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(54) Title: IRON-CONTAINING NANOPARTICLES WITH DOUBLE COATING AND THEIR USE IN DIAGNOSIS AND THERAPY

(57) Abrégé/Abstract:
A nanoparticle compound is provided herein for use in diagnosis and therapy. The nanoparticle compound is an iron-containing core comprising iron material, namely magnetite or maghemite. A synthesis polymer coats the iron-containing core, the synthesis polymer being a dextran or a derivative of dextran. A targeting polymer is non-covalently bonded to, and envelopes, the synthesis polymer to form a second coating. In this way, the targeting polymer is not exposed to synthesis conditions.

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ABSTRACT

A nanoparticle compound is provided herein for use in diagnosis and therapy. The nanoparticle compound is an iron-containing core comprising iron material, namely magnetite or maghemite. A synthesis polymer coats the iron-containing core, the synthesis polymer being a dextran or a derivative of dextran. A targeting polymer is non-covalently bonded to, and envelopes, the synthesis polymer to form a second coating. In this way, the targeting polymer is not exposed to synthesis conditions.

(a) TITLE OF THE INVENTION

IRON-CONTAINING NANOPARTICLES WITH DOUBLE COATING AND THEIR USE IN DIAGNOSIS AND THERAPY

(b) TECHNICAL FIELD TO WHICH THE INVENTION RELATES

This invention relates to iron-containing nanoparticles having a modular structure, methods for their production, and their use for diagnostic and therapeutic purposes.

(c) BACKGROUND ART

Substances that show maximum magnetization even at a low field strength (high saturation magnetization) but no remanence after the external magnetic field is switched off, as the thermal energy counteracts the permanent alignment of spontaneously magnetized Weiss' domains, are called superparamagnetic substances. This category includes iron-containing crystals that are developed as parenteral MR contrast materials. A characteristic property of said substances is their strong impact on proton relaxation times and thus their great efficacy as a contrast medium in this diagnostic procedure. In medical diagnostics, the focus of examining superparamagnetic substances was placed on iron oxides having a "magnetite-like" crystal structure of the kind found in magnetite or maghemite (spinel, inverse spinel).

The superparamagnetic iron oxides to be used as MR contrast materials have similar properties in that they strongly influence proton relaxation in their close range (high relaxivity), and that they are particles having a "magnetite-like" crystal structure.

A great number of methods have been described for the production of iron-containing crystals (iron oxides) having superparamagnetic properties. These methods can be classified according to various aspects. Two basic methods to produce superparamagnetic crystals can be distinguished between: sintering at high temperatures and subsequent mechanical comminution, or wet chemical synthesis in solution. Up to now, only those particles that were produced by wet synthesis have been investigated for

medical applications, while the sintering method has been described for the manufacture of iron oxides for technological (sound carriers, paint pigments and toners) and biotechnological applications such as the magnetic separating method [Schostek S, Beer A; DE 3,729,697 A1; Borelli NF, Luderer AA, Panzarino JN; US 4,323,056; Osamu I, Takeshi H, Toshihiro M et al.; JP 60,260,463 A2]. Wet chemical synthesis can be subcategorized. There is "two-pot synthesis", which first produces an iron-containing core (iron oxide) to which a stabilizer is added to ensure the physical and galenic quality. The production of an iron core using ion exchangers is a variant of "two-pot synthesis". With "single-pot synthesis", the iron oxides are produced in the presence of the stabilizer which already coats the cores during nucleation and precipitation of the iron salts, thereby preventing aggregation and sedimentation of the nanocrystals.

Apart from distinguishing "two-pot" and "single-pot" methods according to the processes involved, there is another distinction based on the type of solvent used, namely between aqueous [Hasegawa M, Hokukoku S; US 4,101,435; Fuji Rebio K.K.; JP 59,195,161] and non-aqueous methods [Porath J, Mats L; EP 179,039 A2; Shigeo A, Mikio K, Toshikatzu M; J. Mater. Chem. 2(3); 277-280; 1992; Norio H, Saturo O; JP 05,026,879 A2].

