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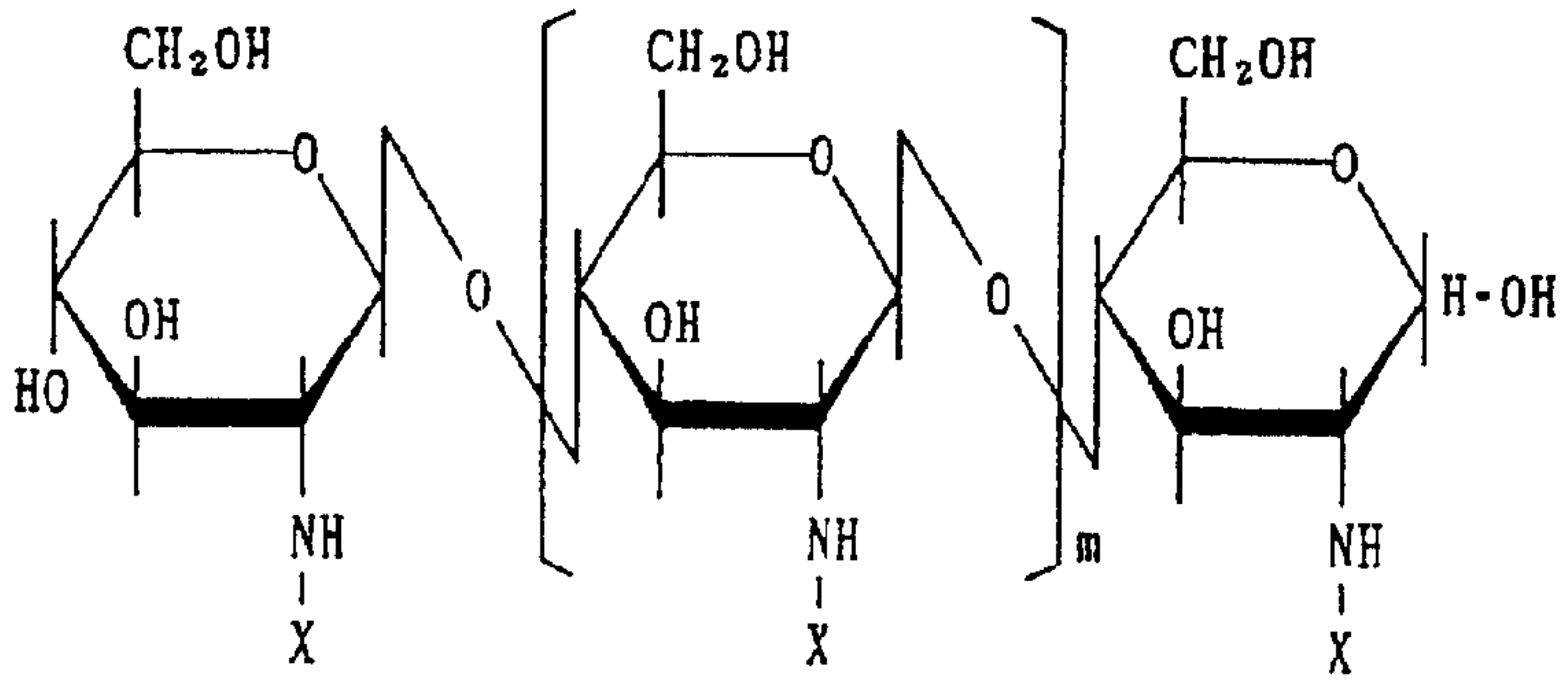

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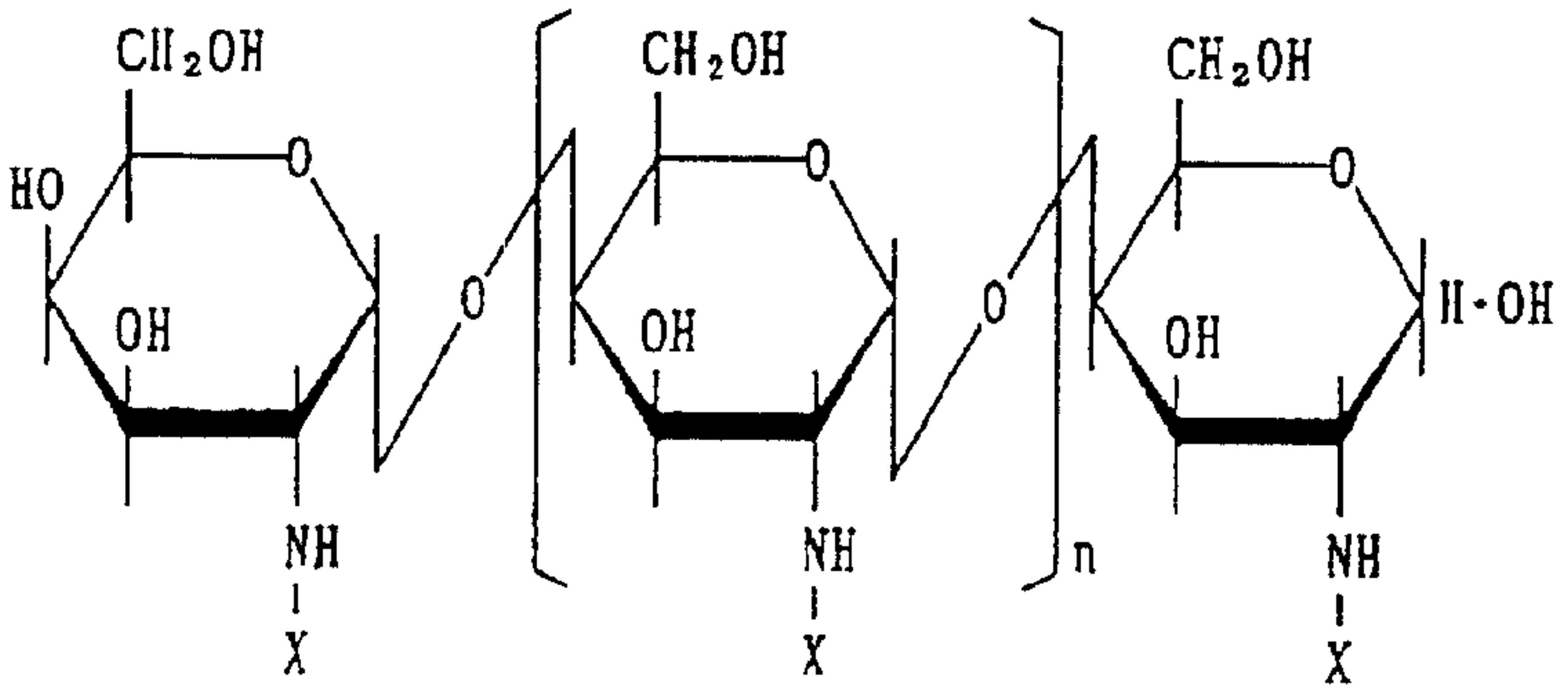

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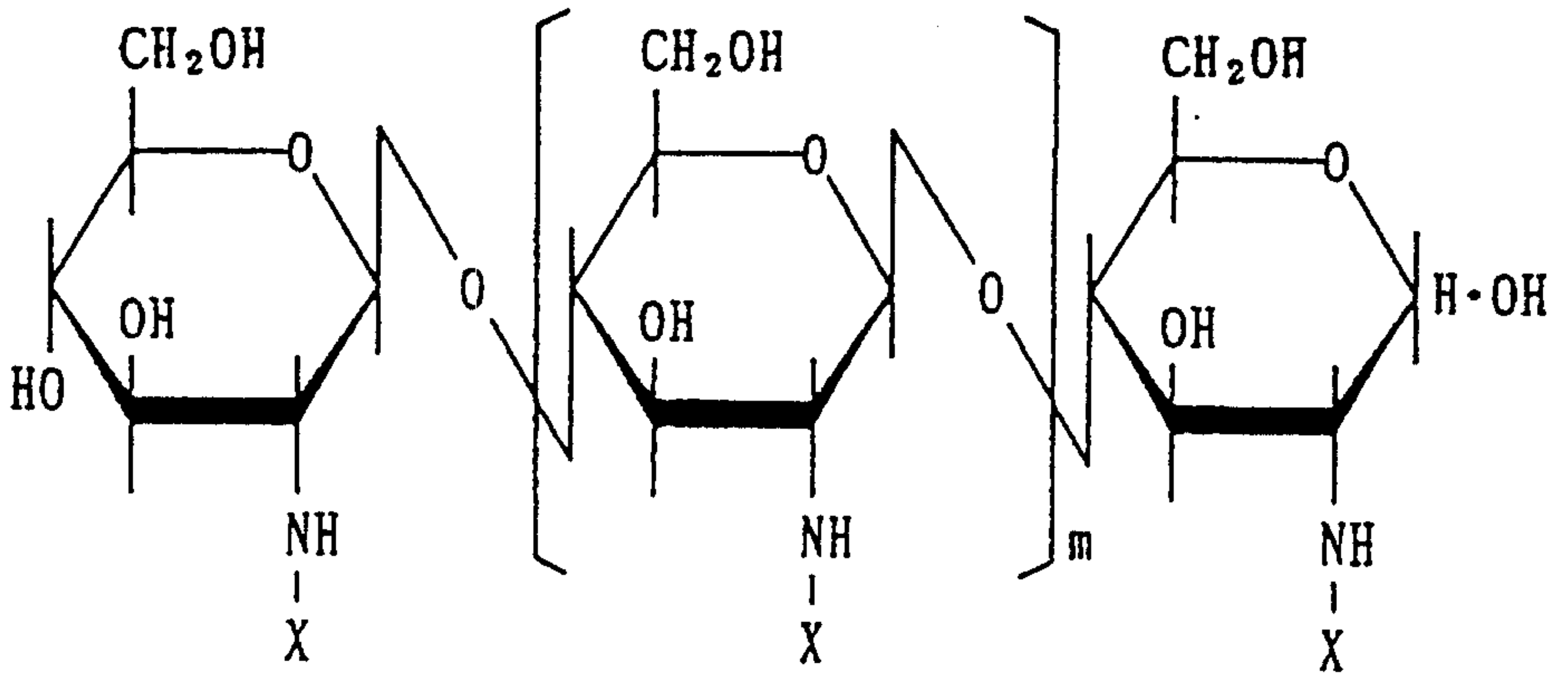
(57) **Abrégé/Abstract**:

