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(19) (CA) CANADIAN PATENT (12)
(54) Hyaluronic Acid Esters and Salts
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NO DRAWING
ABSTRACT OF THE DISCLOSURE

Novel esters of hyaluronic acid of the Formula

\[ \text{Hy(COOR)}_n \]

wherein COOR represents the carboxylic acid moiety on the hyaluronic acid (Hy) molecule radical, R is derived from an alcohol which is selected from the group consisting of an aliphatic alcohol having a chain of 2 to 34 carbons, an araliphatic alcohol a cycloaliphatic alcohol or a heterocyclic alcohol, and \( n \) is a large number, are provided herein. All or only a portion of the carboxylic groups of the acid are esterified. The salts of the partial esters with metals or with pharmacologically-acceptable organic bases are also provided herein. Pharmaceutical preparation are also provided which contain, as an active ingredient, one or more of the above-described hyaluronic acid esters, or of one or more of the salts thereof. Such pharmaceutical preparation contain a pharmacologically-active substance or an association of pharmacologically-active substances, and a carrying vehicle containing the total or partial esters of hyaluronic acid or salts of such partial esters as described above. The novel esters described above possess bioplastic and pharmaceutical properties and are useful in many fields, including cosmetic surgery and medicine.
The present invention relates to new esters of hyaluronic acid and their use in the pharmaceutical and cosmetic fields, and in the field of biodegradable plastic materials. The invention therefore includes new medicaments, cosmetic, medical and surgical articles, and processes for their production.

The term "hyaluronic acid" (also referred to as "HY" hereinafter) is used in the literature to designate an acidic polysaccharide with various molecular weights constituted by residues of D-glucuronic acid and N-acetyl-D-glucosamine, which naturally occur in cellular surfaces, in the basis extracellular substances of the connective tissues of vertebrates, in the synovial fluid of joints, in the vitreous humor of the eye, in the tissue of the human umbilical cord and in cocks' combs.

Hyaluronic acid plays an important role in the biological organism, firstly as a mechanical support of the cells of many tissues, e.g., the skin, the tendons, the muscles and cartilage, and it is therefore the main component of the intracellular matrix. Hyaluronic acid also performs other functions in the biological processes, e.g., the hydration of tissues, lubrication, cellular migration, cell function and differentiation. (See, for example, A. Balazs, et al, Cosmetics & Toiletries, No. 5/84, pages 8-17 published in 1984). Hyaluronic acid may be extracted from the above-mentioned natural tissues, e.g., from cocks' combs, or also from certain bacteria. Today, hyaluronic acid may also be prepared by microbiological processes.
The molecular weight of whole hyaluronic acid obtained by extraction is in the region of 8-13 million. However, the molecular chain of the polysaccharide can be degraded quite easily under the influence of various physical and chemical factors, e.g., mechanical influences or under the influence of radiation, hydrolyzing, oxidizing or enzymatic agents. For this reason, or often in the ordinary purification procedures of original extracts, degraded fractions with a lower molecular weight are obtained. (See Balazs, et al cited above). Hyaluronic acid, its molecular fractions and the respective salts have been used as medicaments and their use is also proposed in cosmetics (see, for example, the above-mentioned article by Balazs, et al and French Patent No. 2478468).

As a therapeutic agent, hyaluronic acid and its salts have been used especially in therapy for arthropathies, e.g., in veterinary medicine for the cure of arthritis in horses [Acta Vet. Scand. 167, 379 (1976)].

As a therapeutic agent, hyaluronic acid and its salts have been used especially in therapy for arthropathies, e.g., in veterinary medicine for the cure of arthritis in horses [Acta Vet. Scand. 167, 379 (1976)]. As an auxiliary and substitutional therapeutic agent for natural tissues and organs, hyaluronic acid and its molecular fractions and their salts have been used in ophthalmic surgery (see, for example, Balazs, et al, "Modern Problems in Ophthalmology", Vol. 10, 1970, p. 3 - E.B. Strieff, S. Karger eds. Basel; "Viscosurgery and the Use of Sodium Hyaluronate During
Intraocular Lens Implantation", Paper presented at the International Congress and First Film Festival on Intraocular Implantation, Cannes, 9179; U.S. Patent No. 4,328,803 with a summary of the literature on the uses of HY in ophthalmology; and U.S. Patent No. 4,141,973 issued February, 1974 to Balazs).

In application W086/6728 published November 1986, a molecular fraction of hyaluronic acid is described which can be used, for example, as its sodium salt, for intraocular and intraarticular injections suitable for the substitution and intraarticular injections suitable for the substitution of internal fluids of the eye and in arthropathy therapies, respectively.

Hyaluronic acid may also be used as an additive for a wide variety of polymeric materials used for medical and surgical articles, e.g., polyurethanes, polyesters, polyolefins, polyamides, polysiloxanes, vinylic and acrylic polymers and carbon fibers with the effect of rendering these materials biocompatible. In this case, the addition of HY or one of its salts is effected, for example, by covering the surface of such materials, by dispersion in the same, or by both of these procedures. Such materials may be used for the manufacture of various sanitary and medical articles, e.g., cardiac values, intraocular lenses, vascular clips, and pacemakers (see, for example, U.S. Patent No. 4,500,676).

Although the term "hyaluronic acid" is commonly used in an improper sense, meaning, as can be seen from above,
a whole series of polysaccharides with alternations of residues of D-glucuronic acid and N-acetyl-D-glucosamine with varying molecular weights or even degraded fractions of the same, and although the plural form "hyaluronic acids" may seem more appropriate, the discussion herein shall continue to use the singular form to refer to hyaluronic acid in its various forms, including its molecular fractions. The abbreviation "HY" will also often be used to describe this collective term.

Relative to the esters of hyaluronic acid, there is a description in the literature of the total methyl ester of a hyaluronic acid with a high molecular weight obtained by extraction from human umbilical cords [Jeanloz, et al., J. Biol. Chem. 186 (1950), 495-511, and Jager, et al., J. Bacteriology 1065-1067 (1979)]. This ester was obtained by treatment of free hyaluronic acid with diazomethane in ether solution and in it substantially all the carboxylic groups proved to be esterified. Furthermore, methyl esters of oligomers of HY with between 5 and 15 disaccharide units have also been described [see Biochem. J. (1977) 167, 711-716]. Also described is a methyl ester of hyaluronic acid etherified with methyl alcohol in a part of the hydroxyl alcohol groups [Jeanloz, et al., J. Biol. Chem. 194 (1952), 141-150; and Jeanloz, et al., Helvetica Chimica Acta 35 (1952), 262-271]. No biological activity, and therefore no pharmaceutical use, has been reported for these esters.
An object of one aspect of the present invention, then, is to provide esters of hyaluronic acid with specified organic alcohols, in which all or only a part of the carboxylic groups of the acid are esterified, and the salts of the partial esters with metals or with organic bases, which are biocompatible or acceptable from a pharmacological point of view, such esters and salts possessing bio-plastic and pharmaceutical properties, so that they may be used in innumerable fields, including cosmetics, surgery and medicine.

An object of another aspect of this invention is to provide such esters which are considerably more stable, especially regarding the action of the natural enzymes responsible for the degradation of the polysaccharide molecule in the organism, e.g., especially hyaluronidase, and they, therefore, conserve the above-mentioned physical-chemical, pharmacological and therapeutic properties, which are qualitatively the same as for hyaluronic acid, for very long periods.

By one broad aspect of this invention, an ester is provided of hyaluronic acid of the formula

\[ \text{Hy(COOR)}_n \]

wherein COO represents the carboxylic acid moiety on the hyaluronic acid (Hy) molecule, R is derived from an alcohol which is selected from the group consisting of an aliphatic alcohol having a chain of 2 to 34 carbon atoms, an araliphatic
alcohol, a cycloaliphatic alcohol and a heterocyclic alcohol, and n is a large number.

By important variants of this invention, these esters may be in the form of a total ester, or in the form of a partial ester or in the form of a salt of such partial ester with an inorganic base or with an organic base.

In either the total ester form, or the partial ester form or the salt form, by variants of such ester, the alcohol is an aliphatic alcohol which is substituted by one or two functional groups which are selected from the group consisting of amino, hydroxy, mercapto, aldehyde, keto, carboxyl, hydrocarbyl, dihydrocarbylamino, ether, ester, thioether, thioester, acetal, ketal, carbalkoxy, unsubstituted carbamide groups and carbamide groups which are substituted by one or two alkyl groups, the hydrocarbyl radicals in these functionally-modified groups having a maximum of 6 carbon atoms, and in which the carbon atom chain may be interrupted by heteroatoms which are selected from the group consisting of oxygen, sulphur and nitrogen.

In a variation thereof, the alcohol is ethyl alcohol, propyl alcohol, isopropyl alcohol, normal butyl alcohol, isobutyl alcohol, tert-butyl alcohol, amyl alcohol, pentyl alcohol, hexyl alcohol, octyl alcohol, ethylene glycol, propylene glycol, butylene glycol or glycerin.

In either the total ester form, or the partial ester form or the salt form, by other variants of such ester, the alcohol
is an araliphatic alcohol with only one benzene residue, in which the aliphatic chain has a maximum of 4 carbon atoms, and in which the benzene residue is unsubstituted or is substituted by 1 to 3 methyl groups or by 1 to 3 hydroxy groups, or by 1 to 3 halogen atoms, and in which the aliphatic chain may be substituted by one or two functions which are selected from the group consisting of free amino groups, monoethylamino groups, diethylamino groups, pyrrolidinyl groups and piperidinyl groups. In a variation thereof, the alcohol is benzyl alcohol, phenethyl alcohol, ephedrine or adrenalin.

In either the total ester form, or the partial ester form or the salt form, by other variants of such ester, the alcohol is a cycloaliphatic alcohol or an aliphatic-cycloaliphatic alcohol and derives from a monocyclic hydrocarbide or from a polycyclic hydrocarbide with a maximum of 34 carbon atoms.

In either the total ester form, or the partial ester form or the salt form, by still other variants of such ester, the alcohol is a polycyclic alcohol which is selected from the group consisting of sterols, chloric acids, steroid alcohols, groups of the estrane and pregnane series and their unsaturated derivatives. In a variation thereof, the alcohol is cortisone, hydrocortisone, prednisone, prednisolone, fluorocortisone, dexamethasone, betamethasone, corticosterone, deoxycorticosterone, paramethasone, flumethasone,
fluocinolone, fluocinolone acetonide, fluprednylidene, clobetasol, or beclomethasone.

In a variant of a salt form of a partial ester of hyaluronic acid, the salt is formed with an alkali metal, or with an alkaline earth metal, or with magnesium, or with aluminum, or with ammonia. In a variation thereof, the salt is a sodium salt.

In a variant thereof, in either a total ester of hyaluronic acid, a partial ester of hyaluronic acid or a salt of a partial ester of hyaluronic acid, the hyaluronic acid derives from hyaluronic acid which is obtained by first dehydrating cocks’ combs with acetone and then exposing them to enzymatic digestion with papain.

In a further variant thereof, in either a total ester of hyaluronic acid, a partial ester of hyaluronic acid or a salt of a partial ester of hyaluronic acid, the hyaluronic acid has been obtained by first dehydrating cocks’ combs with acetone and then exposing them to enzymatic digestion with papain, followed by molecular ultrafiltration, and further purification of the hyaluronic acid fraction so-obtained.

In yet another variant thereof, in either a total ester of hyaluronic acid, a partial ester of hyaluronic acid or a salt of a partial ester of hyaluronic acid, such hyaluronic acid ester derives from an integral hyaluronic acid, or from one of its salts which is obtained by extraction from cocks’
combs, and which has a molecular weight of between 8 and 13 million.

In a further variant thereof, in either a total ester of hyaluronic acid, a partial ester of hyaluronic acid or a salt of a partial ester of hyaluronic acid, such hyaluronic acid ester derives from a hyaluronic acid fraction with a molecular weight of between 90 and 0.23% of the molecular weight of an integral hyaluronic acid with a molecular weight of 13 million.

In still another variant thereof, in either a total ester of hyaluronic acid, a partial ester of hyaluronic acid or a salt of a partial ester of hyaluronic acid, such hyaluronic acid ester derives from a hyaluronic acid molecular fraction having a molecular weight of between 50,000 and 100,000 and which is substantially-free of hyaluronic acid having a molecular weight of less than 30,000.

In yet another variant thereof, in either a total ester of hyaluronic acid, a partial ester of hyaluronic acid or a salt of a partial ester of hyaluronic acid, such hyaluronic acid ester derives from a hyaluronic acid molecular fraction having a molecular weight of between 500,000 and 730,000 and which is substantially-free of hyaluronic acid having a molecular weight of less than 30,000.

Specific embodiments of such esters of hyaluronic acid include the following:

The total ethyl ester of hyaluronic acid.
The total propyl ester of hyaluronic acid.
The total pentyl ester of hyaluronic acid.
The total isopentyl ester of hyaluronic acid.
The total benzyl ester of hyaluronic acid.
The total phenethyl ester of hyaluronic acid.

The mixed ethanol-cortisone ester of hyaluronic acid with 80% of the carboxyl groups esterified with ethanol and with 20% of the carboxyl groups esterified with cortisone.

The mixed ethanol-hydrocortisone ester of hyaluronic acid with 80% of the carboxyl groups esterified with ethanol and with 20% of the carboxyl groups esterified with hydrocortisone.

The mixed ethanol-fluorocortisone ester of hyaluronic acid with 80% of the carboxyl groups esterified with ethanol and with 20% of the carboxyl groups esterified with fluorocortisone.

The mixed ethanol-deoxycorticosterone ester of hyaluronic acid with 80% of the carboxyl groups esterified with ethanol and with 20% of the carboxyl groups esterified with deoxycorticosterone.

A salt of a partial propyl ester of hyaluronic acid with 50% of the carboxyl groups esterified and with 50% of the carboxyl groups salified with sodium.

A salt of a partial isopropyl ester of hyaluronic acid with 50% of the carboxyl groups esterified and with 50% of the carboxyl groups salified with sodium.
A salt of a partial propyl ester of hyaluronic acid with 85% of the carboxylic groups esterified and with 15% of the carboxylic groups salified with sodium.

A salt of a partial ethyl ester of hyaluronic acid with 75% of the carboxyl groups esterified and with 25% of the carboxyl groups salified with sodium.

A salt of a partial methyl ester of hyaluronic acid with 75% of the carboxyl groups esterified and with 25% of the carboxyl groups salified with sodium.

A salt of a partial butyl ester of hyaluronic acid with 50% of the carboxylic groups salified with sodium.

A salt of the partial ethoxycarbonylmethyl ester of hyaluronic acid with 75% of the carboxyl groups esterified and with 25% of the carboxyl groups salified with sodium.

A salt of a partial cortisone ester of hyaluronic acid with 20% of the carboxyl groups esterified and with 80% of the carboxyl groups salified with sodium.

A salt of a partial hydrocortisone ester of hyaluronic acid with 20% of the carboxyl groups esterified and with 80% of the carboxyl groups salified with sodium.

A salt of the partial fluorocortisone ester of hyaluronic acid with 20% of the carboxyl groups esterified and with 80% of the carboxyl groups salified with sodium.

A salt of a deoxycorticosterone ester of hyaluronic acid with 20% of the carboxyl groups esterified and with 80% of the carboxyl groups salified with sodium.
A salt of a partial and mixed ethanol-deoxycorticosterone ester of hyaluronic acid with 40% of the carboxyl groups esterified with ethanol, with 20% of the carboxyl groups esterified with deoxycorticosterone and with the remaining 40% of the carboxyl groups salified with sodium.

A salt of a partial and mixed ethanol-cortisone ester of hyaluronic acid with 40% of the carboxyl groups esterified with ethanol, with 20% of the carboxyl groups esterified with cortisone, and with the remaining 40% of the carboxyl groups salified with sodium.

A salt of a partial and mixed ethanol-hydrocortisone ester of hyaluronic acid with 40% of the carboxyl groups esterified with ethanol, with 20% of the carboxyl groups esterified with hydrocortisone, and with the remaining 40% of the carboxyl groups salified with sodium.

A salt of a partial and mixed ethanol-fluorocortisone ester of hyaluronic acid with 40% of the carboxyl groups esterified with ethanol, with 20% of the carboxyl groups esterified with fluorocortisone, and with the remaining 40% of the carboxyl groups salified with sodium.

A salt of a hyaluronic acid ester of streptomycin with 75% of the carboxyl groups esterified with ethanol and with 25% of the carboxyls salified with streptomycin.

A salt of a hyaluronic acid ester of erythromycin with 75% of the carboxyl groups esterified with ethanol and with 25% of the carboxyls salified with erythromycin.
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A salt of a hyaluronic acid ester of neomycin with 75% of the carboxyl groups esterified with ethanol and with 25% of the carboxyls salified with neomycin.

A salt of a hyaluronic acid ester of gentamycin with 75% of the carboxyl groups esterified with ethanol and with 25% of the carboxyls salified with gentamycin.

A salt of a hyaluronic acid ester of kanamycin with 75% of the carboxyl groups esterified with ethanol and with 25% of the carboxyls salified with kanamycin.

A salt of a hyaluronic acid ester of pilocarpine with 75% of the carboxyl groups esterified with ethanol and with 25% of the carboxyls salified with pilocarpine.

A salt of a hyaluronic acid ester of pilocarpine with 85% of the carboxyl groups esterified with ethanol and with 15% of the carboxyls salified with pilocarpine.

The hyaluronic acid ester of amikacin with 75% of the carboxyl groups esterified with ethanol and with 25% of the carboxyls salified with amikacin.

By another aspect of this invention, a process is provided for the preparation of an ester of hyaluronic acid of the Formula

\[ \text{Hy} (\text{COOR})_n \]

wherein COO represents the carboxylic acid moiety on the hyaluronic acid (Hy) molecule, R is derived from an alcohol which is selected from the group consisting of an aliphatic alcohol having a chain of 2 to 34 carbons, an araliphatic
alcohol, a cycloaliphatic alcohol and a heterocyclic alcohol, and n is a large number, which process comprises:

(I) providing a total ester of hyaluronic acid with such alcohol by carrying out one of the following esterification reactions: (a) treating free hyaluronic acid with a sufficient amount of a selected alcohol as defined above in the presence of a catalyzing substance; or (b) treating free hyaluronic acid with a sufficient amount of an etherifying agent which is capable of introducing the desired alcoholic residue of an alcohol, as defined above, in the presence of an inorganic base or an organic base; or (c) treating a metal salt of hyaluronic acid or an organic azotized base of hyaluronic acid with a sufficient amount of a selected alcohol, as defined above, in the presence of a catalyzing substance; or (d) treating a quaternary ammonium salt of hyaluronic acid with a sufficient amount of an etherifying agent capable of introducing the desired alcoholic residue of an alcohol, as defined above, in the presence of an inorganic base or an organic base; or (II) providing a partial ester of hyaluronic acid with such alcohol, by carrying out one of the following reactions: (a) treating free hyaluronic acid with an insufficient amount of a selected alcohol as defined above in the presence of a catalyzing substance; or (b) treating free hyaluronic acid with an insufficient amount of an etherifying agent which is capable of introducing the desired alcoholic residue of an alcohol, as defined above, in the
presence of an inorganic base or an organic base; or (c) treating a metal salt of hyaluronic acid or an organic azotized base of hyaluronic acid with an insufficient amount of a selected alcohol, as defined above, in the presence of a catalyzing substance; or (d) treating a quaternary ammonium salt of hyaluronic acid with an insufficient amount of an etherifying agent which is capable of introducing the desired alcoholic residue of an alcohol, as defined above, in the presence of an inorganic base or an organic base; or (III) providing a salt of a partial ester of hyaluronic acid with such alcohol, by carrying out one of the following reactions: (a) treating free hyaluronic acid with an insufficient amount of a selected alcohol, as defined above, in the presence of a catalyzing substance; or (b) treating free hyaluronic acid with an insufficient amount of an etherifying agent which is capable of introducing the desired alcoholic residue of an alcohol, as defined above, in the presence of an inorganic base or an organic base; or (c) treating a metal salt of hyaluronic acid or an organic azotized base of hyaluronic acid with an insufficient amount of a selected alcohol, as defined above, in the presence of a catalyzing substance; or (d) treating a quaternary ammonium salt of hyaluronic acid with an insufficient amount of an etherifying agent capable of introducing the desired alcoholic residue of an alcohol, as defined above, in the presence of an inorganic base or an organic base; and then salifying the remaining carboxylic
groups, or part of such carboxylic groups, in the partial ester so formed, with an inorganic base or with an organic base.

By a variant of such process, the catalyzing substance is a strong inorganic acid or an ionic exchanger of the acid type.

By another variant of such process, the etherifying agent is selected from the group consisting of an ester of an inorganic acid, an ester of an organic sulphonie acid, a hydracid, a hydrocarbyl halogenide, a neutral sulphate, a hydrocarboxyl acid, an alifite, a carbonate, a silicate, a phosphite, and a hydrocarbyl sulphonate.

By still another variant of such process, the process takes place in a suitable solvent which is selected from the group consisting of a polar solvent, a non-polar solvent and an aprotic solvent.

By yet another variant of such process, the process takes place in a solvent which is selected from the group consisting of a dialkylsulphoxide, a dialkylcarboxamide, a lower alkyl dialkylamide of a lower aliphatic acid, an alcohol, an ether, a ketone, and an ester.

By a further variant of such process, the base is a hydrate of an alkali metal, a hydrate of an alkaline earth metal, magnesium oxide, silver oxide, a basic salt of an alkali metal, of an alkaline earth metal, of magnesium or of silver, of a carbonate of an alkali metal, of an alkaline
earth metal, of magnesium or of silver, an organic base, a tertiary azotized base, or an ionic exchanger of the basic type. By a variation of such process, the metal salt is a salt of an alkali metal or a salt of an alkaline earth metal. By another variation of such process, the organic azotized base is an ammonium salt or an ammonium-substituted salt.

By yet another variant of such process, the process is carried out at temperature of 0°C-100°C.

By variations of any of the aspects or variants described above, the alcohol is an aliphatic alcohol which is substituted by one or two functional groups which are selected from the group consisting of amino, hydroxy, mercapto, aldehyde, keto, carboxyl, hydrocarbaryl, dihydrocarbarylamino, ether, ester, thioether, thioester, acetal, ketal, carbalkoxy, unsubstituted carbamide groups or carbamidic groups which are substituted by one or two alkyl groups, the hydrocarbaryl radicals in these functionally-modified groups having a maximum of 6 carbon atoms, and in which the carbon atom chain may be interrupted by heteroatoms which are selected from the group consisting of oxygen, sulphur and nitrogen. By a specific variation thereof, the alcohol is ethyl alcohol, propyl alcohol, isopropyl alcohol, normal butyl alcohol, isobutyl alcohol, tert-butyl alcohol, amyl alcohol, pentyl alcohol, hexyl alcohol, octyl alcohol, ethylene glycol, propylene glycol, butylene glycol or glycerin.
By some variations of any of the aspects or variants described above, the alcohol is an aliphatic alcohol with only one benzene residue, in which the aliphatic chain has a maximum of 4 carbon atoms, and in which the benzene residue may be substituted by 1 to 3 methyl groups, or by 1 to 3 hydroxy groups or by 1 to 3 halogen atoms, and in which the aliphatic chain may be substituted by one or two functional groups which are selected from the group consisting of free amino groups, monoethylamino groups, diethylamino groups, pyrroolidinyl groups and piperidinyl groups. By a specific variation thereof, the alcohol is benzyl alcohol, phenethyl alcohol, ephedrine or adrenalin.

By other variations of any of the aspects or variants described above, the alcohol is a cycloaliphatic alcohol or an aliphatic-cycloaliphatic alcohol and derives from a monocyclic hydrocarbide or from a polycyclic hydrocarbide with a maximum of 34 carbon atoms. By a specific variation thereof, the alcohol is a polycyclic alcohol which is selected from the group consisting of sterols, colic acids, steroid alcohols, groups of the estrane and pregnane series and their unsaturated derivatives. By another specific variation thereof, the alcohol is cortisone, hydrocortisone, prednisone, prednisolone, fluorocortisone, dexamethasone, betamethasone, corticosterone, deoxycorticosterone, paramethasone, flumethasone, fluocinolone, fluocinolone acetonide, fluprednylidene, clobetasol, or beclomethasone.
By another variant thereof, the salt is formed with an alkali metal, with an alkaline earth metal, with magnesium, with aluminum, or with ammonia. Preferably the salt is a sodium salt or an ammonia salt. By a variation thereof, the salt is formed with a therapeutically-acceptable ammonium base, aliphatic base, araliphatic base, cycloaliphatic base or heterocyclic base.

Specific embodiments of such processes comprise the following reactions:

Reacting hyaluronic acid with ethyl alcohol.
Reacting hyaluronic acid with n-propyl alcohol.
Reacting the tetrabutylammonium salt of hyaluronic acid with n-pentyl bromide.
Reacting the tetrabutylammonium salt of hyaluronic acid with isopentyl bromide.
Reacting the tetrabutylammonium salt of hyaluronic acid with benzyl bromide.
Reacting the tetrabutylammonium salt of hyaluronic acid with 2-bromoethylbenzene.
Reacting the tetrabutylammonium salt of hyaluronic acid with ethyl iodide and with 21-bromo-4-pregnene-17α-ol-3,11,20-trione, whereby 80% of the carboxyl groups are esterified with ethanol and whereby 20% of the carboxyl groups are esterified with cortisone.

Reacting the tetrabutylammonium salt of hyaluronic acid with ethyl iodide and with 21-bromo-4-pregnene-11β,17α-diol-
3,20-dione, and then salifying with sodium ions, whereby 80% of the carboxyl groups are esterified with ethanol and whereby 20% of the carboxyl groups are esterified with hydrocortisone.

Reacting the tetrabutylammonium salt of hyaluronic acid with ethyl iodide and with 9β-fluoro-21-bromo-4-pregnen-11β,17α-diol-3,20-dione, and salifying with sodium ions, whereby 80% of the carboxyl groups are esterified with ethanol and whereby 20% of the carboxyl groups are esterified with fluorocortisone.

Reacting hyaluronic acid with propyl alcohol and then salifying with sodium ions, whereby 50% of the carboxyl groups are esterified and whereby 50% of the carboxyl groups are salified with sodium.

Reacting hyaluronic acid with isopropyl alcohol and then salifying with sodium ions, whereby 50% of the carboxyl groups are esterified and whereby 50% of the carboxyl groups are salified with sodium.

Reacting hyaluronic acid with n-propyl alcohol and then salifying with sodium ions, whereby 85% of the carboxylic groups are esterified and whereby 15% of the carboxylic groups are salified with sodium.

Reacting hyaluronic acid with ethyl alcohol and then salifying with sodium ions, whereby 75% of the carboxyl groups are esterified and whereby 25% of the carboxyl groups are salified with sodium.
Reacting hyaluronic acid with n-butyl alcohol and then salifying with sodium ions, whereby 50% of the carboxylic groups are salified with sodium.

Reacting the tetrabutylammonium salt of hyaluronic acid with ethyl chloroacetate and then salifying with sodium ions, whereby 75% of the carboxyl groups are esterified and whereby 25% of the carboxyl groups are salified with sodium.

Reacting the tetrabutylammonium salt of hyaluronic acid with 21-bromo-4-pregnene-17α-ol-3,11,20-trione and then salifying with sodium ions, whereby 20% of the carboxyl groups are esterified and whereby 80% of the carboxyl groups are salified with sodium.

Reacting the tetrabutylammonium salt of hyaluronic acid with 21-bromo-4-pregnene-11β,17α-diol-3,20-dione and then salifying with sodium ions, whereby 20% of the carboxyl groups are esterified and whereby 80% of the carboxyl groups are salified with sodium.

Reacting the tetrabutylammonium salt of hyaluronic acid with 9-fluoro-21-bromo-4-pregnene-11β,17α-diol-3,20-dione and then salifying with sodium ions, whereby 20% of the carboxyl groups are esterified and whereby 80% of the carboxyl groups are salified with sodium.

Reacting the tetrabutylammonium salt of hyaluronic acid with 21-bromo-4-pregnene-3,20-dione and then salifying with sodium ions, whereby 20% of the carboxyl groups are esterified
and whereby 80% of the carboxyl groups are salified with sodium.

Reacting the tetrabutylammonium salt of hyaluronic acid with ethyl iodide and with 21-bromo-4-pregnene-3,20-dione, and then salifying with sodium ions, whereby 80% of the carboxyl groups are esterified with ethanol and whereby 20% of the carboxyl groups are esterified with deoxycorticosterone and whereby the remaining 40% of the carboxyl groups are salified with sodium.

Reacting the tetrabutylammonium salt of hyaluronic acid with ethyl iodide and with 21-bromo-4-pregnene-3,20-dione, and then salifying with sodium ions, whereby 40% of the carboxyl groups are esterified with ethanol, whereby 20% of the carboxyl groups are esterified with deoxycorticosterone, and whereby the remaining 40% of the carboxyl groups are salified with sodium.

Reacting the tetrabutylammonium salt of hyaluronic acid with ethyl iodide and with 21-bromo-4-pregnene-17α-ol-3,11,20-trione, and then salifying with sodium ions, whereby 40% of the carboxyl groups esterified with ethanol, whereby 20% of the carboxyl groups are esterified with cortisone and whereby the remaining 40% of the carboxyl groups are salified with sodium.

Reacting the tetrabutylammonium salt of hyaluronic acid with ethyl iodide and with 21-bromo-4-pregnene-11β,17α-diol-3,20-dione, and then salifying with sodium ions, whereby 40%
of the carboxyl groups are esterified with ethanol, whereby 20% of the carboxyl groups are esterified with hydrocortisone and whereby the remaining 40% of the carboxyl groups are salified with sodium.

Reacting the tetrabutylammonium salt of hyaluronic acid with ethyl iodide and with 9β-fluoro-21-bromo-4-pregnene-11β,17α-diol-3,20-dione, and then salifying with sodium ions, whereby 40% of the carboxyl groups are esterified with ethanol, whereby 20% of the carboxyl groups are esterified with fluorocortisone and whereby the remaining 40% of the carboxyl groups are salified with sodium.

Reacting the tetrabutylammonium salt of hyaluronic acid with ethyl iodide and with 9β-fluoro-21-bromo-4-pregnene-11β,17α-tiol-3,20-dione, and then salifying with sodium ions, whereby 40% of the carboxyl groups are esterified with ethanol, whereby 20% of the carboxyl groups are esterified with fluorocortisone and whereby the remaining 40% of the carboxyl groups are salified with sodium.

Reacting the 75% ethyl ester/25% sodium salt of hyaluronic acid with streptomycin, whereby 75% of the carboxyls are esterified with ethanol and whereby 25% of the carboxyls are salified with streptomycin.

Reacting the 75% ethyl ester/25% sodium salt of hyaluronic acid with erythromycin base, whereby 75% of the carboxyls are esterified with ethanol and whereby 25% of the carboxyls are salified with erythromycin.
Reacting the 75% ethyl ester/25% sodium salt of hyaluronic acid with neomycin, whereby 75% of the carboxyls are esterified with ethanol and whereby 25% of the carboxyls are salified with neomycin.

Reacting the 75% ethyl ester/25% sodium salt of hyaluronic acid with gentamycin, whereby 75% of the carboxyls are esterified with ethanol and whereby 25% of the carboxyls are salified with gentamycin.

Reacting the 75% ethyl ester/25% sodium salt of hyaluronic acid with amikacin, whereby 75% of the carboxyls are esterified with ethanol and whereby 25% of the carboxyls are salified with amikacin.

Reacting the 75% ethyl ester/25% sodium salt of the carboxyls are esterified with ethanol and whereby 25% of the carboxyls are salified with kanamycin.

Reacting the 75% ethyl ester/25% sodium salt of hyaluronic acid with pilocarpine, whereby 75% of the carboxyls are esterified with ethanol and whereby 25% of the carboxyls are salified with pilocarpine.

Reacting an 85% propyl ester/15% tetrabutylammonium salt of hyaluronic acid with pilocarpine, whereby 85% of the carboxyls are esterified with propanol and whereby 15% of the carboxyls are salified with pilocarpine.

By variants of any of the variations or embodiments described above, the hyaluronic acid ester derives from hyaluronic acid which is obtained by first dehydrating cocks'
combs with acetone and then by exposing them to enzymatic digestion with papain.

By other variants of any of the variations or embodiments described above, the hyaluronic acid ester derives from hyaluronic acid which is obtained by first dehydrating cocks' combs with acetone and then by exposing them to enzymatic digestion with papain, followed by molecular ultrafiltration, and further purification of the hyaluronic acid fraction obtained.

By still other variants of any of the variations or embodiments described above, the hyaluronic acid ester derives from integral hyaluronic acid or from one of its salts, which is obtained by extraction from cocks' combs and having a molecular weight of between 8 and 13 million.

By further variants of any of the variations or embodiments described above, the hyaluronic acid ester derives from a hyaluronic acid fraction with a molecular weight of between 90 and 0.23% of the molecular weight of an integral hyaluronic acid having a molecular weight of 13 million.

By yet further variants of any of the variations or embodiments described above, the hyaluronic acid ester derives from a molecular fraction having a molecular weight of between 50,000 and 100,000 and which is substantially-free of hyaluronic acid having a molecular weight of less than 30,000.

By still further variants of any of the variations or embodiments described above, the hyaluronic acid ester derives
from a molecular fraction having a molecular weight of between 500,000 and 730,000 and which is substantially-free of hyaluronic acid having a molecular weight of less than 30,000.

By yet still variants of any of the variations or embodiments described above, the process includes the steps of: molecular ultrafiltration; and further purification of the hyaluronic acid fraction so-obtained.

By a preferred variant, the hyaluronic acid may derive from a molecular fraction, which is identified by the Trademark HYALASTINE™ of Fidia SpA, having a molecular weight of between 50,000 and 100,000 and which is substantially-free of hyaluronic acid having a molecular weight of less than 30,000.

By another preferred variant thereof, the hyaluronic acid may derive from a molecular fraction, which is identified by the Trade-mark HYALECTIN™ of Fidia SpA, having a molecular weight of between 500,000 and 730,000, and which is substantially-free of hyaluronic acid having a molecular weight of less than 30,000.