Particles that were produced in a "two-pot" process using non-aqueous solvents are mainly used in engineering. Magnetic iron oxides for use as contrast materials in human diagnostics require an aqueous dispersing agent for medical and toxicological reasons. A special place is held in this categorization by those particles that were produced in a non-aqueous solvent but can be stable when dispersed in an aqueous medium after production. Such particles are currently used, in general, in ex-vivo

diagnostics, e.g. in magnetic separation engineering [Chagnon MS, Groman EV, Josephson L, et al.; US 4,554,088] but have also been proposed for in-vivo diagnostics [Pilgrimm H; US 5,160,725].

Particles produced in a "two-pot" process were mainly used in the early experimental examinations up to the mid-1980s, while today's tests involving iron oxides are described only for materials produced by a "single-pot synthesis". The "single-pot" method has been generally accepted for the production of superparamagnetic iron-containing oxides for human diagnostic applications as they are superior to those produced by a "two-pot" method from the point of view of their physical and chemical quality as well as pharmaceutical/galenic stability.

Pharmaceutically stable suspensions/solutions of particles produced in aqueous media according to the "single-pot method" may be subdivided into iron oxides of different sizes. Biotechnological applications were proposed for particles in the micrometer range [Schröder U, Mosbach K; WO 83/01738 or Schröder U; WO 83/03426], and their application even claimed in in-vivo diagnostics and therapy [Widder KJ, Senyei AE; US 4,247,406 or Jacobsen T, Klaveness J; WO 85/04330]. For approaches in medical diagnostics, however, particles in the nanometer range are the main ones described today. This range may also be subdivided according to the preferred use into "large" (overall diameter ca. > 50 nm) and "small" (overall diameter ca. < 50 nm) particles. MR diagnostics of the liver and the spleen is the main field of application, as particles of this size are rapidly and nearly completely taken up by the macrophages of these organs [Kresse M, Pfefferer D, Lawaczeck R; EP 516,252 A2 or Groman EV, Josephson L; US 4,770,183]. Furthermore, proposals were made for uses as reinforcing substances in clinical

hyperthermia[Hasegawa M, Hirose K, Hokukoku S, et al.; WO 92/22586 A1 and Gordon RT; US 4,731,239].

Nearly all the particles currently proposed for medical applications are iron oxides that were produced in the presence of dextran as the stabilizing substance [Bacic G, Niesmann MR, Magin RL et al.; SMRM - Book of abstracts 328; 1987; Ohgushi M, Nagayama K, Wada A et al.; J. Magnetic Resonance 29; 599-601; 1978; Pouliquen D, Le Jeune JJ, Perdrisot R et al.; Magnetic Resonance Imaging 9; 275-283; 1991 or Ferrucci JT and Stark DD; AJR 155; 311-325; 1990] but the use of other polysaccharides has also been described, for example, for arabinogalactan [Josephson L, Groman EV, Menz E et al; Magnetic Resonance Imaging 8; 616-637; 1990], starch [Fähl vik AK, Holtz E, Schröder U et al; Invest. Radiol. 25; 793-797; 1990], glycosaminoglycans [Pfefferer D, Schimpfky C, Lawaczeck R; SMRM - Book of abstracts 773; 1993], or proteins [Widder DJ, Grief WL, Widder KJ et al.; AJR 148; 399-404; 1987].

The exact conditions for synthesis such as those involving iron salts, temperature, coating polymer (stabilizer), titration rate, alkali selection, purification, etc. affect the chemical and physical properties of the products and, therefore, their pharmaceutical and galenic quality as well as medical value.