The present invention is directed to an imaging agent for diagnosis comprising a compound composed of a polynuclear type compound of the formula I or II: (see formula I and formula II) wherein each X is a hydrogen atom or a bifunctional ligand, at least one of them is a bifunctional ligand and m or n is an integer of 1 to 6, and at least one metal ion being coordinated with at least one bifunctional ligand moiety, said metal ion being selected from the group consisting of metal ions having an atomic number of 21-29, 31, 32, 37-39, 42-44, 49 and 56-83.

Abstract 2079493

The present invention is directed to an imaging agent for diagnosis comprising a compound composed of a polynuclear type compound of the formula I or II:



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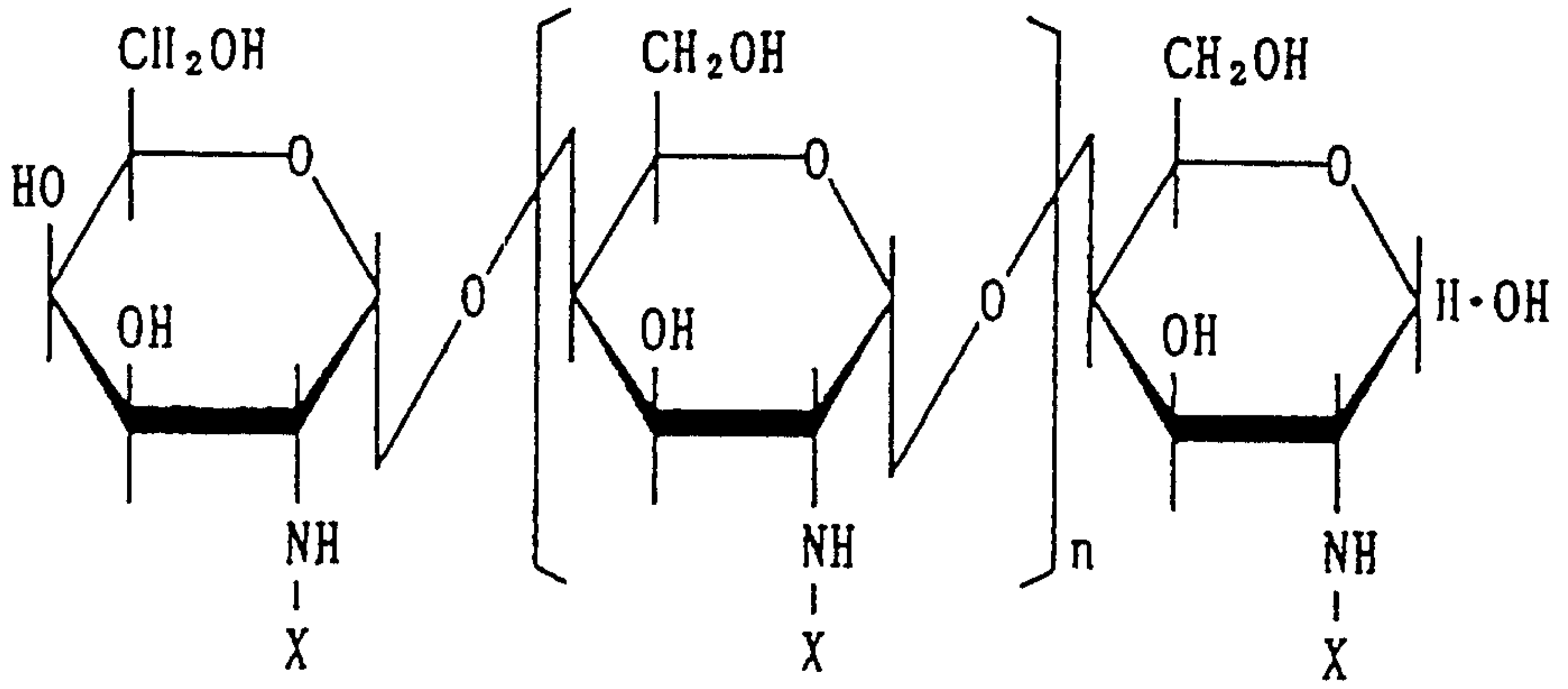

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wherein each X is a hydrogen atom or a bifunctional ligand, at least one of them is a bifunctional ligand and m or n is an integer of 1 to 6, and at least one metal ion being coordinated with at least one bifunctional ligand moiety, said metal ion being selected from the group consisting of metal ions having an atomic number of 21-29, 31, 32, 37-39, 42-44, 49 and 56-83.

IMAGING AGENT FOR DIAGNOSIS

The present invention relates to an imaging agent for diagnosis, in particular, to an imaging agent for diagnosis containing a polynuclear type metal complex compound.

(Diethylenetriaminepentaacetic acid)gadolinate (hereinafter abbreviated as to "DTPA-Gd") is the only practical pharmaceutical which is presently known as a nuclear magnetic resonance imaging (hereinafter sometimes abbreviated as MRI) agent for diagnosis [JP-A 58-29718] and it is considered that use thereof as an imaging agent for diagnosis in the brain or spinal regions has been almost established. Since, however, DTPA-Gd is complexed, the relaxivity showing the image display index is lower (about 1/2) than that of Gd itself. Therefore, it is necessary to compensate this lowered relaxivity by increasing the dose. In addition, DTPA-Gd is rapidly excreted into the urine after administration [Hiroki Yoshikawa et al., Gazoshindan, 6, pages 959-969 (1986)]. This is very disadvantageous when it is desirable to image several parts of the body by reflecting them in the blood stream (blood vessel distribution, blood stream distribution, distribution volume, permeation and the like in a lesion) with a single injection of the pharmaceutical. Further, such rapid excretion also makes distribution properties of DTPA-Gd disadvantageous.

To solve the above-described problems (improvement in the relaxivity), some attempts at polynuclearization by repetition of the mononuclear complex are described in JP-A 63-41468, JP-A 2-196776 and the like. Since, however, the polynuclearization is limited at best to dinuclearization or trinuclearization, a significant improvement in relaxivity cannot be accomplished.

Thereafter, the use of a polynuclear type metal complex compound obtained by introducing a plurality of metal complexes into a carrier polymer material as an imaging agent for diagnosis has been investigated. As a result, an MRI agent for diagnosis having a carrier selected from human serum albumin (abbreviated as "HSA") [Ogan, M.D., et al., Invest. Radiol., 22, pages 665-671 (1987)], dextran [Brash, R.C., et al., Radiology, 175, pages 483-488 (1990)], starch [JP-A 61-501571], polylysine [JP-A 64-54028] or the like has been proposed and has succeeded in improving the relaxivity. These polymer polynuclear type metal complex compounds are localized in blood vessels for a constant period of time immediately after administration and have common distribution properties including retention in the blood vessels for a relatively long period of time. This improves the rapid excretion and penetration properties of DTPA-Gd.

However, the polymer carriers which can act as a backbone for these polynuclear type metal complexes, regardless of whether or not they are a natural or a synthetic material, is a heterogeneous compound the molecular weight of which has no mono-dispersion and is dealt with as an average value having a certain distribution width. Thus, pharmaceutical uniformity cannot be attained and this can be a problem. For this reason, it is very difficult to control the number of metal ions to be introduced at a constant and, therefore, heterogeneity arises inevitably in the desired physicochemical properties. Further, since all of the above-described polymers have a molecular weight of more than tens of thousands, they have an unnecessarily long retention time in the blood, e.g. from ten and a few hours to a few days, and have biological acceptability problems, e.g. retention in the body, antigenicity and the like.

The main object of the present invention is to provide an imaging agent for diagnosis comprising a polynuclear type metal complex compound which can eliminate the above-described problems in the known imaging agents for diagnosis containing a polymer polynuclear type metal complex compound. Namely, the main object of the present invention is to provide an image agent for diagnosis having a plurality of metal ions which are stably introduced in a desired number, good homogeneity, good solubility, physiologically acceptability and suitable retention time in blood for image diagnosis.