To elaborate on the processes of broad aspects of the invention described above, the esters of hyaluronic acid according to aspects of this invention may be prepared by novel processes including the use of procedural methods known per se for the esterification of carboxylic acids. Examples of such known procedures include treatment of free hyaluronic acid with the desired alcohol or alcohols in the presence of catalyzing substances, e.g., strong inorganic acids or ionic...
exchangers of the acid type, or with an etherifying agent which is capable of introducing the desired alcoholic residue of the alcohol, in the presence of an inorganic base or an organic base. As etherifying agents, it is possible to use those known in literature, e.g., especially the esters of various inorganic acids or of various organic acids, e.g., sulphonic acids, or hydracids, e.g., hydrocarbyl halogenides, e.g., methyl iodide or ethyl iodide, or neutral sulphates or hydrocarbyl acids, alfits, carbonates, silicates, phosphites or hydrocarbyl sulphonates, e.g., methylbenzene sulphonate, p-toluene sulphonate, methyl chlorosulphonate or ethyl chlorosulphonate. The reaction may take place in a suitable solvent, for example, an alcohol, preferably one that corresponds to the alkyl group to be introduced in the carboxyl group. The reaction may also take place in non-polar solvents, e.g., ketones, ethers, e.g., dioxane, or aprotic solvents, e.g., dimethylsulphoxide. As a base used as a catalyzing substance, it is possible to use, for example, a hydrate of an alkali metal or of an alkaline earth metal, or of magnesium oxide, or of silver oxide, or of a basic salt of one of these metals, e.g., a carbonate, and of the organic bases, e.g., tertiary azotized base, e.g., pyridine or collidine. In the place of the base, it is also possible to use an ionic exchanger of the basic type.

Another esterification procedure involves the use of the metal salts or salts with organic azotized bases, for example,
ammonium salts or ammonium-substitute salts. Preferably, salts of the alkali metals or of the alkaline earth metals are used, but any other metallic salt may also be used. The esterifying agents which may be used are also the same as those mentioned above, and the same applies to the solvents. It is preferable to use aprotic solvents, for example, dimethylsulphoxide or dimethylformamide.

In the esters of aspects of this invention which are obtained according to this procedure or according to the other procedures described hereinafter, free carboxylic groups of the partial esters may be salified, by means of a procedures known per se.

The hyaluronic esters of aspects of the present invention may, furthermore, be prepared according to a second process which may be generally applied to the preparation of carboxylic esters of acidic polysaccharides with carboxyl groups. This process consists of treating a quaternary ammonium salt of an acidic polysaccharide containing carboxyl groups with an etherifying agent, preferably in an aprotic organic solvent. As starting acidic polysaccharides, it is possible to use, for example, apart from hyaluronic acid, other acidic polysaccharides of animal or vegetable origin and synthetically modified derivatives thereof, e.g., acid hemicellulose, obtainable from the alkaline extracts of certain plants and after precipitation of xylans, whose disaccharide components are made up of D-glucuronic acid and
D-xylulopyranose, (see "The Carbohydrates" by W. Pigman, pages 668-669 - R.L. Whistler, W.M. Corbett), the pectins and acidic polysaccharides obtainable therefrom, that is, galacturonan, acidic polysaccharides obtainable from plant gum (exudates), e.g., gum arabic, gum tragacanth, and finally acidic polysaccharides derived from seaweed, e.g., agar and carrageenans. As starting material, it is, of course, possible also to use the molecular fractions obtained by degradation of any of the above-mentioned polysaccharides.

As organic solvents, it is preferably to use aprotic solvents, e.g., dialkylsulphoxides, dialkylcarboxamides, e.g., in particular lower alkyl dialkylsulphoxides, especially dimethylsulphoxide, and lower alkyl dialkylamides of lower aliphatic acids, e.g., dimethylformamide, diethylformamide, dimethylacetamide, or diethylacetamide.

Other solvents, however, which may be used are not always aprotic, e.g., alcohols, ethers ketones, esters, especially aliphatic alcohols or heterocyclic alcohols, and ketones with a lower boiling point, e.g., hexafluoroisopropanol, trifluoroethanol, and N-methylpyrroolidone.

The reaction is preferably effected at a temperature range of between 0°C and 100°C, especially between 25°C and 75°C, for example, at 30°C.

The esterification preferably is carried out by adding the esterifying agent by degrees to the above-mentioned
ammonium salt in one of the above-mentioned solvents, for example, in dimethylsulphoxide.

As an alkylating agent, it is possible to use those mentioned above, especially the hydrocarbyl halogens, for example, alkyl halogens. As starting quaternary ammonium salts, it is preferable to use the lower ammonium tetraalkylates, with alkyl groups having preferably between 1 and 6 carbon atoms. Most preferably, the hyaluronate of tetrabutylammonium is used. It is possible to prepare these quaternary ammonium salts by reacting a metallic salt of acidic polysaccharide, preferably one of those mentioned above, especially the sodium or potassium salts, in aqueous solution with a salified sulphonic resin with a quaternary ammonium base.

The tetraalkylammonium salt of the acidic polysaccharide can be obtained by freeze-drying the eluate. The tetraalkylammonium salts of acidic polysaccharides used as starting compounds of the process of aspects of the present invention and deriving from lower alkyls, especially alkyls with between 1 and 6 carbon atoms, are new and provide another aspect of the present invention. Surprisingly, such salts have proven to be soluble in the above-mentioned organic solvents, and for this reason the esterification of acidic polysaccharides according to the above-mentioned second novel process is particularly easy and gives generous yields. It is, therefore, only by using this kind of procedure that one
can exactly dose the number of carboxylic groups of acidic polysaccharide which are to be esterified.

The above-described second process is very suitable especially for the preparation of hyaluronic esters according to other aspects of the present invention. In particular, therefore, as starting compounds for this second new process, the quaternary ammonium salts of hyaluronic acid, especially those deriving from lower alkyls, and especially from alkyls with between 1 and 6 carbon atoms, are new and provide a particular aspect of the present invention.

One variation of the previously described process consists in reacting the potassium salt of acidic polysaccharide sodium, which is suspended in a suitable solution, e.g., in dimethylsulphoxide, with a suitable alkylating agent in the presence of catalytic quantities of a quaternary ammonium salt, e.g., the iodide of tetrabutylammonium.

For the preparation of the new esters according to other aspects of the present invention, it is possible to use hyaluronic acids of any origin, for example, the acids extracted from the above-mentioned natural starting materials, for example, from cocks' combs. The preparation of such acids is described in the literature: preferably, purified hyaluronic acids are used. These aspects of the invention have been described above. Especially useful hyaluronic acids comprise molecular fractions of the integral acids obtained
directly by extraction of the organic materials, with molecular weights varying within a wide range, for example from 90% to 80% (MW = 11.7 to 10.4 million) to 0.23% (MW = 30,000) of the molecular weight of the integral acid having a molecular weight of 13 million, preferably between 5% and 0.2%. Such fractions may be obtained by means of various procedures described in the literature, e.g., by hydrolysing, oxidizing, enzymatic or physical procedures, e.g., mechanical or rotational procedures. Primordial extracts are therefore often formed during these same purification procedures (for example, see the article by Balazs, et al., quoted above in "Cosmetics & Toiletries"). The separation and purification of the molecular fractions obtained are brought about by known techniques, for example, by molecular filtration.

One fraction of purified hyaluronic acid which is suitable for use according to aspects of the invention is, for example, that known as "non-inflammatory-NIF-NaHA sodium hyaluronic" described by Balazs in the booklet "Healon - A Guide To Its Use In Ophthalmic Surgery", D. Miller & R. Stegmann, eds, John Wiley & Sons N.Y. 81983: p.5.

Particularly important as starting materials for the preparation of esters of aspects of the present invention are two purified fractions which are obtainable from hyaluronic acid, for example the ones extracted from cocks' combs, known by the trade-marks HYALASTINE™ and HYALECTIN™ of Fidia S.p.A. The fraction HYALASTINE™ has an average molecular weight of
50,000 to 100,000, while the fraction HYALECTIN\textsubscript{TM} has an average molecular weight of between 500,000 and 730,000. A combined fraction of these two fractions has also been isolated and is characterized as having an average molecular weight of 250,000 to 350,000. This combined fraction may be obtained with a yield of 80% of total hyaluronic acid available in the particular starting material, while the fraction HYALECTIN\textsubscript{TM} may be obtained with a yield of 30% and the fraction HYALASTINE\textsubscript{TM} with a yield of 50% of the starting HY. The preparation of these fractions is described in Examples A-C hereinunder.

The salification of hyaluronic acid with the above metals, for the preparation of starting salts for the particular esterification procedure of aspects of the present invention described above, is performed in a manner which is known \textit{per se}, for example, by reacting hyaluronic acid with the calculated base quantity, for example, with alkali metal hydrates or with alkaline earth metal hydrates or with basic salts of such metals, e.g., carbonates or bicarbonates.

In the process of aspects of this invention for preparing the partial esters of aspects of the present invention, it is possible to salify all the remaining carboxylic groups or only part of them, dosing the base quantities so as to obtain the desired stoichiometric degree of salification. With the correct degree of salification it is possible to obtain esters with a wide range of different dissociation constants and
which therefore give the desired pH, in solution in situ at the time of therapeutic application.

The present invention also includes amongst its other aspects, modifications of the above-described processes for the production of the new esters and their salts, in which a process is interrupted at any given stage, or started with an intermediate compound on which the remaining stages are carried out, or in which the starting products are formed in situ.

As described hereinabove, the total esters of hyaluronic acid, or partial esters of hyaluronic acid, or the salts of the partial esters of hyaluronic acid of aspects of this invention, may derive from integral hyaluronic acid or from one of its salts obtained by extraction from cocks' combs, and having a molecular weight of between 8 and 13 million. By one variant thereof, the hyaluronic ester may derive from hyaluronic acid which is obtained by first dehydrating the cocks' combs with acetone and then exposing them to enzymatic digestion with papain, followed by, if desired, molecular ultrafiltration, and further purification of the hyaluronic acid fraction obtained. By another variant thereof, the hyaluronic ester may derive from a hyaluronic acid fraction with a molecular weight of between 90 to 80% to 0.23% of the molecular weight of an integral hyaluronic acid with a molecular weight of 13 million. By a preferred variant thereof, the hyaluronic ester may derive from a molecular
fraction identified by the Trade-mark HYALASTINE™ of Fidia SpA having a molecular weight of between 50,000 and 100,000 and which is substantially-free of hyaluronic acid having a molecular weight of less than 30,000. By another preferable variant thereof, the hyaluronic ester may derive from a molecular fraction identified by the Trade-mark HYALECTIN™ of Fidia SpA having a molecular weight of between 500,000 and 730,000 and which is substantially-free of hyaluronic acid having a molecular weight of less than 30,000.

By yet another aspect of this invention, a pharmaceutical preparation is provided comprising: an effective amount of at least one ester of hyaluronic acid of the Formula

\[ \text{Hy(COOR)}_n \]

wherein COO represents the carboxylic acid moiety on the hyaluronic acid (Hy) molecule, R is derived from an alcohol selected from the group consisting of an aliphatic alcohol having a chain of 2 to 34 carbons, an araliphatic alcohol, a cycloaliphatic alcohol and a heterocyclic alcohol, and n is a large number; and a pharmaceutically-acceptable carrier.

By important variants of such pharmaceutical preparation of this aspect of the invention, the hyaluronic acid ester may be a total ester, or a partial ester or a salt of a partial ester with an organic base or with an inorganic base.

By other variants of such pharmaceutical preparation as described above, the alcohol in such ester is an aliphatic alcohol which is substituted by one or two functional groups
which are selected from the group consisting of amino, hydroxy, mercapto, aldehyde, keto, carboxyl, hydrocarbyl, dihydrocarbylamino, ether, ester, thioether, thioester, acetal, ketal, carbalkoxy, unsubstituted carbamide groups and carbamide groups which are substituted by one or two alkyl groups, the hydrocarbyl radicals in these functionally-modified groups having a maximum of 6 carbon atoms, and in which the carbon atom chain may be interrupted heteroatoms which are selected from the group consisting of oxygen, sulphur and nitrogen. By a specific variation thereof, the alcohol is ethyl alcohol, propyl alcohol, isopropyl alcohol, normal butyl alcohol, isobutyl alcohol, tert-butyl alcohol, amyl alcohol, pentyl alcohol, hexyl alcohol, octyl alcohol, ethylene glycol, propylene glycol, butylene glycol or glycerin.

By still other variants of such pharmaceutical preparation as described above, the alcohol in such ester is an araliphatic alcohol with only one benzene residue, in which the aliphatic chain has a maximum of 4 carbon atoms, and in which the benzene residue may be substituted by 1 to 3 methyl groups or by 1 to 3 hydroxy groups, or by 1 to 3 halogen atoms, and in which the aliphatic chain may be substituted by one or two functions which are selected from the group consisting of free amino groups, monoethylamino groups, diethylamino groups, pyrrolidinyl groups and piperidinyl.
groups. By a specific variation thereof, the alcohol is benzyl alcohol, phenethyl alcohol, ephedrine or adrenalin.

By yet other variants of such pharmaceutical preparation as described above, the alcohol in such ester is a cycloaliphatic alcohol or an aliphatic-cycloaliphatic alcohol and derives from a monocyclic hydrocarbide or a polycyclic hydrocarbide with a maximum of 34 carbon atoms. By a variation thereof, the alcohol is a polycyclic alcohol which is selected from the group consisting of sterols, colic acids, steroid alcohols, and groups of the estrane and pregnane series and their unsaturated derivative. By specific variations of such variations, the alcohol is cortisone, hydrocortisone, prednisone, prednisolone, fluorocortisone, dexamethasone, betamethasone, corticosterone, deoxycorticosterone, paramethasone, flumethasone, fluocinolone, fluocinolone acetonide, fluprednylidene, clobetasol, or beclomethasone.

By yet further variants of such pharmaceutical preparation as described above, such ester is in the form of a salt of such partial ester with an alkali metal, or with an alkaline earth metal, or with magnesium, or with aluminum, or with ammonia. By a variation thereof, the salt is a sodium salt.

Preferred examples of such pharmaceutical preparations as described above comprise the following:

An effective amount of the salt of the partial propyl ester of hyaluronic acid with 50% of the carboxyl groups
esterified and with 50% of the carboxyl groups salified with sodium; and a pharmaceutically-acceptable carrier.

An effective amount of the salt of the partial isopropyl ester of hyaluronic acid with 50% of the carboxyl groups esterified and with 50% of the carboxyl groups salified with sodium; and a pharmaceutically-acceptable carrier.

An effective amount of the salt of the partial propyl ester of hyaluronic acid with 85% of the carboxylic groups esterified and with 15% of the carboxylic groups salified with sodium; and a pharmaceutically-acceptable carrier.

An effective amount of the salt of the partial ethyl ester of hyaluronic acid with 75% of the carboxyl groups esterified and with 25% of the carboxyl groups salified with sodium; and a pharmaceutically-acceptable carrier.

An effective amount of the salt of the partial methyl ester of hyaluronic acid with 75% of the carboxyl groups esterified and with 25% of the carboxyl groups salified with sodium; and a pharmaceutically-acceptable carrier.

An effective amount of the total ethyl ester of hyaluronic acid; and a pharmaceutically-acceptable carrier.

An effective amount of the total propyl ester of hyaluronic acid; and a pharmaceutically-acceptable carrier.

An effective amount of the salt of the partial butyl ester of hyaluronic acid with 50% of the carboxylic groups salified with sodium; and a pharmaceutically-acceptable carrier.
An effective amount of the salt of the partial ethoxycarbonylmethyl ester of hyaluronic acid with 75% of the carboxyl groups esterified and with 25% of the carboxyl groups salified with sodium; and a pharmaceutically-acceptable carrier.

An effective amount of the salt of the partial cortisone ester of hyaluronic acid with 20% of the carboxyl groups esterified and with 80% of the carboxyl groups salified with sodium; and a pharmaceutically-acceptable carrier.

An effective amount of the salt of the partial hydrocortisone ester of hyaluronic acid with 20% of the carboxyl groups esterified and with 80% of the carboxyl groups salified with sodium; and a pharmaceutically-acceptable carrier.

An effective amount of the salt of the partial fluorocortisone ester of hyaluronic acid with 20% of the carboxyl groups esterified and with 80% of the carboxyl groups salified with sodium; and a pharmaceutically-acceptable carrier.

An effective amount of the salt of the deoxycorticosterone ester of hyaluronic acid with 20% of the carboxyl groups esterified and with 80% of the carboxyl groups salified with sodium; and a pharmaceutically-acceptable carrier.

An effective amount of the mixed ethanol-cortisone ester of hyaluronic acid with 80% of the carboxyl groups esterified
with ethanol and with 20% of the carboxyl groups esterified with cortisone; and a pharmaceutically-acceptable carrier.

An effective amount of the mixed ethanol-hydrocortisone ester of hyaluronic acid with 80% of the carboxyl groups esterified with ethanol and with 20% of the carboxyl groups esterified with hydrocortisone; and a pharmaceutically-acceptable carrier.

An effective amount of the mixed ethanol-fluorocortisone ester of hyaluronic acid with 80% of the carboxyl groups esterified with ethanol and with 20% of the carboxyl groups esterified with fluorocortisone; and a pharmaceutically-acceptable carrier.

An effective amount of the mixed ethanol-deoxycorticosterone ester of hyaluronic acid with 80% of the carboxyl groups esterified with ethanol and with 20% of the carboxyl groups esterified with fluorocortisone; and a pharmaceutically-acceptable carrier.

An effective amount of the salt of the partial and mixed ethanol-deoxycorticosterone ester of hyaluronic acid with 40% of the carboxyl groups esterified with ethanol, with 20% of the carboxyl groups esterified with deoxycorticosterone and with the remaining 40% of the carboxyl groups salified with sodium; and a pharmaceutically-acceptable carrier.

An effective amount of the salt of the partial and mixed ethanol-cortisone ester of hyaluronic acid with 40% of the carboxyl groups esterified with ethanol, with 20% of the
carboxyl groups esterified with cortisone, and with the remaining 40% of the carboxyl groups salified with sodium; and a pharmaceutically-acceptable carrier.

An effective amount of the salt of the partial and mixed ethanol-hydrocortisone ester of hyaluronic acid with 40% of the carboxyl groups esterified with ethanol, with 20% of the carboxyl groups esterified with hydrocortisone, and with the remaining 40% of the carboxyl groups salified with sodium; and a pharmaceutically-acceptable carrier.

An effective amount of the salt of the partial and mixed ethanol-fluorocortisone ester of hyaluronic acid with 40% of the carboxyl groups esterified with ethanol, with 20% of the carboxyl groups esterified with fluorocortisone, and with the remaining 40% of the carboxyl groups salified with sodium; and a pharmaceutically-acceptable carrier.

An effective amount of the total pentyl ester of hyaluronic acid; and a pharmaceutically-acceptable carrier.

An effective amount of the total isopentyl ester of hyaluronic acid; and a pharmaceutically-acceptable carrier.

An effective amount of the total benzyl ester of hyaluronic acid; and a pharmaceutically-acceptable carrier.

An effective amount of the total phenethyl ester of hyaluronic acid; and a pharmaceutically-acceptable carrier.

An effective amount of a salt of a hyaluronic acid ester with 75% of the carboxyl groups esterified with ethanol and
with 25% of the carboxyls salified with streptomycin; and a pharmaceutically-acceptable carrier.

An effective amount of a salt of a hyaluronic acid ester with 75% of the carboxyl groups esterified with ethanol and with 25% of the carboxyls salified with erythromycin; and a pharmaceutically-acceptable carrier.

An effective amount of a salt of a hyaluronic acid ester with 75% of the carboxyl groups esterified with ethanol and with 25% of the carboxyls salified with neomycin; and a pharmaceutically-acceptable carrier.

An effective amount of a salt of a hyaluronic acid ester with 75% of the carboxyl groups esterified with ethanol and with 25% of the carboxyls salified with gentamycin; and a pharmaceutically-acceptable carrier.

An effective amount of a salt of a hyaluronic acid ester with 75% of the carboxyl groups esterified with ethanol and with 25% of the carboxyls salified with amikacin; and a pharmaceutically-acceptable carrier.

An effective amount of a salt of a hyaluronic acid ester with 75% of the carboxyl groups esterified with ethanol and with 25% of the carboxyls salified with kanamycin; and a pharmaceutically-acceptable carrier.

An effective amount of a salt of a hyaluronic acid ester with 75% of the carboxyl groups esterified with ethanol and with 25% of the carboxyls salified with pilocarpine; and a pharmaceutically-acceptable carrier.
An effective amount of a salt of a hyaluronic acid ester with 85% of the carboxyl groups esterified with ethanol and with 15% of the carboxyls salified with pilocarpine; and a pharmaceutically-acceptable carrier.

By variations of any of the variants and embodiments described above, the hyaluronic ester derives from hyaluronic acid which is obtained by first dehydrating cocks' combs with acetone and the exposing them to enzymatic digestion with papain.

By other variations of any of the variants and embodiments described above, the hyaluronic ester derives from hyaluronic acid which is obtained by first dehydrating cocks' combs with acetone and the exposing them to enzymatic digestion with papain, followed by molecular ultrafiltration, and further purification of the hyaluronic acid fraction obtained.

By still other variations of any of the variants and embodiments described above, the hyaluronic ester derives from an integral hyaluronic acid or from one of its salts, which is obtained by extraction from cocks' combs, and having a molecular weight of between 8 and 13 million.

By still further variations of any of the variants and embodiments described above, the hyaluronic ester derives from a hyaluronic acid fraction with a molecular weight of between 90 and 0.23% of the molecular weight of an integral hyaluronic acid with a molecular weight of 13 million.
By yet further variants of any of the variants and embodiments described above, the hyaluronic ester derives from a hyaluronic acid fraction having a molecular weight of between 50,000 and 100,000, and which is substantially-free of hyaluronic acid having a molecular weight of less than 30,000.

By still other variant of any of the variants and embodiments described above, the hyaluronic ester derives from a hyaluronic acid fraction having a molecular weight of between 500,000 and 730,000, and which is substantially-free of hyaluronic acid having a molecular weight of less than 30,000.

By yet another aspect of this invention, a medicament is provided comprising: (a) at least one pharmacologically-active substance; and (b) a carrying vehicle comprising an ester of hyaluronic acid of the formula

$$\text{Hy(COOR)}_n$$

wherein COO represents the carboxylic acid moiety on the hyaluronic acid (Hy) molecule, R is derived from an alcohol which is selected from the group consisting of an aliphatic alcohol having a chain of 2 to 34 carbons, an araliphatic alcohol, a cycloaliphatic alcohol and a heterocyclic alcohol, and n is a large number.

By still another aspect of this invention, a medicament is provided comprising: (a) at least one pharmacologically-active substance; (b) a carrying vehicle comprising an ester
of hyaluronic acid of the Formula

\[ \text{Hy(COOR)}_n \]

wherein \( \text{COO} \) represents the carboxylic acid moiety on the hyaluronic acid (Hy) molecule, \( R \) is derived from an alcohol which is selected from the group consisting of an aliphatic alcohol having a chain of 2 to 34 carbons, an araliphatic alcohol, a cycloaliphatic alcohol and a heterocyclic alcohol, and \( n \) is a large number; and (c) common excipients for pharmaceutical preparations.

By variants of such aspects of medicaments, as described above, such hyaluronic acid ester may be a total ester, or a partial ester or a salt of a partial ester with an organic base or with an inorganic base.

By other variants of such aspects of medicaments as described above, the alcohol is an aliphatic alcohol which is substituted by one or two functional groups which are selected from the group consisting of amino, hydroxy, mercapto, aldehyde, keto, carboxyl, hydrocarbonyl, dihydrocarbonylamino, ether, ester, thioether, thioester, acetal, ketal, carbalkoxy, unsubstituted carbamide groups and carbamide groups which are substituted by one or two alkyl groups, the hydrocarbonyl radicals in these functionally-modified groups having a maximum of 6 carbon atoms, and in which the carbon atom chain may be interrupted by heteroatoms which are selected from the group consisting of oxygen, sulphur and nitrogen. By a specific variation thereof, the alcohol is ethyl alcohol,
propyl alcohol, isopropyl alcohol, normal butyl alcohol, isobutyl alcohol, tert-butyl alcohol, amyl alcohol, pentyl alcohol, hexyl alcohol, octyl alcohol, ethylene glycol, propylene glycol, butylene glycol or glycerin.

By further variants of such aspects of medicaments as described above, the alcohol is an araliphatic alcohol with only one benzene residue, in which the aliphatic chain has a maximum of 4 carbon atoms, and in which the benzene residue may be substituted by 1 to 3 methyl groups or by 1 to 3 hydroxy groups, or by 1 to 3 halogen atoms, and in which the aliphatic chain may be substituted by one or two functions which are selected from the group consisting of free amino groups, monoethylamino groups, diethylamino groups, pyrrolidinyl groups and piperidinyl groups. By a specific variation thereof, the alcohol is benzyl alcohol, phenethyl alcohol, ephedrine or adrenalin.

By another variant of the medicament as described above, the alcohol is a cycloaliphatic alcohol or an aliphatic-cycloaliphatic alcohol and derives from a monocyclic hydrocarbide or a polycyclic hydrocarbide with a maximum of 34 carbon atoms. By a variation thereof, the alcohol is a polycyclic alcohol which is selected from the group consisting of sterols, colic acids, steroid alcohols, and groups of the estrane and pregnane series and their unsaturated derivative. By a specific variation thereof, the alcohol is cortisone, hydrocortisone, prednisone, prednisolone, fluorocortisone,
dexamethasone, betamethasone, corticosterone, deoxycorticosterone, paramethasone, flumethasone, fluocinolone, fluocinolone acetonide, fluprednylidene, clobetasol, or beclomethasone.

By yet other variants of such aspects of medicaments as described above, the ester is in the form of a salt of a partial ester with an alkali metal, or with an alkaline earth metal, or with magnesium, or with aluminum, or with ammonia.

By a variation thereof, the salt is a sodium salt.

By still other variants of such aspects of medicaments as described above, the pharmacologically-active substance (a) is selected from the group consisting of an anaesthetic, an analgesic, an anti-inflammatory, a vasoconstrictor, an antibiotic-antibacterial, and an antiviral. By a specific variation thereof, the pharmacologically-active substance (a) is topically active.

By yet other variants of aspects of such medicaments as described above, the carrying vehicle (b) contains an ester of hyaluronic acid with a pharmacologically-inactive alcohol, or the carrying vehicle (b) contains an ester of hyaluronic acid with a pharmacologically-active alcohol.

By still other variants of aspects of such medicaments as described above, the carrying vehicle (b) is of a basic nature and contains a partial ester of hyaluronic acid, the unesterified groups of which are salified with the pharmacologically-active substance.
By still other aspects of this invention, various uses are provided for the above described esters. Thus, one use is in cosmetic articles, as a carrying vehicle.

Another use is in sanitary and surgical articles, as a carrying vehicle. In one variation of such use, the sanitary and surgical articles are in the form of microcapsules for subcutaneous, intramuscular or intravenous injection. In another variation of such use, the sanitary and surgical articles are in the form of solid inserts to be removed after a certain length of time. In still another variation of such use, the sanitary and surgical articles are in the form of sponges for the medication of wounds and various lesions.

Yet another use is in films or threads, as a carrying vehicle. In one variation of such use, the films or threads are for the medication of wounds and in surgery or as suture threads in surgical operations. In another variation of such use, the films are in the form of artificial skin for use in surgical dermatology.

Still another use is as films or threads in the production of gauze, as a carrying vehicle. In one variation of such use, such threads provide a gauze for the medication of wounds and in surgery. In another variation of such use, such threads provide a gauze in the form of sponges for the medication of wounds or in surgery.

A further use of such ester of hyaluronic acid is for the preparation of films or threads which includes the steps of:
dissolving such hyaluronic ester in an organic solvent; making the solution into sheet form or thread form respectively; and then eliminating the organic solvent by treatment with another suitable organic or aqueous solvent which is soluble in the first solvent.

Yet a still further use of such ester of hyaluronic acid is for the preparation of films or threads which includes the steps of: dissolving such hyaluronic ester in an organic solvent; making the solution into sheet form or thread form respectively; and then eliminating the organic solvent by treatment with a current of a suitably heated inert gas.

Thus, as noted above, a first group of esters of HY according to a first aspect of the present invention, useful in therapy as well as in the other above-mentioned fields, is represented by those in which the qualities of
hyaluronic acid itself dominate and may be exploited. Such esters derive from alcohols of the above-mentioned series which do not themselves possess a notable pharmacological action, for example, the saturated alcohols of the aliphatic series or simple alcohols of the cycloaliphatic series.

A second group of esters of HY according to a second aspect of this invention, also useful in therapy, is represented, on the other hand, by the esters in which the pharmacological qualities of the alcohol component dominate. That is, it relates to esters of HY with pharmacologically-active alcohols, e.g., steroid alcohols, i.e., those of the cortisone type with an anti-inflammatory action. These compounds possess properties qualitatively similar to those of the alcohol, but with a more differentiated range of action, compared also to the already-known esters, ensuring a better balanced, constant and regular pharmacological action, and usually obtaining a marked retard effect.

A third group of esters of HY according to a third aspect of the present invention, and which represent a particularly original and useful aspect, is respected by the esters with a more mixed character compared to the two previous groups. That is, it relates to esters of HY in which a part of the carboxylic groups of HY is esterified with a pharmacologically-active alcohol and in which another part or the carboxylic group of HY is esterified with a pharmacologically-indifferent alcohol, or with one
whose activity is negligible. By suitably dosing percentages of the two types of alcohols as esterifying components, it is possible to obtain esters with the same pharmacological activity as the pharmacologically-active alcohol, without the specific activity of hyaluronic acid, but having those above-mentioned qualities of better stability and bioavailability, with respect to the activity desired and characteristic of the pharmacologically-active alcohol and due to the ester groups of the pharmacologically-inert alcohol.

A fourth group of esters of HY according to a fourth aspect of this invention is represented by those of a mixed character in which the ester groups derive from two different therapeutically-active substances. In this case also the esters may be partial or total, that is, only some carboxylic groups may be esterified by two different therapeutically-active alcohols, for example, by one cortisone steroid and by an antibiotic, or by a phenothiazine, while the carboxylic groups may be free or salified, for example, with alkali metals, particularly with sodium, or all the carboxylic groups may be esterified with the above-mentioned alcohols. It is, however, also possible to prepare esters with three or more alcohol components, e.g., esters in which a part of the carboxylic groups are esterified with one therapeutically-active alcohol, another part of the carboxylic groups are esterified with another therapeutically-active alcohol, a third part of the carboxylic groups are esterified with a
therapeutically-inactive alcohol and a fourth part of the carboxylic groups are possibly salified with a metal or with a therapeutically-active or inactive base or which may comprise carboxylic groups in a free form.

In the above-mentioned partial esters of HY in which some of the carboxylic acid groups remain free, these may be salified with metals or organic bases, e.g., with alkali metals or with alkaline earth metals or with ammonia or with nitrogenous organic bases.

Most of the esters of Hy, unlike Hy itself, present a certain degree of solubility in organic solvents. This solubility depends on the percentage of esterified carboxylic groups and on the type of alkyl group linked with the carboxyl. Therefore an ester of HY with all its carboxylic groups esterified presents, at room temperature, good solubility, for example, in dimethylsulfoxide (the benzyl ester of HY dissolves in DMSO in a measure of 200 mg/ml). Most of the total esters of HY also present, unlike HY and especially its salts, poor solubility in water.

The previously mentioned solubility characteristics, together with particular and notable viscoelastic properties, allow the use of HY esters to obtain sanitary and medical preparations which are insoluble in saline and which have the particular desired form. These materials may be obtained by preparing a solution of an HY ester in an organic solvent, forming the very viscous solution so-provided into the form of the desired article, and then
extracting the organic solvent with another solvent which mixes with the first solvent, but in which the HY ester is insoluble.

Thus, by another aspect of this invention, a process is provided for the preparation of films or threads of hyaluronic esters with an alcohol of the aliphatic, araliphatic, cycloaliphatic or heterocyclic series, with the exception of the total methyl ester of hyaluronic acid, which comprises: dissolving such hyaluronic ester in an organic solvent; making the solution into sheet or thread form respectively; and then eliminating the organic solvent by treatment with another suitable organic solvent or with an aqueous solvent which is soluble in said first solvent.

By a further aspect of this invention, a process is provided for the preparation of films or threads of hyaluronic esters with an alcohol of the aliphatic, araliphatic, cycloaliphatic or heterocyclic series, with the exception of the total methyl ester of hyaluronic acid which comprises: dissolving such hyaluronic ester in an organic solvent; making the solution into sheet or thread form respectively; and then eliminating the organic solvent by treatment with a current of a suitably heated inert gas.

By yet another aspect of this invention, a process is provided for the preparation of films or threads of total hyaluronic esters with an alcohol of the aliphatic, araliphatic, cycloaliphatic or heterocyclic series, with the exception of the total methyl ester of hyaluronic acid which comprises: dissolving such hyaluronic ester in an
organic solvent; making the solution into sheet or thread form respectively; and then eliminating the organic solvent by treatment with another suitable organic solvent or with an aqueous solvent which is soluble in the first solvent.

By a still further aspect of this invention, a process is provided for the preparation of film or threads of salts of partial esters of hyaluronic acid with an alcohol of the aliphatic, araliphatic, cycloaliphatic or heterocyclic series, with the exception of the total methyl ester of hyaluronic acid which comprises: dissolving such hyaluronic ester in an organic solvent; making the solution into sheet or thread form respectively; and then eliminating the organic solvent by treatment with a current of a suitably heated inert gas.

By another aspect of this invention, a process is provided for the preparation of films or threads of partial esters with an alcohol of the aliphatic, araliphatic, cycloaliphatic or heterocyclic series which comprises: dissolving the hyaluronic ester in an organic solvent; making the solution into sheet or thread form respectively; and then eliminating the organic solvent by treatment with another suitable organic solvent or with an aqueous solvent which is soluble in the first solvent.

By yet another aspect of this invention, a process is provided for the preparation of films or threads of partial esters with an alcohol of the aliphatic, araliphatic, cycloaliphatic or heterocyclic series which comprises: dissolving such hyaluronic ester in an organic solvent;
making the solution into sheet or thread form respectively; and then eliminating the organic solvent by treatment with a current of a suitably heated inert gas.

By a still further aspect of this invention, a process is provided for the preparation of films or threads of salts of partial esters of hyaluronic acid with an alcohol of the aliphatic, araliphatic, cycloaliphatic or heterocyclic series, with an inorganic base or with an organic base which comprises: dissolving such salt in an organic solvent; making the solution into sheet or thread form respectively; and then eliminating the organic solvent by treatment with another suitable organic solvent or with an aqueous solvent which is soluble in the first solvent.