An important step in the development leading to an effective use in specific applications was made by Weissleder and Papisov [Weissleder R; Papisov MI; Reviews of Magnetic Resonance in Medicine 4; 1-20; 1992] who were able to show that the "targetability" of the magnetic iron oxides is reciprocally proportional to particle size. A problem in this respect is the fact that efficacy (MR effect) decreases with smaller particle sizes. The production of particularly small magnetic iron oxides

without any fractionating stages has recently been described [Hasegawa M, Ito Y, Yamada H, et al.; JP 4,227,792]. Experiments on "functional imaging" were reported for particularly small particles called MIONs. The dextran coating of said particles (magnetic labels) were oxidized using periodate and then coupled with specific molecules (antimyosin; polyclonal antibody) [Weissleder R, Lee AS, Khaw BA et al.; Radiology 182; 381-385; 1992, or Weissleder R, Lee AS, Fishman A et al.; Radiology 181; 245-249; 1991].

A special course is taken by Menz et al. [Menz ET, Rothenberg JM, Groman EV, et al.; WO 90/01295] who coat their large nanometer particles with polymers (arabinogalactan) having physiological effector cells and claim a specific uptake mechanism via receptor-mediated endocytosis just like Gordon [Gordon RT; US 4,735,796], who oxidizes dextran-stabilized particles using periodate and then couples them with transferrin by reductive amination.

Production of "large" superparamagnetic iron oxides for use as contrast materials in MR diagnostics of the liver and the spleen is the state of the art, and the diagnostic benefit of said materials has been proved. Some of these iron oxides are being developed for clinical purposes (AMI-25; Advanced Magnetics Inc.; Cambridge; Mass.; USA; Phase III/IV and SHU 555A; Schering AG Berlin; Germany; Phase II). The importance of the hydrodynamic diameters of iron oxides for specific (extrahepatic) approaches such as MR lymphography or MR angiography are known and are being examined. The half-life in the blood should increase with smaller diameters for particles that are otherwise identical. Synthesis variants for producing small iron oxides are known from the literature.

An essential problem encountered in the development of specific contrast materials based on superparamagnetic iron oxides is that it has been impossible so far to improve targeting properties, i.e. accumulation and distribution in the target tissue, without having to accept simultaneous drawbacks in physical and chemical parameters, as the stabilizers that are most suitable for producing iron-containing cores are very limited for targeting purposes. In addition, reaction conditions during synthesis (acid to alkaline pH, temperatures, redox reactions involving the iron salts) reduce the choice of potential stabilizers in that whole groups of important and highly specific molecules (proteins, peptides, oligonucleotides, but also most oligo- and polysaccharides) cannot be used as stabilizers in the manufacturing phase whenever said substances have to retain any targeting properties (bioactivity) after the synthesis.

It is known from the (chemically) "insensitive" polymers used up to now, mainly from dextran, that various non-controllable reactions occur in synthesis conditions, for example, depolymerization in the acidic range of pH values (low-molecular weight dextran, for example, is yielded in technical quality by acid hydrolysis) and various other reactions that may result in complete destruction of the (glyco-) polymer in the alkaline range (precipitation step). It may be assumed, taking into account sucro-/glycochemistry and the reaction conditions required, that state-of-the-art "dextran magnetites" are not dextran magnetites at all because dextran was used for stabilizing, but no dextran remained after synthesis. If this is viewed from the pharmaceutical and approval point of view, this means that an essential ingredient - as the stabilizer forms the coat and thus determines

biological behavior to a major extent - is unknown or undeclared.

Another practical problem resulting therefrom is that surface properties cannot be optimized during future development if the surface itself is unknown.

A specific application such as MR lymphography, which has been studied the best, can be used to show that size optimization of state-of-the-art particles using dextran as the stabilizer (nothing has been published so far about other polymers for the production of small iron oxides) does, on the one hand, improve applicability, since it facilitates considerable particle accumulation in the lymphatic tissue, but that its distribution throughout lymphatic nodes, on the other, is not sufficiently homogeneous for clinical application [Taupitz, M et al.; SMRM - Book of abstracts 500; New York; USA; 1993]. This strong but inhomogeneous accumulation makes additional improvement by repeated optimization of the hydrodynamic diameters not very likely.

The small size of the target organ is an important problem for developing specific diagnostic substances. The overall weight of the lymphatic nodes, for example, makes up less than 1 percent of the body weight. Diagnostic substances must therefore have substantial accumulation potential in the target tissue (specificity) and facilitate a strong contrast-enhancing effect at low concentrations.