This object as well as other objects and advantages of the present invention will become apparent to those skilled in the art from the following description with reference to the accompanying drawings.

In drawings that illustrate preferred embodiments of the present invention:

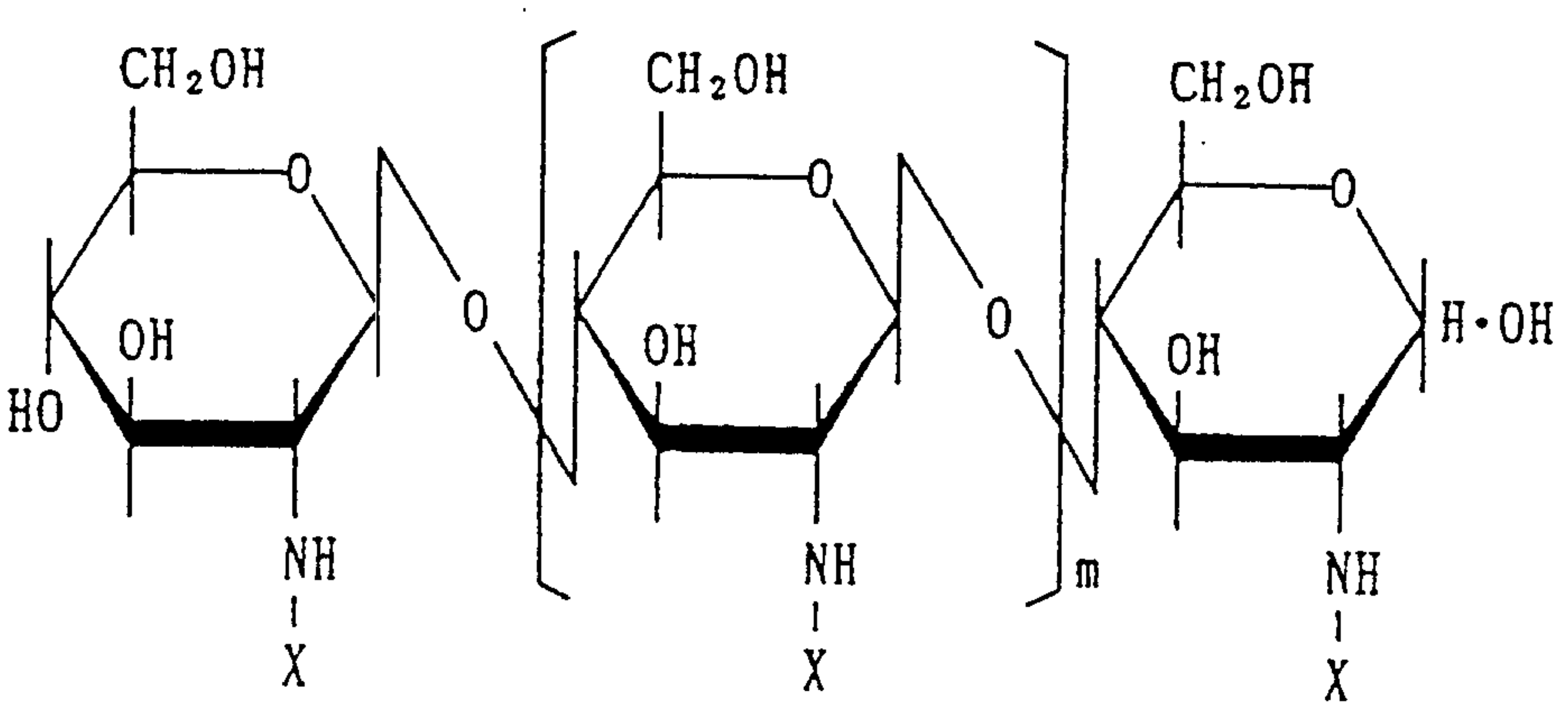
Figure 1 is an MRI showing a transverse view of the chest region including the heart of a rat sacrificed 1 hour after administration of a (galactosamino-pentamer)-[1-(p-isothiocyanatebenzyl)-diethylenetriaminepentaacetic acid]gadolinate (abbreviated as "GPEN-DTES-Gd") solution.

Figure 2 is an MRI showing a transverse view of the chest region including the heart of a rat sacrificed 1 hour after administration of DTPA-Gd (MAGNEVIST®).

In order to accomplish the above-described objects, the present inventors have studied extensively. As a result, it has been found that a polynuclear type metal complex compound having as a backbone a chitosan-oligosaccharide or galactosamino-oligosaccharide is suitable and has a clinically effective retention time in the blood.

For example, the present inventors have investigated in vitro or in vivo relaxivity and contrast effect of a polynuclear type metal complex compound GPEN-DTES-Gd, wherein 1-(p-isothiocyanatebenzyl)-DTPA (abbreviated as to "DTES") [Martin, W.B., et al., Inorg. Chem., 25, pages 2772-2781 (1986)] is chemically bonded as a bifunctional ligand to galactosamino-pentamer (abbreviated as "GPEN") and Gd is coordinated therewith as a metal ion. As a result, it has been confirmed that T_1 relaxivity in water (magnetic field intensity: 6.35T, 25°C) is remarkably increased to 7.6 $(mM \cdot S)^{-1}$, being about two times that of DTPA-Gd. Further, it has been confirmed that the contrast effects (magnetic field intensity: 1.5T, T_1 weighted imaging by spin echo method) in the heart of a rat at 1 hour after administration is enhanced by about 1.8 times that of DTPA-Gd imaged under the same conditions. Furthermore, GPEN-DTES-In-111 labeled with a radioactive metal ion, In-111, has a half-life period in blood of about 55 minutes in the distribution test in rats. This half-life period in blood is sufficiently longer than that of DTPA-In-111, and shows good retention in blood.

The present invention has been completed based on these findings and provides an imaging agent for diagnosis comprising a compound composed of a polynuclear type compound of the formula I or II:



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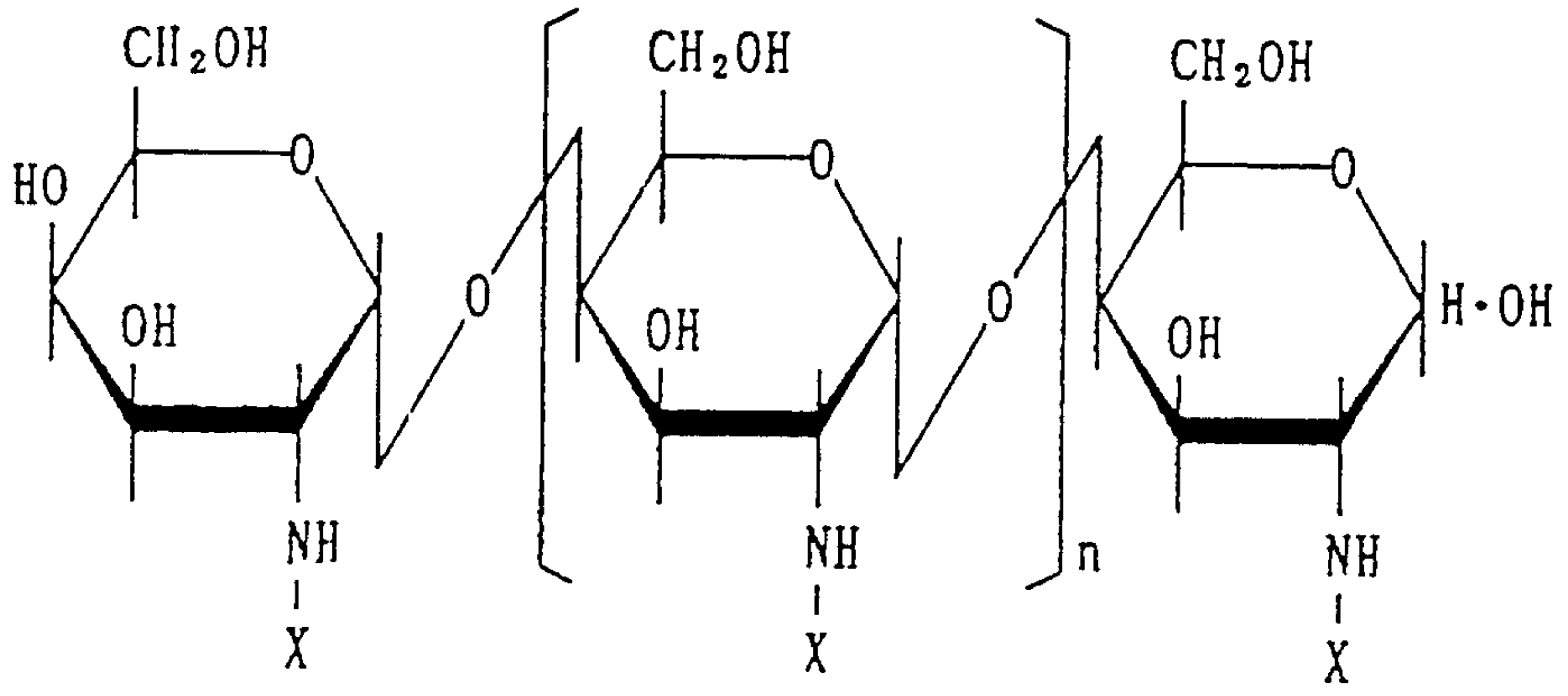

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wherein each X is a hydrogen atom or a bifunctional ligand, at least one being a bifunctional ligand and each of m and n is an integer of 1 to 6, and at least one metal ion being coordinated with at least one bifunctional ligand moiety, said metal ion being selected from the group consisting of metal ions having an atomic number of 21-29, 31, 32, 37-39, 42-44, 49 and 56-83.