By yet another aspect of this invention, a process is provided for the preparation of films or threads of salts of partial esters with an alcohol of the aliphatic, araliphatic, cycloaliphatic or heterocyclic series, with an inorganic base or with an organic base which comprises: dissolving such salt in an organic solvent; making the solution into sheet or thread form respectively; and then eliminating the organic solvent by treatment with a current of a suitably heated inert gas.

The esters of hyaluronic acid, in the many various aspects as described above according to aspects of the present invention, are all new, except for the aforementioned total methyl ester of hyaluronic acid extracted from human umbilical cords, and the methyl esters of the above-mentioned oligomers of HY. Also, new, however, are
partial esters of hyaluronic acid with methyl alcohol and their salts with metal bases or with organic bases. The biological and pharmacological activities of the above-mentioned partial methyl esters described in literature, were unknown, as were their excellent bioplastic qualities and high stability. Also unknown, therefore, was the use of such esters for the preparation of medicaments, cosmetics, sanitary and surgical articles and other new products discussed above which are provided as part of other aspects of the present invention.

This disclosure also provides a teaching of a process for the preparation of acidic polysaccharide esters containing carboxyl groups, which process comprises: treating a quaternary ammonium salt of the polysaccharide with an esterifying agent in an aprotic solvent, and, if desired, salifying free carboxyl groups in the partial esters obtained; or if desired, releasing salified groups in them.

The acidic polysaccharides used in the above-described process may be of animal or vegetable origin. They may also contain sulfonic acid groups.

Hyaluronic acid or one of its molecular particles, as described above for aspects of the present invention, is preferably used. Dimethylsulfoxide is preferably used as the aprotic solvent. A lower tetraalkylammonium salt is used as the starting salt; preferably tetraalkylammonium salt of acidic polysaccharide is used.
This disclosure also provide tetraalkylammonium salts of an acidic polysaccharide containing carboxyl groups deriving from alkyls with between 1 and 6 carbon atoms, especially where the acidic polysaccharide is hyaluronic acid or one of its molecular fractions.

As referred to hereinabove, alcohols of the aliphatic series to be used as esterifying components of the carboxylic groups of hyaluronic acid according to aspects of the present invention are, for example, those with a maximum of 34 carbon atoms, which may be saturated or unsaturated and which may possibly also be substituted by other free functional or functionally-modified groups, e.g., amine, hydroxyl, aldehyde, ketone, mercaptan, or carboxyl groups or by groups derived from these e.g., hydrocarbyl or dihydrocarbylamine groups [from now on the term "hydrocarbyl" will be used to refer not only to monovalent radicals of hydrocarbons, e.g., the C\textsubscript{n}H\textsubscript{2n+1} type, but also bivalent or trivalent radicals, e.g., "alkylenes" C\textsubscript{n}H\textsubscript{2n} or "alkylidenes" C\textsubscript{n}H\textsubscript{2n}], ether or ester groups, acetal or ketal groups, thioether or thioester groups, and esterified carboxyl or carbamide groups and carbamide substituted by one or more hydrocarbyl groups, by nitrile groups or by halogens.

Of the above-mentioned groups containing hydrocarbyl radicals, these are preferably lower aliphatic radicals, e.g., alkyls, with a maximum of 6 carbon atoms. Such alcohols may also be interrupted in the carbon atom chain by heteroatoms, e.g., oxygen, nitrogen and sulfur atoms.
Preferred are alcohols substituted with one or two of such functional groups.

Alcohols of the above-mentioned group which are preferably to be used within the bounds of preferred aspects of the present invention are those with a maximum of 12, and especially 6 carbon atoms, and in which the hydrocarbyl atoms in the above-mentioned amine, ether, ester, thioether, thioester, acetal, ketal groups represent alkyl groups with a maximum of 4 carbon atoms, and also in the esterified carboxyl or substituted carbamide groups the hydrocarbyl groups are alkyls with the same number of carbon atoms, and in which in the amine or carbamide groups may be alkylamine or alkylencarbamide groups with a maximum of 8 carbon atoms. Of these alcohols for use in the total esters of HY, special mention should be given to those which are saturated and not substituted, e.g., the ethyl, propyl, and isopropyl alcohols, normal butyl alcohol, isobutyl alcohol, tertiary butyl alcohol, the amyl, pentyl, hexyl, octyl, monyl and dodecyl alcohols and, above all, those with a linear chain, e.g., normal octyl and dodecyl alcohols. Of the substituted alcohols of this group, the bivalent alcohols should be included, e.g., ethylene glycol, propylene glycol and butylene glycol, the trivalent alcohols, e.g., glycerine, the aldehyde alcohols, e.g., tartronic alcohol, the carboxylic alcohols, e.g., lactic acids, glycolic acid, malic acid, the tartaric acids, citric acid, the amino alcohols, e.g., normal aminoethanol, aminopropanol, normal aminobutanol and their
dimethylated and diethylated derivatives in the amine function, choline, pyrrolidinylethanol, piperidinylethanol, piperazinylethanol and the corresponding derivatives of normal propyl or normal butyl alcohol, monothioethyleneglycol or its alkyl derivatives, e.g., the ethyl derivative in the mercaptan function.

Of the higher saturated aliphatic alcohols, the following should be mentioned: cetyl alcohol and myricyl alcohol, but for the aim of the present invention in its other aspects, the higher unsaturated alcohols with one or two double bonds are especially important, e.g., especially those contained in many essential oils and with affinity to terpene, e.g., cirronellol, geraniol, nerol, nerolidol, linalool, farnesol, and phytol. Of the unsaturated lower alcohols, it is necessary to consider allyl alcohol and propargyl alcohol. Of the araliphatic alcohols, special attention should be given to those with only one benzene residue and in which the aliphatic chain has a maximum of 4 carbon atoms, in which the benzene residue can be substituted by between 1 and 3 methyl or hydroxyl groups or by halogen atoms, especially by chlorine, bromine or iodine, and in which the aliphatic chain may be substituted by one or more functions selected from the group consisting of those containing free amine groups or mono- or dimethylated or by pyrrolidine or piperidine groups. Of these alcohols, special attention should be given to benzyl alcohol and phenethyl alcohol.
The alcohols of the cycloaliphatic or aliphatic-cycloaliphatic series may derive from mono- or polycyclic hydrocarbons, may preferably have a maximum of 34 carbon atoms, may be unsubstituted or may contain one or more substituents, e.g., those mentioned above for the aliphatic alcohols. Of the alcohols derived from cyclic monoannular hydrocarbons, special mention should be given to those with a maximum of 12 carbon atoms, the rings preferably between 5 and 7 carbon atoms, which may be substituted, for example, by between one and three lower alkyl groups, e.g., methyl, ethyl, propyl or isopropyl groups. As specific alcohols of this group the following can be mentioned: cyclohexanol, cyclohexanediol, 1,2,3-cyclohexanetriol, 1,3,5-cyclohexanetriol (phloroglucitol), inositol, and the alcohols which derive from p-methane, e.g., carbomenthol, menthol, and α-γ terpineol, l-terpineol, 4-terpineol and piperitol, or the mixture of these alcohols known as "terpineol", 1,4- and 1,8 terpin. Of the alcohols which derive from hydrocarbons with condensed rings, e.g., those of thujane, pinane or camphane, the following can be mentioned: thujanol, sabinol, pinol hydrate, D- and L-borneol and D- and L-isoborneol.

Aliphatic-cycloaliphatic polycyclic alcohols which may be used for the esters of aspects of the present invention are sterols, colic acids and steroids, e.g., sexual hormones and their synthetic analogues, especially corticosteroids and their derivatives. It is therefore possible to use: cholesterol, dihydrocholesterol,
epidihydrocholesterol, coprostanol, epicoprostanol, sitosterol, stigmasterol, ergosterol, colic acid, deoxycholic acid, lithocholic acid, estriol, estradiol, equilenin, equilin and their alkylate derivatives, as well as their ethynyl or propynyl derivatives in position 17, e.g., 17α-ethynyl-estradiol or 7α-methyl-17α-ethynyl-estradiol, pregnenolone, pregnanediol, testosterone and its derivatives, e.g., 17α-methyltestosterone, 1,2-dehydrotestosterone and 17α-methyl-1,2-dehydrotestosterone, the alkynylate derivatives in position 17 of testosterone and 1,2-dehydrotestosterone, e.g., 17α-ethynyltestosterone, 17α-propynyltestosterone, norgestrel, hydroxyprogesterone, corticosterone, deoxycorticosterone, 19-nortestosterone, 19-nor-17α-methyltestosterone and 19-nor-17α-ethynyltestosterone, antihormones, e.g., cyproterone, cortisone, hydrocortisone, prednisone, prednisolone, fluorocortisone, dexamethasone, betamethasone, paramethasone, flumethasone, fluocinolone, fluprednylidene, clobetasol, beclomethasone, aldosterone, deoxycorticosterone, alfazolone, alfadolone, bolasterone. As esterifying components for the preparation of esters of aspects of the present invention, the following are useful: genins (aglycons) of the cardioactive glucosides, e.g., digitoxigenin, gitoxigenin, digoxigenin, strophanthidin, tigogenin and saponins.

Other alcohols which may be used according to the process of aspects of this invention are the vitamins, e.g., axerophthol, vitamins D₂ and D₃, aneurine,
lactoflavine, ascorbic acid, riboflavin, thiamine, or pantothenic acid.

In the heterocyclic acids, the following can be considered as derivatives of the above-mentioned cycloaliphatic or aliphatic-cycloaliphatic alcohols if their linear or cyclic chains are interrupted by one or more heteroatoms, for example, by between one and three heteroatoms, for instance selected from the group consisting of -O-, -S-, -N-, -NH-. In these derivatives, there may be one or more unsaturated bonds, for example, double bonds, in particular between one and three, thus also including heterocyclic compounds with aromatic structures. For example, the following should be mentioned: furfuryl alcohol, alkaloids and derivatives thereof, e.g., atropine, scopolamine, cinchonine, la cinchonidine, quinine, morphine, codeine, nalorphine, N-butylscopolammonium bromide, and ajmaline; phenylethylamines, e.g., ephedrine, isoprotenerol, and epinephrine; phenothiazine drugs, e.g., perphenazine, pipothiazine, carphenazine, homofenazine, acetophenazine, fluophenazine, and N-hydroxyethylpromethazine chloride; thioxanthene drugs, e.g., flupenthixol and clopenthixol; anticonvulsants, e.g., meprophendiol; antipsychotics, e.g., opipramol; antiemetetics, e.g., oxypendyl; analgesics, e.g., carbetidine, phenoperidine and methadol; hypnotics, e.g., etodroxazine; anorexics, e.g., benidrol and diphemethoxidine; minor tranquilizers, e.g., hydroxyzine; muscle relaxants, e.g., cinnamedrine, diphyllyline, mephenesin,
methocarbamol, chlorphenesin, 2,2-diethyl-1,3-propanediol, quaifenesin, and hydrocileamide; coronary vasodilators, e.g., dipyridamole and oxyfedrine; adrenergic blockers, e.g., propanolol, timolol, pindolol, bupranolol, atenolol, metoprolol, and practolol; antineoplastics, e.g., 6-azauridine, cytarabine, and floxuridine; antibiotics, e.g., chloramphenicol, thiamphenicol, erythromycin, oleandomycin, and lincomycin; antivirals, e.g., idoxuridine; peripheral vasodilators, e.g., isonicotinyl alcohol; carbonic anhydrase inhibitors, e.g., sulocarbilate; anti-asthmatic and antiinflammatories, e.g., tiaramide; and sulfamidics, e.g., 2-p-sulfanilonoethanol.

As discussed above, in some cases hyaluronic acid esters may be of interest where the ester groups derive from two or more therapeutically-active hydroxylic substances, and naturally all possible variants may be obtained. Especially interesting are the substances in which two types of different ester groups deriving from drugs of a hydroxylic character are present and in which the remaining carboxyl groups are free, or are salified with metals or with one or various bases listed later, or possibly also bases which are themselves therapeutically-active, for example, with the same or similar activity as that of the esterifying component. In particular, it is possible to have hyaluronic esters deriving, on the one hand, from an antiinflammatory steroid, e.g., one of those mentioned previously, and on the other hand from a vitamin,
from an alkaloid or from an antibiotic, e.g., one of those mentioned previously.

The degree of esterification of hyaluronic acid with the above-mentioned alcohols depends firstly on the special properties to be obtained in the various fields of application, for example, a greater or lesser lipophilia or hydrophilia with respect to certain tissues, e.g., the skin.

Normally, a high degree of esterification up to total esterification of hyaluronic acid increases its lipophilic character and therefore lessens its solubility in water. For a therapeutic use of the new esters of aspects of this invention, for example, it is especially important to regulate the degree of esterification in order to ensure, despite a good and increased level of lipophilia compared to hyaluronic acid per se or its sodium salt, sufficient hydrosolubility, for example, a solubility of 10 mg/ml. Naturally, it is necessary to bear in mind the influence of the molecular size of the same esterifying component, which usually influences hydrosolubility in an inversely proportional manner. As has already been mentioned, the esterification of the carboxylic groups of hyaluronic acid may play various roles, which may be useful in different fields, for example, in medicine using the esters as therapeutic agents or in surgery using them as plastic articles. For use in therapy, it has already been mentioned that it is possible to consider the esterification of an alcohol which is itself
therapeutically-active, e.g., an antiinflammatory cortisteroid, with hyaluronic acid as a means to improve its therapeutic efficacy.

Regarding similar therapeutically-active alcohols, hyaluronic acid therefore acts as a particularly efficient vehicle which is preferably compatible with the biological environment. In the above list of alcohols which may be used for esterification according to the process of aspects of the present invention, there appear several of these pharmacologically-active alcohols and therefore the possible applications of the corresponding esters are apparent as the indications are the same as those for the free alcohols. Again, as has already been mentioned, in partial esters with therapeutically-active alcohols, it is possible to esterify part or all of the remaining carboxylic groups of the hyaluronic component with a pharmacologically-inert alcohol, e.g., the saturated lower aliphatic alcohols, e.g., ethyl or isopropyl alcohol.

A particularly interesting aspect of the process of another aspect of the present invention is the possibility of preparing more stable drugs than those available at present. It is possible therefore, on the one hand, to prepare esters of hyaluronic acid with therapeutically-inactive alcohols for use in typical indications of hyaluronic acid itself, e.g., for intra-articular injections, where the ester acts as a lubricant. Due to the improved stability of the esters relative to hyaluronidase as compared to the free acid, it is possible
to obtain quite a considerable prolonged action. On the other hand, it is possible to obtain drugs with a "retard" action for the above-mentioned esters of HY with therapeutically-active alcohols, possibly also salified with therapeutically-active bases. The liberation of the active alcohols due to esterase and that of the salified groups due to the hydrolytic action is very slow.

For cosmetic use, it is preferable to use total or partial esters of hyaluronic acid with pharmacologically-inert alcohols, e.g., saturated or unsaturated aliphatic alcohols, for example, non-substituted alcohols of this type with a straight or ramified chain, for example, with between 1 and 8 carbon atoms, e.g., those specifically mentioned previously. Particularly interesting are those unsaturated alcohols, e.g., with one or more double bonds, e.g., vinyl or allyl alcohols and their condensed derivatives, e.g., especially polyvinyl alcohols or polyvalent alcohols, e.g., glycerine. In this case also it is possible to use, according to the intended purpose, mixed esters.

Also useful are cycloaliphatic alcohols, e.g., derivatives of cyclopentane or cyclohexane and their derivatives which are substituted by lower alkyl groups, e.g., alkyls with between 1 and 4 carbon atoms, especially by methyl groups. Of particular interest also are esters with cycloaliphatic and aliphatic alcohols - cycloaliphatics derived from terpene, e.g., those mentioned above.
and from therapeutically-active alcohols, and which may also be used in cosmetics.

The alcohols which may be used preferably to make articles for sanitary and surgical use are essentially the same as those listed above for cosmetic use. In the partial esters of HY according to aspects of the present invention, the percentage of esterified groups may vary greatly in relation to the use for which the product is intended, and that is above all with regard to the use in the various fields of application mentioned above.

Of particular interest, however, are those partial esters of HY which at least 5% and at most 90% of all the carboxylic groups of HY are esterified, and especially those with an esterified percent of between 50 and 80%.

The ratio between the number of different types of ester groups may obviously also vary in the mixed partial esters. For example, in the case of two types of such groups, the ratio varies preferably between 0.1:1 and 1:01, and the same is true of total esters. For the esters intended for therapeutic use, the ratio varies preferably between 0.5:1 and 1:0.5. Such ratios are preferably also valid for total esters and, in the partial esters, they are to be taken preferably with reference to the percentages mentioned above regarding the inclusive number of esterified groups.

In the partial esters of HY of aspects of this invention, the non-esterified carboxylic groups may be kept free or may be salified. For the formation of such salts,
the bases are selected according to the criterion of these for which the product is intended. It is possible to form inorganic salts deriving from alkali metals, e.g., potassium and especially sodium, or from ammonium, or deriving from alkaline earth metals, e.g., calcium, or magnesium, or from aluminum salts.

Particularly interesting are the salts with organic bases, especially nitrogenized bases and therefore aliphatic, aylaliphatic, cycloaliphatic or heterocyclic amines.

These ammoniac salts may derive from therapeutically-acceptable but inactive amines or from amines with therapeutic action. Of the former, the aliphatic amines should be considered, e.g., mono-, di-, and tri-alkylamines with alkyl groups having a maximum of 18 carbon atoms or aylalkylamines with the same number of carbon atoms in the aliphatic part and where ayl means a benzene group, possibly substituted by 1 to 3 methyl groups or halogen atoms or hydroxyl groups. The biologically-inactive bases for the formation of salts may also be cyclic, e.g., monocyclic alkylenamines with rings of between 4 and 6 carbon atoms, possibly interrupted in the ring by heteroatoms selected from the group consisting of nitrogen, oxygen and sulfur, e.g., piperidine or morpholine, and may be substituted, for example, by aminic or hydroxylic functions, e.g., aminoethanol, ethylendiamine, ephedrine or choline.
It is also possible to form the quaternary ammonium salts of the partial esters of HY, for example, tetralkylammonium salts with the above-mentioned number of carbon atoms and preferably salts of such a type in which the fourth alkyl group has between 1 and 4 carbon atoms, for example, a methyl group.

Among the biologically-active amines whose therapeutic actions may be put to use, are included all the nitrogenized and basic drugs, e.g., those included in the following groups: alkaloids, peptides, phenothiazines, benzodiazepines, thioxanthenes, hormones, vitamins, anticonvulsants, antipsychotics, antiemetics, anaesthetics, hypnotics, anorexics, tranquilizers, muscle relaxants, coronary vasocilators, antineoplastics, antibiotics, antibacterials, antivirals, antimalarials, carbonic anhydrase inhibitors, non-steroid antiinflammatory agents, vasoconstrictors, cholinergic agonists, cholinergic antagonists, adrenergic agonists, adrenergic antagonists, and narcotic antagonists.

All those drugs with basic nitrogenized groups listed hereinabove and regarding the use of the esters may be quoted as examples.

According to a particular aspect of the present invention, the new hyaluronic esters and their salts may be used as an excellent vehicle for therapeutically-active substances. To this end, it is possible to use the total esters, or the partial esters, or the salified partial esters in the remaining carboxylic groups, for example,
with one of the above-mentioned substances therapeutically-acceptable but no biologically-active, above all with alkali metals, for example sodium. The above-mentioned medicaments may therefore be made by two associated components, namely:

Component (1) - a pharmacologically-active substance or an association of two or more active substances; and

Component (2) - a carrying vehicle comprising a partial or total ester of hyaluronic acid of aspects of the present invention with an alcohol, or the salts of such partial esters with an organic base or with an organic base, optionally with the addition of hyaluronic acid or a salt thereof with an inorganic base or with an organic base.

The hyaluronic esters which may be used in these medicaments are those in which the esterifying alcohol is not itself pharmacologically-active, for example, a simple aliphatic alcohol, as described above. Medicaments of this type in which the ester also is pharmacologically-active are not excluded from this aspect of the invention, as, for example, in the case of one of the esters described above deriving from alcohols with pharmacological action.

In the same way, the invention in another of its aspects also includes medicaments of this type in which the esters of Component (2) are also salified with therapeutically-active substances vehicled in the hyaluronic ester, and the mixture in this case, as described below, therefore contains salts of a partial ester of hyaluronic
acid with therapeutically-active bases, possibly in the presence of an excess of active base Component (1). The vehicled substance may not be of a basic nature, and free carboxylic groups in the hyaluronic ester may still be salified with therapeutically-active bases.

The use of hyaluronic esters as a vehicle therefore allows the preparation of the new medicaments of further aspects of this invention described above, including (1) a pharmacologically-active substance or an association of two or more of such substances, and (2) a hyaluronic ester as described above or one of its salts. In such medicaments, if partial esters of HY are used, the possible salification of the remaining carboxylic groups is preferably carried out with therapeutically-neutral inorganic bases or organic bases, especially with alkali metals e.g., sodium, or with ammonium. Should the active substance component (1) or a corresponding association of substances have basic groups, e.g., for example, antibiotics containing amine groups, and if partial esters of hyaluronic acid should be used with remaining free carboxylic groups, the corresponding salts are formed between the carboxylic groups and these basic substances. The new medicaments therefore include, in particular, partial esters of hyaluronic acid partially or totally salified with pharmacologically-active substances and of a basic character. As described above, particularly important are the associated medicaments of the type described here, in which Component (1) is a pharmacologically-active substance for topical use.
The use of hyaluronic esters as a vehicle for drugs to be applied topically is particularly useful in ophthalmology where a particular compatibility is to be observed for the new products with the corneal epithelium, and therefore excellent tolerability, without any sensitization effects. Furthermore, when the medicaments are administered in the form of concentrated solutions with elastic-viscous characteristics or in solid form, it is possible to achieve homogeneous and stable films which are preferably transparent and adhesive on the corneal epithelium, guaranteeing prolonged bioavailability of the drug and therefore representing excellent preparations with a retard effect.

Such ophthalmic medicaments are particularly valuable in the veterinary field, considering, for example, that at present there are no veterinary specialities for oculistic use containing chemotherapeutic agents. Indeed, preparations intended for human use are usually used, and these to not always guarantee a specific range of action if they do not make allowances for the particular conditions in which the treatment must take place. This, for example, is the case in therapy for infective keratoconjunctivitis, pink eye or IBK, an infection which usually affects cattle, sheep and goats. Presumably for these three species, there are specific etiological factors and more particularly: in cattle, the main microorganism involved seems to be Moraxella bovis (even though other agents of a viral origin should not be excluded, e.g., for example Rinotracheitis
virus); in sheep, Micoplasma, Rickettsiae and Clamidia; and in goats, Rickettsiae. The disease manifests itself in acute form and tends to spread rapidly: in the initial stages the symptomatology is characterized by blepharospasm and excessive lachrymation, followed by purulent exudate, conjunctivitis and keratitis, often accompanied by fever, loss of appetite and milk production. Particularly serious are the corneal lesions which in the final stages may even cause perforation of the cornea itself. The clinical progress of the disease varies from a few days to several weeks.

A vast selection of chemotherapeutic agents are used for treatment, administered both topically (often in association with steroid anti-inflammatory agents), and systemically, including: tetracyclines, e.g., oxytetracycline, penicillins, e.g., cloxacillin and benzylpenicillin, sulfonamides, polymyxin B (associated with miconazole and prednisolone), chloramphenicol, tylosin and chloromycetin. Topical treatment of the disease, despite its apparent simplicity, is still an unsolved problem, since with the oculistic preparations used so far, it has not been possible for one reason or another, to obtain therapeutically-efficient concentrations of antibiotics of sulphamides in the lachrymal secretion. This is quite understandable in the case of solutions, considering the mainly inclined position of the head in these animals, but the same is also true of semisolid medicaments, as the commonly used excipients do not possess the necessary
qualities of adhesiveness to the corneal surface, since they do not usually have a high enough concentration of active substance and cannot achieve optimum distribution of the same (i.e., there is a presence of a distribution gradient). These defects of conventional colliriums in ophthalmic use have, for example, been described by Slatter, et al., Austr. Vet. J., 1982, 59 (3), pp. 69 - 72.

With the esters of HY aspects of the present invention, these difficulties can be overcome. The presence of the hyaluronic acid ester as a vehicle for ophthalmic drugs in fact allows the formulation of excellent preparations with no concentration gradients of the active substance and they are therefore homogeneous, with transparency and excellent adhesiveness to the corneal epithelium, with no sensitization effects, with excellent vehicling of the active substance and possibly a retard effect.

The above-mentioned properties of the new medicaments of aspects of this invention may, of course, also be exploited in fields other than ophthalmology. They may be used in dermatology and in diseases of the mucous membranes, for example, in the mouth. Furthermore, they may be used to obtain a systemic effect due to the effect of transcutaneous absorption, e.g., in suppositories. All these applications are possible both in human and veterinary medicine. In human medicine, the new medicaments are particularly suitable for use in
pediatrics. The present invention therefore teaches any of these therapeutic applications.

For the sake of brevity, from now on when the active substance of Component (1) according to aspects of this invention is mentioned, it is to be understood to also include the association of one or more active substances.

The Component (1) described above may be defined in regard to its use in the various fields of therapy, starting with the distinction between human and veterinary medicine, and then specifying the various sectors of application with regard to the organs or tissues to be treated, e.g., with reference to topical use, ophthalmology, dermatology, otorhinolaryngology, gynaecology, angiology, neurology or any type of pathology of internal organs which may be treated with topical applications, for example, with rectal applications.

The vechicling action of the hyaluronic esters also applies to associated medicaments of the type mentioned above in which the active substance acts not only topically or by nasal or rectal absorption, for example, by nasal sprays or preparations for inhalation for the oral cavity or the pharynx, but also by oral or parenteral route, for example, by intramuscular, subcutaneous or intravenous route, as it favours absorption of the drug into the application site. The new medicaments can therefore be applied, apart from in the fields already mentioned, in practically all sectors of medicine, e.g., internal medicine, for example in pathologies of the cardiovascular
system, in infections of the respiratory system, the
digestive system, the renal system, in diseases of an
derocrinological nature, in oncology, and in psychiatry,
etc. They may also be classified therefore from the point
of view of their specific action, being perhaps
anaesthetics, analgesics, antiinflammatories, wound
healers, antimicrobics, adrenergic agonists and
antagonists, cytostatics, antirheumatics, antihypertensive,
diuretics, sexual hormones, immunostimulants or
immunosuppressants, for example, one of the drugs having
the activity already described for the therapeutically-
active alcohols which may be used as an esterifying
component according to an aspect of the present invention,
or for the therapeutically-active bases which may be used
for the salification of the free carboxyclic groups.

Component (1) of the above-mentioned medicaments may
also be, according to other aspects of this invention, an
association of two or more active substances, as contained
in many known medicaments.

Regarding the field of ophthalmology, the indications
may be, for example: the miotic, antiinflammatory, wound
healing and antimicrobial effects.

Examples of pharmacologically-active substances which
may be used in ophthalmic medicaments according to aspects
of the present invention are: basic and non-basic
antibiotics, e.g., aminoglycosides, macrolides,
tetracyclines and peptides, e.g., gentamycin, neomycin,
streptomycin, dihydrostreptomycin, kanamycin, amikacin,
tobramycin, spectinomycin, erythromycin, oleandomycin, carbomycin, spiramycin, oxytetracycline, rolitetracycline, bacitracin, polymyxin B, gramicidin, colistin, chloramphenicol, lincomycin, vancomycin, novobiocin, ristocetin, clindamycin, amphotericin B, griseofulvin, nystatin, and possibly their salts, e.g., sulfate or nitrate, or associations of the same between themselves or with other active ingredients, e.g., those mentioned below.

Other ophthalmic drugs which may be used to advantage accordingly to aspects of the present invention are: other antiinfectives, e.g., diethylcarbamazine, or mebendazole; sulfamidics, e.g., sulfacetamide, sulfadiazone, or sulfisoxazole; antivirals and antitumorals, e.g., iododeoxyuridine, adenine arabinoside, trifluorothymidine, acyclovir, ethyldeoxyuridine, bromovinyldeoxyuridine, or 5-iodo-5′-amino-2′,5-dideoxy-uridine; steroid antiinflammatories, e.g., dexamethosone, hydrocortisone, prednisolone, fluorometholone, or medrysone and possibly their esters, for example, phosphoric acid; non-steroid antiinflammatories, e.g., indomethacin, oxyphenbutazone, or flurbiprofen; wound healers, e.g., epidermal growth factor, EGF; local anaesthetics, e.g., benoxinate, or proparacaine and possibly their salts; cholinergic agonists, e.g., pilocarpine, methylcholine, carbomylcholine, aceclidine, physostigmine, neostigmine, or demecarium and possibly their salts; cholinergic antagonist drugs, e.g., atropine and their salts; adrenergic agonist drugs, e.g., noradrenaline, adrenaline, naphazoline, or methoxamine and
possibly their salts; adrenergic antagonist drugs, e.g., propanolol, timolol, pindolol, bupranolol, atenolol, metoprolol, oxprenolol, practolol, butaoxamine, sotalol, butathrin, or labetolol and possibly their salts.

Examples of the active substances which may be used alone or in association among themselves or with other active principles in dermatology are: therapeutic agents, e.g., antiinfective agents, antibiotics, antimicrobials, antiinflammatory, cytostatic, cytotoxic, antiviral, anaesthetic agents, and propylactic agents, e.g., sun screens, deodorants, antiseptics and disinfectants. Of the antibiotics, particularly important are: erythromycin, bacitracin, gentamicin, neomycin, aureomycin, gramicidin and their associations, of the antibacterials and disinfectants: nitrofurazone, mafenide, chlorhexidine, and derivatives of 81-hydroxyquinoline and possibly their salts; of the anti-inflammatory agents, above all, the corticosteroids, e.g., prednisolone, dexamethazone, flumethasone, clobetasol, triamcinolone acetonide, or betamethasone and their esters, e.g., valerates, benzoated, or dipropionates; of the cytotoxic group: fluorouracil, or methotrexate, or the anaesthetics dibucaine, lidocain, or benzocaine.

This list, of course, only gives some examples and any other agents described in the literature may be used.

As associations of drugs to be used in dermatology, the various antibiotics should be mentioned, e.g., erythromycin, gentamycin, neomycin, gramicidin, polymyxin
B, among themselves, or associations of these antibiotics with anti-inflammatory agents, e.g., corticosteroids, for example, hydrocortisone + neomycin, hydrocortisone + neomycin + polymyxin B + gramicidin, dexamethasone + neomycin, fluorometholone + neomycin, prednisolone + neomycin, triamcinolone + neomycin + gramicidin + nystatin, or any other association used in conventional preparations or dermatology.

The association of various active substances are not of course limited to this field, but in each of the above-mentioned sectors of medicine it is possible use associations similar to those already in use for the known pharmaceutical preparations of the art.

In the above case of the use of a Component (1) of a basic character, the salts which are formed with a partial hyaluronic ester (since the latter is used to excess) may be of various types, that is, all the remaining carboxylic groups may be salified or only an aliquot part, thereby producing esters - acid salts, or esters - neutral salts. The number of acid groups which are to be kept free may be of importance for the preparation of medicaments with a particular pH. Vice versa, it is possible to use an excess of basic Component (1), in which case all the carboxylic groups available in the hyaluronic ester are salified with the base.

According to a particular aspect of the invention, it is possible to prepare the medicaments of this type starting from previously isolated and possibly purified
salts, in their solid anhydrous state, as amorphous powders, which form an aqueous solution on contact with the tissue to be treated, characterized by viscosity and elastic properties. These qualities are maintained even at stronger dilutions and it is possible therefore to use, in place of the above-mentioned anhydrous salts, more or less concentrated solutions in water or saline, possibly with the addition of other excipients or additives, e.g., other mineral salts to regulate the pH and osmotic pressure. It is of course possible to use the salts also for the preparation of gels, inserts, creams or ointments, also containing other excipients or ingredients used in traditional formulations of these pharmacological preparations.

According to a major aspect of the invention, however, the medicaments containing the hyaluronic ester or their salts with therapeutically-active or inactive substances as a vehicle are used alone (except possibly with an aqueous solvent). Also included in according to other aspects of this invention are the mixtures obtainable from all the types of medicaments described here, mixtures of the same medicaments, and also possibly mixtures of the new hyaluronic esters with free hyaluronic acid or mixtures of their salts, for example, sodium salts.

Component (1) in the composition according to an aspect of this invention may also be associations or mixtures of two or more such drugs and possibly also with other agents. For example, in ophthalmology, a drug may be
associated with an antibiotic or antiphlogistic substance and a vasoconstrictor or with several antibiotics, one or more antiphlogistic substances, or with one or more antibiotics, a mydriatic or a miotic or wound healing or antiallergic agent, etc. For example, the following associations of ophthalmic drugs may be used: kanamycin + phenylephrine + dexamethasone phosphate; kanamycin + betamethasone phosphate + phenylephrine; or similar associations with other antibiotics used in ophthalmology, e.g., rolitetracycline, neomycin, gentamicin, or tetracycline.

If in the place of just one active substance Component (1), associations of active substances are used, e.g., those mentioned above, the salts of the basic active substances and the partial ester of hyaluronic acid may be mixed salts of one or more of such basic substances or possibly mixed salts of this type with a certain number of other acid groups of the polysaccharides salified with metals or bases mentioned above. For example, it is possible to prepare salts of a partial ester of hyaluronic acid or of one of the molecular fractions HYALASTINE™ or HYALECTIN™ with a pharmacologically-inactive alcohol, for example, a lower alkanol and with a certain percentage of salified acid groups with the antibiotic kanamycin, another percentage of carboxylic groups salified with the vasoconstrictor phenylephrine, and a remaining percentage of acid groups may be, for example, free of salified with sodium or one of the other above-mentioned metals. It is
also possible to mix this type of mixed salt with free hyaluronic acid or its fractions or their metallic salts, as indicated above for the medicaments containing salts of one single active substance with the aforementioned polysaccharide esters.

From the examples discussed for ophthalmology and dermatology, it is possible to understand by analogy which medicaments according to aspects of the present invention are to be used in the above-mentioned fields of medicine, e.g., for example, in otorhinolaryngology, odontology or in internal medicine, for example, in endocrinology. Such preparations may, therefore be, for example, antiinflammatories, vasoconstrictors, or vasocompressors, e.g., those already mentioned for ophthalmology, vitamins, antibiotics, e.g., those mentioned above, hormones, chemotherapics, antibacterials, etc., also as mentioned above for use in dermatology.