As superparamagnetic iron oxides currently represent the group of substances having the strongest contrast in MR, these particles appear particularly appropriate for specific applications. The crystal core of the iron oxides, which causes the particular character of said substances, is a problem, however, as particle size has an

essential influence on biological behavior. Smaller particle sizes improve targetability, but the efficiency of the contrast material diminishes due to the interdependency of particle size and magnetic moment, so that a compromise must be found between the (physical) contrastive effect and (biological) targetability. As a rule, the iron-containing core should be required to be as large as possible to achieve high efficacy, whereas the overall diameter should be kept small.

(d) DESCRIPTION OF THE INVENTION

An object of a broad aspect of this invention is to provide iron-containing nanoparticles that match physical and biological requirements with a specific nanoparticle in optimum fashion.

A first broad aspect of this invention provides a nanoparticle compound for use in diagnosis and therapy. The nanoparticle compound includes an iron-containing core comprising iron material which is selected from the group consisting of magnetite and maghemite. A synthesis polymer coats the iron-containing core, the synthesis polymer being selected from the group consisting of dextrans and derivatives of dextrans. A targeting polymer is non-covalently bonded to, and envelopes, the synthesis polymer to form a second coating. In this way, the targeting polymer is not exposed to synthesis conditions.

By a first variant of this first broad aspect of the invention, the iron-containing core comprises 0.1 to 25% weight of non-iron metallic ions. By a first variation thereof, the non-iron metallic ions may be paramagnetic ions or diamagnetic ions.

By a second variant of this first broad aspect of the invention and/or the above variants thereof, the iron-

containing core and the synthesis polymer coating have a diameter less than 100 nanometres.

By a third variant of this first broad aspect of the invention and/or the above variants thereof, the nanoparticle compound has a hydrodynamic diameter of less than 10 times the diameter of the iron-containing core.

By a fourth variant of this first broad aspect of the invention and/or the above variants thereof, the targeting polymer has a weight that is between 0.5 times to 50 times the weight of the non-iron metallic ions.

By a fifth variant of this first broad aspect of the invention and/or the above variants thereof, the nanoparticle compound further includes an optical absorption-permitting peptide substance which is selected from the group consisting of RRTVKHHVN, RRSRHH and RSKRGR.

By a sixth variant of this first broad aspect of the invention and/or the above variants thereof, the nanoparticle compound further includes a pharmaceutically-active compound.

By a seventh variant of this first broad aspect of the invention and/or the above variants thereof, the targeting polymer may be dextran, dextran derivatives, laminarin, transferrin, and endothelin-receptor-specific heptapeptide.

A second broad aspect of this invention provides a nanoparticle compound for use in diagnosis and therapy. The nanoparticle compound includes an iron-containing core comprising an iron compound which may be magnetite or maghemite, and a plurality of non-iron metallic ions. A synthesis polymer coats the iron-containing core, the

synthesis polymer having a weight ratio of 0.1 to 1.0 to the iron compound. A targeting polymer is non-covalently bonded to, and envelopes, the synthesis polymer to form a second coating. The targeting polymer is not subject to synthesis conditions and has a weight that is between 0.5 times to 50 times the weight of the non-iron metallic ions.

By a first variant of this second broad aspect of this invention, the targeting polymer may be dextran, dextran derivatives, laminarin, transferrin, or endothelin-receptor-specific heptapeptide.

By a second variant of this second broad aspect of this invention, and/or the above variant or variants thereof, the synthesis polymer has a molecular weight less than 100,000 Da.

By a third variant of this second broad aspect of this invention, and/or the above variant or variants thereof, the synthesis polymer may be dextran or derivatives of dextran.