The term "polynuclear type" as used herein means a structure wherein a plurality of metal ions are introduced therein via a complexing agent per unit molecule.

The compound used as a backbone for polynuclearization in the present invention is an amino oligosaccharide, more particularly, a chitosan-oligosaccharide or galactosamino-oligosaccharide. In particular, an oligomer having a repetition number of component monosaccharides of 3 to 6 (m or n is 1 to 4 in the formula I or II) is advantageously used. The chitosan-oligosaccharide is an oligosaccharide wherein D-glucosamine monomers are bonded through β-1,4 bond. The chitosan-oligosaccharide to be used can be obtained, for example, by hydrochloric acid-hydrolyzing or enzymatically degrading chitosan prepared from natural crab shell. On the other hand, the galactosamino-oligosaccharide has a structure wherein D-galactosamine monomers are polymerized through α-1,4 bond. The galactosamino-oligosaccharide to be used can be obtained, for example, by hydrolyzing natural polygalactosamine produced by imperfect fungi, Paecilomyces with an acid or enzyme. Since both chitosan and galactosamino-oligosaccharide are reactive molecules having a highly reactive amino group at the 2-position in the component monosaccharide, a complicated derivation is not required to bond with a ligand. As a result, reaction with a bifunctional ligand can be completed in a single step.

Respective oligosaccharides are fractionated in high purity by chromatography according to the degree of polymerization and these oligosaccharides having uniform molecular weight are commercially available. Therefore, the number of bifunctional ligands and metal ions to be introduced can be precisely controlled and it is possible to prepare a pharmaceutically homogenous polynuclear type metal complex compound. In addition, both have high compatibility with the living body and physiological acceptability.

As the bifunctional ligand, there can be used linear or cyclic polyaminopolycarboxylic acids having a cross-linking chain moiety which can bond to the amino group at the 2-position of the amino oligosaccharide as a backbone. The preferred bifunctional ligand is a ligand having as a coordinating partial structure the skeleton of DTPA or a derivative thereof, or the skeleton of 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (abbreviated as "DOTA") or a derivative thereof. As the reactive group in the cross-linking chain part of the bifunctional ligand which can bond to the amino group at the 2-position, i.e., the reactive functional group, active halogen, alkoxyester, succinimidiester, isothiocyanate, acid anhydride and the like are preferred. More particularly, there are 1-(p-isothiocyanatebenzyl)-DTPA [Martin, W.B., et al., Inorg. Chem., 25, pages 2772-2781 (1986), DTPA anhydride, 2-(p-isothiocyanatebenzyl)-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid [U.S. Patent No. 4,678,667] and the like.

The bond between the amino-oligosaccharide and the bifunctional ligand can be formed according to a known method. For example, reaction of the bifunctional ligand having as the cross-linking chain terminal an acid anhydride [Hnatowich, D.J., et al., Int. J. Appl. Rad. Isot., 33, pages 327-332 (1982)], isothiocyanate [Esteban, J.M., et al., J. Nucl. Med., 28, pages 861-870 (1987)], alkoxyester [Washburn, L.C., et al., Nucl. Med. Biol., 18, pages 313-321 (1991)] or active halogen [Fourie, P.J., et al., Eur. J. Nucl. Med., 4, pages 445-448 (1979)] with the amino-oligosaccharide can be carried out according to the description in the above cited publications.

In the present invention, the metal ion is selected from the group consisting of metal ions having the atomic number of 21-29, 31, 32, 37-39, 42-44, 49 and 56-83 depending upon a particular use of image diagnosis. When the polynuclear type metal complex of the present invention is used for MRI diagnosis, the metal ion must be paramagnetic and is selected from the ions of atomic number 26 and lanthanide having atomic numbers of 57-70. The metal ion is preferably an ion of Gd, Dy, Tb, Ho, Er or Fe. When used for X-ray diagnosis, the metal ion is selected from the lanthanide element ions having atomic numbers of 57-70 and the ions of the element having an atomic number of 56, 76, 82 and 83. The metal ion is preferably an ion of Bi, Pb or Os. For radiation diagnosis, the metal ion must be radioactive and is suitably a radioactive metal ion such as Co, Cu, Ga, Ge, Sr, Y, Tc, In, Sm, Gd, Yb, Re or Ir. As the metal ion, there can be used a metal itself or an inorganic compound thereof (for example, chloride, oxide). Complexation can be carried out using a conventional method.

In the polynuclear type metal complex compound thus obtained, at least one, preferably, two or more bifunctional ligands are chemically bonded to chitosan-oligosaccharide or galactosamino-oligosaccharide and the metal ions are bonded to this coordinating moiety through a complexing bond.

The polynuclear type metal complex compound can be formulated into an imaging agent for diagnosis in any suitable dosage form by mixing with any suitable pharmaceutically acceptable additive according to a conventional method and, preferably, formulated into an imaging agent for diagnosis in a solution form by dissolving it in a physiclogically acceptable aqueous solvent.

When the polynuclear type metal complex compound of the present invention is used as an imaging agent for diagnosis, the dose to be used is selected depending upon the particular type of image diagnosis. For example, for MRI diagnosis, the dose is generally 0.0001 to 10 mmol/kg, preferably, 0.005 to 0.5 mmol/kg in terms of the metal ion. For X-ray diagnosis, the dose is 0.01 to 20 mmol/kg, preferably, 0.1 to 10 mmol/kg in terms of the metal ion. Further, for radiation diagnosis, the dose is 370-18500 MBq in terms of radioactivity. Usually, the imaging agent is administered intravenously and, in some cases, can be administered orally or intra-arterially.

Retention in the blood of the polynuclear type metal complex compound of the present invention is in a clinically effective range (half-life period in the blood of 0.5 to 5 hours). Thus, it is possible to suitably combine the imaging agent with a particular MRI apparatus having a different magnetic field intensity by appropriately selecting the polymerization degree of the amino oligosaccharide. For example, in the case of low magnetic field intensity MRI apparatus, the use of the imaging agent for diagnosis having a relatively long retention time in the blood is preferred in order to improve the collection efficacy of the proton relaxation effect by the imaging agent. In addition, the polynuclear type metal complex compound of the present invention has the advantage of having a higher contrast efficacy per unit dose. For example, when Gd is contained as the metal ion, the shortening effect of the relaxation time per molecule is superior to that of DTPA-Gd, the polynuclear type metal complex compound can be used advantageously as an MRI diagnostic agent. This improves the detection efficacy in another sense in the diagnosis by low magnetic field MRI apparatus having a low collection efficacy of proton relaxation effect, resulting in a shortening of the imaging time. Further, when the same contrast effect as that of DTPA-Gd in an apparatus having the same magnetic field intensity is required, the polynuclear type metal complex compound of the present invention can be administered in a smaller dose than DTPA-Gd and, therefore, becomes more advantageous from the viewpoint of safety. To the contrary, at the same dose, the polynuclear metal complex compound of the present invention provides more information about the living body than DTPA-Gd, resulting in an improvement in the clinical usefulness. Therefore, the present invention can provide an imaging agent having suitable retention in the blood, matching with the magnetic field intensity of an MRI apparatus and imaging conditions, as well as effective contrast effect.

Further, since the polynuclear type metal complex compound of the present invention shows suitable retention in blood, evaluation of the blood vessel distribution image (vascularity) becomes possible. Therefore, the imaging agent for diagnosis of the present invention can image the blood vessel without pulse sequence which is particularly necessary for recently remarkably advanced MR angiography, and the agent is also useful as a diagnostic imaging agent for intravenous injection.

Since the polynuclear type metal complex compound of the present invention has good solubility in water, the compound itself can be prepared as a solution containing the compound in a high concentration. Accordingly, a solubilizer is not necessarily required upon preparation of the solution. In addition, the metal complex compound of the present invention is a polynuclear compound and, therefore, can decrease the total molality in the preparation of a solution in comparison with the mononuclear compound, which results in a decrease in osmotic pressure. This alleviates the load to volume on the circulatory system or body fluid equilibrium upon administration in the living body, resulting in increased safety.

As described hereinabove, the imaging agent of the present invention comprises a polynuclear type metal complex wherein a plurality of metal ions are chemically bonded thereto via a plurality of bifunctional ligands which are chemically bonded to the chitosan-oligosaccharide or galactosamino-oligosaccharide. By using this novel and special polynuclear type metal complex compound, image diagnosis, e.g. MRI diagnosis, X-ray diagnosis, radiation diagnosis and the like can be efficiently carried out.