The associated medicaments of a hyaluronic ester with a pharmacologically active substance may contain other pharmaceutical vehicles, e.g., those mentioned below for the pharmaceutical preparations containing only hyaluronic esters, and may appear in the form of ointments, creams, pastilles, gelatine capsules, capsules, aqueous or oily solutions, sprays, suppositories, etc. However, according to a particular aspect of the present invention, it is preferable to use medicaments containing an association of Components (1) and (2), with Component (2) as the sole
vehicle (apart from a possible solvent, e.g., an aqueous solvent).

Of the medicaments of aspects of this invention, the following are of particular importance, according to each case, those with a degree of acidity suitable for the environment to which they are to be applied, that is with a physiologically tolerable pH. The adjustment of the pH, for example, in the above-mentioned salts of the partial ester of hyaluronic acid with a basic active substance, may be done by suitably regulating the quantities of polysaccharide, or of its salts or of the basic substance itself. Thus, for example, if the acidity of a salt of the partial ester of hyaluronic acid with a basic substance is too high, the excess of free acid groups can be neutralized with the above-mentioned inorganic bases, for example, with the hydrate of sodium, or of potassium, or of ammonia.

Of the new products of aspects of the present invention, of particular importance are the esters of HY and their salts described above and those described in the following illustrative Examples, which illustrate the invention.

Preparation Examples

The following Examples A - C describe some procedures for preparing the preferred hyaluronic acids fractions utilized in the preparation of the ester of hyaluronic acid of various aspects of the present invention.
Example A - Method for Obtaining a Mixture of HYALASTINE<sub>TM</sub> and HYALECTIN<sub>TM</sub> Fractions Having No Inflammatory Activity.

Fresh or frozen cocks' combs, (3000 g) are minced in a meat mincer and then carefully homogenized in a mechanical homogenizer. The paste thus obtained is placed in a stainless steel container AISI 316 or in glass and treated with 10 volumes of anhydrous acetone. The whole is agitated for 6 hours at a speed of 50 rpm. It is left to separate for 12 hours and the acetone is discarded by syphoning. The acetone extraction is repeated until the discarded acetone has reached the correct degree of humidity (Karl-Fischer method). The whole is then centrifuged and vacuum dried at a suitable temperature for 5 - 8 hours. In this way, 500 - 600 g of dry powdered cocks' combs are obtained.

300 gr. of dry powder are exposed to enzymatic digestion with papain (0.2 g) in aqueous conditions, buffered with phosphate buffer in the presence of a suitable quantity of hydrochloride cysteine. The resultant is agitated for 24 hours at 60 rpm keeping the temperature constant at 60 - 65°C. It is then cooled at 25°C and a diatomaceous earth filter aid known by the trade-mark CELITE<sub>TM</sub> of Johns Manville Product Corporation (60 gr) is added maintaining the agitation for another hour. The resulting mixture is filtered until a clear liquid is obtained. The clear liquid then undergoes molecular ultrafiltration using membranes with a molecular exclusion.
limit of 30,000 in order to retain on the membrane those molecules with a molecular weight greater than 30,000.

The product is ultrafiltered from 5 to 6 original volumes adding distilled water continually to the product in ultrafiltration. The addition of water is suspended and the ultrafiltration is continued until the volume is reduced to 1/3 of the original volume.

The residue liquid is rendered 0.1M by the addition of sodium chloride and the temperature is brought to 50°C. Under agitation at 60 rpm, 45 g of cetylpyridinium chloride are added. It is agitated for 60 minutes and then 50 g of CELITE™ are added. Under agitation, the temperature of the whole is brought to 25°C and the precipitate formed by centrifugation is gathered. The precipitate obtained is suspended in a 0.01M solution in sodium chloride (5 litres) containing 0.05% of cetylpiridinium chloride. The resulting suspension is agitated for 60 minutes at 50°C; the temperature is then brought to 25°C and the precipitate is centrifuged. The washing operation is repeated 3 times after which the precipitate is gathered in a receptacle containing 3 litres of a 0.05M solution of sodium chloride containing 0.05% of cetylpyridinium chloride. It is agitated at 60 rpm for 60 minutes and the temperature is kept constant at 25°C for two hours. The supernatant is eliminated by centrifugation. The procedure is repeated several times with solutions of 0.1M sodium chloride containing 0.05% of cetylpyridinium chloride. The mixture is centrifuged and the supernatant is discarded. The
precipitate is dispersed in a solution of 0.30M sodium chloride containing 0.05% of cetylpyridinium chloride (3 litres). The mixture is agitated and both the precipitate and the clear liquid are gathered. Extraction is repeated three more times on the precipitate, each time using 0.5 litre of the same aqueous solution.

Finally the precipitate residue is eliminated and the clear liquids are all placed together in a single container. The temperature of the liquid is brought to 50°C under constant agitation. The liquid is then brought to 0.23M with sodium chloride. 1 gr of cetylpyridinium chloride is added, and it is maintained in agitation for 12 hours.

The mixture is cooled at 25°C and then filtered first on CELITE™ pack and then through a filter. It then undergoes molecular ultrafiltration again, on a membrane with a molecular exclusion limit of 30,000 ultrafiltering three initial volumes with the addition of a solution of 0.33M sodium chloride. The addition of sodium chloride solution is interrupted and the volume is reduced to 1/4 of the initial volume. The solution thus concentrated is precipitated under agitation (60 rpm) at 25°C with 3 volumes of ethanol (95%). The precipitate is gathered by centrifugation and the supernatant is discarded. The precipitate is dissolved in 1 L of 0.01M solution in sodium chloride and the precipitation is repeated with 3 volumes of ethanol 95%.
The precipitate is gathered and washed first with 75% ethanol (3 times), then with absolute ethanol (3 times), and lastly with absolute acetone (3 times).

The product thus obtained (HYALASTINE\textsuperscript{TM} + HYALECTIN\textsuperscript{TM} fractions) has an average molecular weight of between 250,000 and 350,000.

The yield of HY is 0.6% of the original fresh tissue.

Example B - Method for Obtaining the Fraction HYALATINE\textsuperscript{TM} from the Mixture Obtained by the Method Described in Example A.

The mixture obtained by the method described in Example A is dissolved in twice distilled apyrogenic water at the rate of 10 mg of product to each 1 ml of water. The solution obtained is exposed to molecular filtration through filter membranes with a molecular exclusion limit of 200,000, following a concentration technique on the membrane without the addition of water. During the ultrafiltration process through membranes with a molecular exclusion limit of 2000,000, the molecules with a molecular weight of more than 200,000 do not pass through, while the smaller molecules pass through the membrane together with the water. During the filtration procedure no water is added, so that the volume decreases, and there is therefore an increase in the concentration of molecules with a molecular weight of more than 200,000. The product is ultrafiltered until the volume on top of the membrane is reduced to 10% of the initial volume. Two volumes of apyrogenic twice distilled water are added and
it is then ultrafiltered again until the volume is reduced to 1/3. The operation is repeated twice more. The solution passed through the membrane is brought to 0.1M with sodium chloride and then precipitated with 4 volumes of ethanol at 95%. The precipitate is washed 3 times with ethanol at 75% and then vacuum dried.

The product thus obtained (HYALASTINE\textsuperscript{TM} fraction) has an average molecular weight of between 50,000 and 100,000. The yield of Hy is equal to 0.4% of the original fresh tissue.

Example C - Method of Obtaining the Fraction HYALECTIN\textsuperscript{TM}.

The concentrated solution gathered in the container on top of the ultrafiltration membrane with a molecular exclusion of 200,000 as in Example B, is diluted with water until a solution containing 5 mg/ml of hyaluronic acid is obtained, as determined by quantitative analysis based on the dosage of glucuronic acid.

The solution is brought to 0.1M in sodium chloride and then precipitated with 4 volumes of ethanol at 95%. The precipitate is washed 3 times with ethanol at 75% and then vacuum dried.

The product thus obtained (HYALECTIN\textsuperscript{TM} fraction) has an average molecular weight of between 500,000 and 730,000. This corresponds to a specific fraction of hyaluronic acid with a defined length of molecular chain of about 2,500 to 3,500 saccharide units with a high degree of purity. The yield of HY is equal to 0.2% of the original fresh tissue.
Example D - Preparation of the Salt of Tetrabutylammonium of Hyaluronic Acid (HY).

4.02 g of HY sodium salt (10 m.Eq.) are solubilized in 400 ml of distilled H₂O. The solution is then eluted in a thermostatic column at 4°C containing 15 ml of sulphonic resin (known by the trade-mark DOWEX<sub>TM</sub> 50 x 8 of The Dow Chemical Company) in tetrabutylammonium form. The eluate, free from sodium, is instantly frozen and freeze-dried. Yield: 6.18 g.

Example 1 - Preparation of the (Partial) Propyl Ester of Hyaluronic Acid (HY).

- 50% of the esterified carboxylic groups
- 50% of the salified carboxylic groups (Na)

12.4 g of HY tetrabutylammonium salt with a molecular weight 170,000 corresponding to 20 m.Eq. of a monomeric unit are solubilized in 620 ml of dimethylsulfoxide at 25°C, 1.8 g (10.6 m.Eq.) of propyl iodide are added and the resulting solution is kept at a temperature of 30°C for 12 hours.

A solution containing 62 ml of water and 9 g of sodium chloride is added and the resulting mixture is slowly poured into 3,500 ml of acetone under constant agitation. A precipitate is formed which is filtered and washed three times with 500 ml of acetone/water 5:1 and three times with acetone and finally vacuum dried for eight hours at 30°C.

The product is then dissolved in 550 ml of water containing 1% of sodium chloride and the solution is slowly poured into 3,000 ml of acetone under constant agitation.
A precipitate is formed which is filtered and washed twice with 500 ml of acetone/water (5:1) and three times with 500 ml of acetone and finally vacuum dried for 24 hours at 30°C. 7.9 g of the partial propyl esters compound in the title are obtained. Quantitative determination of the ester groups is carried out using the method of R.H. Cundiff and P.C. Markunas [Anal. Chem. 33, 1028 - 1030, (1961)].

Example 2 - Preparation of the (Partial) Isopropyl Ester of Hyaluronic Acid (HY) - 50% of Esterified Carboxylic Groups - 50% of Salified Carboxylic Groups (Na).

12.4 g of HY tetrabutylammonium salt with a molecular weight of 160,000 corresponding to 20 m.Eq. of a monomeric unit are solubilized in 620 ml of dimethylsulfoxide at 25°C, 1.8 g (10.6 m.Eq.) of isopropyl iodide are added and the resulting solution is kept for 12 hours at 30°C.

A solution containing 62 ml of water and 9 g of sodium chloride is added and the resulting mixture is slowly poured into 3,500 ml of acetone under constant agitation. A precipitate is formed which is filtered and washed three times with 500 ml of acetone/water 5:1 and three times with acetone and finally vacuum dried for eight hours at 30°C.

The product is then dissolved in 550 ml of water containing 1% of sodium chloride and the solution is slowly poured into 3,000 ml of acetone under constant agitation. A precipitate is formed which is filtered and washed twice with 500 ml of acetone/water 5:1 and three times with 500 ml of acetone and finally vacuum dried for 24 hours at
30°C. 7.9 g of the partial isopropyl ester compound in the
title are obtained. Quantitative determination of the
ester groups is carried out using the method of R.H.
Cundiff and P.C. Markunas [Anal. Chem. 33, 1028 - 1030,
(1961).

Example 3 - Preparation of the (Partial) Ethyl Ester of
Hyaluronic Acid (HY) - 75% of Esterified Carboxylic
Groups - 25% of Salified Carboxylic Groups (Na).

12.4 g of HY tetrabutylammonium salt with a molecular
weight of 250,000 corresponding to 20 m.Eq. of a monomeric
unit are solubilized in 620 ml of dimethylsulfoxide at
25°C. 2.5 g (15.9 m.Eq.) of ethyl iodide are added and the
resulting solution is kept for 12 hours at 30°C.

A solution containing 62 ml of water and 9 g of sodium
chloride is added and the resulting mixture is slowly
poured into 3,500 ml of acetone under constant agitation.
A precipitate is formed which is filtered and washed three
times with 500 ml of acetone/water 5:1 and three times with
acetone and finally vacuum dried for eight hours at 30°C.

The product is then dissolved in 550 ml of water
containing 1% of sodium chloride and the solution is slowly
poured into 3,000 ml of acetone under constant agitation.
A precipitate is formed which is filtered and washed twice
with 500 ml of acetone/water 5:1 and three times with 500
ml of acetone and finally vacuum dried for 24 hours at
30°C. 7.9 g of the partial ethyl ester compound in the
title are obtained. Quantitative determination of the
ester groups is carried out using the method of R.H.
Example 4 - Preparation of the (Partial) Methyl Ester of Hyaluronic Acid (HY) - 75\% of Esterified Carboxylic Groups - 25\% of Salified Carboxylic Groups (Na).

12.4 of HY tetrabutylammonium salt with a molecular weight of 80,000 corresponding to 20 m.Eq. of a monomeric unit are solubilized in 620 ml of dimethylsulfoxide at 25°C, 2.26 g (15.9 m.Eq.) of methyl iodide are added and the resulting solution is kept for 12 hours at 30°C.

A solution containing 62 ml of water and 9 g of sodium chloride is added and the resulting mixture is slowly poured into 3,500 ml of acetone under constant agitation. A precipitate is formed which is filtered and washed three times with 500 ml of acetone/water 5:1 and three times with acetone and finally vacuum dried for eight hours at 30°C.

The product is then dissolved in 550 ml of water containing 1\% of sodium chloride and the solution is slowly poured into 3,000 ml of acetone under constant agitation. A precipitate is formed which is filtered and washed twice with 500 ml of acetone/water 5:1 and three times with 500 ml of acetone and finally vacuum dried for 24 hours at 30°C. 7.9 g of the partial methyl ester compound in the title are obtained. Quantitative determination of the ester groups is carried out using the method of R.H. Cundiff and P.C. Markunas [Anal. Chem. 33, 1028 - 1030, (1961)].
Example 5 - Preparation of the Methyl Ester of Hyaluronic Acid (HY).

12.4 g of HY tetrabutylammonium salt with a molecular weight of 120,000 corresponding to 20 m.Eq. of a monomeric unit are solubilized in 620 ml of dimethylsulfoxide at 25°C, 3 g (21.2 m.Eq.) of methyl iodide are added and the solution is kept for 12 hours at 30°C.

The resulting mixture is slowly poured into 3,500 ml of ethyl acetate under constant agitation. A precipitate is formed which is filtered and washed four times with 500 ml of ethyl acetate and finally vacuum dried for twenty-four hours at 30°C.

8 g of the ethyl ester product in the title are obtained. Quantitative determination of the ester groups is carried out using the method of R.H. Cundiff and P.C. Markunas [Anal. Chem. 33, 1028 - 1030, (1961)].

Example 6 - Preparation of the Ethyl Ester of Hyaluronic Acid (HY).

12.4 g of HY tetrabutylammonium salt with a molecular weight of 85,000 corresponding to 20 m.Eq. of a monomeric unit are solubilized in 620 ml of dimethylsulfoxide at 25°C, 3.3 g (21.2 m.Eq.) of ethyl iodide are added and the solution is kept for 12 hours at 30°C.

The resulting mixture is slowly poured into 3,500 ml of ethyl acetate under constant agitation. A precipitate is formed which is filtered and washed four times with 500 ml of ethyl acetate and finally vacuum dried for twenty-four hours at 30°C.
8 g of the ethyl ester product in the title are obtained. Quantitative determination of the ester groups is carried out using the method of R.H. Cundiff and P.C. Markunas [Anal. Chem. 33, 1028 - 1030, (1961)].

Example 7 - Preparation of the Propyl Ester of Hyaluronic Acid (HY).

12.4 g of HY tetrabutylammonium salt with a molecular weight of 170,000 corresponding to 20 m.Eq. of a monomeric unit are solubilized in 620 ml of dimethylsulfoxide at 25°C, 3.6 g (21.2 m.Eq.) of propyl iodide are added and the solution is kept for 12 hours at 30°C.

The resulting mixture is slowly poured into 3,500 ml of ethyl acetate under constant agitation. A precipitate is formed which is filtered and washed four times with 500 ml of ethyl acetate and finally vacuum dried for twenty-four hours at 30°C.

8.3 g of the propyl ester product in the title are obtained. Quantitative determination of the ester groups is carried out using the method of R.H. Cundiff and P.C. Markunas [Anal. Chem. 33, 1028 - 1030, (1961)].

Example 8 - Preparation of the (Partial) Butyl Ester of Hyaluronic Acid (HY) - 50% of Esterified Carboxylic Groups - 50% of Salified Carboxylic Groups (Na).

12.4 g of HY tetrabutylammonium salt with a molecular weight of 620,000 corresponding to 20 m.Eq. of a monomeric unit are solubilized in 620 ml of dimethylsulfoxide at 25°C, 1.95 g (10.6 m.Eq.) of n-butyl iodide are added and the resulting solution is kept for 12 hours at 30°C.
A solution containing 62 ml of water and 9 g of sodium chloride is added and the resulting mixture is slowly poured into 3,500 ml of acetone under constant agitation. A precipitate is formed which is filtered and washed three times with 500 ml of acetone/water 5:1 and three times with acetone and finally vacuum dried for eight hours at 30°C.

The product is then dissolved in 550 ml of water containing 1% of sodium chloride and the solution is slowly poured into 3,000 ml of acetone under constant agitation. A precipitate is formed which is filtered and washed twice with 500 ml of acetone/water 5:1 and three times with 500 ml of acetone and finally vacuum dried for 24 hours at 30°C. 8 g of the partial butyl ester compound in the title are obtained. Quantitative determination of the ester groups is carried out using the method of R.H. Cundiff and P.C. Markunas [Anal. Chem. 33, 1028 - 1030, (1961)].

Example 9 - Preparation of the (Partial) Ethoxycarbonylmethyl Ester of Hyaluronic Acid (HY) - 75% of Esterified Carboxylic Groups - 25% of Salified Carboxylic Groups (Na).

12.4 g of HY tetrabutylammonium salt with a molecular weight of 180,000 corresponding to 20 m.Eq. of a monomeric unit are solubilized in 620 ml of dimethylsulfoxide at 25°C, 2 g of tetrabutylammonium iodide and 1.84 g (15 m.Eq.) of ethyl chloroacetate are added and the resulting solution of kept for twenty-fours hours at 30°C.

A solution containing 62 ml of water and 9 g of sodium chloride is added and the resulting mixture is slowly poured into 3,500 ml of acetone under constant agitation.
A precipitate is formed which is filtered and washed three times with 500 ml of acetone/water 5:1 and three times with acetone and finally vacuum dried for eight hours at 30°C.

The product is then dissolved in 550 ml of water containing 1% of sodium chloride and the solution is slowly poured into 3,000 ml of acetone under constant agitation. A precipitate is formed which is filtered and washed twice with 500 ml of acetone/water 5:1 and three times with 500 ml diacetone and finally vacuum dried for 24 hours at 30°C. 10 g of the partial ethoxycarbonylmethyl ester compound in the title are obtained. Quantitative determination of the ester groups is carried out using the method of R.H. Cundiff and P.C. Markunas [Anal. Chem. 33, 1028 - 1030, (1961)].

Example 10 - Preparation of the (Partial) Cortisone Ester (C_{21}) of Hyaluronic Acid (HY) - 20% of Esterified Carboxylic Groups - 80% of Salified Carboxylic Groups (Na).

6.2 g of HY tetrabutylammonium salt with a molecular weight of 105,000 corresponding to 10 m.Eq. of a monomeric unit are solubilized in 310 ml of dimethylsulfoxide at 25°C, 0.850 g (2 m.Eq.) of 21-bromo-4-pregnene-17α-ol-3,11, 20-trione are added and the resulting solution is kept for 24 hours at 30°C.

A solution containing 100 ml of water and 5 g of sodium chloride is added and the resulting mixture is slowly poured into 2,000 ml of acetone under constant agitation. A precipitate is formed which is filtered and washed three times with 100 ml of acetone/water 5:1 and
three times with acetone and finally vacuum dried for eight hours at 30°C.

The product is then dissolved in 300 ml of water containing 1% of sodium chloride and the solution is slowly poured into 1,500 ml of acetone under constant agitation. A precipitate is formed which is filtered and washed twice with 100 ml of acetone/water 5:1 and three times with 100 ml of acetone and finally vacuum dried for 24 hours at 30°C. 4.5 g of the partial cortisone ester compound in the title are obtained. Quantitative determination of cortisone, after mild alkaline hydrolysis with a hydroalcoholic solution of Na₂CO₃ and extraction with chloroform, is carried out according to British Pharmacopea, 1980, p. 127.

Example 11 - Preparation of the (Partial) Hydrocortisone Ester C₂₁ of Hyaluronic Acid (HY) - 20% of Esterified Carboxylic Groups - 80% of Salified Carboxylic Groups (Na).

6.2 g of HY tetrabutylammonium salt with a molecular weight of 80,000 corresponding to 20 m.Eq. of a monomeric unit are solubilized in 310 ml of dimethylsulfoxide at 25°C, 0.850 g (2 m.Eq.) of 21-bromo-4-pregnene-11β,17α-diol-3,20-dione are added and the resulting solution is kept for 24 hours at 30°C.

A solution is then added containing 100 ml of water and 5 g of sodium chloride and the resulting mixture is slowly poured into 2,000 ml of acetone under constant agitation. A precipitate is formed which is filtered and washed three times with 100 ml of acetone/water 5:1 and
three times with acetone and finally vacuum dried for eight hours at 30°C.

The product is then dissolved in 300 ml of water containing 1% of sodium chloride and the solution is slowly poured into 1,500 ml of acetone under constant agitation. A precipitate is formed which is filtered and washed twice with 100 ml of acetone/water 5:1 and three times with 100 ml of acetone and finally vacuum dried for 24 hours at 30°C. 4.4 g of the partial hydrocortisone ester compound in the title are obtained. Quantitative determination of hydrocortisone, after mild alkaline hydrolysis with hydroalcoholic solution of Na₂CO₃ and extraction with chloroform, is carried out according to British Pharmacopea, 1980, p.224.

Example 12 - Preparation of the (Partial) Fluorocortisone Ester (C₂₁) of Hyaluronic Acid (HY) - 20% of Esterified Carboxylic Groups - 80% of Salified Carboxylic Groups (Na).

6.2 g of HY tetrabutylammonium salt with a molecular weight of 80,000 corresponding to 10 m.Eq. of a monomeric unit are solubilized in 310 ml of dimethylsulfoxide at 25°C, 0.89 g (2 m.Eq.) of 9-fluoro-21-bromo-4-pregnene-11β-17α-diol-3,20-dione are added and the resulting solution is kept for 12 hours at 30°C.

A solution is then added containing 62 ml of water and 5 g of sodium chloride and the resulting mixture is slowly poured into 2,000 ml of acetone under constant agitation. A precipitate is formed which is filtered and washed three
times with 100 ml of acetone/water 5:1 and three times with acetone and finally vacuum dried for eight hours at 30°C.

The product is then dissolved in 300 ml of water containing 1% of sodium chloride and the solution is slowly poured into 1,500 ml of acetone under constant agitation. A precipitate is formed which is filtered and washed twice with 100 ml of acetone/water 5:1 and three times with 100 ml of acetone and finally vacuum dried for 24 hours at 30°C. 4.6 g of the partial fluorocortisone compound in the title are obtained. Quantitative determination of hydrocortisone, after mild alkaline hydrolysis with hydroalcoholic solution of Na₂CO₃ and extraction with chloroform, is carried out according to British Pharmacopea, 1980, p.196.

Example 13 - Preparation of the (Partial) Deoxycorticosterone Ester (C₈) of Hyaluronic Acid (HY) - 20% of Esterified Carboxylic Groups - 80% of Salified Carboxylic Groups (Na).

6.21 g of HY tetrabutylammonium salt with a molecular weight of 105,000 corresponding to 10 m.Eq. of a monomeric unit are solubilized in 310 ml of dimethylsulfoxide at 25°C, 0.661 g (2 m.Eq.) of 21-bromo-4-pregnene-3,20-dione are added and the resulting solution is kept for 24 hours at 30°C.

A solution is then added containing 100 ml of water and 5 g of sodium chloride and the resulting mixture is slowly poured into 2,000 ml of acetone under constant agitation. A precipitate is formed which is filtered and
washed three times with 100 ml of acetone/water 5:1 and three times with acetone and finally vacuum dried for eight hours at 30°C.

The product is then dissolved in 300 ml of water containing 1% of sodium chloride and the solution is slowly poured into 1,500 ml of acetone under constant agitation. A precipitate is formed which is filtered and washed twice with 100 ml of acetone/water 5:1 and three times with 100 ml of acetone and finally vacuum dried for 24 hours at 30°C. 4.5 g of the partial desoxycorticosterone ester compound in the title are obtained. Quantitative determination of cortisone, after mild alkaline hydrolysis with a hydroalcoholic solution of Na₂CO₃ and extraction with chloroform, is carried out according to British Pharmacopea, 1980, p. 137.

Example 14 - Preparation of the (Mixed Ethanol and Cortisone Ester (C₂₁) of Hyaluronic Acid (HY) - 80% of the Carboxylic Groups Esterified With Ethanol - 20% of the Carboxylic Groups Esterified With Cortisone (C₂₁).

6.2 g of HY tetrabutylammonium salt with a molecular weight of 70,000 corresponding to 10 m.Eq. of a monomeric unit are solubilized in 310 ml of dimethylsulfoxide at 25°C, 1.25 g (8 m.Eq.) of ethyl iodide are added and the resulting solution is kept for 12 hours at 30°C.

0.85 g (2 m.Eq.) of 21-bromo-4-pregnene-17α-ol-3-11,20-trione are added and the solution is kept for 24 hours at 30°C.
A solution is then added containing 100 ml of water and 5 g of sodium chloride and the resulting mixture is slowly poured into 2,000 ml of acetone under constant agitation. A precipitate is formed which is filtered and washed three times with 100 ml of acetone/water 5:1 and three times with acetone and finally vacuum dried for eight hours at 30°C.

4.6 of the mixed ethanol and cortisone ester compound in the title are obtained. Quantitative determination of cortisone, after mild alkaline hydrolysis with a hydroalcoholic solution of Na₂CO₃ and extraction with chloroform, is carried out according to British Pharmacopea, 1980.

Quantitative determination of the ethoxyls is carried out according to R.H. Cundiff and P.C. Markunas [Anal. Chem. 33, 1028 - 1030, (1961)].

Example 15 - Preparation of the (Mixed) Ethanol and Hydrocortisone Ester (C₂₁) of Hyaluronic Acid (HY) - 80% of Carboxylic Groups Esterified With Ethanol - 20% of Carboxylic Groups Esterified With Hydrocortisone (LC₂₁).

6.2 g of HY tetrabutylammonium salt with a molecular weight of 125,000 corresponding to 10 ml m.Eq. of a monomeric unit are solubilized in 310 ml of dimethylsulfoxide at 25°C, 1.25 g (8 m.Eq.) of ethyl iodide are added and the solution is kept at 30°C for 12 hours.

0.85 g (2 m.Eq.) of 21-bromo-4-pregnene-11β,17α-diol-3,20-dione are added and the solution is kept for 24 hours at 30°C.
A solution is then added containing 100 ml of water and 5 g of sodium chloride and the resulting mixture is slowly poured into 2,000 ml of acetone under constant agitation. A precipitate is formed which is filtered and washed three times with 100 ml of acetone/water 5:1 and three times with acetone and finally vacuum dried for eight hours at 30°C.

4.6 of the mixed ethanol and hydrocortisone ester compound in the title are obtained. Quantitative determination of hydrocortisone, after mild alkaline hydrolysis with a hydroalcoholic solution of Na₂CO₃, and extraction with chloroform, is carried out according to British Pharmacopea, 1980.

Quantitative determination of the ethoxyls is carried out according to R.H. Cundiff and P.C. Markunas [Anal. Chem. 33, 1028 - 1030, (1961)].

Example 16 - Preparation of the (Mixed) Ethanol and Fluorocortisone Ester (C₇₈) of Hyaluronic Acid (HY) - 80% of Carboxylic Groups Esterified With Ethanol - 20% of Carboxylic Groups Esterified With Fluorocortisone (C₇₈).

6.2 g of HY tetrabutylammonium salt with a molecular weight of 70,000 corresponding to 10 m.Eq. of a monomeric unit are solubilized in 310 of dimethylsulfoxide at 25°C, 1.25 g (8 m.Eq.) of ethyl iodide are added and the solution is kept for 24 hours at 30°C.

0.89 g (2 m.Eq.) of 9α-fluoro-21-bromo-4-pregnene-11β,17α-diol-3,20-dione are added and the solution is kept for 24 hours at 30°C.
A solution is then added containing 100 ml of water and 5 g of sodium chloride and the resulting mixture is slowly poured into 2,000 ml of acetone under constant agitation. A precipitate is formed which is filtered and washed three times with 100 ml of acetone/water 5:1 and three times with acetone and finally vacuum dried for eight hours at 30°C.

4.6 of the mixed ethanol and fluorocortisone ester compound featured in the title are obtained. Quantitative determination of fluorocortisone, after mild alkaline hydrolysis with a hydroalcoholic solution of Na₂CO₃ and extraction with chloroform, is carried out according to British Pharmacopeia, 1980.

Quantitative determination of the ethoxyls is carried out according to R.H. Cundiff and P.C. Markunas [Anal. Chem. 33, 1028 - 1030, (1961)].

Example 17 - Preparation of the (Mixed) Ethanol and Deoxycorticosterone Ester (C₇) of Hyaluronic Acid (HY) - 80% of Carboxylic Groups Esterified With Ethanol - 20% of Carboxylic Groups Esterified With Deoxycorticosterone (C₇).

6.2 g of HY tetrabutylammonium salt with a molecular weight of 70,000 corresponding to 10 m.Eq. of a monomeric unit are solubilized in 310 ml of dimethylsulfoxide at 25°C, 1.25 g (8 m.Eq.) of ethyl iodide are added and the resulting solution is kept for 12 hours at 30°C.

0.661 g (2 m.Eq.) of 21-bromo-4-pregnene-3-20-dione are added and the solution is kept for 24 hours at 30°C. A solution is then added containing 100 ml of water and
5 g of sodium chloride and the resulting mixture is slowly poured into 2,000 ml of acetone under constant agitation. A precipitate is formed which is filtered and washed three times with 100 ml of acetone/water 5:1 and three times with acetone and finally vacuum dried for eight hours at 30°C.

4.6 of the mixed ethanol and desoxycorticosterone ester compound in the title are obtained. Quantitative determination of desoxycortisone, after mild alkaline hydrolysis with a hydroalcoholic solution of Na₂CO₃ and extraction with chloroform, is carried out according to British Pharmacopea, 1980.

Quantitative determination of the ethoxyls is carried out according to R.H. Cundiff and P.C. Markunas [Anal. Chem. 33, 1028 - 1030, (1961)].

Example 18 - Preparation of the (Partial and Mixed) Ethanol and Desoxycorticosterone Ester of Hyaluronic Acid (HY) - 40% of Carboxylic Groups Esterified With Desoxycorticosterone (C₂₁) - 40% of Salified Carboxylic Groups (Na).

6.2 g of HY tetrabutylammonium salt with a molecular weight of 125,000 corresponding to 10 m.Eq. of a monomeric unit are solubilized in 310 ml of dimethylsulfoxide at 25°C, 0.62 g (4 m.Eq.) of ethyl iodide are added and the solution is kept for 24 hours at 30°C.

0.85 g (2 m.Eq.) of 21-bromo-4-pregnene-3,20-dione are added and the solution is kept for 24 hours at 30°C. A solution is then added containing 100 ml of water and 5 g of sodium chloride and the resulting mixture is slowly poured into 2,000 ml of acetone under constant agitation.
A precipitate is formed which is filtered and washed three times with 100 ml of acetone/water 5:1 and three times with acetone and finally vacuum dried for eight hours at 30°C.

4.5 g of the partial and mixed ethanol and desoxycorticosterone ester compound in the title are obtained.

Quantitative determination of desoxycortisone, after mild alkaline hydrolysis with a hydroalcoholic solution of Na₂CO₃, and extraction with chloroform, is carried out according to British Pharmacopea, 1980.

Quantitative determination of the ethoxyls is carried out according to R.H. Cundiff and P.C. Markunas [Anal. Chem. 33, 1028 - 1030, (1961)].

**Example 19 - Preparation of the (Partial and Mixed) Ethanol and Cortisone Ester of Hyaluronic Acid (HY) - 40% of Carboxylic Groups Esterified With Ethanol - 20% of Carboxylic Groups Esterified With Cortisone (C₉) - 40% of Salified Carboxylic Groups (Na).**

6.2 g of HY tetrabutylammonium salt with a molecular weight of 125,000 corresponding to 10 m.Eq. of a monomeric unit are solubilized in 310 ml of dimethylsulfoxide at 25°C, 0.62 g (4 m.Eq.) of ethyl iodide are added and the solution is kept for 24 hours at 30°C.

0.85 g (2 m.Eq.) of 21-bromo-4-pregnene-17α-ol-3-11,20-trione are added and the solution is kept for 24 hours at 30°C.

A solution is then added containing 100 ml of water and 5 g of sodium chloride and the resulting mixture is slowly poured into 2,000 ml of acetone under constant
agitation. A precipitate is formed which is filtered and washed three times with 100 ml of acetone/water 5:1 and three times with acetone and finally vacuum dried for eight hours at 30°C.

4.5 g of the partial and mixed ethanol and cortisone compound in the title are obtained. Quantitative determination of cortisone, after mild alkaline hydrolysis with a hydroalcoholic solution of Na₂CO₃, and extraction with chloroform, is carried out according to British Pharmacopea, 1980.

Quantitative determination of the ethoxyls is carried out according to R.H. Cundiff and P.C. Markunas [Anal. Chem. 33, 1028 - 1030, (1961)].

Example 20 - Preparation of the (Partial and Mixed) Ethanol and Hydrocortisone Ester (LC₃) of Hyaluronic Acid (HY) - 40% of Carboxylic Groups Esterified With Ethanol - 20% of Carboxylic Groups Esterified With Hydrocortisone (C₃H) - 40% of Salified Carboxylic Groups (Na).

6.2 g of HY tetrabutylammonium salt with a molecular weight of 70,000 corresponding to 10 m.Eq. of a monomeric unit are solubilized in 310 ml of dimethylsulfoxide at 25°C, 0.62 g (4 m.Eq.) of ethyl iodide are added and the solution is kept for 24 hours at 30°C.

0.85 g (2 m.Eq.) of 21-bromo-4-pregnene-11B-17α-ol-3-11,20-trione are added and the solution is kept for 24 hours at 30°C.