A third broad aspect of this invention provides a method for the fabrication of a nanoparticle compound for use in diagnosis and therapy. The method includes mixing an iron-containing compound with a synthesis polymer in the presence of a base to create a first mixture. The synthesis polymer may be dextran or derivatives of dextran. The first mixture is subjected to desorption to create a second mixture with an iron-containing core and a synthesis polymer coating, the second mixture having a polymer-to-iron weight ratio of 0.1:1. A targeting polymer is non-covalently bonded to the second mixture to create a nanoparticle compound comprising the iron-containing core surrounded by the synthesis polymer coating which is surrounded and enveloped by the

targeting polymer coating. In this way, the targeting polymer coating is not exposed to synthesis conditions.

By a first variant of the method of this third broad aspect of this invention, the method further includes mixing an iron(II) salt and an iron (III) salt to produce the iron-containing compound with the ratio of divalent to trivalent iron being between 1:1 and 1:20.

By a second variant of the method of this third broad aspect of this invention, and/or the above variant or variants thereof, the method further includes reacting an iron(III) salt mixture with a reducing agent to produce the iron-containing compound while selecting a quantity of reducing agent that generates an iron(II) to iron(III) ratio between 1:1 and 1:20.

By a third variant of the method of this third broad aspect of this invention, and/or the above variant or variants thereof, the base which is selected is a 0.1:10N base used to precipitate the iron compounds.

By a fourth variant of the method of this third broad aspect of this invention, and/or the above variant or variants thereof, the base may be ammonia gas, ammonia salt, an amine, an amine derivative or a volatile buffer.

By a fifth variant of the method of this third broad aspect of this invention, and/or the above variant or variants thereof, the method includes selecting the iron-containing core to comprise 0.1 to 25% weight of non-iron metallic ions. By a first variation thereof, the non-iron metallic ions may be selected from paramagnetic ions or diamagnetic ions.

By a sixth variant of the method of this third broad aspect of this invention, and/or the above variant or

variants thereof, the method includes selecting the iron-containing core and the synthesis polymer coating to have a diameter less than 100 nanometres.

By a seventh variant of the method of this third broad aspect of this invention, and/or the above variant or variants thereof, the method includes selecting the nanoparticle compound to have a hydrodynamic diameter of less than 10 times the diameter of the iron-containing core.

By an eighth variant of the method of this third broad aspect of this invention, and/or the above variant or variants thereof, the method includes selecting the targeting polymer to have a weight that is between 0.5 times to 50 times the weight of the non-iron metallic ions.

By a ninth variant of the method of this third broad aspect of this invention, and/or the above variant or variants thereof, the method further comprises adding an optical absorption permitting peptide substance which may be RRTVKHHVN, RRSRHH or RSKRGR, prior to adding the targeting polymer.

By a tenth variant of the method of this third broad aspect of this invention, and/or the above variant or variants thereof, the method further comprises adding a pharmaceutically-active compound prior to adding the targeting polymer.

By an eleventh variant of the method of this third broad aspect of this invention, and/or the above variant or variants thereof, the method includes selecting the targeting polymer to be selected from dextran, dextran derivatives, laminarin, transferrin, or endothelin-receptor-specific heptapeptide.

By a twelfth variant of the method of this third broad aspect of this invention, and/or the above variant or variants thereof, the method further includes removing most of the synthesis polymer during desorption.

A fourth broad aspect of this invention provides the use, in diagnosis and therapy, of a nanoparticle disclosed hereinabove.

A fifth broad aspect of this invention provides the use, in imaging atherosclerosis, of a nanoparticle disclosed hereinabove.

A sixth broad aspect of this invention provides the use for the manufacture of a composition for diagnosis and therapy, of a nanoparticle disclosed hereinabove.

A seventh broad aspect of this invention provides the use for the manufacturing of a diagnostic composition for imaging atherosclerosis, of a nanoparticle disclosed hereinabove.

Surprisingly, it was found that the targeting capabilities of the nanoparticles of embodiments of aspects of the invention are superior to those of state-of-the-art iron oxide particles. Contrast materials and/or therapeutic substances/supporting systems of unprecedented "targetability" can be produced by combining physical quality with improved targeting capabilities of the nanoparticles.