The following Examples and Tests further illustrate the present invention in detail but are not to be construed to limit the scope thereof.

The abbreviations used in the Examples and Tests are defined as follows:

GPEN: galactosamino-pentamer

CHEX: chitosan-hexamer

GTRI: galactosamino-trimer

CPEN: chitosan-pentamer

DTPA: diethylenetriaminepentaacetic acid

DTES: 1-(p-isothiocyanatebenzyl)-diethylenetriaminepentaacetic acid

DOTA: 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid

ICB-DOTA: 2-(p-isothiocyanatebenzyl)-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid.

Example 1

Synthesis of GPEN-DTES

GPEN (0.39 g; 0.43 mmol) was dissolved in 0.1M phosphate buffer (pH 7.0) (2 ml) and DTES (0.70 g; 1.3 mmol) was added thereto. 10N Aqueous solution of sodium hydroxide was added thereto to adjust pH to about 12, and the mixture was reacted at room temperature for 24 hours with stirring. To the reaction mixture neutralized by adding 7N hydrochloric acid to obtain crude GPEN-DTES.

A portion of the reaction mixture (50 µl) was removed and 0.1M citrate buffer (pH 5.9) (100 µl) and a solution (50 µl) of indium chloride (In-111) were admixed with the reaction mixture. The ratio of GPEN-DTES-In-111 and DTES-In-111 was determined by thin layer chromatography and it was confirmed that 1.4 molecules of DTES were bonded per

GPEN molecule.

The above reaction mixture was concentrated and purified by preparative thin layer chromatography (silica gel) to obtain GPEN-DTES (0.24 g).

Proton-nuclear magnetic resonance (NMR) spectrum (solvent/D_2O, 270MHz): 2.10-3.37 ppm (10H, m, CH_2), 3.49-4.55 ppm, 4.88-5.59 ppm (m, CH, CH_2 and NH), 4.22 ppm(1H, bs, N-CH), 7.07-7.40 ppm (4H, m, benzene ring)

Infrared absorption (IR) spectrum (KBr tablet): $810cm^{-1}$ (CH of benzene ring), $1100cm^{-1}$ (OH), $1400cm^{-1}$ (CH_2), $1590cm^{-1}$ (COOH)

Example 2

Synthesis of GTRI-DTES

GTRI (6.4 mg; 0.01 mmol) was dissolved in 0.1M phosphate buffer (pH 7.0) (1 ml) and DTES (17.4 mg; 0.03 mmol) was added thereto. 10 N Aqueous solution of sodium hydroxide was added thereto to adjust pH to about 12, and the mixture was reacted at room temperature for 24 hours with stirring. To this reaction mixture was added 7N hydrochloric acid to neutralize to obtain crude GTRI-DTES.

A portion of the reaction mixture (50 μl) was removed and 0.1M citrate buffer (pH 5.9) (100 μl) and a solution (50 μl) of indium chloride (In-111) were admixed with the reaction mixture. The ratio of GTRI-DTES-In-111 and DTES-In-111 was determined by thin layer chromatography and it was confirmed that 3 molecules of DTES were bonded per GTRI molecule.

The above reaction mixture was concentrated and purified by preparative thin layer chromatography (silica gel) to obtain GTRI-DTES (11.0 mg).

Proton-NMR spectrum (solvent/D_2O, 270MHz): 2.20-3.58 ppm (10H, m, CH_2), 3.58-4.63 ppm, 4.95-5.65 ppm (m, CH, CH_2 and NH), 4.30 ppm (1H, bs, N-CH), 7.15-7.45 ppm (4H, m, benzene ring)

IR spectrum (KBr tablet): 810cm^{-1} (CH of benzene ring), 1070cm^{-1} (OH), 1400cm^{-1} (CH_2), 1625cm^{-1} (COOH)

Example 3

Synthesis of CPEN-DTPA

CPEN (0.08 g; 0.08 mmol) was dissolved in water (2 ml) and 4N aqueous solution (1.2 ml) of sodium hydroxide was added thereto. DTPA anhydride (0.57 g; 1.59 mmol) was added thereto immediately, and the mixture was reacted at room temperature for 3 hours with stirring to obtain crude CPEN-DTPA.

A portion of the reaction mixture (0.2 ml) was removed and 0.1M citrate buffer (pH 5.9) (0.2 ml) and a solution (0.025 ml) of indium chloride (In-111) were admixed with the reaction mixture. The ratio of CPEN-DTPA-In-111 and DTPA-In-111 was determined by thin layer chromatography and it was confirmed that 4.5 molecules of DTPA were bonded per CPEN molecule.

The above reaction mixture was concentrated and purified by preparative thin layer chromatography (silica gel) to obtain CPEN-DTPA (0.08 g).

Proton-NMR spectrum (solvent/D_2O, 270MHz): 2.0 ppm (H, s, CH_2), 3.1-3.3 ppm (m, CH_2), 3.4-3.6 ppm (m, CH_2), 3.8 ppm(4H, s, CH_2)

IR spectrum (KBr tablet): 1090cm^{-1} (OH), 1400cm^{-1} (CH_2), 1600cm^{-1} (COOH)

Example 4

Synthesis of CPEN-(ICB-DOTA)

CPEN and ICB-DOTA were dissolved in 0.1M phosphate buffer (pH 7.0), and the solution was reacted at room temperature while maintaining pH at 12 to obtain CPEN-(ICB-DOTA).

Example 5

Preparation of GPEN-DTES-Gd solution

GPEN-DTES (0.30 g; 0.18 mmol) was dissolved in distilled water (2 ml). Gadolinium chloride hexahydride (0.06 g; 0.17 mmol) was added thereto and the mixture was reacted at room temperature with stirring to obtain GPEN-DTES-Gd. The absence of free Gd was confirmed by a color developing reaction using Xylenol Orange as a pigment indicator.

Gd concentration (ICP emission analysis): 75.1 mM

Example 6

Synthesis of Gd complex

Gd complex of the relevant compound was obtained using the same method as that described in Example 5 except that GPEN-DTES was substituted by GTRI-DTES, CPEN-DTPA and CPEN-(ICB-DOTA).

Example 7

Preparation of GPEN-DTES-In-111 solution

GPEN-DTES (10 mg) was dissolved in distilled water (0.5 ml) and 0.1M citrate buffer (pH 5.9) (1 ml) was added thereto. A solution (0.5 ml; 148MBeq) of indium chloride (In-111) was admixed to obtain GPEN-DTES-In-111. Its radiochemical purity was 100%.

Example 8

Synthesis of CHEX-DTPA-Bi

CHEX-DTPA (0.45 g; 0.13 mmol) synthesized according to the same method as that described in Example 3 was dissolved in distilled water (30 ml). Bismuth chloride (0.28 g; 0.88 mmol) was added thereto, pH was adjusted to about neutral by the addition of 4N aqueous solution of sodium hydroxide. The mixture was reacted at 60°C for 18 hours with stirring. The insoluble materials were filtered off and the filtrate was purified through a desalting apparatus (manufactured by Asahikasei K.K., Japan). The purified solution was concentrated and dried to obtain CHEX-DTPA-Bi (0.54 g). The absence of free Bi was confirmed by a color developing reaction using Xylenol Orange as a pigment indicator.

IR spectrum (KBr tablet): 1070cm^{-1} (OH), 1393cm^{-1} (CH_2), 1458cm^{-1} (CONH), 1582cm^{-1} (COO^-)

Quantitative analysis of Bi (ICP emission analysis): 0.11 g

Test 1

Relaxivity of GPEN-DTES-Gd and GTRI-DTES-Gd (*in vitro* test)

An appropriate amount of each of GPEN-DTES-Gd and GTRI-DTES-Gd was dissolved in distilled water. The relation to water proton exposed to these compounds was determined as a proton relaxation time (T_1 and T_2, msec) at room temperature (24 to 26°C) using NMR (6.35T, manufactured by Nihondenshi K.K., Japan). Respective relaxation times are shown in Tables 1 and 2.

Table 1

Relaxation time of GPEN-DTES-Gd

Concentration (mM)	T_1 (msec)	T_2 (msec)
2.3	55	26
0	3275	2208

Table 2

Relaxation time of GTRI-DTES-Gd

Concentration (mM)	T_1 (msec)	T_2 (msec)
2.9	46	26
0	3275	2208

GPEN-DTES-Gd (2.3mM) shortened remarkably the T_1 value of water by about 60 times and the T_2 value of water by 85 times. And GTRI-DTES-Gd (2.9mM) shortened remarkably the T_1 value of water by about 70 times and the T_2 value by about 85 times. The relaxivity on T_1 and T_2 [each R_1 and R_2, $(mM \cdot S)^{-1}$] was calculated based on the values in Tables 1 and 2. The results are shown in Table 3.

Table 3

Relaxivity of GPEN-DTES-Gd and GTRI-DTES-Gd

Compound	R_1 $(mM \cdot S)^{-1}$	R_2 $(mM \cdot S)^{-1}$
GPEN-DTES-Gd	7.6	16.2
GTRI-DTES-Gd	7.4	13.1
DTPA-Gd	3.9	4.8

GPEN-DTES-Gd and GTRI-DTES-Gd have good *in vitro* relaxation effect and the effect determined according to the same manner is predominantly higher than that of DTPA-Gd (also shown in Table 3) which is a mononuclear complex. The results clearly show the effectiveness of GPEN-DTES-Gd and GTRI-DTES-Gd.