A solution is then added containing 200 ml of water and 5 g of sodium chloride and the resulting mixture is
slowly poured into 2,000 ml of acetone under constant agitation. A precipitate is formed which is filtered and washed three times with 100 ml of acetone/water 5:1 and three times with 100 ml of acetone and finally vacuum dried for eight hours at 30°C.

4.5 g of the partial and mixed ethanol and hydrocortisone ester compound in the title are obtained. Quantitative determination of hydrocortisone, after mild alkaline hydrolysis with a hydroalcoholic solution of Na₂CO₃ and extraction with chloroform, is carried out according to British Pharmacopea, 1980.

Quantitative determination of the ethoxyls is carried out according to R.H. Cundiff and P.C. Markunas [Anal. Chem. 33, 1028 - 1030, (1961)].

**Example 21 - Preparation of the (Partial and Mixed) Ethanol and Fluorocortisone Esters (C₂₁) of Hyaluronic Acid (HY) - 40% of Carboxylic Groups Esterified With Ethanol - 20% of Carboxylic Groups Esterified With Fluorocortisone (C₂₁) - 40% of Salified Carboxylic Groups (Na).**

6.2 g of HY tetrabutylammonium salt with a molecular weight of 65,000 corresponding to 20 m.Eq. of a monomeric unit are solubilized in 310 ml of dimethylsulfoxide at 25°C, 0.62 g (4 m.Eq.) of ethyl iodide are added and the solution is kept for 24 hours at 30°C.

0.89 g (2 m.Eq.) of 9α-fluoro-21-bromo-4-pregnene-11β,17α-diol-3,20-dione are added and the solution is kept for 24 hours at 30°C.
A solution is then added containing 100 ml of water and 5 g of sodium chloride and the resulting mixture is slowly poured into 2,000 ml of acetone under constant agitation. A precipitate is formed which is filtered and washed three times with 100 ml of acetone/water 5:1 and three times with 100 ml of acetone and finally vacuum dried for eight hours at 30°C.

4.6 g of the partial and mixed ethanol and fluorocortisone ester compound in the title are obtained.

Quantitative determination of fluorocortisone, after mild alkaline hydrolysis with a hydroalcoholic solution of Na₂CO₃ and extraction with chloroform, is carried out according to British Pharmacopeia, 1980.

Quantitative determination of the ethoxyls is carried out according to R.H. Cundiff and P.C. Markunas, [Anal. Chem. 33, 1028 - 1030, (1961)].

Example 22 - Preparation of the N-Pentyl Ester of Hyaluronic Acid (HY).

12.4 g of Hy tetrabutylammonium salt with a molecular weight of 620,000, corresponding to 20 m.Eq. of a monomeric units, are solubilized in 620 ml of dimethylsulfoxide at 25°C, 3.8 g (25 m.Eq.) of n-pentyl bromide and 0.2 of iodide tetrabutylammonium are added, and the solution is kept for 12 hours at 30°C.

The resulting mixture is slowly poured into 3,500 ml of ethyl acetate under constant agitation. A precipitate is formed which is filtered and washed four times with 500
ml of ethyl acetate and finally vacuum dried for twenty-four hours at 30°C.

8.7 g of the n-pentyl ester product in the title are obtained. Quantitative determination of the ester groups is carried out using the method described on pages 169 - 172 of Siggia S. and Hann J.G., "Quantitative Organic Analysis Via Functional Groups" 4th edition, John Wiley and Sons.

Example 23 - Preparation of the Isopentyl Ester of Hyaluronic Acid (HV).

12.4 g of HV tetrabutylammonium salt with a molecular weight of 170,000, corresponding to 20 m.Eq. of a monomeric unit, are solubilized in 620 ml of dimethylsulfoxide at 25°C, 3.8 g (24 M.Eq.) of isopentyl bromide and 0.2 g of tetrabutylammonium iodide are added, and the solution is kept for 12 hours at 30°C.

The resulting mixture is slowly poured into 3,500 ml of ethyl acetate under constant agitation. A precipitate is formed which is filtered and washed four times with 500 ml of ethyl acetate and finally vacuum dried for twenty-four hours at 30°C.

8.6 g of the isopentyl ester product featured in the title are obtained. Quantitative determination of the ester groups is carried out using the method described on pages 169 - 172 of Siggia S. and Hann J.G., "Quantitative Organic Analysis Via Functional Groups" 4th edition, John Wiley and Sons.
Example 24 - Preparation of the Benzylester of Hyaluronic Acid (HY).

12.4 g of HY tetrabutylammonium salt with a molecular weight of 170,000, corresponding to 20 m.Eq. of a monomeric unit, are solubilized in 620 ml of dimethylsulfoxide at 25°C, 4.5 g (25 m.Eq.) of benzyl bromide and 0.2 g of tetrabutylammonium iodide are added, and the solution is kept for 12 hours at 30°C.

The resulting mixture is slowly poured into 3,500 ml of ethyl acetate under constant agitation. A precipitate is formed which is filtered and washed four time with 500 ml of ethyl acetate and finally vacuum dried for twenty-four hours at 30°C.

9 g of the benzyl ester product in the title are obtained. Quantitative determination of the ester groups is carried out according to the method described on pages 169 - 172 of Siggia S. and Hanna J.G., "Quantitative Organic Analysis Via Functional Groups", 4th Edition, John Wiley and Sons.

Example 25 - Preparation of the β-Phenylethyl Ester of Hyaluronic Acid (HY).

12.4 g of HY tetrabutylammonium salt with a molecular weight of 125,000, corresponding to 20 m.Eq. of a monomeric unit, are solubilized in 620 ml of dimethylsulfoxide at 25°C, 4.6 g (25 m.Eq.) of 2-bromoethylbenzene bromide and 185 mg of tetrabutylammonium iodide are added, and the solution is kept for 12 hours at 30°C.
The resulting mixture is slowly poured into 3,500 ml of ethyl acetate under constant agitation. A precipitate is thus formed which is then filtered and washed four times with 500 ml of ethyl acetate and finally vacuum dried for twenty-four hours at 30°C.

9.1 g of the β-phenylethyl ester in the title are obtained. Quantitative determination of the ester groups is carried out according to the method described on pages 169 - 172 of Siggia S. and Hanna J.G., "Quantitative Organic Analysis Via Functional Groups", 4th Edition, John Wiley and Sons.

Example 26 - Preparation of the Benzyl Ester of Hyaluronic Acid (HY).

3 g of the potassium salt of HY with a molecular weight of 162,000 are suspended in 200 ml of dimethyl-sulfoxide; 120 mg of tetrabutylammonium iodide and 2.4 g of benzyl bromide are added.

The suspension is kept in agitation for 48 hours at 30°C. The resulting mixture is slowly poured into 1,000 ml of ethyl acetate under constant agitation. A precipitate is formed which filtered and washed four times with 150 ml of ethyl acetate and finally vacuum dried for twenty four hours at 30°C.

3.1 g of the benzyl ester product in the title are obtained. Quantitative determination of the ester groups is carried out according to the method described on pages 169 - 172 of Siggia S. and Hanna J.G., "Quantitative

Example 27 - Preparation of Streptomycin Salt of Hyaluronic Acid (HY) Partially Esterified With Ethanol - 75% of Carboxylic Groups Esterified With Ethanol - 25% of Carboxylic Groups Salified With Streptomycin.

243 mg of streptomycin sulphate (1 m.Eq.) are solubilized in 20 ml of water. The solution is eluted in a thermostatic column at 5°C containing 2 ml of a quaternary ammonium resin (e.g., that known by the Trade-mark DOWEX\textsuperscript{TM} 1 x 8) in OH\textsuperscript{-} form.

The sulphate-free eluate is gathered in a thermostatic container at a temperature of 5°C.

1.6 of a 75% ethyl ester of HY and 25% sodium salt (corresponding to 1 m.Eq. of a monomeric unit relative to the non-esterified carboxyl), are solubilized in 400 ml of water. The solution is eluted in a thermostatic column at 20°C and containing 2 ml of a sulphonic resin (DOWEX\textsuperscript{TM} 50 X 8) in H\textsuperscript{+} form.

The sodium-free eluate is gathered under agitation in the solution of streptomycin base. The resulting solution is instantly frozen and freeze-dried. Yield: 1.7 g.

Microbiological determination on B.subtilis ATCC 6633 in comparison with streptomycin standard, shows a content of 10.9% in weight of streptomycin base, corresponding to the theoretically calculated content.
Example 28 - Preparation of the Erythromycin salt of
Hyaluronic Acid (HY) Partially Esterified With Ethanol -
75% of Carboxylic Groups Esterified With Ethanol - 25% of
Carboxylic Groups Salified With Erythromycin.

1.6 of a 75% ethyl ester of HY and sodium salt at 25%
corresponding to 1 m.Eq. of a monomeric unite relative to
the non-esterified carboxyl), are solubilized in 400 ml of
water. The solution is eluted in a thermostatic column at
20°C containing 2 ml of sulfonic resin (DOWEX™ 50 x 8) in
H⁺ form.

To the sodium-free eluate are added 734 mg of
erthyromycin base (1 m.Eq.). The resulting solution is
instantly frozen and freeze-dried. Yield: 2.1 g.

Microbiological determination on St. aureus ATCC 6538
in comparison to standard erythromycin, shows a content of
31.7% in weight of erythromycin base, corresponding to the
theoretically calculated weight.

Example 29 - Preparation of the Neomycin Sal of a
Hyaluronic Acid (HY) Partially Esterified With Ethanol -
75% of Carboxylic Groups Esterified With Ethanol - 25% of
Carboxylic Groups salified With Neomycin.

152 mg of neomycin sulfate (1 m.Eq.) are solubilized
in 20 ml of water. The solution is eluted in a
thermostatic column at 5°C containing 2 ml of quaternary
ammonium resin (DOWEX™ 1 x 8) in OH form.

The sulphate-free eluate is gathered in a thermostatic
container at a temperature of 5°C.
1.6 g of a 75% ethyl ester of HY and sodium salt at 25% (corresponding to 1 m.Eq. of monomeric unit relative to the non-esterified carboxyl), are solubilized in 400 ml of water. The solution is eluted in a thermostatic column at 20°C and containing 2 ml of sulfonylic resin (DOWEX<sub>TM</sub> 1 x 8) in H<sup>+</sup> form.

The sodium-free eluate is gathered under agitation in the solution of neomycin base. The resulting solution is instantly frozen and freeze-dried. Yield: 1.65 g.

Microbiological determination carried out on St. aureus ATCC 6538 in comparison to standard neomycin, shows a content of 6.1% in weight of neomycin base, corresponding to the theoretically calculated value.

Example 30 - Preparation of the Gentamicin Salt of Hyaluronic Acid (HY) Partially Esterified With Ethanol - 75% of Carboxylic Groups Esterified With Ethanol - 25% of Carboxylic Groups Salified With Gentamicin.

145 mg of gentamicin sulfate are solubilized in 10 ml of water. The solution is eluted in a thermostatic column at 5°C containing 2 ml of quaternary ammonium resin (DOWEX<sub>TM</sub> 1 x 8) in OH<sup>-</sup> form.

The sulphate-free eluate is gathered in a thermostatic container at a temperature of 5°C.

1.6 g of a 75% ethyl ester of HY and sodium salt at 25% (corresponding to 1 m.Eq. of a monomeric unit relative to the non-esterified carboxyl), are solubilized in 400 ml of water. The solution is eluted in a thermostatic column
at 20°C and containing 2 ml of sulfonic resin (DOWEX\textsuperscript{TM} 50 x 8) in H\textsuperscript{+} form.

The sodium-free eluate is gathered under agitation in the solution of gentamicin base. The resulting solution is instantly frozen and freeze-dried. Yield: 1.7 g.

Microbiological determination carried out on S. epidermidus ATCC 12228 in comparison to standard gentamicin, shows a content of 6.5% in weight of gentamicin base, corresponding to the theoretically calculated value.

**Example 31 - Preparation of the Amikacin Salt of Hyaluronic Acid (HY) Partially Esterified With Ethanol - 75% of Carboxylic Groups Esterified With Ethanol - 25% of Carboxylic Groups Salified With Amikacin 147 mg of Amikacin Base (1 m.Eq.) are Solubilized in 20 ml of Water.**

147 mg of amikacin (1 m.Eq.) are solubilized in 20 ml of water.

1.6 g of a 75% ethyl ester of HY and Sodium salt at 25% (corresponding to 1 m.Eq.) of a monomeric unit relative to the non-esterified carboxyl), are solubilized in 400 ml of water. The solution is eluted in a thermostatic column at 20°C and containing 2 ml of sulfonic resin (DOWEX\textsuperscript{TM} 50 x 8) in H\textsuperscript{+} form.

The sodium-free eluate is gathered under agitation in the solution of amikacin base. The resulting solution is instantly frozen and freeze-dried. Yield: 1.70 g.

Microbiological determination carried out on St. aureus ATCC 29737 in comparison to standard amikacin, shows
a content of 8.5% in weight of amikacin base, corresponding to the theoretically calculated value.

Example 32 - Preparation of the Kanamycin Salt of Hyaluronic Acid (HY) Partially Esterified With Ethanol - 75% of Carboxylic Groups Esterified With Ethanol - 25% of Carboxylic Groups Salified With Kanamycin.

146 mg of kanamycin sulfate (1 m.Eq.) are solubilized in 20 ml of water. The solution is eluted in a thermostatic column at 5°C containing 2 ml of quaternary ammonium resin (DOWEX\textsuperscript{TM} 1 x 8) in OH\textsuperscript{-} form.

The sulphate-free eluate is gathered in a thermostatic container at a temperature of 5°C.

1.6 g of a 75% ethyl ester of HY and sodium salt at 25% (corresponding to 1 m.Eq.) of a monomeric unit relative to the non-esterified carboxyl), are solubilized in 400 ml of water. The solution is eluted in a thermostatic column at 20°C and containing 2 ml of a sulfonic resin (DOWEX\textsuperscript{TM} 50 x 8) in H\textsuperscript{+} form.

The sodium-free eluate is gathered under agitation in the solution of kanamycin base. The resulting solution is instantly frozen and freeze-dried. Yield: 1.5 g.

Microbiological determination carried out on B. subtilis ATCC 6633 in comparison to standard kanamycin, shows a content of 7% in weight of kanamycin base, corresponding to the theoretically calculated value.
Example 33 - Preparation of the Pilocarpine Salt of Hyaluronic Acid (HY) Partially Esterified With Ethanol - 75% of Carboxylic Groups Esterified with Ethanol - 25% of Carboxylic Groups Salified With Pilocarpine.

245 mg of pilocarpine hydrochloride (1 m.Eq.) are solubilized in 20 ml of water. The solution is eluted in a thermostatic column at 5°C containing 2 ml of quaternary ammonium resin (DOWEX™ 1 x 8) in OH- form.

The chloride-free eluate is gathered in a thermostatic container at 5°C.

1.6 g of a 75% ethyl ester of HY and sodium salt at 25% (corresponding to 1 m.Eq. of a monomeric unit relative to the non-esterified carboxyl), are solubilized in 400 ml of water. The solution is eluted in a thermostatic column at 20°C and containing 2 ml of sulfonic resin (DOWEX™ 50 x 8) in H+ form.

The sodium-free eluate is gathered under agitation in the solution of pilocarpine base. The resulting solution is instantly frozen and freeze-dried. Yield: 1/89 g.

Example 34 - Preparation of the (Partial Propyl) Ester of Hyaluronic Acid (HY) - 85% of Esterified Carboxylic Groups - 15% of Salified Carboxylic Groups (Na).

12.4 g of HY tetrabutylammonium salt with a molecular weight of 165,000, corresponding to 20 m.Eq. of a monomeric unit are solubilized in 620 ml of dimethylsulfoxide at 25°C, 2.9 g (17 m.Eq.) of propyl iodide are added and the resulting solution is kept for 12 hours at 30°C.
A solution is then added containing 62 ml of water 9g of sodium chloride and the resulting mixture is slowly poured into 3,500 ml of acetone under constant agitation. A precipitate is formed which is filtered and washed three times with 500 ml of acetone/water 5:1 and three times with acetone and finally vacuum dried for eight hours at 30°C.

The product is then dissolved in 550 ml of water containing 1% of sodium chloride and the solution is slowly poured into 3,000 ml of acetone under constant agitation. A precipitate is formed which is filtered and washed twice with 500 ml of acetone/water 5:1 and three times with 500 ml of acetone and finally vacuum dried for 24 hours at 30°C. 8 g of the partial propyl ester compound in the title are obtained. Quantitative determination of the ester groups is carried out using the method of R.H. Cundiff and P.C. Markunas, [Anal. Chem. 33, 1038 - 1030, (1961)].

Example 35 - Preparation of the Pilocarpine salt of Hyaluronic Acid (HY) Partially Esterified With N-Propanol - 85% of Carboxylic Groups Esterified With N-Propanol - 15% of Carboxylic Groups Salified With Pilocarpine.

245 mg of pilocarpine hydrochloride (1 m.Eq.) are solubilized in 10 ml of water. The solution is eluted in a thermostatic column at 5°C containing 2 ml of quaternary ammonium resin (DOWEX™ 1 x 8) in OH⁻ form.

The chloride-free eluate is gathered in a thermostatic container at 5°C.
4.1 g of the propyl ester of HY 85% and tetrabutylammonium salt at 15% (corresponding to 1 m.Eq. of a monomeric unit relative to the non-esterified carboxyl) are solubilized in 100 ml of dimethylsulfoxide. The solution is eluted in a thermostatic column at 20°C containing 2 ml of damp sulfonic resin (DOWEX™ 50 x 8) in H⁺ form.

The eluate is gathered under agitation in the solution of pilocarpine base. The resulting solution is precipitated with ethyl acetate (600 ml).

The precipitate is filtered and washed four times with 200 ml of ethyl acetate and finally vacuum dried for 24 hours at 30°C. 3.5 g of the compound featured in the title are obtained.
Example 36 - Preparation of the ethyl ester of an acidic polysaccharide produced by Rhinocladiella eliatar.


![Chemical structure](image)

5.2 g of the potassium salt of this acidic polysaccharide, corresponding to 20 mEq of a monomeric unit, are suspended in 250 ml of dimethylsulfoxide. While the mixture is kept in agitation, 200 mg of tetrabutylammonium iodide are added at 35°C and then slowly 3.5 g of methyl iodide. The mixture is kept in agitation for 48 hours at 35°C, after which it is
slowly poured into 800 ml of ethyl acetate, keeping it under constant agitation. A precipitate is formed which is filtered and washed four times with 150 ml of ethyl acetate and lastly vacuum dried. 4 g of the ethyl ester product in the title are thus obtained, in which all the carboxylic groups are esterified. Quantitative determination of the ester groups is carried out by the method of R.H. Cundiff and P.C. Markanas Anal. Chem. 33, 1028 - 1030 (1961).

Example 37 - Preparation of the Ethyl Ester of Acid Polysaccharide Produced by Rhinocladiella Eliator.

10.0 g of the tetrabutylammonium salt of the acidic polysaccharide used as starting substance in Example 36, corresponding to 20 m.Eq. of a monomeric unit, are treated with 800 ml of dimethylsulfoxide at 30°C. 3.3 g (21.2 m.Eq.) of ethyl iodide are added and the solution is kept under agitation for 48 hours at 30°C. The resulting mixture is slowly poured into 4000 ml of ethyl acetate while kept under constant agitation. A precipitate is formed which is filtered and washed four times with 500 ml of ethyl acetate and lastly vacuum dried.

3 g of the ethyl ester product in the title are obtained, in which all the carboxylic groups are esterified.

Quantitative determination of the ester groups is carried out by the method of R.H. Cundiff and P.C. Markanas, [Anal. Chem. 33, 1028 - 1030, (1961)].
Example 38 - Preparation of the Ethyl Ester of the Acidic Polysaccharide Produced by Rhinocladiella Mansonii.


![Chemical structure diagram](image)

18.2 g of tetrabutylammonium salt of this acidic polysaccharide, corresponding to 20 m.Eq. of a monomeric unit, are treated with 1000 ml of dimethylsulfoxide at 30°C. Under agitation, 3.3 g (21.2 m.Eq.) of ethyl iodide and the solution is kept at 30°C for 24 hours, after which it is slowly poured into 4000 ml of ethyl acetate, keep it under constant agitation. A precipitate is formed which is filtered and washed four times with 500 ml of ethyl acetate and lastly vacuum dried.

11 g of the product featured in the title are obtained, in which all the carboxylic groups are
esterified. Quantitative determination of the ester groups is carried out according to the method of R.H. Cundiff and P.C. Markanas, [Anal. Chem. 33, 1028 - 1030, (1961)].

**Biological Activity Studies**

1) Antiinflammatory Activity Studies

The technical effect of the new esters and of the new medicaments according to aspects of the present invention may be demonstrated, for example, by placing in evidence the antiinflammatory activity of some partial esters of hyaluronic acid with antiphlogistic corticosteroids, measured in the model of exudative phlogosis induced by dextran in rabbit eye.

9 hyaluronic esters of cortisolone, hydrocortisone and fluorocortisone (9-fluorohydrocortisone) identified by the code names HYC1-HYC9 were tested. Table 1 describes these compounds and gives the percentages of the number of carboxylic groups of HY which are esterified with the above corticosteroids, and where applicable the percentage esterified with simple aliphatic alcohols and those salified with alkaline metals (Na):

The activity of the compounds of Table 1 was compared with the corresponding cortisones.
<table>
<thead>
<tr>
<th>COMPOUND</th>
<th>% CARBOXYLS ESTERIF. WITH CORTICOSTEROIDS</th>
<th>CORTICOSTEROID ASSAY (p/p)</th>
<th>% CARBOXYLS ESTERIF. WITH ALIPHATIC ALCOHOL</th>
<th>ALIPHATIC ALCOHOL ASSAY (p/p)</th>
<th>% CARBOXYLS SALIFIED WITH Na</th>
</tr>
</thead>
<tbody>
<tr>
<td>HYC1</td>
<td>20 CORTISONE</td>
<td>15.5%</td>
<td>-</td>
<td>-</td>
<td>80</td>
</tr>
<tr>
<td>HYC2</td>
<td>20 HYDROCORTISONE</td>
<td>15.6%</td>
<td>-</td>
<td>-</td>
<td>80</td>
</tr>
<tr>
<td>HYC3</td>
<td>20 FLUDROCORTISONE</td>
<td>16.2%</td>
<td>-</td>
<td>-</td>
<td>80</td>
</tr>
<tr>
<td>HYC4</td>
<td>20 CORTISONE</td>
<td>15.3%</td>
<td>80 ETHANOL</td>
<td>7.84%</td>
<td>/</td>
</tr>
<tr>
<td>HYC5</td>
<td>20 HYDROCORTISONE</td>
<td>15.4%</td>
<td>80 ETHANOL</td>
<td>7.83%</td>
<td>/</td>
</tr>
<tr>
<td>HYC6</td>
<td>20 FLUDROCORTISONE</td>
<td>16.1%</td>
<td>80 ETHANOL</td>
<td>7.77%</td>
<td>/</td>
</tr>
<tr>
<td>HYC7</td>
<td>20 CORTISONE</td>
<td>15.4%</td>
<td>40 ETHANOL</td>
<td>3.94%</td>
<td>40</td>
</tr>
<tr>
<td>HYC8</td>
<td>20 HYDROCORTISONE</td>
<td>15.5%</td>
<td>40 ETHANOL</td>
<td>3.94%</td>
<td>40</td>
</tr>
<tr>
<td>HYC9</td>
<td>20 FLUDROCORTISONE</td>
<td>16.1%</td>
<td>40 ETHANOL</td>
<td>3.91%</td>
<td>40</td>
</tr>
</tbody>
</table>
All the derivatives, except for HYC4, HYC5 and HYC6 (dissolved in DMSO) were dissolved in saline (2 mg/ml).

Method

Aseptic (exudative) phlogosis was induced in 48 rabbits by intraocular injection of dextran (1% in saline, 0.1 ml). The various products were administered by instillation in the right eye (RE) of the rabbits, while in the left eye (LE) only vehicle was instilled.

The treatment (3 drops every 6 hours) was begun immediately after the injection of dextran and was continued for 16 days.

Ophthalmic Examination

Both eyes of each rabbit were observed through a slit lamp. In particular the following were examined: the state of the conjunctiva and corneal epithelium, the anterior chamber (presence of Tyndall effect), state of the iris and of the posterior segment of the eye. With a Goldmann lens, the state of the back of the eye was examined. The presence of signs of inflammation (hyperaemia, exudate, cloudiness of the liquids, etc.) was recorded. The percentage of the eyes which did not present any signs of phlogosis was then calculated.

Results

As can be observed from the results reported in Table 2, hereinafter, the HYC derivatives all proved to possess a considerable anti-inflammatory activity consistently superior to that of the corresponding cortisones tested in parallel, reduced not only the percentage of eyes with
phlogosis on each day of observation, but also reducing the duration of inflammation. The most efficient of these derivatives seem to be HYC4, HYC5 and HYC6, presumably because they are more lipophilic.
Table 2

Antiinflammatory effect of the HYC derivatives (hyaluronic esters) on dextran-induced aseptic (exudative) phlogosis in rabbit eye

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Days from start of phlogosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Cortisone (4)</td>
<td>0.0</td>
</tr>
<tr>
<td>Vehicle (4)</td>
<td>0.0</td>
</tr>
<tr>
<td>Hydrocortisone (4)</td>
<td>0.0</td>
</tr>
<tr>
<td>Vehicle (4)</td>
<td>0.0</td>
</tr>
<tr>
<td>Fluctocortis. (4)</td>
<td>0.0</td>
</tr>
<tr>
<td>Vehicle (4)</td>
<td>0.0</td>
</tr>
<tr>
<td>HYC1 (4)</td>
<td>0.0</td>
</tr>
<tr>
<td>Vehicle (4)</td>
<td>0.0</td>
</tr>
<tr>
<td>HYC2 (4)</td>
<td>0.0</td>
</tr>
<tr>
<td>Vehicle (4)</td>
<td>0.0</td>
</tr>
<tr>
<td>HYC3</td>
<td>0.0</td>
</tr>
<tr>
<td>Vehicle (4)</td>
<td>0.0</td>
</tr>
<tr>
<td>HYC4 (4)</td>
<td>0.0</td>
</tr>
<tr>
<td>Vehicle (4)</td>
<td>0.0</td>
</tr>
<tr>
<td>HYC5 (4)</td>
<td>0.0</td>
</tr>
<tr>
<td>Vehicle (4)</td>
<td>0.0</td>
</tr>
<tr>
<td>HYC6 (4)</td>
<td>0.0</td>
</tr>
<tr>
<td>Vehicle (4)</td>
<td>0.0</td>
</tr>
</tbody>
</table>
Table 2 (cont'd)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Days from the start of phlogosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Hyc7 (4)</td>
<td>0.0</td>
</tr>
<tr>
<td>Vehicle (4)</td>
<td>0.0</td>
</tr>
<tr>
<td>Hyc8 (4)</td>
<td>0.0</td>
</tr>
<tr>
<td>Vehicle (4)</td>
<td>0.0</td>
</tr>
<tr>
<td>Hyc9 (4)</td>
<td>0.0</td>
</tr>
<tr>
<td>Vehicle (4)</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Values are expressed as percentages (number of eyes without signs of phlogosis out of the total number of eyes treated). In brackets are the number of treated eyes.
2) Absorption and Bioavailability Studies

The technical effect of the new products according to aspects of the present invention may be demonstrated by a study of the absorption and of the bioavailability of some derivatives of hydrocortisone with hyaluronic acid. The derivatives used are those described above and identified as HYC2, HYC5 and HYC8.

Materials and Methods

Animals

Male Sprague-Dawley rats, with a body weight of 250 - 350 gr were used, obtained from Charles River-Calco (Como), fed ad libitum with water and compound feed in pellets, with the code name of 4RF 21, produced by "Italiana Mangimi", licensee of Charles River.

Treatment

Hydrocortisone was administered in the form of sodium hemisuccinate salt at the dose of 1.34 mg/kg (corresponding to 1 mg/kg of hydrocortisone base) by general intravenous route and at the dose of 1.34 mg/kg and 2.68 mg/kg (corresponding to 2 mg/kg of hydrocortisone base) by subcutaneous route (the i.v. route was considered in order to determine the pharmacokinetic parameters which serve as a comparison for the evaluation of absorption of any other administration route).

The three HYC derivatives were administered by subcutaneous route at the dose of 6.5 and 13 mg/kg (doses corresponding to 1 and 2 mg/kg in hydrocortisone base). All the various products were dissolved in sterile saline,
except for HYC5 which, being insoluble in completely aqueous solutions, was first solubilized with the addition of the minimum quantity necessary of dimethylsulphoxide, and then brought to the right volume with saline. All the compounds were injected at a constant volume of 1 ml/kg.

Gathering of the Plasma Samples

After administration, 0.3 ml of blood was drawn from each animal by cardiac puncture in the presence of anticoagulant (sodium heparin).

Blood drawing times were as follows: *15 mins, 30 mins, 60 mins, 120 mins, 180 mins, 300 mins, 360 mins, 420 mins, 480 mins (*limited to the intravenous route).

Dosage of Hydrocortisone

The hydrocortisone was dosed by a radioimmunoassay method (using a kit known by the Trade-mark CORTISOLO™ Kit, Biodata, cod. 10394) using iodate tracing. The precision and accuracy of the method, determined on six repeats (double) of a control serum with a known control assay, proved to be 3.03% and 0.021% respectively. The linearity of the method comes between 1 and 1000 ng/ml. The observation limit is 1 ng/ml.

The dosage of the cortisolemia in the rat is not influenced either by the base levels or by the circadian rhythms of this hormone, as the metabolic pattern of the endogenous glucocorticoid and not cortisol (see E.L. Green: "Biology of the Laboratory Mouse").

Preliminary proof has demonstrated that the dosage method is specific only for free cortisol. The
anticortisol antibody does not present any form of competition towards any of the three Hyc derivatives.

**Results**

In Table 3 are reported the results of the average plasma levels of hydrocortisone, after i.v. and s.c. injection (1 and 2 mg/kg). It should be emphasized that, after s.c. injection, there is a quite rapid absorption of the product (Tmax evaluated at 30 mins, Cmax the same as the i.v. route levels at the same dose). In Table 3 hereinbelow are reported the average levels of cortisol after subcutaneous administration of the three Hyc derivatives at doses of 6.5 and 13 mg/kg (corresponding to 2 mg/kg in hydrocortisone base). In Table 4 hereinbelow are reported the pharmacokinetic parameters relative to cortisol calculated graphically from the plasmatic decline curves with the method of residues from the plasma absorption curve of the three Hyc products.

It should be noted that the kinetics of hydrocortisone released by the three derivatives with hyaluronic acid are not linear; that is, no direct relationship exists between the dose-dependent parameters, e.g., the area beneath the plasma decline curve and the plasma levels. Since the kinetics of cortisol are themselves linear and a first rate model results, it can be deduced that the saturation process in the case of the Hyc derivatives is the hydrolysis of the ester bond between hyaluronic acid and cortisolo. This phase (tending towards zero rate kinetics) is not itself connected with the absorption of the active
principle and therefore the kinetics of the three HYC's were likewise resolved according to a first rate model.
TABLE 3

Average plasmatic levels of hydrocortisone after s.c. administration of the derivatives HJC, HJC, HJC, (6.5 and 13 mg/kg) in comparison to the corresponding doses (7 - 2 mg/Kg) of hydrocortisone (average of 4 values)

<table>
<thead>
<tr>
<th>TIME MINUTES</th>
<th>AVERAGE PLASMATIC LEVELS (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>s.c. HYDROCORTISONE</td>
</tr>
<tr>
<td>i.v. 1mg/kg</td>
<td>1mg/kg 2mg/kg 6.5mg/kg 13mg/kg</td>
</tr>
<tr>
<td>15min 154.57</td>
<td>88.50 86.29 196.32 27.02 32.32</td>
</tr>
<tr>
<td>30min 59.62</td>
<td>61.27 142.12 35.67 50.51 49.52</td>
</tr>
<tr>
<td>60min 46.97</td>
<td>49.10 84.34 42.32 62.42 60.52</td>
</tr>
<tr>
<td>120min 39.35</td>
<td>23.44 50.02 37.35 58.44 61.17</td>
</tr>
<tr>
<td>180min 29.78</td>
<td>11.98 24.53 32.27 51.68 -----</td>
</tr>
<tr>
<td>300min 24.49</td>
<td>9.96 20.15 26.96 48.17 42.44</td>
</tr>
<tr>
<td>360min 21.08</td>
<td>8.18 16.81 24.24 44.67 30.43</td>
</tr>
<tr>
<td>420min 18.31</td>
<td>7.61 14.81 18.92 39.41 25.85</td>
</tr>
<tr>
<td>480min -----</td>
<td>------ ------ ------ ------ ------</td>
</tr>
</tbody>
</table>

1341276
TABLE 4

Pharmacokinetic parameters of hydrocortisone after subcutaneous administration of HYC₂, HYC₅, HYC₈ at a dose of 6.5 mg/kg in comparison to the corresponding dose of hydrocortisone (1 mg/kg)

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>HYDROCORTISONE</th>
<th>HYC₂</th>
<th>HYC₅</th>
<th>HYC₈</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kₑ</td>
<td>0.15 h⁻¹</td>
<td>0.17 h⁻¹</td>
<td>0.24 h⁻¹</td>
<td>0.19 h⁻¹</td>
</tr>
<tr>
<td>t 1/2 clfm.</td>
<td>4.5 h</td>
<td>4.08 h</td>
<td>2.89 h</td>
<td>3.65 h</td>
</tr>
<tr>
<td>kcal</td>
<td>0.86 h⁻¹</td>
<td>0.65 h⁻¹</td>
<td>0.94 h⁻¹</td>
<td></td>
</tr>
<tr>
<td>tₘax</td>
<td>30 min.</td>
<td>2.35 h</td>
<td>2.4 h</td>
<td>2.13 h</td>
</tr>
<tr>
<td>([AUC]₀⁻口头)(cm²)</td>
<td>192.00 ng/ml h</td>
<td>241.06 ng/ml h</td>
<td>278.92 ng/ml h</td>
<td>250 ng/ml h</td>
</tr>
<tr>
<td>Bioavailability</td>
<td>70.3</td>
<td>88.3 %</td>
<td>100 %</td>
<td>91 %</td>
</tr>
</tbody>
</table>
Conclusions

The bioavailability, as compared to hydrocortisone, of the three products in examination, proves to be complete and even superior to that of the quick release preparation. Regarding this, however, the absorption is slower (maximum times 2 hours) and maximum concentrations equal to those of subcutaneously administered cortisol are not reached. The plasmatic cortisolemia proves, however, on average to be higher several hours after administration. Esterification with hyaluronic acid therefore determines slower release of hydrocortisone, and this is the desired objective.