The nanoparticles of embodiments of aspects of the invention are produced from individual blocks (modular design), which ensures maximum flexibility when the iron-containing cores (physical effect; contrast) are combined with the target component (biological behaviour). This modular structure is advantageous in that it allows

"just-in-time" assembly of the complete nanoparticle of embodiments of aspects of the invention from a component that can be stored (iron-containing core) and targeting molecules that may be highly sensitive. This similarity with cold kits known from clinical radiopharmacy also facilitates, for example, the use of individual patients' serum components as target molecules (e.g. autologous antibodies).

The nanoparticles of embodiments of aspects of the invention can also be detected visually due to their intense colouring, which is desirable, for example, when they are used as a visual labelling substance in surgery.

Furthermore, the nanoparticles of embodiments of aspects of the invention are also suitable for therapeutic uses, for example, for magnetic targeting using external magnets above a target volume in conjunction with a magnetically linked release of active substances. Nanoparticles of embodiments of aspects of the invention may, for example, accumulate in tumours, and thus be used as specific reinforcing agents in local hyperthermia.

The nanoparticles of embodiments of aspects of the invention consist of an iron-containing core, a primary coat (synthesis polymer) which is responsible for optimal sized nanoparticles, and a secondary coat (targeting polymer) and, optionally, pharmaceutical adjuvants, pharmaceuticals and/or adsorption mediators/enhancers.

The iron-containing core can have the form of a particle, a colloid or a crystal. The nanoparticles of embodiments of aspects of the invention contain synthesis polymer from the production of the core which coats the core as a primary coat and is required during production for control of the physical and/or pharmaceutical/galenic quality. The ratio of synthesis polymer to iron is then

adjusted to a desired value by means of a desorption procedure. A targeting polymer is adsorbed for use in specific diagnostics that represents the surface of the nanoparticles of embodiments of aspects of the invention and envelopes the basic structural unit of the core and primary coat. Adsorption mediators/enhancers may be present between the primary and the secondary coat for improved adsorption. Other ingredients of the nanoparticles of embodiments of aspects of the invention may be pharmaceutical adjuvants or drugs.

The hydrodynamic diameter of the basic structural unit (iron-containing core plus primary coat) in solution is smaller than 100 nm, preferably smaller than 50 nm, and not more than five times the diameter of the iron-containing core.

The nanoparticles of embodiments of aspects of the invention are further
characterized in that they are available in the form of stable colloidal sols, which is preferred, but they can also be formulated as lyophilized powders which can easily be put into solution again using solvents common in medicine (electrolyte solution, plasma expander, glucose solution, physiological saline, etc.), or in that the basic structural unit as well as targeting component and optional adjuvants are separate solutions or lyophilizates that can be mixed at any desired point in time to obtain the solution for administration.

The iron-containing core has a magnetic moment greater than that of iron(II) or iron(III) ions. The iron-containing core, due to its magnetic properties, facilitates the contrast-enhancing effect when the substance is used as contrast material in MR tomography. It should be superparamagnetic, or at least contain superparamagnetic portions, to achieve optimum contrast rendering. This means that the core should either be a crystal or a polyatomic complex ("particle") as this type of magnetism only occurs in solid matter.

The iron-containing core of the nanoparticles of embodiments of aspects of the invention may consist of, or contain, magnetite or maghemite.

Up to 25 percent of the iron by weight contained in the core may be substituted by other metallic ions.

Such non-iron metallic ions are paramagnetic, diamagnetic, or a mixture of both.

The nanoparticles of embodiments of aspects of the invention are further
characterized in that the iron-containing core comprises a diameter determined by way of electron microscopy that is smaller than 30 nm, preferably smaller than 15 nm, contains a minimum of 50 metallic atoms with a particle size distribution in which at least 90% of the iron-containing cores are in the range of 0.7 x average to 1.3 x average.

The nanoparticles of embodiments of aspects of the invention contain a quantity of synthesis polymer

between 0.01 to 1 times the total weight of metallic ions present. The preferred quantity is between 0.25 and 0.75 times that weight.

A monomeric or polymeric substance, or a mixture of these substances or derivatives, or derivatives comprising functional groups, or derivatives that were additionally substituted and have a molecular weight less than 100,000 Da are used as synthesis polymer. Preferred substances have molecular weights less than 10,000 or 5000 Da.