Test 2

Relaxation time of GPEN-DTES-Gd in blood in a mouse after intravenous administration (*ex vivo* test)

GPEN-DTES-Gd (Gd concentration: 75 mM) (0.025 mmol/kg in terms of Gd) was administered to a thiopental anesthetized ICR female mouse (body weight: 44 g) through the tail vein. At 15 minutes after administration, the blood was taken from the aorta descendence, and the relaxation time (T_1, msec) of the blood at room temperature (24 to 26°C) was determined with a 6.35T NMR apparatus (manufactured by Nihondenshi K.K., Japan).

As a control, blood was taken from the aorta descendence of a thiopental anesthetized ICR female mouse (body weight: 55 g) and, according to the same manner, the relaxation time was determined. The results are shown in Table 4.

Table 4

Relaxation time of GPEN-DTES-Gd in blood

Administered compound	T_1 in blood (msec)
GPEN-DTES-Gd	769
control	1769

T_1 relaxation time of GPEN-DTES-Gd in blood is about 2.3 times effect compared with that of the control mouse and it has been found that the relaxation time of the blood is effectively shortened.

Test 3

Contrast enhancement of the heart in a rat 1 hour after intravenous administration of GPEN-DTES-Gd (*in vivo* test)

A solution of GPEN-DTES-Gd (Gd concentration: 75.1 mM) (0.094 mmol/kg in terms of Gd) was administered to a thiopental anesthetized Sprague-Dawley female rat (214 g, 9-weeks old) through a cannula fixed to the femoral vein. At 1 hour after administration, the animal was sacrificed by administration of pentobarbital solution (1 ml) through the above cannula, fixed at prone position in the magnetic field of an MRI spectrometer. MRI measurement (transverse sectional view) of the chest region including the heart was carried out.

As a control, DTPA-Gd (MAGNEVIST®) was administered to a Sprague-Dawley female rat (body weight: 204 g, 9-weeks old) through a cannula fixed at the femoral vein (0.1 mmol/kg) and the measurement (transverse sectional view) of the chest region including the heart was carried out as described above.

The apparatus used was SIGMA* (manufactured by GE, U.S.A.) with a magnetic field intensity of 1.5T and, as an imaging coil, a 26 cm ϕbird-cage type head QD coil was used. Imaging was carried out according to the spin echo method of T_1 weighted (TR/TE, 600/30 msec) under the condition of 10 mm in slice thickness, a resolution of 256 x 128.

The signal intensity from the rat to which GPEN-DTES-Gd was administered was found to be about 1.8 times higher than that of the rat to which MAGNEVIST® was administered when comparing the signal intensity from the same part of the heart. The superiority in retention in the blood of GPEN-DTES-Gd over that of DTPA-Gd together with the

*Trade mark

dose of Gd demonstrated the advantages of the present invention.

Test 4

Radioactivity distribution in blood and urine after intravenous administration of GPEN-DTES-In-111 (*in vivo* test)

Sprague-Dawley female rats (three rats/mesurement time) (body weight: 110 to 130 g) were anesthetized with thiopental and GPEN-DTES-In-111 solution prepared in Example 7 was administered through the tail vein (50 μl/rat). The animals were sacrificed by dehematization at 0.25, 0.5, 1, 3, 6 and 24 hours after administration. The blood and bladder were removed and the radioactivity was measured. The radioactivity distribution ratio in blood and urine at each measurement time are shown in Table 5.

Table 5

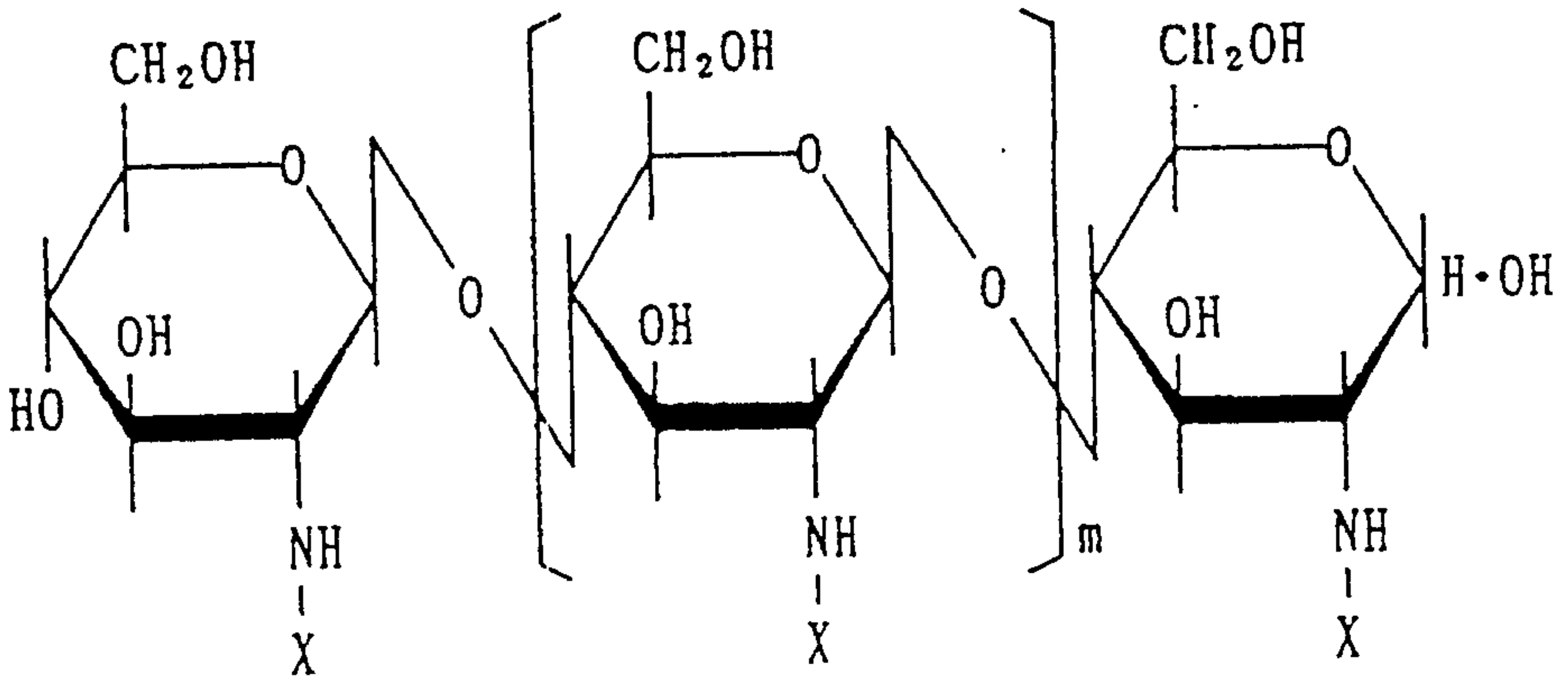
Radioactivity distribution ratio of GPEN-DTES-In-111 in blood and urine

Time (hr)	Blood (%/dose)	Urine (%/dose)
0.25	4.63±1.65	51.23±1.40
0.5	2.63±0.86	66.07±3.45
1.0	2.72±0.40	77.13±3.36
3.0	1.92±1.06	81.43±6.23
6.0	0.67±0.35	87.04±4.68
24.0	0.16±0.12	90.12±3.57