3) Skin Hydration Studies

Hydrolysis of the ester bond, as has already been said, is a saturation process; that is, it tends towards zero grade kinetics. This, for a retard form, is a very desirable factor, since, by definition, a controlled release preparation is "a preparation which determines the release of a constant aliquot of active principle in a given time" and this is the condition reached by zero grade kinetics.

The skin, due to the complex nature of its physiological functions, cannot be considered as exclusively a passive covering organ, but rather as a dynamic, polyvalent organ. The complete functional capacity of the skin is fundamentally guaranteed by the presence of an intact hydrolipidic covering and this requires a correct humidity content in the horny layer, which varies a great deal according to its storage capacity
(values vary between 10% and 60% of water content). The humidity of the skin depends on a series of endogenous and exogenous factors.

Cutaneous humidity fundamentally influences the formation of the specific hydrolipidic film of the skin which modifies and stores the substances it eliminates, thus forming the basis for the realization of its protective functions.

The means of defense used so far to restore the maximum degree of hydration for the skin involve the use of highly hygroscopic substances, e.g., glycerine, sodium lactate and propylene glycol. These substances, however, had the disadvantage, in dry conditions, of drawing humidity from the skin itself instead of from the external environment, thus making the skin even drier.

For this reason at present there is a preference for biological substances whose origins lie, for their particular characteristics, to the natural hydrating factors mentioned before. In this context is included the considerable interest in the use of hyaluronic acid.

The hydration of the skin and its nourishment seem closely related to the HY content of the cutaneous tissue. It has in fact been demonstrated that the exogenous contribution of HY contributes noticeably to the state of hydration of the cutaneous tissue.

These particular characteristics of hyaluronic acid are also found, and to an even greater degree, in the esterified derivative of HY according to aspects of the
present invention, and for this reason they may be used to a great extent in the field of cosmetics.

In order to establish a comparison between hyaluronic acid and its derivatives of aspects of the present invention, some experiments were carried out to evaluate instrumentally, after topical application, the hydrating properties of the compounds in examination.

Materials

As hyaluronic derivatives according to aspects of the present invention the following esters were used.

HYAFF₂  hyaluronic acid esterified by 75% with methanol
HYAFF₇  hyaluronic acid esterified by 75% with ethanol
HYAFF₈  hyaluronic acid esterified by 50% with isopropanol
HYAFF₉  hyaluronic acid esterified by 50% with n-propanol
HYAFF₁₀ hyaluronic acid esterified by 50% with n-butanol

Hyaluronic acid sodium salt (HYALASTINEᵀᴹ fraction).

All the compounds were vehicled at a concentration of 0.2% in an ointment the composition of which was as follows:

- Polyethyleneglycol monostearate 400, gr. 10.000
- CETIOL Vᵀᴹ (the trade-mark for a cetyl alcohol), gr. 5.000
- LANETTE SXᵀᴹ (the trade-mark for an emulsifying wax containing cetostearyl alcohol and sodium lauryl sulphate or sodium salts of similar sulphated higher primary aliphatic alcohols), gr. 2.000
- Paraoxybenzoate of methyl, gr. 0.075
- Paraoxybenzoate of propyl, gr. 0.050
- Sodium dehydroacetate, gr. 0.100
- Glycerine F.U., gr. 1.000
- Sorbitol 70 (hexahydric alcohol), gr. 1.500
- Test cream, gr. 0.050
- Water for injectable prepar. q.b.a., gr. 100.00

The placebo formulation contained only vehicle.

Method

Study Sample

The study was carried out on 10 healthy volunteers (6 women and 4 men not suffering from any form of skin disease), aged between 20 and 60 years.

Treatment

Each volunteer was treated (single administration) with all the formulations in examination, which were applied (1 gr./ointment) to the inside surface of each forearm, distinguishing, with a demographic pencil, the application zone (25 cm²) of each product and standardizing the procedure as far as possible. To the right forearm were applied the compounds known as HYAFF₇, HYAFF₇, HYAFF₉, HYAFF₉, while to the left were applied HYAFF₁₀, placebo and hyaluronic acid.

Evaluation Parameters

At the established times (0, 3, 6 and 24 hours after treatment) the degree of hydration of the horny layer of each application zone was measured with a corneometer.

Most particularly, the dielectric strength of the water was measured (in 0.8 seconds), after application of the sensor (condenser) to the skin surface. The value thus obtained, the measurement unit of which corresponds to
0.07 mg of water (normal values are between 90 and 100 units), was read on the dial of the instrument.

Registrations were carried out in constant humidity conditions.

Results

As can be seen from the results reported in Table 5, hereinbelow, treatment with the compounds of the HYAFF series induced, in all cases, a notable increase in the degree of hydration of the horny layer, which was particularly evident not only during the hours immediately following application, but also from the last registrations. This effect proved to be superior both to that of the placebo formulation and to the formulation containing hyaluronic acid sodium salt. Of the compounds tested, the derivatives HYAFF₁ and HYAFF₂, appeared particularly interesting.

Conclusions

On the basis of the results obtained it was possible to conclude that the esterified HYAFF derivatives do in fact determine a notable and prolonged hydrating effect at the skin level, which is superior to that observed with the formulation containing hyaluronic acid, thus guaranteeing the integrity and physiological efficiency of the hydrolipidic film. These satisfactory results form therefore a valid basis for the use of these compounds in the prevention (or treatment) of chapped skin, the treatment of burns and scalds and the maintaining of physiological nourishment and elasticity of the skin.
TABLE 5

Effect of the compounds of the HYAFF series on the degree of hydration of the corneal layer (each value represents the average for 10 subjects)

<table>
<thead>
<tr>
<th>PRODUCT</th>
<th>1st hr</th>
<th>3rd hr</th>
<th>6th hr</th>
<th>24th hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLACEBO</td>
<td>41.5</td>
<td>28.3</td>
<td>13.4</td>
<td>2.2</td>
</tr>
<tr>
<td>HYALURONIC ACID</td>
<td>54.7</td>
<td>37.6</td>
<td>19.7</td>
<td>5.3</td>
</tr>
<tr>
<td>HYAFF (_2)</td>
<td>66.6</td>
<td>43.1</td>
<td>24.5</td>
<td>6.1</td>
</tr>
<tr>
<td>HYAFF (_1)</td>
<td>91.5</td>
<td>60.7</td>
<td>30.1</td>
<td>11.4</td>
</tr>
<tr>
<td>HYAFF (_9)</td>
<td>70.0</td>
<td>51.4</td>
<td>26.2</td>
<td>8.7</td>
</tr>
<tr>
<td>HYAFF (_9)</td>
<td>81.0</td>
<td>59.9</td>
<td>29.0</td>
<td>9.6</td>
</tr>
<tr>
<td>HYAFF (_10)</td>
<td>68.8</td>
<td>57.0</td>
<td>27.5</td>
<td>0.3</td>
</tr>
</tbody>
</table>
4) Enzyme Stability and Oxygen Permeability Studies

Materials

The valuable properties of the new esters according to aspects of the present invention, already partially described, which form their technical advantages over the already known products in the respective fields are further illustrated by the following results on the stability of the enzymes and the permeability to oxygen of the films obtained with the following compounds:

- $\text{HYAFF}_2$: hyaluronic acid esterified by 100% with methanol
- $\text{HYAFF}_7$: hyaluronic acid esterified by 100% with isopropanol
- $\text{HYAFF}_9$: hyaluronic acid esterified by 100% with n-propanol
- $\text{HYAFF}_{11}$: hyaluronic acid esterified by 100% with benzylic alcohol
- $\text{HYAFF}_{20}$: hyaluronic acid esterified by 100% with $\beta$-phenylethyl alcohol
- $\text{HYAFF}_{22}$: hyaluronic acid esterified by 100% with isopentyl alcohol

The films may be prepared according to the procedure described in Example 39.

Stability to Enzymes of the HYAFF Films

Stability to Serum Esterase

Each film (weighing 20 mg.) was placed in a polyethylene capsule together with 5 ml of rabbit serum and kept at a constant temperature (37°C).
The evaluation parameter was the time taken (in hours) for the film to dissolve. The results are reported in Table 6 hereinbelow.

**Stability to Hyaluronidase**

Each film (weighing 20 mg.) was placed in a polyethylene capsule together with pH 5 buffer (acetate 0.1M, NaCl 0.15M) or pH 7.2 (phosphate 0.1M, NaCl 0.15M) containing 100 U of enzyme (testicle hyaluronidase from Miles batch 8062, activity 342 turbidometric units/mg) in each ml and kept at a constant temperature (37°C). The evaluation parameter was the time taken (in hours) for the film to dissolve. The results are reported in Table 6 hereinbelow.

**Table 6**

<table>
<thead>
<tr>
<th>COMPOUNDS</th>
<th>STABILITY (hrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SERUM ESTERASE</td>
<td>HYALURONIDASE pH 5</td>
</tr>
<tr>
<td>HYAFF₂</td>
<td>72</td>
</tr>
<tr>
<td>HYAFF₉</td>
<td>120</td>
</tr>
<tr>
<td>HYAFF₇</td>
<td>90</td>
</tr>
<tr>
<td>HYAFF₁₁</td>
<td>60</td>
</tr>
<tr>
<td>HYAFF₂₀</td>
<td>130</td>
</tr>
<tr>
<td>HYAFF₂₂</td>
<td>130</td>
</tr>
</tbody>
</table>
Permeability to Oxygen of the Films of the HYAFF Series

Each film was placed in a container having 2 compartments separated by the membrane itself. One compartment (volume = 1.2 cc) was filled with partially degassed water (PO₂=45mm of Hg at 23°C), into the other was introduced a flow of O₂ and CO₂ (95% and 5% respectively), kept constant (1 bubble/second) in time. The whole system was insulated in nitrogen.

At the established times (15, 30, 60, 90, 120, 240 minutes) a suitable aliquot of water was drawn off (1.2 cc) and determination of the partial pressure of O₂ was effected by an analyzer known by the Trade-mark GAS SYSTEM™ 1302 of the Instrumentation Laboratories. The saturation pressure (550 mg of Hg) was taken as reference value and calculated, in the previously described experimental conditions, by insufflating the O₂ atmosphere.

The tests were carried out in comparison to an impermeable membrane and an organo-silicon synthetic rubber known by the Trade-mark SILASTIC™ of The Dow Chemical Company (in Lepetit Cat. No. 500-1). The results are reported in Table 7 hereinafter.
<table>
<thead>
<tr>
<th>COMPOUNDS</th>
<th>PRESSURE OF O₂ (mm Hg at 23°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0min</td>
</tr>
<tr>
<td>Non Perm. membrane</td>
<td>45</td>
</tr>
<tr>
<td>Plastic</td>
<td>45</td>
</tr>
<tr>
<td>HYAF 2</td>
<td>45</td>
</tr>
<tr>
<td>HYAF 3</td>
<td>45</td>
</tr>
<tr>
<td>HYAF 4</td>
<td>45</td>
</tr>
<tr>
<td>HYAF 5</td>
<td>45</td>
</tr>
<tr>
<td>HYAF 6</td>
<td>45</td>
</tr>
<tr>
<td>HYAF 7</td>
<td>45</td>
</tr>
<tr>
<td>HYAF 8</td>
<td>45</td>
</tr>
<tr>
<td>HYAF 9</td>
<td>45</td>
</tr>
<tr>
<td>HYAF 10</td>
<td>45</td>
</tr>
<tr>
<td>HYAF 11</td>
<td>45</td>
</tr>
</tbody>
</table>
Pharmaceutical Preparations

The present invention also provides pharmaceutical preparations of another aspect of this invention, containing one or more of the above-mentioned esters of hyaluronic acid and salts thereof, of other aspects of this invention, or one or more medicaments resulting from the association of one of such esters with a pharmacologically-active substance for topical application, of other aspects of this invention, as described above. That is, the invention provides medicaments in which the hyaluronic ester acts as a vehicle for the active substance.

The pharmaceutical preparations of other aspects of this invention containing the hyaluronic esters of other aspects of this invention as an active principle, both in the case of esters with therapeutically-inactive alcohols destined for the same uses as hyaluronic acid itself, and esters of HY with therapeutically-active alcohols intended for the usual uses of such alcohols, contain the usual excipients and may be employed for oral, rectal, parenteral, subcutaneous, local or intradermal use. They are therefore in solid or semisolid form, for example, as pastilles, tablets, gelatin capsules, capsules, suppositories, or soft gelatin capsules. For parenteral and subcutaneous use it is possible to use forms intended for intramuscular or intradermal administration, or suitable for infusions or intravenous injections. It is possible therefore to provide solutions of the active compounds or freeze-dried powders of the active compounds.
to be added to one or more pharmaceutically-acceptable excipients or diluents, convenient for the above-mentioned uses and with osmolarity compatible with the physiological liquids. For local use, preparations in spray form may be used; for example, nasal sprays, creams or ointments for topical use of specially prepared sticking plasters for intradermal administration. The solubility of the hyaluronic esters in organic solvents with low boiling points makes them particularly suitable for the manufacture of "sprays".

The preparations of aspects of the present invention may be administered to man or animals. They contain preferably between 0.01% and 10% of active component for the solutions, sprays, ointments and creams and between 1% and 100%, preferably between 5% and 50%, of active compound for the preparations in solid form. The dosage to be administered will depend on individual needs, on the desired effect and on the chosen administration route. The daily dosage of such preparations may be decided according to that use for the corresponding known preparations both of hyaluronic acid for the corresponding cures; for example, for the cure of arthritis, for example, in man or horse; and of the therapeutically-active alcohol, the action of which is to be put to use. Thus, for example, the dosage of a hyaluronic ester with cortisone may be derived from its content of this steroid and from its usual dosage in the known pharmaceutical preparations.
One particular form of pharmaceutical preparations is represented by the above-mentioned medicaments containing the association of an hyaluronic ester and of one or more active substances. These may also be in solid form, for example, as freeze-dried powders containing only the two Components (1) and (2), together or separate. This galenic form is especially suitable for topical use. Indeed these solid medicaments form, on contact with the surfaces to be treated, more or less concentrated solutions according to the nature of the particular epithelium, with the same characteristics of the solutions previously prepared in vitro and which represent another particularly important aspect of the present invention. Such solutions are preferably in distilled water or sterile saline and preferably contain no other pharmaceutical vehicle apart from the hyaluronic ester or one of its salts. The concentrations of such solutions may also vary within ample limits, for example, between 0.01 and 75% both for each of the two components taken separately, and for their mixtures or salts. Particular preference is given to solutions with a pronounced elastic-viscous character, for example, with a content of 10% to 90% of the medicament or of each of its components.

Particularly important are the medicaments of this type, both in anhydrous form (freeze-dried powder) or as solutions, concentrated or diluted in water or saline, possibly with the addition of additive or auxiliary substances, e.g., in particular disinfectant substances or
mineral salts acting as a buffer or others, for ophthalmic use.

Of the medicaments of aspects of the invention, particularly important, as the case may be, are those with a degree of acidity suitable for the environment to which they are to be applied, that is with a physiologically-tolerable pH. Adjustment of the pH, for example in the above-mentioned salts of the esters of hyaluronic acid with a basic active substance, may be effected by suitably regulating the quantities of polysaccharide, of the salts or of the basic substance itself. Thus, for example, if the acidity of a salt of a hyaluronic ester with a basic substance is too high, the excess of free acid groups is neutralized with the above-mentioned inorganic bases, for example, with sodium, potassium or ammonium hydrate.

The preparation of the salts according to aspects of the invention may be effected by a procedure known per se by placing in contact solutions, aqueous solutions or organic solutions, of the two Components (1) and (2), and possibly bases or basic salts of the above-mentioned alkaline or alkaline earth metals or magnesium or aluminum in the right quantities and isolating the salts in an amorphous anhydrous form according to known techniques. It is possible, for example, to prepare first aqueous solutions of the two Components (1) and (2), freeing such components from aqueous solutions of their salts with suitable ionic exchangers, and mixing the two solutions at a low temperature, for example, between 0°C and 20°C. If
the salt thus obtained is easily soluble in water, it can be freeze-dried, while the salts which are difficult to solubilize may be separated by centrifugation, filtration or decantation and possibly subsequently dried.

For these associated medicaments too, the dose is based on that of the active principles used singly and may therefore easily be determined by a skilled person, taking into consideration the dosage recommended for the corresponding known drugs.

In the cosmetic articles according to aspects of the invention the hyaluronic esters and their salts are mixed with the excipients commonly used in the field and are, for example, those already listed above for the pharmaceutical preparations. Mostly used are creams, ointments, and lotions for topical use in which the hyaluronic ester or one of its salts may represent the cosmetic active agent, possibly with the addition of other cosmetically-active agents, e.g., steroids, for example, pregnenolone, or one of the agents mentioned above. In such preparations, the hyaluronic ester is preferably an ester with an alcohol with no cosmetic action, e.g., a lower aliphatic alcohol, e.g., one of those already mentioned. The effect is due to the intrinsic cosmetic properties of the polysaccharide component, e.g., in the case of free hyaluronic acid or of its salts.

The cosmetic articles may, however, be based on substances with specific actions other than those of hyaluronic acid, for example, disinfectant substances,
sunshields, waterproofing or regenerating or antiwrinkle substances, or odorants, especially perfumes. In this case, the hyaluronic ester may itself be the active ingredient and derives from alcohols with the same properties, for example, from higher aliphatic alcohols or terpenic alcohols in the case of perfumes or acts as a vehicle for substances with those properties associated with it.

Particularly important therefore are cosmetic compositions, according to other aspects of this invention, similar to the medicaments described above in which the pharmaceutically-active component (1) or its relative salts is substituted by a cosmetic factor.

The use of the above-mentioned esters of HY deriving from alcohols used in the perfume industry represent an important step forward in technology, since they allow a slow, constant and protracted release of the scented ingredients.

An important application of aspects of the present invention regards sanitary and surgical articles which have already been described above, and to the procedures for their manufacture and use. The invention therefore includes all the articles similar to those already on the market, based on the hyaluronic acid but containing a hyaluronic ester of aspects of this invention or one of its salts of aspects of this invention in place of the free acid or one of its salts, for example inserts or ophthalmic lenses.
Completely new surgical and sanitary articles according to aspects of the present invention are represented by the esters of hyaluronic acid regenerated as such by appropriate organic solutions from which it is possible to obtain, by means of suitable procedures, films, thin sheets or threads to be used in surgery, as aids or substitutes of the skin in case of serious damage to this organ, e.g., following burns, as a suture in surgical operations. The invention in other aspects includes particularly these uses and a procedure for the preparation of such articles consisting in the formation of a solution of hyaluronic ester of aspects of this invention or of one of its salts of aspects of this invention in a suitable organic solvent, e.g., an amide of a carboxylic acid, especially a dialkylamide of an aliphatic acid with between 1 and 5 carbon atoms and deriving from alkyl groups with between 1 and 6 carbon atoms, and above all from an organic sulphoxide, that is, a dialkylsulphoxide with alkyl groups with a maximum of 6 carbon atoms, e.g., dimethylsulphoxide or diethylsulphoxide and again most importantly by a fluorinated solvent with a lower boiling point such as especially hexafluoroisopropanol. The invention, in other aspects, includes turning such solutions into sheet or thread form and in removing the organic solvent by contact with another organic or aqueous solvent which can be mixed with the first solvent and in which the hyaluronic ester is not soluble, especially a lower aliphatic alcohol, for example ethyl alcohol (wet spinning), or, should a solvent
with a not-too-high boiling point be used to prepare the solution of the hyaluronic derivative, in removing such a solvent in dry conditions with a current of gas, especially with suitably heated nitrogen (dry spinning). It is also possible to obtain excellent results with dry-wet spinning.

The threads of other aspects of the invention obtained with hyaluronic acid esters of aspects of the invention may be used for the preparation of gauze for the medication of wounds and in surgery. The use of such gauze has the exceptional advantage of the biodegradability thereof in the organism, made possible by the enzymes which they contain. These enzymes divide the ester into hyaluronic acid and the corresponding alcohol, and therefore into a compound already present in the organism, and into a harmless compound, e.g., an alcohol, should a hyaluronic ester be used which derives from a therapeutically-acceptable alcohol, e.g., ethyl alcohol, made possible by the enzymes which they contain.

These gauzes and also the aforementioned threads may therefore be left inside the organism after surgery, since they are slowly absorbed thanks to the above-described degradation.

During the preparation of the sanitary and surgical articles of aspects of the invention described above, it is possible to add plastifying materials which improve their mechanical characteristics, e.g., in the case of the threads, to improve their resistance to knots. These plastifying materials may be, for example, alkaline salts
of fatty acids, for examples, sodium stearate or sodium palmitate, the esters of organic acids with many carbon atoms, etc.

Another application of the new esters of HY of aspects of the invention using to advantage their biodegradability due to the esterases present in the organism, is represented by the preparation of capsules of aspects of the invention for subcutaneous implantation of medicaments or of microcapsules for injection, for example, by subcutaneous or intramuscular route. For the applications of subcutaneous medicaments for obtaining a slow release and therefore a "retard" action, capsules made of silicone materials have mostly been used up till now, with the disadvantage that the capsule is liable to move about inside the organism and it is not possible to recover it. Evidently with the new hyaluronic esters of aspects of this invention this danger is no longer exists.

Of great importance is also the preparation of microcapsules of aspects of this invention made with hyaluronic esters of aspects of this invention, eliminating the problems regarding their use which up till now has been limited, for the same reasons as those mentioned above and which opens up a vast field of application where a "retard" effect is sought be an injected route.

A further application in the sector of medicine and surgery of the new esters of HY concerns the preparation of a large variety of solid inserts, e.g., plates, discs, sheets, etc. substituting those in metallic form or those
made of synthetic plastic materials already in use, in the case of inserts intended for removal after a certain period of time. Preparations made of animal collagen, being of a proteic nature, often provoke undesirable side effects e.g., inflammation or rejection. In the case of animal, and not human, hyaluronic acid, this danger does not exist, as there is no incompatibility between the polysaccharides of different animal species.

Another application relates to the use to augment and correct soft tissue defects. The need for a safe and effective biomaterial by which to replace missing or damaged soft tissue has long been recognized. Several alloplastic materials, including paraffin, a paste of the polytetrafluoroethylene known by the Trade-mark TEFLON, du Pont, silicone and bovine collagen have been used to replace lost soft tissue. However, these materials have been associated with permanent undesirable textural changes in the skin, with migration from the site of implantation and with adverse treatment reactions. Thus, the need for a versatile biomaterial in medicine continues. The hyaluronic acid esters of aspects of this invention can be used safely and effectively to augment and correct such soft tissue defects as acne scars, atrophy post surgical irregularities, moths chemosurgery, cleft lip scars and age-related wrinkles.

Part of the application in the field of medicine and surgery of the new esters according to aspects of the present invention, are represented by expansive materials,
especially in the form of sponges, for the medication of wounds and various lesions.

The following are particular exemplary pharmaceutical preparations according to aspects of the invention.

**Formulation 1** - Collirium containing cortisone of which 100 ml contain:

- partial ester of hyaluronic acid with cortisone (Ex.10), gr. 0.200
- ethyl p. hydroxybenzoate, gr. 0.010
- methyl p. hydroxybenzoate, gr. 0.050
- sodium chloride, gr. 0.900
- water for injectable preparations/q.b.a., ml. 100

**Formulation 2** - Injectable solution containing hydrocortisone of which 100 ml contain:

- partial ester of hyaluronic acid with hydrocortisone (Ex. 11), gr. 0.1
- sodium chloride, gr. 0.9
- water for injectable preparations/q.b.a., ml. 100

**Formulation 3** - Cream containing a partial ester of hyaluronic acid with ethyl alcohol (Ex. 3), of which 100 gr. contain:

- partial ester of hyaluronic acid with ethyl alcohol, gr. 0.2
- polyethyleneglycol monostearate 400, gr. 10.000
- CETIOL V<sub>TM</sub>, gr. 5.000
- LANETTE SX<sub>TM</sub>, gr. 2.000
- paraoxybenzoate of methyl, gr. 0.075
- paraoxybenzoate of propyl, gr. 0.050
- sodium dihydroacetate, gr. 0.100
- glycerine F.U., gr. 1.500
- sorbitol 75, gr. 1.500
- test cream, gr. 0.050
- water for injectable preparations/q.b.a., gr. 100.00

The following are exemplary material products utilizing the hyaluronic esters of aspects of the present invention.

Example 39 – Preparation of Films Using Esters of Hyaluronic Acid.

A solution is prepared in dimethylsulfoxide of the n-propyl ester of HY (MW 130,000) with a concentration of 180 mg/ml.

By means of a stratifier, a thin layer of solution is spread on a glass sheet; the thickness must be 10 times greater than the final thickness of the film. The glass sheet is immersed in ethanol which absorbs the dimethylsulfoxide but does not solubilize the HY ester which becomes solid. The film is detached from the glass sheet, is repeatedly washed with ethanol, then with water and then again with ethanol.

The resulting sheet is dried in a press for 48 hours at 30°C.

Example 40 – Preparation of Threads Using Esters of Hyaluronic Acid

A solution is prepared in dimethylsulfoxide of the benzyl ester of HY (MW 165,000) with a concentration of 200
mg/ml. The solution thus obtained is pressed by means of a pump through a threader with 0.5 mm holes.

The threader is immersed in ethanol/dimethylsulfoxide 80:20 (this concentration is kept constant by continuous addition of ethanol); when the solution in dimethylsulfoxide is soaked in this way it tends to lose most of the dimethylsulfoxide and the thread solidifies.

The thread is stretched while it still has a content of dimethylsulfoxide, is then repeatedly stretched and washed with ethanol. The thread is dried in nitrogen current.

Example 41 - Preparation of a Spongy Material Made With Hyaluronic Acid Esters.

1 g of benzyl ester of hyaluronic acid with a molecular weight of 170,000 in which all the carboxylic groups are esterified (obtained for example as described in Example 14) are dissolved in 5 ml of dimethylsulfoxide. To each 10 ml of solution prepared, a mixture of 31.5 g of sodium chloride with a degree of granularity corresponding to 300 μ, 1.28 g of sodium bicarbonate and 1 g of citric acid is added and the whole is homogenized in a mixer.

The pasty mixture is stratified in various ways, for instance, by means of a mange consisting of two rollers which turn opposite each other at an adjustable distance between the two. Regulating this distance the paste is passed between the rollers together with a strip of silicone paper which acts as a support to the layer of paste thus formed. The layer is cut to the desired
dimensions of length and breadth, removed from the silicone, wrapped in filter paper and emerged in a suitable solvent, e.g., water. The sponges thus obtained are washed with a suitable solvent, e.g., water and possibly sterilized with gamma rays.

Example 42 - Preparation of a Spongy Material Made With Hyaluronic Acid Esters

In the manner described in Example 41, it is possible to prepare spongy materials with other hyaluronic acid esters of aspects of the present invention. In the place of dimethylsulfoxide it is possible to use, if desired, any other solvent capable of dissolving the chosen ester of HY of aspects of the present invention. In the place of sodium chloride it is possible to use any other solid compound which is insoluble in the solvent used to dissolve the hyaluronic acid ester of aspects of the present invention, but which is however soluble in the solvent used to precipitate the hyaluronic ester of aspects of the present invention after the above-mentioned mechanical treatment, and finally which has the correct degree of granularity to obtain the type of pores desired in the sponge material.

In the place of sodium bicarbonate and citric acid it is possible to use other couples of similar compounds, that is, compounds, which react to each other in suspension or solution of the solvent used to dissolve hyaluronic acid in such a way as to form a gas, e.g., carbon dioxide, which has the effect of producing a less compact spongy material.
In this way it is possible to use, in the place of sodium bicarbonate, other bicarbonates or alkaline or alkaline earth carbonates and in the place of citric acid other acids in solid form., e.g., tartaric acid.
CLAIMS:

1. An ester of hyaluronic acid of the Formula
   \[ \text{Hy}(\text{COOR})_n \]
   wherein \( \text{COO} \) represents the carboxylic acid moiety on the
   hyaluronic acid (\( \text{Hy} \)) molecule, \( \text{R} \) is derived from an alcohol
   which is selected from the group consisting of an aliphatic
   alcohol having a chain of 2 to 34 carbons, an araliphatic
   alcohol, a cycloaliphatic alcohol and a heterocyclic alcohol,
   and \( n \) is a large number.

2. The ester of claim 1, which is in the form of a total
   ester.

3. The ester of claim 1, which is in the form of a
   partial ester.

4. The ester of claim 1, which is in the form of a salt
   of a partial ester with an inorganic base or with an organic
   base.

5. A total ester of claim 2, in which said alcohol is an
   aliphatic alcohol which is substituted by one or two
   functional groups which are selected from the group consisting
   of amino, hydroxy, mercapto, aldehyde, keto, carboxyl,
   hydrocarbonyl, dihydrocarbonylamino, ether, ester, thioether,
   thioester, acetal, ketal, carbalkoxy, unsubstituted carbamide
   groups and carbamide groups which are substituted by one or
   two alkyl groups, the hydrocarbonyl radicals in said
   functionally-modified groups having a maximum of 6 carbon
   atoms, and in which the carbon atom chain may be interrupted
by heteroatoms which are selected from the group consisting of oxygen, sulphur and nitrogen.

6. A total ester of hyaluronic acid according to claim 5, in which said alcohol is ethyl alcohol, propyl alcohol, isopropyl alcohol, normal butyl alcohol, isobutyl alcohol, tert-butyl alcohol, amyl alcohol, pentyl alcohol, hexyl alcohol, octyl alcohol, ethylene glycol, propylene glycol, butylene glycol or glycerin.

7. A total ester of hyaluronic acid according to claim 2, in which said alcohol is an araliphatic alcohol with only one benzene residue, in which the aliphatic chain has a maximum of 4 carbon atoms, and in which the benzene residue is unsubstituted or is substituted by 1 to 3 methyl groups, by 1 to 3 hydroxy groups or by 1 to 3 halogen atoms, and in which the aliphatic chain may be substituted by one or two functions which are selected from the group consisting of free amino groups, monoethylamino groups, diethylamino groups, pyrrolidinyl groups and piperidinyl groups.

8. A total ester of hyaluronic acid according to claim 7, in which said alcohol is benzyl alcohol, phenethyl alcohol, ephedrine or adrenalin.

9. A total ester of hyaluronic acid according to claim 2, in which said alcohol is a cycloaliphatic alcohol or an aliphatic-cycloaliphatic alcohol and derives from a monocyclic hydrocarbide or from a polycyclic hydrocarbide with a maximum of 34 carbon atoms.
10. A total ester of hyaluronic acid according to claim 9, in which said alcohol is a polycyclic alcohol which is selected from the group consisting of sterols, chloric acids, steroid alcohols, groups of the estrane and pregnane series and their unsaturated derivatives.

11. A total ester of hyaluronic acid according to claim 10, in which said alcohol is cortisone, hydrocortisone, prednisone, prednisolone, fluorocortisone, dexamethasone, betamethasone, corticosterone, deoxycorticosterone, paramethasone, flumethasone, fluocinolone, fluocinolone acetonide, fluprednylidene, clobetasol, or beclometasone.

12. A partial ester of claim 3, in which said aliphatic alcohol is substituted by one or two functional groups which are selected from the group consisting of amino, hydroxy, mercapto, aldehyde, keto, carboxyl, hydrocarbyl, dihydrocarbylamino, ether, ester, thioether, thioester, acetal, ketal, carbalkoxy, unsubstituted carbamide groups and carbamide groups which are substituted by one or two alkyl groups, the hydrocarbyl radicals in said functionally-modified groups having a maximum of 6 carbon atoms, and in which the carbon atom chain may be interrupted by heteroatoms which are selected from the group consisting of oxygen, sulphur and nitrogen.

13. A partial ester of hyaluronic acid according to claim 12, in which said alcohol is methyl alcohol, ethyl alcohol, propyl alcohol, isopropyl alcohol, normal butyl alcohol, isobutyl alcohol, tert-butyl alcohol, amyl alcohol,
pentyl alcohol, hexyl alcohol, octyl alcohol, ethylene glycol, propylene glycol, butylene glycol or glycerin.

14. A partial ester of hyaluronic acid according to claim 3, in which said alcohol is an araliphatic alcohol with only one benzene residue, in which the aliphatic chain has a maximum of 4 carbon atoms, and in which the benzene residue is unsubstituted or is substituted by 1 to 3 methyl groups or hydroxy groups, or by one or more halogen atoms, and in which the aliphatic chain may be substituted by one or two functions selected from the group consisting of free amino groups, or monoethylamino groups or diethylamino groups or by pyrrolidinyl or by piperidinyl groups.

15. A partial ester of hyaluronic acid according to claim 14, in which said alcohol is benzyl alcohol, phenethyl alcohol, ephedrine or adrenalin.

16. A partial ester of hyaluronic acid according to claim 3, in which said alcohol is a cycloaliphatic alcohol or an aliphatic-cycloaliphatic alcohol and derives from a monocyclic hydrocarbide or from a polycyclic hydrocarbide with a maximum of 34 carbon atoms.

17. A partial ester of hyaluronic acid according to claim 16, in which said alcohol is a polycyclic alcohol which is selected from the group consisting of sterols, chloic acids, steroid alcohols, groups of the estrane and pregnane series and their unsaturated derivatives.

18. A partial ester of hyaluronic acid according to claim 17, in which said alcohol is cortisone, hydrocortisone,
prednisone, prednisolone, fluorocortisone, dexamethasone, betamethasone, corticosterone, deoxycorticosterone, paramethasone, flumethasone, fluocinolone, fluocinolone acetonide, fluprednylidene, clobetasol, or beclomethasone.

19. A salt of a partial ester of claim 4, in which said aliphatic alcohol is substituted by one or two functional groups which are selected from the group consisting of amino, hydroxy, mercapto, aldehyde, keto, carboxyl, hydrocarbyl, dihydrocarbylamino, ether, ester, thioether, thioester, acetal, ketal, carbalkoxy, unsubstituted carbamide groups and carbamide groups which are substituted by one or two alkyl groups, the hydrocarbyl radicals in said functionally-modified groups having a maximum of 6 carbon atoms, and in which the carbon atom chain may be interrupted by heteroatoms which are selected from the group consisting of oxygen, sulphur and nitrogen.