A dextran derivative or a mixture of dextran and/or dextran derivatives, are particularly preferred for use as synthesis polymers.

The synthesis polymer may contain in its molecule one or several acid groups, or several functional groups, which preferably contain N, S, P, or O atoms.

The substances or mixtures of substances used as targeting and synthesis polymers may be the same or different, with the targeting polymer having retained its physiological state as it was not exposed to synthesis and the side reactions of the synthesis polymer during synthesis.

The parent substance of the iron-containing core and primary coat determines the physical quality of the nanoparticle of embodiments of aspects of the invention, while the targeting polymer determines the biological behaviour of the nanoparticles of embodiments of aspects of the invention.

The weight of targeting polymer contained in the nanoparticle of embodiments of aspects of the invention is 0.5 times to 50 times, preferably 1 to 25 times the weight of the metallic ions present.

The nanoparticles of embodiments of aspects of the invention may contain adsorption mediators/enhancers in a quantity less than, or equal to, the total weight of metallic ions contained. These adsorption mediators/enhancers reinforce or enable the adsorption of targeting polymer by the basic structural unit consisting of the iron-containing core/(remaining) synthesis polymer.

Preferred adsorption mediators/enhancers are peptides having the following structures: RRTVKHHVN, RRSRHH, or RSKRGR [one-letter code of amino acids], or partial structures thereof.

The hydrodynamic diameter, including all the components of the nanoparticles of embodiments of aspects of the invention is not more than ten times the diameter of the iron-containing core and not more than 20% greater than the diameter of the basic structural unit.

The nanoparticles of embodiments of aspects of the invention are composed of individual modules, e.g., a basic structural unit, a targeting polymer, a pharmacon and an adsorption mediator that can be combined at any time.

The nanoparticle preparations of embodiments of aspects of the invention are low-viscosity, aqueous colloidal solutions or suspensions of stabilized iron-containing particles in the nanometre range. The nanoparticle solution of embodiments of aspects of the invention do not contain any larger aggregates and can be administered

intravenously, which meets with particle size requirements for parenterals found in international pharmacopoeia.

In general, the basic structural unit can be sterilized by heat treatment. The "sterilization" procedure of the final nanoparticles of embodiments of aspects of the invention is dependent on the sensitivity of the secondary coat, but sterile, aseptic manufacture is guaranteed in any case. Sterilization by filtration is always feasible due to the small size of the nanoparticles of embodiments of aspects of the invention. Another way to guarantee a practically-sterile solution for administration is the option that combines a sensitive targeting polymer with the sterilizable basic structural unit shortly before use.

The nanoparticles of embodiments of aspects of the invention are well-tolerated and comprise a very favorable margin of safety between the diagnostic and lethal dose when used, for example, as MR contrast media. The diagnostic dose, depending on the specific application, is between 5 µmol and 200 µmol (iron) per kilogram of body weight, while the approximate lethal dose is between 20 mmol and 50 mmol/kg body weight (in mice). The substances are completely biodegradable. The iron-containing core is dissolved, and the iron is incorporated into the physiological iron pool. The molecules used as synthesis and targeting polymers can in general be catabolized to decomposable elementary units (sugars, amino acids).

The nanoparticle solutions of embodiments of aspects of the invention are very stable; there is no detectable change in physical parameters (particle size, magnetic properties) after their preparation. The nanoparticle solutions of embodiments of aspects of the invention can be stored for an extended period of time when they are produced in sterile conditions; for example, no instabilities, e.g., aggregation or sedimentation, were visible within 12 months.

The nanoparticle solutions or suspensions of embodiments of aspects of the invention have a reddish-brown to black colouring due to the intense colour of the iron-containing crystals. This characteristic colouring can be utilized for visual detection purposes so that such substances can be used for labelling in surgical medicine. The nanoparticles of embodiments of aspects of the invention are superparamagnetic or contain superparamagnetic portions when used as contrast media in MR tomography.