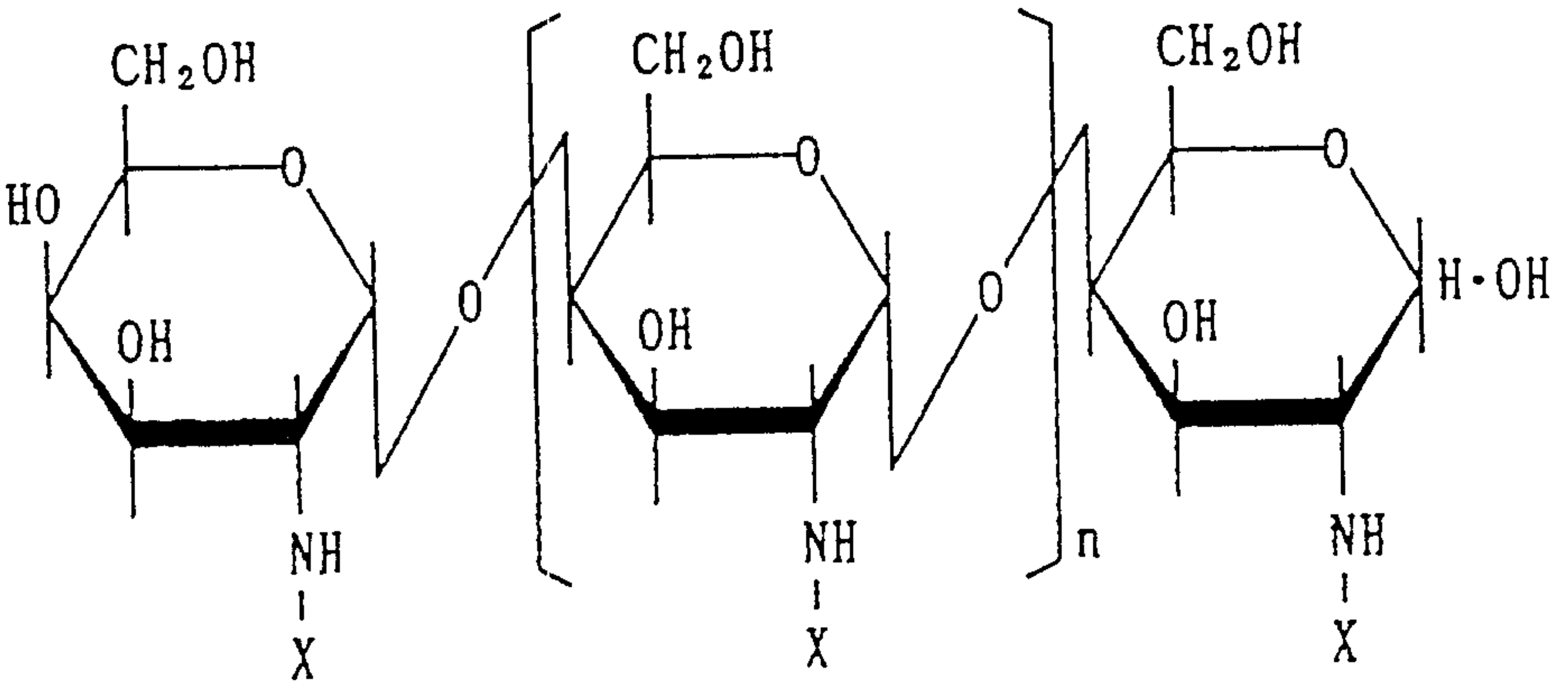
As seen from the results in Table 5, the half-life period of GPEN-DTES-In-111 in blood was about 55 minutes and was found to be clinically effective retention in blood. Since excretion into the urine was good, there was no problem of residence in the body.

Claims:

1. An imaging agent for diagnosis comprising a compound composed of a polynuclear type compound of the formula I or II:



(I)



(II)

wherein each X is a hydrogen atom or a bifunctional ligand, at least one being a bifunctional ligand and each of m and n is an integer of 1 to 6, and at least one metal ion being coordinated with at least one bifunctional ligand moiety, said metal ion being selected from the group consisting of metal ions having an atomic number of 21-29, 31, 32, 37-39, 42-44, 49 and 56-83.

2. The imaging agent for diagnosis according to claim 1, wherein the bifunctional ligand is derived from diethylenetriaminepentaacetic acid or 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid.

3. The imaging agent for diagnosis according to claim 1, which is useful as an imaging agent for nuclear magnetic resonance diagnosis and the metal ion is Gd, Dy, Tb, Ho, Er or Fe ion.

4. The imaging agent for diagnosis according to claim 1, which is useful as an imaging agent for X-ray diagnosis and the metal ion is Bi, Pb or Os ion.

5. The imaging agent for diagnosis according to claim 1, which is useful as an imaging agent for radiation diagnosis and the metal ion is Co, Cu, Ga, Ge, Sr, Y, Tc, In, Sm, Gd, Yb, Re or Ir ion.

6. The imaging agent for diagnosis according to claim 1, the retention time in blood of which is 0.5 to 5 hours as half-life period.