20. A salt of a partial ester of hyaluronic acid according to claim 19, in which said alcohol is methyl alcohol, ethyl alcohol, propyl alcohol, isopropyl alcohol, normal butyl alcohol, isobutyl alcohol, tert-butyl alcohol, amyl alcohol, pentyl alcohol, hexyl alcohol, octyl alcohol, ethylene glycol, propylene glycol, butylene glycol or glycerin.

21. A salt of a partial ester of hyaluronic acid according to claim 4, in which said alcohol is an araliphatic alcohol with only one benzene residue, in which the aliphatic chain has a maximum of 4 carbon atoms, and in which the
benzene residue is unsubstituted or is substituted by 1 to 3 methyl groups, 1 to 3 hydroxy groups or by 1 to 3 halogen atoms, and in which the aliphatic chain may be substituted by one or two functions selected from the group consisting of free amino groups, monoethylamino groups, diethylamino groups pyrrolidinyl groups and piperidinyl groups.

22. A salt of a partial ester of hyaluronic acid according to claim 4, in which said alcohol is benzyl alcohol, phenethyl alcohol, ephedrine or adrenalin.

23. A salt of a partial ester of hyaluronic acid according to claim 4, in which said alcohol is a cycloaliphatic alcohol or an aliphatic-cycloaliphatic alcohol and derives from a monocyclic hydrocarbide or from a polycyclic hydrocarbide with a maximum of 34 carbon atoms.

24. A salt of a partial ester of hyaluronic acid according to claim 4, in which said alcohol is a polycyclic alcohol which is selected from the group consisting of sterols, chloic acids, steroid alcohols, groups of the estrane and pregnane series and their unsaturated derivatives.

25. A salt of a partial ester of hyaluronic acid according to claim 24, in which said alcohol is cortisone, hydrocortisone, prednisone, prednisolone, fluorocortisone, dexamethasone, betamethasone, corticosterone, deoxycorticosterone, paramethasone, flumethasone, fluocinolone, fluocinolone acetonide, fluprednylidene, clobetasol, or beclomethasone.
26. A salt of a partial ester of hyaluronic acid according to any one of claims 4, and 19 to 25, inclusive, with an alkali metal, or with an alkaline earth metal, or with magnesium, or with aluminum, or with ammonia.

27. A salt of a partial ester of hyaluronic acid according to any one of claims 4, and 19 to 25, inclusive, with a therapeutically-acceptable ammonium, aliphatic, araliphatic, cycloaliphatic or heterocyclic base.

28. A sodium salt of a partial ester of hyaluronic acid according to claim 26 or claim 27.

29. A total ester of hyaluronic acid according to any one of claims 2, and 5 to 11, inclusive, wherein said hyaluronic acid derives from hyaluronic acid which is obtained by first dehydrating cocks' combs with acetone and the exposing them to enzymatic digestion with papain.

30. A total ester of hyaluronic acid according to any one of claims 2, and 5 to 11, inclusive, wherein said hyaluronic acid has been obtained by first dehydrating cocks' combs with acetone and then exposing them to enzymatic digestion with papain, followed by molecular ultrafiltration, and further purification of the hyaluronic acid fraction so-obtained.

31. A total ester of hyaluronic acid according to any one of claims 2, and 5 to 11, inclusive, wherein said hyaluronic acid ester derives from an integral hyaluronic acid or from one of its salts which is obtained by extraction from
cocks' combs, and having a molecular weight of between 8 and 13 million.

32. A total ester of hyaluronic acid according to any one of claims 2, and 5 to 11, inclusive, wherein said hyaluronic acid ester derives from a hyaluronic acid fraction with a molecular weight of between 90 and 0.23% of the molecular weight of an integral hyaluronic acid with a molecular weight of 13 million.

33. A total ester of hyaluronic acid according to any one of claims 2, and 5 to 11, inclusive, wherein said hyaluronic acid ester derives from a hyaluronic acid molecular fraction having a molecular weight of between 50,000 and 100,000 and which is substantially free of hyaluronic acid having a molecular weight of less than 30,000.

34. A total ester of hyaluronic acid according to any one of claims 2, and 5 to 11, inclusive, wherein said hyaluronic acid ester derives from a hyaluronic acid molecular fraction having a molecular weight of between 500,000 and 730,000 and which is substantially-free of hyaluronic acid having a molecular weight of less than 30,000.

35. A partial ester of hyaluronic acid according to any one of claims 3, and 12 to 18, inclusive, wherein said hyaluronic acid derives from hyaluronic acid which is obtained by first dehydrating cocks' combs with acetone and then exposing them to enzymatic digestion with papain.

36. A partial ester of hyaluronic acid according to any one of claims 3, and 12 to 18, inclusive, wherein said
hyaluronic acid has been obtained by first dehydrating cocks' combs with acetone and then exposing them to enzymatic digestion with papain, followed by molecular ultrafiltration, and further purification of the hyaluronic acid fraction so-obtained.

37. A partial ester of hyaluronic acid according to any one of claims 3, and 12 to 18, inclusive, wherein said hyaluronic acid ester derives from an integral hyaluronic acid or from one of its salts which is obtained by extraction from cocks' combs, and having a molecular weight of between 8 and 13 million.

38. A partial ester of hyaluronic acid according to any one of claims 3, and 12 to 18, inclusive, wherein said hyaluronic acid ester derives from a hyaluronic acid fraction with a molecular weight of between 90 and 0.23% of the molecular weight of an integral hyaluronic acid with a molecular weight of 13 million.

39. A partial ester of hyaluronic acid according to any one of claims 3, and 12 to 18, inclusive, wherein said hyaluronic acid ester derives from a hyaluronic acid molecular fraction having a molecular weight of between 50,000 and 100,000, and which is substantially-free of hyaluronic acid having a molecular weight of less than 30,000.

40. A partial ester of hyaluronic acid according to any one of claims 3, and 12 to 18, inclusive, wherein said hyaluronic acid ester derives from a hyaluronic acid molecular fraction having a molecular weight of between 500,000 and
730,000, and which is substantially-free of hyaluronic acid having a molecular weight of less than 30,000.

41. A salt of a partial ester of hyaluronic acid according to any one of claims 4, and 19 to 28, inclusive, wherein said hyaluronic acid derives from hyaluronic acid which is obtained by first dehydrating cocks' combs with acetone and then exposing them to enzymatic digestion with papain.

42. A salt of a partial ester of hyaluronic acid according to any one of claims 4, and 19 to 28, inclusive, wherein said hyaluronic acid derives from hyaluronic acid which is obtained by first dehydrating cocks' combs with acetone and then exposing them to enzymatic digestion with papain, followed by molecular ultrafiltration, and further purification of the hyaluronic acid fraction so-obtained.

43. A salt of a partial ester of hyaluronic acid according to any one of claims 4, and 19 to 28, inclusive, wherein said hyaluronic acid ester derives from an integral hyaluronic acid or from one of its salts which is obtained by extraction from cocks' combs, and having a molecular weight of between 8 and 13 million.

44. A salt of a partial ester of hyaluronic acid according to any one of claims 4, and 19 to 28, inclusive, wherein, in said hyaluronic acid ester derives from a hyaluronic acid fraction with a molecular weight of between 90 and 0.23% of the molecular weight of an integral hyaluronic acid with a molecular weight of 13 million.
45. A salt of a partial ester of hyaluronic acid according to any one of claims 4, and 19 to 28, inclusive, wherein said hyaluronic acid ester derives from a molecular fraction having a molecular weight of between 50,000 and 100,000, and which is substantially free of hyaluronic acid having a molecular weight of less than 30,000.

46. A salt of a partial ester of hyaluronic acid according to any one of claims 4, and 19 to 28, inclusive, wherein, said hyaluronic acid ester derives from a hyaluronic acid molecular fraction having a molecular weight of between 500,000 and 730,000, and which is substantially-free of hyaluronic acid having a molecular weight of less than 30,000.

47. The total ethyl ester of hyaluronic acid.

48. The total propyl ester of hyaluronic acid.

49. The total pentyl ester of hyaluronic acid.

50. The total isopentyl ester of hyaluronic acid.

51. The total benzyl ester of hyaluronic acid.

52. The total phenethyl ester of hyaluronic acid.

53. The mixed ethanol-cortisone ester of hyaluronic acid with 80% of the carboxyl groups esterified with ethanol and with 20% of the carboxyl groups esterified with cortisone.

54. The mixed ethanol-hydrocortisone ester of hyaluronic acid with 80% of the carboxyl groups esterified with ethanol and with 20% of the carboxyl groups esterified with hydrocortisone.

55. The mixed ethanol-fluorocortisone ester of hyaluronic acid with 80% of the carboxyl groups esterified
with ethanol and with 20% of the carboxyl groups esterified with fluorocortisone.

56. The mixed ethanol-deoxycorticosterone ester of hyaluronic acid with 80% of the carboxyl groups esterified with ethanol and with 20% of the carboxyl groups esterified with deoxycorticosterone.

57. A salt of a partial propyl ester of hyaluronic acid with 50% of the carboxyl groups esterified and with 50% of the carboxyl groups salified with sodium.

58. A salt of a partial isopropyl ester of hyaluronic acid with 50% of the carboxyl groups esterified and with 50% of the carboxyl groups salified with sodium.

59. A salt of a partial propyl ester of hyaluronic acid with 85% of the carboxylic groups esterified and with 15% of the carboxylic groups salified with sodium.

60. A salt of a partial ethyl ester of hyaluronic acid with 75% of the carboxyl groups esterified and with 25% of the carboxyl groups salified with sodium.

61. A salt of a partial methyl ester of hyaluronic acid with 75% of the carboxyl groups esterified and with 25% of the carboxyl groups salified with sodium.

62. A salt of a partial butyl ester of hyaluronic acid with 50% of the carboxyl groups salified with sodium.

63. A salt of the partial ethoxycarbonylmethyl ester of hyaluronic acid with 75% of the carboxyl groups esterified and with 25% of the carboxyl groups salified with sodium.
64. A salt of a partial cortisone ester of hyaluronic acid with 20% of the carboxyl groups esterified and with 80% of the carboxyl groups salified with sodium.

65. A salt of a partial hydrocortisone ester of hyaluronic acid with 20% of the carboxyl groups esterified and with 80% of the carboxyl groups salified with sodium.

66. A salt of the partial fluorocortisone ester of hyaluronic acid with 20% of the carboxyl groups esterified and with 80% of the carboxyl groups salified with sodium.

67. A salt of a deoxycorticosterone ester of hyaluronic acid with 20% of the carboxyl groups esterified and with 80% of the carboxyl groups salified with sodium.

68. A salt of a partial and mixed ethanol-deoxycorticosterone ester of hyaluronic acid with 40% of the carboxyl groups esterified with ethanol, with 20% of the carboxyl groups esterified with deoxycorticosterone and with the remaining 40% of the carboxyl groups salified with sodium.

69. A salt of a partial and mixed ethanol-cortisone ester of hyaluronic acid with 40% of the carboxyl groups esterified with ethanol, with 20% of the carboxyl groups esterified with cortisone, and with the remaining 40% of the carboxyl groups salified with sodium.

70. A salt of a partial and mixed ethanol-hydrocortisone ester of hyaluronic acid with 40% of the carboxyl groups esterified with ethanol, with 20% of the carboxyl groups esterified with hydrocortisone, and with the remaining 40% of the carboxyl groups salified with sodium.
71. A salt of a partial and mixed ethanol-fluorocortisone ester of hyaluronic acid with 40% of the carboxyl groups esterified with ethanol, with 20% of the carboxyl groups esterified with fluorocortisone, and with the remaining 40% of the carboxyl groups salified with sodium.

72. A salt of a hyaluronic acid ester of streptomycin with 75% of the carboxyl groups esterified with ethanol and with 25% of the carboxyls salified with streptomycin.

73. A salt of a hyaluronic acid ester of erythromycin with 75% of the carboxyl groups esterified with ethanol and with 25% of the carboxyls salified with erythromycin.

74. A salt of a hyaluronic acid ester of neomycin with 75% of the carboxyl groups esterified with ethanol and with 25% of the carboxyls salified with neomycin.

75. A salt of a hyaluronic acid ester of gentamycin with 75% of the carboxyl groups esterified with ethanol and with 25% of the carboxyls salified with gentamycin.

76. A salt of a hyaluronic acid ester of kanamycin with 75% of the carboxyl groups esterified with ethanol and with 25% of the carboxyls salified with kanamycin.

77. A salt of a hyaluronic acid ester of pilocarpine with 75% of the carboxyl groups esterified with ethanol and with 25% of the carboxyls salified with pilocarpine.

78. A salt of a hyaluronic acid ester of pilocarpine with 85% of the carboxyl groups esterified with ethanol and with 15% of the carboxyls salified with pilocarpine.
79. The hyaluronic acid ester of amikacin with 75% of the carboxyl groups esterified with ethanol and with 25% of the carboxyls salified with amikacin.

80. A process for the preparation of an ester of hyaluronic acid of the Formula

\[ \text{Hy(COOR)}_n \]

wherein COO represents the carboxylic acid moiety on the hyaluronic acid (Hy) molecule, R is derived from an alcohol which is selected from the group consisting of an aliphatic alcohol having a chain of 2 to 34 carbons, an araliphatic alcohol, a cycloaliphatic alcohol and a heterocyclic alcohol, and n is a large number, which process comprises:

(I) providing a total ester of hyaluronic acid with said alcohol by carrying out one of the following esterification reactions:

(a) treating free hyaluronic acid with a sufficient amount of a selected alcohol as defined above in the presence of a catalyzing substance; or

(b) treating free hyaluronic acid with a sufficient amount of an etherifying agent which is capable of introducing the desired alcoholic residue of an alcohol, as defined above, in the presence of an inorganic base or an organic base; or

(c) treating a metal salt of hyaluronic acid or an organic azotized base of hyaluronic acid with a sufficient amount of a selected alcohol, as defined
above, in the presence of a catalyzing substance; or
(d) treating a quaternary ammonium salt of hyaluronic acid with a sufficient amount of an etherifying agent capable of introducing the desired alcoholic residue of an alcohol, as defined above, in the presence of an inorganic base or an organic base;

or (II) providing a partial ester of hyaluronic acid with said alcohol, by carrying out one of the following reactions:
(a) treating free hyaluronic acid with an insufficient amount of a selected alcohol as defined above in the presence of a catalyzing substance; or
(b) treating free hyaluronic acid with an insufficient amount of an etherifying agent which is capable of introducing the desired alcoholic residue of an alcohol, as defined above, in the presence of an inorganic base or an organic base; or
(c) treating a metal salt of hyaluronic acid or an organic azotized base of hyaluronic acid with an insufficient amount of a selected alcohol, as defined above, in the presence of a catalyzing substance; or
(d) treating a quaternary ammonium salt of hyaluronic acid with an insufficient amount of an
etherifying agent which is capable of introducing the desired alcoholic residue of an alcohol, as defined above, in the presence of an inorganic base or an organic base;

or (III) providing a salt of a partial ester of hyaluronic acid with said alcohol, by carrying out one of the following reactions:

(a) treating free hyaluronic acid with an insufficient amount of a selected alcohol, as defined above, in the presence of a catalyzing substance; or

(b) treating free hyaluronic acid with an insufficient amount of an etherifying agent which is capable of introducing the desired alcoholic residue of an alcohol, as defined above, in the presence of an inorganic base or an organic base; or

(c) treating a metal salt of hyaluronic acid or an organic azotized base of hyaluronic acid with an insufficient amount of a selected alcohol, as defined above, in the presence of a catalyzing substance; or

(d) treating a quaternary ammonium salt of hyaluronic acid with an insufficient amount of an etherifying agent which is capable of introducing the desired alcoholic residue of an alcohol, as
defined above, in the presence of an inorganic base or an organic base;
and then salifying the remaining carboxylic groups, or part of
said carboxylic groups, in the partial ester so formed with an
inorganic base or with an organic base.

81. The process of claim 80 (I)(a), claim 80 (II)(a), or
claim 80 (iii)(a), wherein said catalyzing substance is a
strong inorganic acid or an ionic exchanger of the acid type.

82. The process of claim 80 (I)(b), claim 80 (II)(b), or
claim 80 (III)(b), wherein said etherifying agent is selected
from the group consisting of an ester of an inorganic acid, an
ester of an organic sulphonic acid, a hydracid, a hydrocarbaryl
halogenide, a neutral sulphate, a hydrocarboxyl acid, an
alfite, a carbonate, a silicate, a phosphite, and a
hydrocarbaryl sulphonate.

83. The process of claim 80 (I)(b), claim 80 (II)(b), or
claim 80 (iii)(b), which takes place in a suitable solvent
which is selected from the group consisting of a polar
solvent, a non-polar solvent and an aprotic solvent.

84. The process of claim 80 (I)(d), claim 80 (II)(d), or
claim 80 (III)(d), which takes place in a solvent which is
selected from the group consisting of a dialkylsulphoxide, a
dialkylcarboxamide, a lower alkyl dialkylamide of a lower
aliphatic acid, an alcohol, an ether, a ketone, and an ester.

85. The process of claim 80 (I)(b), claim 80 (II)(b), or
claim 80 (III)(b), wherein said base is a hydrate of an alkali
metal, a hydrate of an alkaline earth metal, magnesium oxide,
silver oxide, a basic salt of an alkali metal, of an alkaline earth metal, of magnesium or of silver, or a carbonate of an alkali metal, of an alkaline earth metal, of magnesium or of silver, an organic base, a tertiary azotized base, or an ionic exchanger of the basic type.

86. The process of claim 80 (I)(c), claim 80 (II)(c), or claim 80 (III)(c), wherein said metal salt is a salt of an alkali metal or a salt of an alkaline earth metal.

87. The process of claim 80 (I)(c), claim 80 (II)(c), or claim 80 (III)(c), wherein said organic azotized base is an ammonium salt or an ammonium-substituted salt.

88. The process of claim 80 (I)(d), claim 80 (II)(d), or claim 80 (III)(d), which is carried out at temperature of 0°C - 100°C.

89. The process of any one of claims 80 to 88, inclusive, wherein said alcohol is an aliphatic alcohol which is substituted by one or two functional groups which are selected from the group consisting of amino, hydroxy, mercapto, aldehyde, keto, carboxyl, hydrocarbyl, dihydrocarbylamino, ether, ester, thioether, thioester, acetal, ketal, carbalkoxy, unsubstituted carbamide groups and carbamide groups which are substituted by one or two alkyl groups, the hydrocarbyl radicals in said functionally-modified groups having a maximum of 6 carbon atoms, and in which the carbon atom chain may be interrupted by heteroatoms which are selected from the group consisting of oxygen, sulphur and nitrogen.
90. The process of claim 89, wherein said alcohol is ethyl alcohol, propyl alcohol, isopropyl alcohol, normal butyl alcohol, isobutyl alcohol, tert-butyl alcohol, amyl alcohol, pentyl alcohol, hexyl alcohol, octyl alcohol, ethylene glycol, propylene glycol, butylene glycol or glycerin.

91. The process of any one of claims 80 to 88, inclusive, wherein said alcohol is an araliphatic alcohol with only one benzene residue, in which the aliphatic chain has a maximum of 4 carbon atoms, and in which the benzene residue may be substituted by 1 to 3 methyl groups, 1 to 3 hydroxy groups or 1 to 3 halogen atoms, and in which the aliphatic chain may be substituted by one or two functional groups which are selected from the group consisting of free amino groups, monoethylamino groups, diethylamino groups, pyrrolidinyl groups and piperidinyl groups.

92. The process of claim 91, wherein said alcohol is benzyl alcohol, phenethyl alcohol, ephedrine or adrenalin.

93. The process of any one of claims 80 to 88, inclusive, wherein said alcohol is a cycloaliphatic alcohol or an aliphatic-cycloaliphatic alcohol and derives from a monocyclic hydrocarbide or from a polycyclic hydrocarbide with a maximum of 34 carbon atoms.

94. The process of any one of claims 80 to 88, inclusive, wherein said alcohol is a polycyclic alcohol which is selected from the group consisting of sterols, colic acids, steroid alcohols, groups of the estrane and pregnane series and their unsaturated derivatives.
95. The process of claim 94, wherein said alcohol is cortisone, hydrocortisone, prednisone, prednisolone, fluorocortisone, dexamethasone, betamethasone, corticosterone, deoxycorticosterone, paramethasone, flumethasone, fluocinolone, fluocinolone acetonide, fluprednylidene, clobetasol, or beclomethasone.

96. The process of claim 80 (III), wherein said salt is formed with an alkali metal, with an alkaline earth metal, with magnesium, with aluminum, or with ammonia.

97. The process of claim 80 (III), wherein said salt is formed with a therapeutically-acceptable ammonium base, aliphatic base, araliphatic base, cycloaliphatic base or heterocyclic base.

98. The process of claim 96 or claim 97, wherein said salt is formed with sodium or with ammonia.

99. The process for preparing an ester of hyaluronic acid according to claim 80, which process comprises: reacting hyaluronic acid with ethyl alcohol.

100. The process for preparing an ester of hyaluronic acid according to claim 80, which process comprises: reacting hyaluronic acid with n-propyl alcohol.

101. The process for preparing an ester of hyaluronic acid according to claim 80, which process comprises: reacting the tetrabutylammonium salt of hyaluronic acid with n-pentyl bromide.

102. The process for preparing an ester of hyaluronic acid according to claim 80, which process comprises: reacting
the tetrabutylammonium salt of hyaluronic acid with isopentyl bromide.

103. The process for preparing an ester of hyaluronic acid according to claim 80, which process comprises: reacting the tetrabutylammonium salt of hyaluronic acid with benzyl bromide.

104. The process for preparing an ester of hyaluronic acid according to claim 80, which process comprises: reacting the tetrabutylammonium salt of hyaluronic acid with 2-bromoethylbenzene.

105. The process for preparing an ester of hyaluronic acid according to claim 80, which process comprises: reacting the tetrabutylammonium salt of hyaluronic acid with ethyl iodide and with 21-bromo-4-pregnene-17α-ol-3,11,20-trione, whereby 80% of the carboxyl groups are esterified with ethanol and whereby 20% of the carboxyl groups are esterified with cortisone.

106. The process for preparing an ester of hyaluronic acid according to claim 80, which process comprises: reacting the tetrabutylammonium salt of hyaluronic acid with ethyl iodide and with 21-bromo-4-pregnene-11β,17α-diol-3,20-dione, and then salifying with sodium ions, whereby 80% of the carboxyl groups are esterified with ethanol and whereby 20% of the carboxyl groups are esterified with hydrocortisone; or

107. The process for preparing an ester of hyaluronic acid according to claim 80, which process comprises: reacting the tetrabutylammonium salt of hyaluronic acid with ethyl
iodide and with 9β-fluoro-21-bromo-4-pregnene-11β,17α-diol-3,20-dione, and salifying with sodium ions, whereby 80% of the carboxyl groups are esterified with ethanol and whereby 20% of the carboxyl groups are esterified with fluorocortisone.

108. The process for preparing an ester of hyaluronic acid according to claim 80, which process comprises: reacting hyaluronic acid with propyl alcohol and then salifying with sodium ions, whereby 50% of the carboxyl groups are esterified and whereby 50% of the carboxyl groups are salified with sodium.

109. The process for preparing an ester of hyaluronic acid according to claim 80, which process comprises: reacting hyaluronic acid with isopropyl alcohol and then salifying with sodium ions, whereby 50% of the carboxyl groups are esterified and whereby 50% of the carboxyl groups are salified with sodium.

110. The process for preparing an ester of hyaluronic acid according to claim 80, which process comprises: reacting hyaluronic acid with n-propyl alcohol and then salifying with sodium ions, whereby 85% of the carboxylic groups are esterified and whereby 15% of the carboxylic groups are salified with sodium.

111. The process for preparing an ester of hyaluronic acid according to claim 80, which process comprises: reacting hyaluronic acid with ethyl alcohol and then salifying with sodium ions, whereby 75% of the carboxyl groups are esterified
and whereby 25% of the carboxyl groups are salified with sodium.

112. The process for preparing an ester of hyaluronic acid according to claim 80, which process comprises: reacting hyaluronic acid with n-butyl alcohol and then salifying with sodium ions, whereby 50% of the carboxylic groups are salified with sodium.

113. The process for preparing an ester of hyaluronic acid according to claim 80, which process comprises: reacting the tetrabutylammonium salt of hyaluronic acid with ethyl chloroacetate and then salifying with sodium ions, whereby 75% of the carboxyl groups are esterified and whereby 25% of the carboxyl groups are salified with sodium.

114. The process for preparing an ester of hyaluronic acid according to claim 80, which process comprises: reacting the tetrabutylammonium salt of hyaluronic acid with 21-bromo-4-pregnene-17α-ol-3,11,20-trione and then salifying with sodium ions, whereby 20% of the carboxyl groups are esterified and whereby 80% of the carboxyl groups are salified with sodium.

115. The process for preparing an ester of hyaluronic acid according to claim 80, which process comprises: reacting the tetrabutylammonium salt of hyaluronic acid with 21-bromo-4-pregnene-11β,17α-diol-3,20-dione and then salifying with sodium ions, whereby 20% of the carboxyl groups are esterified and whereby 80% of the carboxyl groups are salified with sodium.
116. The process for preparing an ester of hyaluronic acid according to claim 80, which process comprises: reacting the tetrabutylammonium salt of hyaluronic acid with 9-fluoro-21-bromo-4-pregnene-11β,17α-diol-3,20-dione and then salifying with sodium ions, whereby 20% of the carboxyl groups are esterified and whereby 80% of the carboxyl groups are salified with sodium.

117. The process for preparing an ester of hyaluronic acid according to claim 80, which process comprises: reacting the tetrabutylammonium salt of hyaluronic acid with 21-bromo-4-pregnene-3,20-dione and then salifying with sodium ions, whereby 20% of the carboxyl groups are esterified and whereby 80% of the carboxyl groups are salified with sodium.

118. The process for preparing an ester of hyaluronic acid according to claim 80, which process comprises: reacting the tetrabutylammonium salt of hyaluronic acid with ethyl iodide and with 21-bromo-4-pregnene-3,20-dione, and then salifying with sodium ions, whereby 80% of the carboxyl groups are esterified with ethanol and whereby 20% of the carboxyl groups are esterified with deoxycorticosterone and whereby the remaining 40% of the carboxyl groups are salified with sodium.

119. The process for preparing an ester of hyaluronic acid according to claim 80, which process comprises: reacting the tetrabutylammonium salt of hyaluronic acid with ethyl iodide and with 21-bromo-4-pregnene-3,20-dione, and then salifying with sodium ions, whereby 40% of the carboxyl groups are esterified with ethanol, whereby 20% of the carboxyl
groups are esterified with deoxycorticosterone, and whereby the remaining 40% of the carboxyl groups are salified with sodium.

120. The process for preparing an ester of hyaluronic acid according to claim 80, which process comprises: reacting the tetrabutylammonium salt of hyaluronic acid with ethyl iodide and with 21-bromo-4-pregnene-17α-ol-3,11,20-trione, and then salifying with sodium ions, whereby 40% of the carboxyl groups esterified with ethanol, whereby 20% of the carboxyl groups are esterified with cortisone and whereby the remaining 40% of the carboxyl groups are salified with sodium.

121. The process for preparing an ester of hyaluronic acid according to claim 80, which process comprises: reacting the tetrabutylammonium salt of hyaluronic acid with ethyl iodide and with 21-bromo-4-pregnene-11β,17α-diol-3,20-dione, and then salifying with sodium ions, whereby 40% of the carboxyl groups are esterified with ethanol, whereby 20% of the carboxyl groups are esterified with hydrocortisone and whereby the remaining 40% of the carboxyl groups are salified with sodium.

122. The process for preparing an ester of hyaluronic acid according to claim 80, which process comprises: reacting the tetrabutylammonium salt of hyaluronic acid with ethyl iodide and with 9β-fluoro-21-bromo-4-pregnene-11β,17α-diol-3,20-dione, and then salifying with sodium ions, whereby 40% of the carboxyl groups are esterified with ethanol, whereby 20% of the carboxyl groups are esterified with fluorocortisone
and whereby the remaining 40% of the carboxyl groups are salified with sodium.

123. The process for preparing an ester of hyaluronic acid according to claim 80, which process comprises: reacting the tetrabutylammonium salt of hyaluronic acid with ethyl iodide and with 9β-fluoro-21-bromo-4-pregnene-11β,17α-diol-3,20-dione, and then salifying with sodium ions, whereby 40% of the carboxyl groups are esterified with ethanol, whereby 20% of the carboxyl groups are esterified with fluorocortisone and whereby the remaining 40% of the carboxyl groups are salified with sodium.

124. The process for preparing an ester of hyaluronic acid according to claim 80, which process comprises: reacting the 75% ethyl ester/25% sodium salt of hyaluronic acid with streptomycin, whereby 75% of the carboxyls are esterified with ethanol and whereby 25% of the carboxyls are salified with streptomycin.

125. The process for preparing an ester of hyaluronic acid according to claim 80, which process comprises: reacting the 75% ethyl ester/25% sodium salt of hyaluronic acid with erythromycin base, whereby 75% of the carboxyls are esterified with ethanol and whereby 25% of the carboxyls are salified with erythromycin.

126. The process for preparing an ester of hyaluronic acid according to claim 80, which process comprises: reacting the 75% ethyl ester/25% sodium salt of hyaluronic acid with neomycin, whereby 75% of the carboxyls are esterified with
ethanol and whereby 25% of the carboxyls are salified with neomycin.

127. The process for preparing an ester of hyaluronic acid according to claim 80, which process comprises: reacting the 75% ethyl ester/25% sodium salt of hyaluronic acid with gentamycin, whereby 75% of the carboxyls are esterified with ethanol and whereby 25% of the carboxyls are salified with gentamycin.

128. The process for preparing an ester of hyaluronic acid according to claim 80, which process comprises: reacting the 75% ethyl ester/25% sodium salt of hyaluronic acid with amikacin, whereby 75% of the carboxyls are esterified with ethanol and whereby 25% of the carboxyls are salified with amikacin.

129. The process for preparing an ester of hyaluronic acid according to claim 80, which process comprises: reacting the 75% ethyl ester/25% sodium salt of the carboxyls are esterified with ethanol and whereby 25% of the carboxyls are salified with Kanamycin.

130. The process for preparing an ester of hyaluronic acid according to claim 80, which process comprises: reacting the 75% ethyl ester/25% sodium salt of hyaluronic acid with pilocarpine, whereby 75% of the carboxyls are esterified with ethanol and whereby 25% of the carboxyls are salified with pilocarpine.

131. The process for preparing an ester of hyaluronic acid according to claim 80, which process comprises: reacting
an 85% propyl ester/15% tetrabutylammonium salt of hyaluronic acid with pilocarpine, whereby 85% of the carboxyls are esterified with propanol and whereby 15% of the carboxyls are salified with pilocarpine.

132. The process of any one of claims 80 to 131, inclusive, wherein said hyaluronic acid derives from hyaluronic acid which is obtained by first dehydrating cocks' combs with acetone and then exposing them to enzymatic digestion with papain.

133. The process of any one of claims 80 to 131, inclusive, wherein said hyaluronic acid has been obtained by first dehydrating cocks' combs with acetone and then exposing them to enzymatic digestion with papain, followed by molecular ultrafiltration, and further purification of the hyaluronic acid fraction so-obtained.

134. The process of any one of claims 80 to 131, inclusive, wherein said hyaluronic acid ester derives from integral hyaluronic acid or from one of its salts obtained by extraction from cocks' combs and having a molecular weight of between 8 and 13 million.

135. The process of any one of claims 80 to 131, inclusive, wherein said hyaluronic acid ester derives from a hyaluronic acid fraction with a molecular weight of between 90 and 0.23% of the molecular weight of an integral hyaluronic acid having a molecular weight of 13 million.

136. The process of any one of claims 80 to 131, inclusive, wherein said hyaluronic acid ester derives from a
molecular fraction having a molecular weight of between 50,000 and 100,000 and which is substantially-free of hyaluronic acid having a molecular weight of less than 30,000.

137. The process of any one of claims 80 to 131, inclusive, wherein said hyaluronic acid ester derives from a molecular fraction having a molecular weight of between 500,000 and 730,000 and which is substantially-free of hyaluronic acid having a molecular weight of less than 30,000.

138. The process of any one of claims 80 to 132 and 134 to 137, inclusive, including the steps of: molecular ultrafiltration; and further purification of the hyaluronic acid fraction obtained.

139. A pharmaceutical preparation comprising: an effective amount of at least one ester of hyaluronic acid of the Formula

\[
\text{Hy(COOR)}_n
\]

wherein COO represents the carboxylic acid moiety on the hyaluronic acid (Hy) molecule, R is derived from an alcohol which is selected from the group consisting of an aliphatic alcohol having a chain of 2 to 34 carbons, an araliphatic alcohol, a cycloaliphatic alcohol and a heterocyclic alcohol, and \( n \) is a large number; and a pharmaceutically-acceptable carrier.

140. A pharmaceutical preparation comprising: an effective amount of at least one total ester of hyaluronic acid of the Formula

\[
\text{Hy(COOR)}_n
\]
wherein COO represents the carboxylic acid moiety on the hyaluronic acid (Hy) molecule, R is derived from an alcohol which is selected from the group consisting of an aliphatic alcohol having a chain of 2 to 34 carbons, an araliphatic alcohol, a cycloaliphatic alcohol and a heterocyclic alcohol, and n is a large number; and a pharmaceutically-acceptable carrier.

141. A pharmaceutical preparation comprising: an effective amount of at least one partial ester of hyaluronic acid of the Formula

$$\text{Hy(COOR)}_n$$

wherein COO represents the carboxylic acid moiety on the hyaluronic acid (Hy) molecule, R is derived from an alcohol which is selected from the group consisting of an aliphatic alcohol having a chain of 2 to 34 carbons, an araliphatic alcohol, a cycloaliphatic alcohol and a heterocyclic alcohol, and n is a large number; and a pharmaceutically-acceptable carrier.

142. A pharmaceutical preparation comprising: an effective amount of at least one salt of a partial ester of hyaluronic acid of the Formula

$$\text{Hy(COOR)}_n$$

wherein COO represents the carboxylic acid moiety on the hyaluronic acid (Hy) molecule, R is derived from an alcohol which is selected from the group consisting of an aliphatic alcohol having a chain of 2 to 34 carbons, an araliphatic alcohol, a cycloaliphatic alcohol and a heterocyclic alcohol,
and $n$ is a large number; and a pharmaceutically-acceptable carrier.

