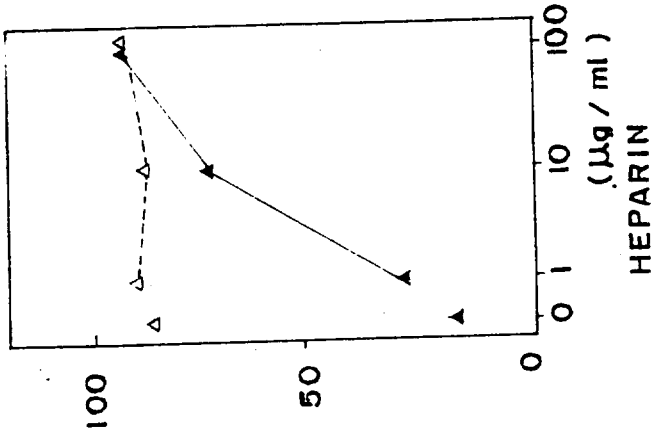
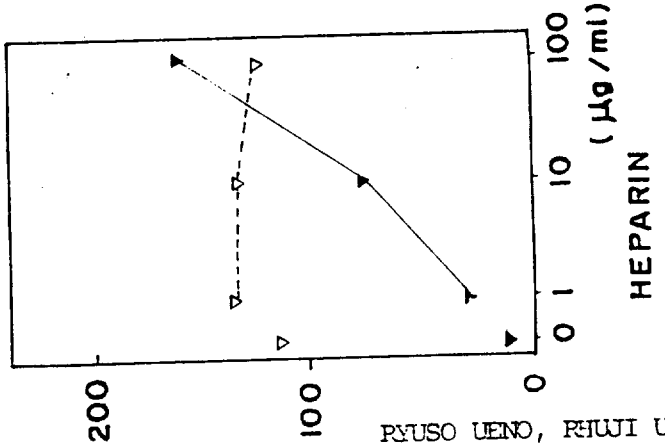


FIG. 46



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