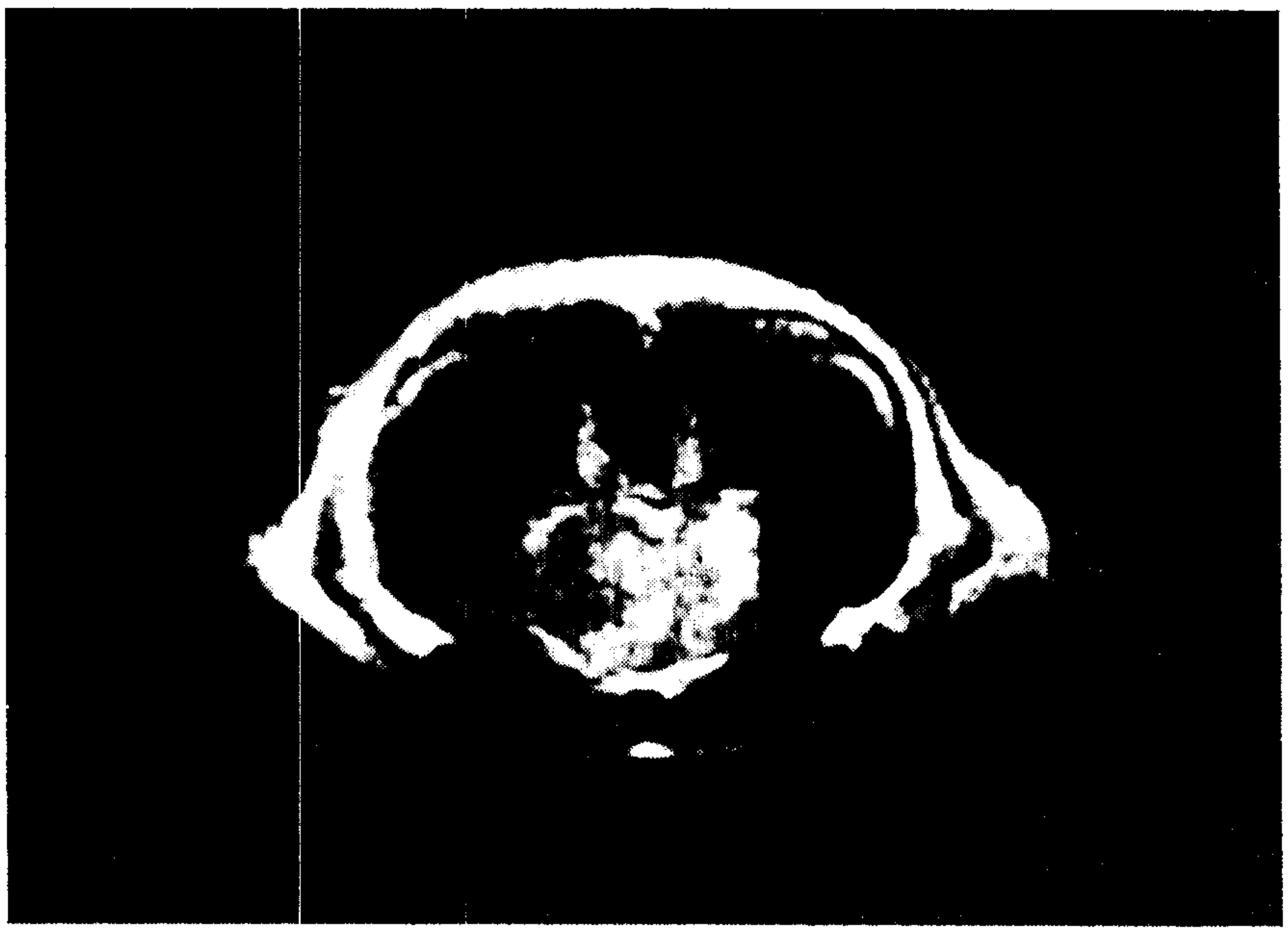


FIG.1

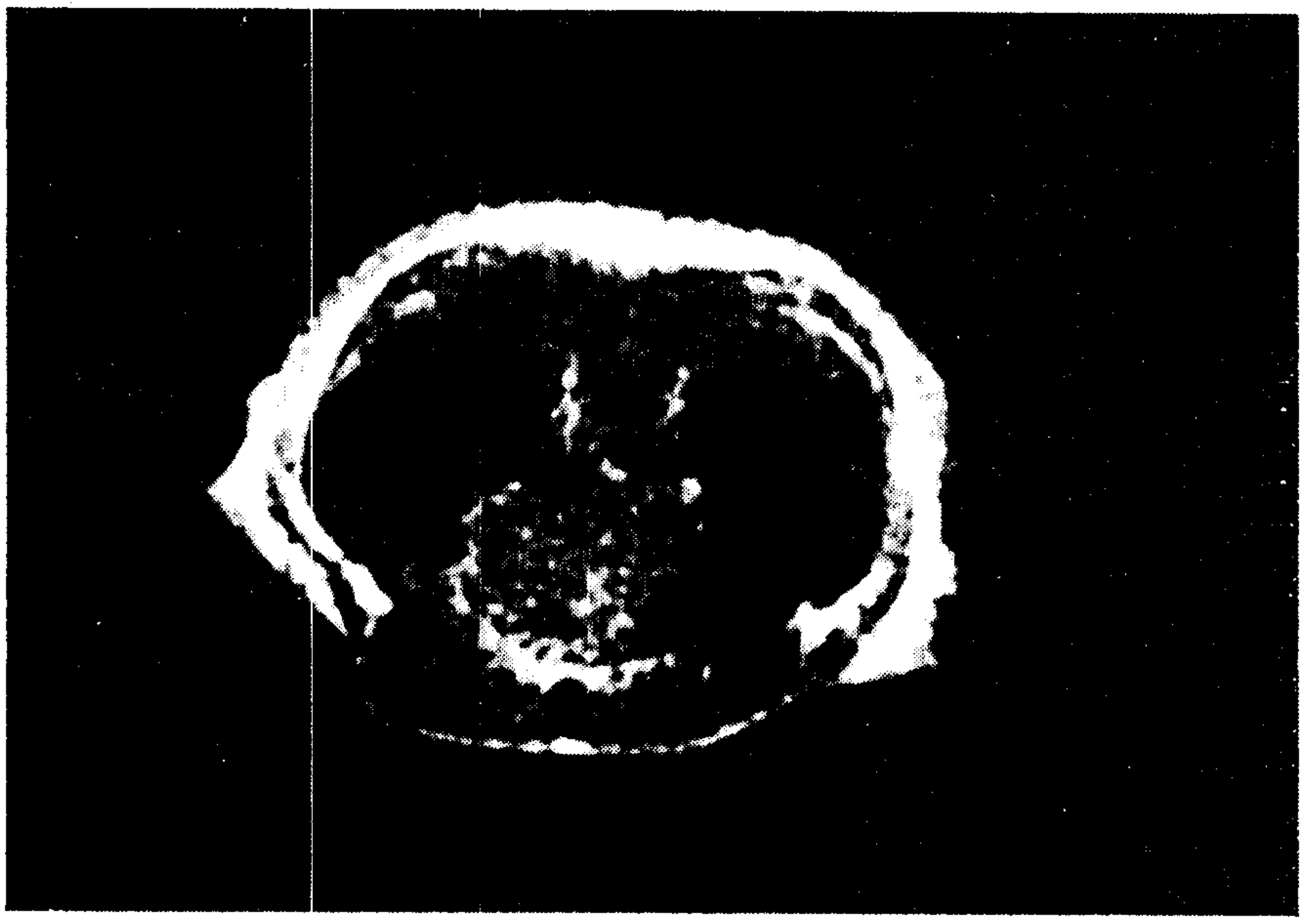


FIG.2

Kirby Eades Gale
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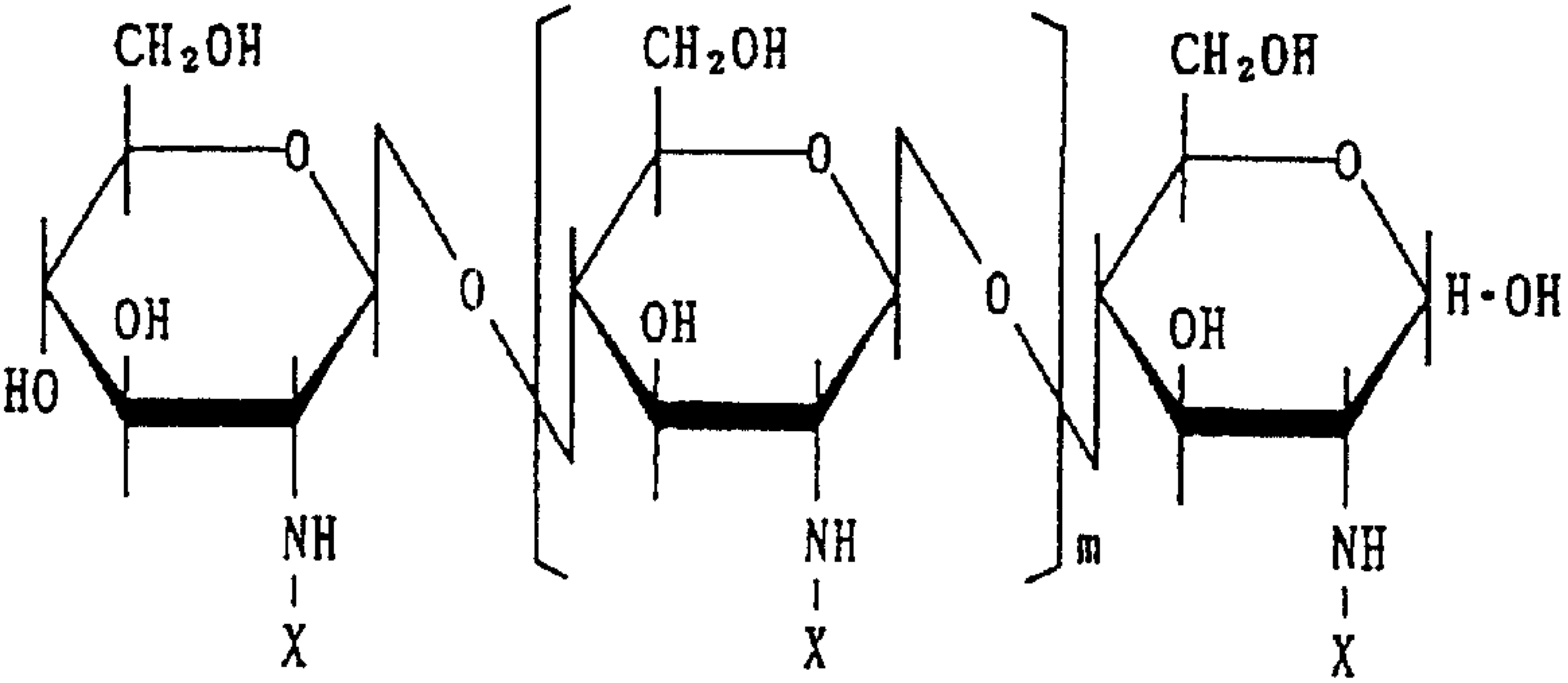
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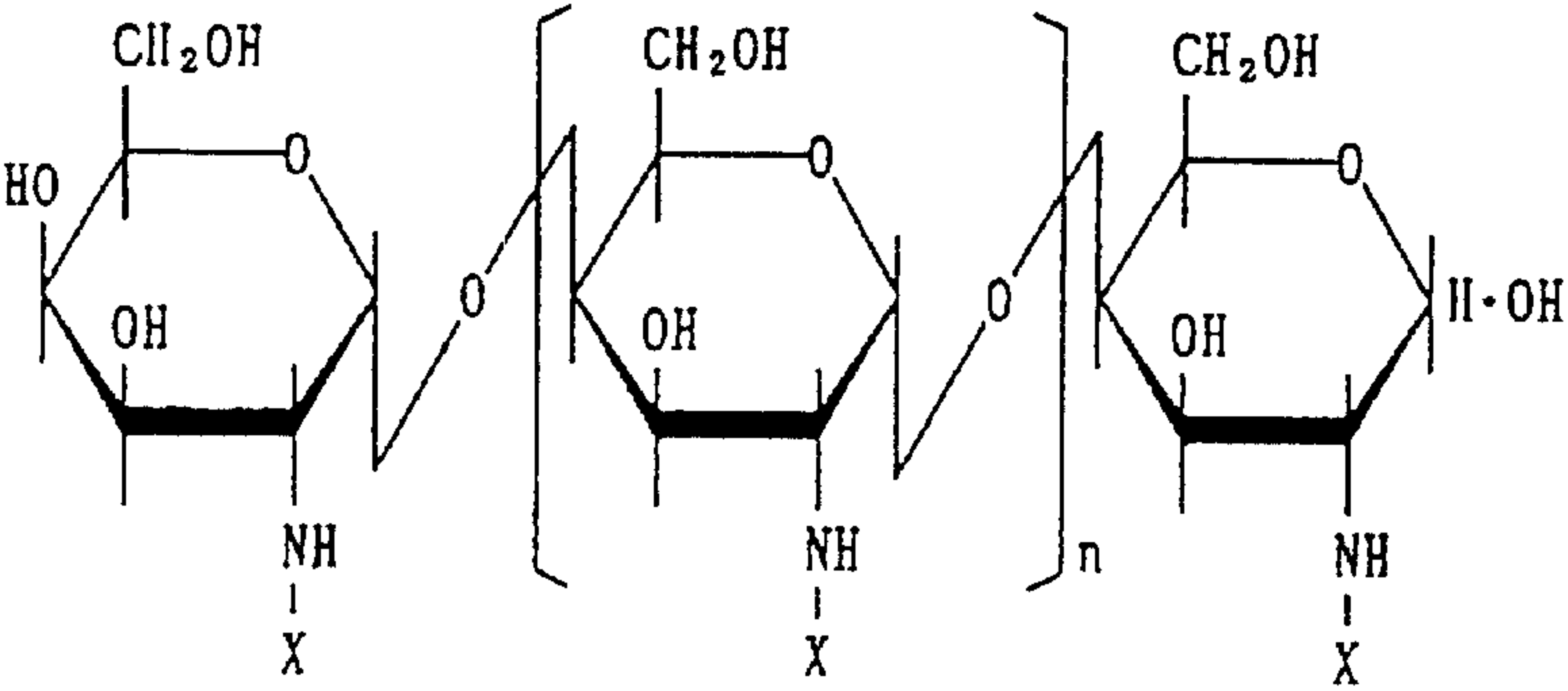
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