143. The pharmaceutical preparation of any one of claims 140 to 142, inclusive, wherein, in said ester of hyaluronic acid, said alcohol is an aliphatic alcohol which is substituted by one or two functional groups which are selected from the group consisting of amino, hydroxy, mercapto, aldehyde, keto, carboxyl, hydrocarbonyl, dihydrocarbonylamino, ether, ester, thioether, thioester, acetal, ketal, carbalkoxy, unsubstituted carbamide groups and carbamide groups which are substituted by one or two alkyl groups, the hydrocarbonyl radicals in said functionally-modified groups having a maximum of 6 carbon atoms, and in which the carbon atom chain may be interrupted by heteroatoms which are selected from the group consisting of oxygen, sulphur and nitrogen.

144. The pharmaceutical preparation of claim 143, wherein said alcohol is ethyl alcohol, propyl alcohol, isopropyl alcohol, normal butyl alcohol, isobutyl alcohol, tert-butyl alcohol, amyl alcohol, pentyl alcohol, hexyl alcohol, octyl alcohol, ethylene glycol, propylene glycol, butylene glycol or glycerin.

145. The pharmaceutical preparation of any one of claims 140 to 142, inclusive, wherein, in said ester of hyaluronic acid, said alcohol is an araliphatic alcohol with only one benzene residue, in which the aliphatic chain has a maximum of 4 carbon atoms, and in which the benzene residue may be substituted by 1 to 3 methyl groups, 1 to 3 hydroxy groups, or
1 to 3 halogen atoms, and in which the aliphatic chain may be substituted by one or two functions which are selected from the group consisting of free amino groups, monoethylamino groups, diethylamino groups, pyrrolidinyl groups and piperidinyl groups.

146. The pharmaceutical preparation of claim 145, wherein said alcohol is benzyl alcohol, phenethyl alcohol, ephedrine or adrenalin.

147. The pharmaceutical preparation of any one of claims 140 to 142, inclusive, wherein, in said ester of hyaluronic acid, said alcohol is a cycloaliphatic alcohol or an aliphatic-cycloaliphatic alcohol and derives from a monocyclic hydrocarbide or a polycyclic hydrocarbide with a maximum of 34 carbon atoms.

148. The pharmaceutical preparation of claim 147, wherein said alcohol is a polycyclic alcohol selected from the group consisting of sterols, colic acids, steroid alcohols, and groups of the estrane and pregnane series and their unsaturated derivative.

149. The pharmaceutical preparation of claim 148, wherein said alcohol is cortisone, hydrocortisone, prednisone, prednisolone, fluorocortisone, dexamethasone, betamethasone, corticosterone, deoxycorticosterone, paramethasone, flumethasone, fluocinolone, fluocinolone acetonide, fluprednylidene, clobetasol, and beclomethasone.

150. The pharmaceutical preparation of claim 142, wherein said salt is a salt with an alkali metal, or with an
alkaline earth metal, or with magnesium, or with aluminum, or with ammonia.

151. The pharmaceutical preparation of claim 150, wherein said salt is a sodium salt of hyaluronic acid.

152. A pharmaceutical preparation comprising: an effective amount of the salt of the partial propyl ester of hyaluronic acid with 50% of the carboxyl groups esterified and with 50% of the carboxyl groups salified with sodium; and a pharmaceutically-acceptable carrier.

153. A pharmaceutical preparation comprising: an effective amount of the salt of the partial isopropyl ester of hyaluronic acid with 50% of the carboxyl groups esterified and with 50% of the carboxyl groups salified with sodium; and a pharmaceutically-acceptable carrier.

154. A pharmaceutical preparation comprising: an effective amount of the salt of the partial propyl ester of hyaluronic acid with 85% of the carboxylic groups esterified and with 15% of the carboxylic groups salified with sodium; and a pharmaceutically-acceptable carrier.

155. A pharmaceutical preparation comprising: an effective amount of the salt of the partial ethyl ester of hyaluronic acid with 75% of the carboxyl groups esterified and with 25% of the carboxyl groups salified with sodium; and a pharmaceutically-acceptable carrier.

156. A pharmaceutical preparation comprising: an effective amount of the salt of the partial methyl ester of hyaluronic acid with 75% of the carboxyl groups esterified and
with 25% of the carboxyl groups salified with sodium; and a pharmaceutically-acceptable carrier.

157. A pharmaceutical preparation comprising: an effective amount of the total ethyl ester of hyaluronic acid; and a pharmaceutically-acceptable carrier.

158. A pharmaceutical preparation comprising: an effective amount of the total propyl ester of hyaluronic acid; and a pharmaceutically-acceptable carrier.

159. A pharmaceutical preparation comprising: an effective amount of the salt of the partial butyl ester of hyaluronic acid with 50% of the carboxylic groups salified with sodium; and a pharmaceutically-acceptable carrier.

160. A pharmaceutical preparation comprising: an effective amount of the salt of the partial ethoxycarbonylmethyl ester of hyaluronic acid with 75% of the carboxyl groups esterified and with 25% of the carboxyl groups salified with sodium; and a pharmaceutically-acceptable carrier.

161. A pharmaceutical preparation comprising: an effective amount of the salt of the partial cortisone ester of hyaluronic acid with 20% of the carboxyl groups esterified and with 80% of the carboxyl groups salified with sodium; and a pharmaceutically-acceptable carrier.

162. A pharmaceutical preparation comprising: an effective amount of the salt of the partial hydrocortisone ester of hyaluronic acid with 20% of the carboxyl groups esterified and with 80% of the carboxyl groups salified with sodium; and a pharmaceutically-acceptable carrier.
163. A pharmaceutical preparation comprising: an effective amount of the salt of the partial fluorocortisone ester of hyaluronic acid with 20% of the carboxyl groups esterified and with 80% of the carboxyl groups salified with sodium; and a pharmaceutically-acceptable carrier.

164. A pharmaceutical preparation comprising: an effective amount of the salt of the deoxycorticosterone ester of hyaluronic acid with 20% of the carboxyl groups esterified and with 80% of the carboxyl groups salified with sodium; and a pharmaceutically-acceptable carrier.

165. A pharmaceutical preparation comprising: an effective amount of the mixed ethanol-cortisone ester of hyaluronic acid with 80% of the carboxyl groups esterified with ethanol and with 20% of the carboxyl groups esterified with cortisone; and a pharmaceutically-acceptable carrier.

166. A pharmaceutical preparation comprising: an effective amount of the mixed ethanol-hydrocortisone ester of hyaluronic acid with 80% of the carboxyl groups esterified with ethanol and with 20% of the carboxyl groups esterified with hydrocortisone; and a pharmaceutically-acceptable carrier.

167. A pharmaceutical preparation comprising: an effective amount of the mixed ethanol-fluorocortisone ester of hyaluronic acid with 80% of the carboxyl groups esterified with ethanol and with 20% of the carboxyl groups esterified with fluorocortisone; and a pharmaceutically-acceptable carrier.
168. A pharmaceutical preparation comprising: an effective amount of the mixed ethanol-deoxycorticosterone ester of hyaluronic acid with 80% of the carboxyl groups esterified with ethanol and with 20% of the carboxyl groups esterified with fluorocortisone; and a pharmaceutically-acceptable carrier.

169. A pharmaceutical preparation comprising: an effective amount of the salt of the partial and mixed ethanol-deoxycorticosterone ester of hyaluronic acid with 40% of the carboxyl groups esterified with ethanol, with 20% of the carboxyl groups esterified with deoxycorticosterone and with the remaining 40% of the carboxyl groups salified with sodium; and a pharmaceutically-acceptable carrier.

170. A pharmaceutical preparation comprising: an effective amount of the salt of the partial and mixed ethanol-cortisone ester of hyaluronic acid with 40% of the carboxyl groups esterified with ethanol, with 20% of the carboxyl groups esterified with cortisone, and with the remaining 40% of the carboxyl groups salified with sodium; and a pharmaceutically-acceptable carrier.

171. A pharmaceutical preparation comprising: an effective amount of the salt of the partial and mixed ethanol-hydrocortisone ester of hyaluronic acid with 40% of the carboxyl groups esterified with ethanol, with 20% of the carboxyl groups esterified with hydrocortisone, and with the remaining 40% of the carboxyl groups salified with sodium; and a pharmaceutically-acceptable carrier.
172. A pharmaceutical preparation comprising: an effective amount of the salt of the partial and mixed ethanol-fluorocortisone ester of hyaluronic acid with 40% of the carboxyl groups esterified with ethanol, with 20% of the carboxyl groups esterified with fluorocortisone, and with the remaining 40% of the carboxyl groups salified with sodium; and a pharmaceutically-acceptable carrier.

173. A pharmaceutical preparation comprising: an effective amount of the total pentyl ester of hyaluronic acid; and a pharmaceutically-acceptable carrier.

174. A pharmaceutical preparation comprising: an effective amount of the total isopentyl ester of hyaluronic acid; and a pharmaceutically-acceptable carrier.

175. A pharmaceutical preparation comprising: an effective amount of the total benzyl ester of hyaluronic acid; and a pharmaceutically-acceptable carrier.

176. A pharmaceutical preparation comprising: an effective amount of the total phenethyl ester of hyaluronic acid; and a pharmaceutically-acceptable carrier.

177. A pharmaceutical preparation comprising: an effective amount of a salt of a hyaluronic acid ester with 75% of the carboxyl groups esterified with ethanol and with 25% of the carboxyls salified with streptomycin; and a pharmaceutically-acceptable carrier.

178. A pharmaceutical preparation comprising: an effective amount of a salt of a hyaluronic acid ester with 75% of the carboxyl groups esterified with ethanol and with 25% of
the carboxyls sallied with erythromycin; and a pharmaceutically-acceptable carrier.

179. A pharmaceutical preparation comprising: an effective amount of a salt of a hyaluronic acid ester with 75% of the carboxyl groups esterified with ethanol and with 25% of the carboxyls sallied with neomycin; and a pharmaceutically-acceptable carrier.

180. A pharmaceutical preparation comprising: an effective amount of a salt of a hyaluronic acid ester with 75% of the carboxyl groups esterified with ethanol and with 25% of the carboxyls sallied with gentamycin; and a pharmaceutically-acceptable carrier.

181. A pharmaceutical preparation comprising: an effective amount of a salt of a hyaluronic acid ester with 75% of the carboxyl groups esterified with ethanol and with 25% of the carboxyls sallied with amikacin; and a pharmaceutically-acceptable carrier.

182. A pharmaceutical preparation comprising: an effective amount of a salt of a hyaluronic acid ester with 75% of the carboxyl groups esterified with ethanol and with 25% of the carboxyls sallied with kanamycin; and a pharmaceutically-acceptable carrier.

183. A pharmaceutical preparation comprising: an effective amount of a salt of a hyaluronic acid ester with 75% of the carboxyl groups esterified with ethanol and with 25% of the carboxyls sallied with pilocarpine; and a pharmaceutically-acceptable carrier.
184. A pharmaceutical preparation comprising: an effective amount of a salt of a hyaluronic acid ester with 85% of the carboxyl groups esterified with ethanol and with 15% of the carboxyls salified with pilocarpine; and a pharmaceutically-acceptable carrier.

185. The pharmaceutical preparation of any one of claims 140 to 184, inclusive, wherein said hyaluronic acid derives from hyaluronic acid which is obtained by first dehydrating cocks' combs with acetone and the exposing them to enzymatic digestion with papain.

186. The pharmaceutical preparation of any one of claims 140 to 184, inclusive, wherein said hyaluronic acid has been obtained by first dehydrating cocks' combs with acetone and then exposing them to enzymatic digestion with papain, followed by molecular ultrafiltration, and further purification of the hyaluronic acid fraction so-obtained.

187. The pharmaceutical preparation of any one of claims 140 to 184, inclusive, wherein said hyaluronic ester derives from an integral hyaluronic acid or from one of its salts which is obtained by extraction from cocks' combs, and having a molecular weight of between 8 and 13 million.

188. The pharmaceutical preparation of any one of claims 140 to 184, inclusive, wherein said hyaluronic ester derives from a hyaluronic acid fraction with a molecular weight of between 90 and 0.23% of the molecular weight of an integral hyaluronic acid with a molecular weight of 13 million.
189. The pharmaceutical preparation of any one of claims 140 to 184, inclusive, wherein said hyaluronic ester derives from a hyaluronic acid fraction having a molecular weight of between 50,000 and 100,000, and which is substantially-free of hyaluronic acid having a molecular weight of less than 30,000.

190. The pharmaceutical preparation of any one of claims 140 to 184, inclusive, wherein said hyaluronic ester derives from a hyaluronic acid fraction having a molecular weight of between 500,000 and 730,000, and which is substantially-free of hyaluronic acid having a molecular weight of less than 30,000.

191. A medicament preparation comprising:

(a) at least one pharmacologically-active substance; and
(b) a carrying vehicle comprising an ester of hyaluronic acid of the Formula

Hy(COOR)$_n$

wherein COO represents the carboxylic acid moiety on the hyaluronic acid (Hy) molecule, R is derived from an alcohol which is selected from the group consisting of an aliphatic alcohol having a chain of 2 to 34 carbons, an araliphatic alcohol, a cycloaliphatic alcohol and a heterocyclic alcohol, and n is a large number.

192. A medicament preparation comprising:

(a) at least one pharmacologically-active substance; and
(b) a carrying vehicle comprising a total ester of hyaluronic acid of the Formula

Hy(COOR)$_n$.
wherein COO represents the carboxylic acid moiety on the hyaluronic acid (Hy) molecule, R is derived from an alcohol which is selected from the group consisting of an aliphatic alcohol having a chain of 2 to 34 carbons, an araliphatic alcohol, a cycloaliphatic alcohol and a heterocyclic alcohol, and n is a large number.

193. A medicament preparation comprising:

(a) at least one pharmacologically-active substance;
and (b) a carrying vehicle comprising a partial ester of hyaluronic acid of the Formula

\[ \text{Hy(COOR)}_n \]

wherein COO represents the carboxylic acid moiety on the hyaluronic acid (Hy) molecule, R is derived from an alcohol which is selected from the group consisting of an aliphatic alcohol having a chain of 2 to 34 carbons, an araliphatic alcohol, a cycloaliphatic alcohol and a heterocyclic alcohol, and n is a large number.

194. A medicament preparation comprising:

(a) at least one pharmacologically-active substance;
and (b) a carrying vehicle comprising a salt of a partial ester of hyaluronic acid of the Formula

\[ \text{Hy(COOR)}_n \]

wherein COO represents the carboxylic acid moiety on the hyaluronic acid (Hy) molecule, R is derived from an alcohol which is selected from the group consisting of an aliphatic alcohol having a chain of 2 to 34 carbons, an araliphatic alcohol, a cycloaliphatic alcohol and a heterocyclic alcohol,
and n is a large number; with an inorganic base or with an organic base.

195. A medicament preparation comprising:
(a) at least one pharmacologically-active substance;
(b) a carrying vehicle comprising an ester of hyaluronic acid of the Formula

$\text{Hy(COOR)}_n$

wherein R COO represents the carboxylic acid moiety on the hyaluronic acid (Hy) molecule, is derived from an alcohol which is selected from the group consisting of an aliphatic alcohol having a chain of 2 to 34 carbons, an araliphatic alcohol, a cycloaliphatic alcohol and a heterocyclic alcohol, and n is a large number; and (c) common excipients for pharmaceutical preparations.

196. A medicament preparation comprising:
(a) at least one pharmacologically-active substance;
(b) a carrying vehicle comprising a total ester of hyaluronic acid of the Formula

$\text{Hy(COOR)}_n$

wherein COO represents the carboxylic acid moiety on the hyaluronic acid (Hy) molecule, R is derived from an alcohol which is selected from the group consisting of an aliphatic alcohol having a chain of 2 to 34 carbons, an araliphatic alcohol, a cycloaliphatic alcohol and a heterocyclic alcohol, and n is a large number; and (c) common excipients for pharmaceutical preparations.

197. A medicament preparation comprising:
(a) at least one pharmacologically-active substance;
(b) a carrying vehicle comprising a partial ester of hyaluronic acid of the Formula

$$\text{Hy(COOR)}_n$$

wherein COO represents the carboxylic acid moiety on the hyaluronic acid (Hy) molecule, R is derived from an alcohol which is selected from the group consisting of an aliphatic alcohol having a chain of 2 to 34 carbons, an araliphatic alcohol, a cycloaliphatic alcohol and a heterocyclic alcohol, and n is a large number;

and (c) common excipients for pharmaceutical preparations.

198. A medicament preparation comprising:
(a) at least one pharmacologically-active substance;
(b) a carrying vehicle comprising a salt of a partial ester of hyaluronic acid of the Formula

$$\text{Hy(COOR)}_n$$

wherein COO represents the carboxylic acid moiety on the hyaluronic acid (Hy) molecule, R is derived from an alcohol which is selected from the group consisting of an aliphatic alcohol having a chain of 2 to 34 carbons, an araliphatic alcohol, a cycloaliphatic alcohol and a heterocyclic alcohol, and n is a large number. with an organic base or with an inorganic base;

and (c) common excipients for pharmaceutical preparations.
199. The medicament preparation of any one of claims 191 to 198, inclusive, wherein said alcohol is an aliphatic alcohol which is substituted by one or two functional groups which are selected from the group consisting of amino, hydroxy, mercapto, aldehyde, keto, carboxyl, hydrocarbyl, dihydrocarbylamino, ether, ester, thioether, thioester, acetal, ketal, carbalkoxy, unsubstituted carbamide groups and carbamide groups which are substituted by one or two alkyl groups, the hydrocarbyl radicals in these functionally-modified groups having a maximum of 6 carbon atoms, and in which the carbon atom chain may be interrupted heteroatoms selected from the group consisting of oxygen, sulphur and nitrogen.

200. The medicament preparation of claim 199, wherein said alcohol is ethyl alcohol, propyl alcohol, isopropyl alcohol, normal butyl alcohol, isobutyl alcohol, tert-butyl alcohol, amyl alcohol, pentyl alcohol, hexyl alcohol, octyl alcohol, ethylene glycol, propylene glycol, butylene glycol or glycerin.

201. The medicament preparation of any one of claims 191 to 198, inclusive, wherein said alcohol is an araliphatic alcohol with only one benzene residue, in which the aliphatic chain has a maximum of 4 carbon atoms, and in which the benzene residue may be substituted by 1 to 3 methyl groups or by 1 to 3 hydroxy groups, or by 1 to 3 halogen atoms, and in which the aliphatic chain may be substituted by one or two functions which are selected from the group consisting of free
amino groups, monoethylamino groups and diethylamino groups, by pyrrolidinyl groups and by piperidinyl groups.

202. The medicament preparation of claim 201, wherein said alcohol is benzyl alcohol, phenethyl alcohol, ephedrine or adrenalin.

203. The medicament preparation of any one of claims 191 to 198, inclusive, wherein said alcohol is a cycloaliphatic alcohol or an aliphatic-cycloaliphatic alcohol and derives from a monocyclic hydrocarbide or a polycyclic hydrocarbide with a maximum of 34 carbon atoms.

204. The medicament preparation of claim 203, wherein said alcohol is a polycyclic alcohol which is selected from the group consisting of sterols, colic acids, steroid alcohols, and groups of the estrane and pregnane series and their unsaturated derivative.

205. The medicament preparation of claim 204, wherein said alcohol is cortisone, hydrocortisone, prednisone, prednisolone, fluorocortisone, dexamethasone, betamethasone, corticosterone, deoxycorticosterone, paramethasone, flumethasone, fluocinolone, fluocinolone acetonide, fluprednylidene, clobetasol, or beclomethasone.

206. The medicament preparation of any one of claims 191 to 198, inclusive, wherein ester is in the form of a salt of a partial ester of hyaluronic acid with an alkali metal, or with an alkaline earth metal, or with magnesium, or with aluminum, or with ammonia.
207. The medicament preparation of claim 206, wherein said salt is a sodium salt of hyaluronic acid.

208. A medicament preparation according to any one of claims 191 to 207, inclusive, wherein said pharmacologically-active substance (a) is selected from the group consisting of an anaesthetic, an analgesic, an anti-inflammatory, a vasoconstrictor, an antibiotic-antibacterial, and an antiviral.

209. A medicament preparation according to any one of claims 191 to 207, inclusive, wherein said pharmacologically-active substance (a) is topically active.

210. A medicament preparation according to any one of claims 191 to 209, inclusive, wherein said carrying vehicle (b) contains an ester of hyaluronic acid with a pharmacologically-inactive alcohol.

211. A medicament preparation according to any one of claims 191 to 209, inclusive, wherein said carrying vehicle (b) contains an ester of hyaluronic acid with a pharmacologically-active alcohol.

212. A medicament preparation according to any one of claims 191 to 209 inclusive, wherein said carrying vehicle (b) is of a basic nature and contains a partial ester of hyaluronic acid, the unesterified groups of which are salified with the pharmacologically-active substance.

213. The preparation of any one of claims 139 to 212, inclusive, wherein said hyaluronic acid derives from hyaluronic acid which is obtained by first dehydrating cocks'
combs with acetone and the exposing them to enzymatic digestion with papain.

214. The preparation of any one of claims 139 to 212, inclusive, wherein said hyaluronic acid has been obtained by first dehydrating cocks’ combs with acetone and then exposing them to enzymatic digestion with papain, followed by molecular ultrafiltration, and further purification of the hyaluronic acid fraction so-obtained.

215. The preparation of any one of claims 139 to 212, inclusive, wherein said hyaluronic acid ester derives from integral hyaluronic acid or from one of its salts obtained by extraction from cocks’ combs and having a molecular weight of between 8 and 13 million.

216. The preparation of any one of claims 139 to 212, inclusive, wherein said hyaluronic acid ester derives from a hyaluronic acid fraction with a molecular weight of between 90 and 0.23% of the molecular weight of an integral hyaluronic acid having a molecular weight of 13 million.

217. The preparation of any one of claims 139 to 212, inclusive, wherein said hyaluronic acid ester derives from a molecular fraction having a molecular weight of between 50,000 and 100,000 and which is substantially-free of hyaluronic acid having a molecular weight of less than 30,000.

218. The preparation of any one of claims 139 to 212, inclusive, wherein said hyaluronic acid ester derives from a molecular fraction having a molecular weight of between
500,000 and 730,000 and which is substantially-free of hyaluronic acid having a molecular weight of less than 30,000.

219. The preparation of any one of claims 139 to 185, 187 to 213 and 215 to 218, inclusive, including the steps of: molecular ultrafiltration; and further purification of the hyaluronic acid fraction obtained.

220. The use, in cosmetic articles, of a carrying vehicle which comprises: an ester of hyaluronic acid of the Formula

\[ \text{Hy(COOR)}_n \]

wherein COO represents the carboxylic acid moiety on the hyaluronic acid (Hy) molecule, R is derived from an alcohol which is selected from the group consisting of an aliphatic alcohol having a chain of 2 to 34 carbons, an araliphatic alcohol, a cycloaliphatic alcohol and a heterocyclic alcohol, and n is a large number, or a salt thereof, as claimed in any one of claims 1 to 5, 7, 9, 10, 12, 14, 17, 19, 21, 23, 24, and 26 to 46, inclusive.

221. The use, in cosmetic articles, of a carrying vehicle which comprises: an ester of hyaluronic acid of the Formula

\[ \text{Hy(COOR)}_n \]

wherein COO represents the carboxylic acid moiety on the hyaluronic acid (Hy) molecule, R is derived from an alcohol which is selected from the group consisting of an aliphatic alcohol having a chain of 2 to 34 carbons, an araliphatic alcohol, a cycloaliphatic alcohol and a heterocyclic alcohol,
and n is a large number, or a salt thereof, as claimed in any one of claims 6, 8, 11, 13, 15, 18, 20, 22, 25 and 52 to 78, inclusive.

222. The use according to claim 220 or claim 221, wherein said carrying vehicle contains an ester of hyaluronic acid with a pharmacologically-inactive alcohol.

223. The use according to claim 220 or claim 221, wherein said carrying vehicle contains an ester of hyaluronic acid with a pharmacologically-active alcohol.

224. The use according to any one of claims 220 to 222, inclusive, wherein said carrying vehicle is of a basic nature and contains a salt of a partial ester of hyaluronic acid.

225. The use, in sanitary and surgical articles, of a carrying vehicle which comprises: an ester of hyaluronic acid of the Formula

$$\text{Hy(\text{COOR})}_n$$

wherein HyCOO represents a hyaluronic acid radical, and R is derived from an alcohol which is selected from the group consisting of an aliphatic alcohol having a chain of 2 to 34 carbons, an araliphatic alcohol, a cycloaliphatic alcohol and a heterocyclic alcohol, and n is a large number, or a salt thereof, as claimed in any one of claims 1 to 5, 7, 9, 10, 12, 14, 17, 19, 21, 23, 24, and 26 to 46, inclusive.

226. The use, in sanitary and surgical articles, of a carrying vehicle which comprises: an ester of hyaluronic acid of the Formula

$$\text{Hy(\text{COOR})}_n$$
wherein COO represents the carboxylic acid moiety on the 
hyaluronic acid (Hy) molecule, R is derived from an alcohol 
which is selected from the group consisting of an aliphatic 
alcohol having a chain of 2 to 34 carbons, an araliphatic 
alcohol, a cycloaliphatic alcohol and a heterocyclic alcohol, 
and n is a large number, or a salt thereof, as claimed in any 
one of claims 6, 8, 11, 13, 15, 18, 20, 22, 25 and 52 to 78, 
inclusive.

227. The use according to claim 225 or claim 226, 
wherein said carrying vehicle contains an ester of hyaluronic 
acid with a pharmacologically-inactive alcohol.

228. The use according to claim 225 or claim 226, 
wherein said carrying vehicle contains an ester of hyaluronic 
acid with a pharmacologically-active alcohol.

229. The use according to any one of claims 225 to 228, 
inclusive, wherein said carrying vehicle is of a basic nature 
and contains a salt of a partial ester of hyaluronic acid.

230. The use according to any one of claims 225 to 229, 
inclusive, wherein said sanitary and surgical articles are in 
the form of microcapsules for subcutaneous, intramuscular or 
intravenous injection.

231. The use according to any one of claims 225 to 229, 
inclusive, wherein said sanitary and surgical articles are in 
the form of solid inserts to be removed after a certain length 
of time.

232. The use according to any one of claims 225 to 229, 
inclusive, wherein said sanitary and surgical articles are in
the form of sponges for the medication of wounds and various lesions.

233. The use, in films or threads, of a carrying vehicle which comprises: an ester of hyaluronic acid of the Formula

\[ \text{Hy(COOR)}_n \]

wherein COO represents the carboxylic acid moiety on the hyaluronic acid (Hy) molecule, R is derived from an alcohol which is selected from the group consisting of an aliphatic alcohol having a chain of 2 to 34 carbons, an araliphatic alcohol, a cycloaliphatic alcohol and a heterocyclic alcohol, and n is a large number, or a salt thereof, as claimed in any one of claims 1 to 5, 7, 9, 10, 12, 14, 17, 19, 21, 23, 24, and 26 to 46, inclusive.

234. The use, in films or threads, of a carrying vehicle which comprises: an ester of hyaluronic acid of the Formula

\[ \text{Hy(COOR)}_n \]

wherein COO represents the carboxylic acid moiety on the hyaluronic acid (Hy) molecule, R is derived from an alcohol which is selected from the group consisting of an aliphatic alcohol having a chain of 2 to 34 carbons, an araliphatic alcohol, a cycloaliphatic alcohol and a heterocyclic alcohol, and n is a large number, or a salt thereof, as claimed in any one of claims 6, 8, 11, 13, 15, 18, 20, 22, 25 and 52 to 78, inclusive.

235. The use according to claim 233 or claim 234, wherein said carrying vehicle contains an ester of hyaluronic acid with a pharmacologically-inactive alcohol.
236. The use according to claim 233 or claim 234, wherein said carrying vehicle contains an ester of hyaluronic acid with a pharmacologically-active alcohol.

237. The use according to any one of claims 233 to 236, inclusive, wherein said carrying vehicle is of a basic nature and contains a salt of a partial ester of hyaluronic acid.

238. The use according to any one of claims 233 to 237, inclusive, wherein said films or threads are for the medication of wounds and for use in surgery.

239. The use according to any one of claims 233 to 237, inclusive, wherein said films is in the form of artificial skin for use in surgical dermatology and as suture threads in surgical operations.

240. The use, as an element in the production of gauze, of films or threads, said films or threads including a carrying vehicle which comprises: an ester of hyaluronic acid of the Formula

\[ \text{Hy(COOR)}_n \]

wherein COO represents the carboxylic acid moiety on the hyaluronic acid (Hy) molecule, R is derived from an alcohol which is selected from the group consisting of an aliphatic alcohol having a chain of 2 to 34 carbons, an araliphatic alcohol, a cycloaliphatic alcohol and a heterocyclic alcohol, and \( n \) is a large number, or a salt thereof, as claimed in any one of claims 1 to 5, 7, 9, 10, 12, 14, 17, 19, 21, 23, 24, and 26 to 46, inclusive.
241. The use, as an element in the production of gauze, of films or threads, said films or threads including a carrying vehicle which comprises: an ester of hyaluronic acid of the Formula

\[ \text{Hy(COOR)}_n \]

wherein COO represents the carboxylic acid moiety on the hyaluronic acid (Hy) molecule, R is derived from an alcohol which is selected from the group consisting of an aliphatic alcohol having a chain of 2 to 34 carbons, an araliphatic alcohol, a cycloaliphatic alcohol and a heterocyclic alcohol, and \( n \) is a large number, or a salt thereof, as claimed in any one of claims 6, 8, 11, 13, 15, 18, 20, 22, 25 and 52 to 78, inclusive.

242. The use according to claim 240 or claim 241, wherein said carrying vehicle contains an ester of hyaluronic acid with a pharmacologically-inactive alcohol.

243. The use according to claim 240 or claim 241, wherein said carrying vehicle contains an ester of hyaluronic acid with a pharmacologically-active alcohol.

244. The use according to any one of claims 240 to 243, inclusive, wherein said carrying vehicle is of a basic nature and contains a salt of a partial ester of hyaluronic acid.

245. The use according to any one of claims 240 to 244, inclusive, for providing a gauze for the medication of wounds and for use in surgery.
246. The use according to any one of claims 240 to 244, inclusive, for providing a gauze in the form of sponges for the medication of wounds or in surgery.

247. The use of an ester of hyaluronic acid of the Formula

\[ \text{Hy(COOR)}_n \]

wherein COO represents the carboxylic acid moiety on the hyaluronic acid (Hy) molecule, R is derived from an alcohol which is selected from the group consisting of an aliphatic alcohol having a chain of 2 to 34 carbons, an araliphatic alcohol, a cycloaliphatic alcohol and a heterocyclic alcohol, and \( n \) is a large number, or a salt thereof, as claimed in any one of claims 1 to 5, 7, 9, 10, 12, 14, 17, 19, 21, 23, 24, and 26 to 46, inclusive, for the preparation of films or threads which includes the steps of: dissolving said hyaluronic ester in an organic solvent; making the solution into sheet or thread form respectively; and then eliminating the organic solvent by treatment with another suitable organic or aqueous solvent which is soluble in said first solvent.

248. The use of an ester of hyaluronic acid of the Formula

\[ \text{Hy(COOR)}_n \]

wherein COO represents the carboxylic acid moiety on the hyaluronic acid (Hy) molecule, R is derived from an alcohol which is selected from the group consisting of an aliphatic alcohol having a chain of 2 to 34 carbons, an araliphatic alcohol, a cycloaliphatic alcohol and a heterocyclic alcohol,
and \( n \) is a large number, or a salt thereof, as claimed in any one of claims 6, 8, 11, 13, 15, 18, 20, 22, 25 and 52 to 78, inclusive, for the preparation of films or threads which includes the steps of: dissolving said hyaluronic ester in an organic solvent; making the solution into sheet or thread form respectively; and then eliminating the organic solvent by treatment with another suitable organic or aqueous solvent which is soluble in said first solvent.

249. The use of an ester of hyaluronic acid of the Formula

\[
\text{Hy(COOR)}_n
\]

wherein \( \text{COO} \) represents the carboxylic acid moiety on the hyaluronic acid (\( \text{Hy} \)) molecule, \( R \) is derived from an alcohol which is selected from the group consisting of an aliphatic alcohol having a chain of 2 to 34 carbons, an araliphatic alcohol, a cycloaliphatic alcohol and a heterocyclic alcohol, and \( n \) is a large number, or a salt thereof, as claimed in any one of claims 1 to 5, 7, 9, 10, 12, 14, 17, 19, 21, 23, 24, and 26 to 46, inclusive, for the preparation of films or threads which includes the steps of: dissolving the hyaluronic ester in an organic solvent; making the solution into sheet or thread form respectively; and then eliminating the organic solvent by treatment with a current of a suitably heated inert gas.

250. The use of an ester of hyaluronic acid of the Formula

\[
\text{Hy(COOR)}_n
\]
wherein COO represents the carboxylic acid moiety on the hyaluronic acid (Hy) molecule, R is derived from an alcohol which is selected from the group consisting of an aliphatic alcohol having a chain of 2 to 34 carbons, an araliphatic alcohol, a cycloaliphatic alcohol and a heterocyclic alcohol, and n is a large number, or a salt thereof, as claimed in any one of claims 6, 8, 11, 13, 15, 18, 20, 22, 25 and 52 to 78, inclusive, for the preparation of films or threads which includes the steps of: dissolving the hyaluronic ester in an organic solvent; making the solution into sheet or thread form respectively; and then eliminating the organic solvent by treatment with a current of a suitably heated inert gas.

251. The use of an ester of hyaluronic acid, or one of its salts, according to any one of claims 1 to 5, 7, 9, 10, 12, 14, 17, 19, 21, 23, 24, and 26 to 46, inclusive, as, or in the preparation of, a medicament for use in ophthalmology.

252. The use of an ester of hyaluronic acid, or one of its salts, according to any one of claims 6, 8, 11, 13, 15, 18, 20, 22, 25 and 52 to 78, inclusive, as, or in the preparation of, a medicament for use in ophthalmology.

253. The use of an ester of hyaluronic acid, or one of its salts, according to any one of claims 1 to 5, 7, 9, 10, 12, 14, 17, 19, 21, 23, 24, and 26 to 46, inclusive, as, or in the preparation of, a medicament for use in dermatology, in otorhinology, in odontology, in angiology, in gynaecology, in neurology, or in any type of internal medical pathologies.
254. The use of an ester of hyaluronic acid, or one of its salts, according to any one of claims 6, 8, 11, 13, 15, 18, 20, 22, 25 and 52 to 78, inclusive, as, or in the preparation of, a medicament for use in dermatology, in otorhinology, in odontology, in angiology, in gynaecology, in neurology, or in any type of internal medical pathologies.