The nanoparticles of embodiments of aspects of the invention show high saturation magnetization even at low field strengths and have no remanence after the external magnet has been switched off.

The nanoparticles of embodiments of aspects of the invention are formulated as solutions (suspensions) and can be applied without further preparation. As the nanoparticle solutions of embodiments of aspects of the invention are compatible with common medical solvents, e.g., physiological saline, electrolyte solution or glucose solution, nanoparticles of embodiments of aspects of the invention can be diluted as may be desired and injected or infused for specific applications.

Lyophilizates are an alternative to formulating a solution from the point of view of storage. Either the basic structural unit is lyophilized and later resuspended in the dissolved targeting polymer, or the basic structural unit and nanoparticle of embodiments of aspects of the invention are lyophilized after adsorption and dissolved again before use in physiological saline or sterile water for injection. Another way of keeping a stock of such substances is to store the basic structural unit and the targeting polymer in separate solutions and mix them before use.

The particular or colloidal iron-containing cores are produced from unimolecularly dissolved iron precursors by changing, following single-pot synthesis, their pH value and causing them to precipitate in the presence of a stabilizer substance (synthesis polymer). The synthesis polymer separates the crystal cores during production and may therefore be used to control particle size. The synthesis polymer is responsible for the physical and pharmaceutical/galenic properties not only of the crystal core but of the whole nanoparticle of embodiments of aspects of the invention. The synthesis polymer facilitates a stable solution (suspension) as the cores are separated to such an extent that aggregation cannot occur (steric stabilization).

When the iron-containing core particles are obtained, the synthesis polymer is adjusted by desorption to a given ratio of synthesis polymer to iron. The solution (suspension) of the iron core and the synthesis polymer residue that envelops and stabilizes the iron-containing core as a primary coat represent the basic structural unit of the modular system. This basic structural unit is characterized by high physical and galenic quality.

A second important component is the targeting polymer that is adsorbed by the basic structural unit after synthesis and envelops the core and primary coat as a secondary coat. The secondary coat is the surface of the nanoparticles and determines *in-vivo* behavior. The basic structural unit and targeting polymer can be mixed at any time, which also allows "just-in-time" production.

The adsorption procedure between synthesis polymer residue and targeting polymer may be improved or facilitated by an intermediate step: addition of adsorption mediators/enhancers. An adsorption mediator/enhancer can also be added to a mixture with the targeting polymer. Pharmaceutical adjuvants or drugs may be added at any time in a similar way.

The special advantages of embodiments of aspects of the invention are obvious as the production method according to embodiments of aspects of the invention makes it possible for the first time to meet both physical and biological requirements for a specific nanoparticle of embodiments of aspects of the invention in an optimum way.

The synthesis polymer having the most appropriate physical properties may be chosen for synthesis because of the modular structure of the particle, i.e., separate production of the basic structural unit (iron-containing core + primary coat) and targeting polymer (secondary coat), without any limitation by the desired biological targetability of the nanoparticles of embodiments of aspects of the invention; so, for the first time, no compromises must be made regarding the physical and pharmaceutical quality of the nanoparticles of embodiments of aspects of the invention and their desired biological effect. The targeting polymer is not exposed to the destructive synthesis conditions. This allows the use of many substances for targeting that are ruled out

as a primary coat. Similarly, there is no need for post-synthetic chemical reactions that would require adequate reaction conditions and reduce the integrity of the ligand; for example, there are no redox reactions that involve proteins containing disulphide bridges as in periodate oxidation and subsequent reductive amination: biological activity is maintained.

Another essential advantage is the fact that there is no need for purification of the basic structural unit/targeting polymer, as no reaction solutions, e.g., periodate, have to be separated. The method of embodiments of aspects of the invention allows instant production, including "just-in-time" production of nanoparticles of embodiments of aspects of the invention immediately before use, which may be required or desirable, for example, for "individual" contrast media (e.g., autologous antibodies) or if the targeting polymer remains stable in solution only for a short time.

The method of embodiments of aspects of the invention has advantages for further optimization as the "surface" of the nanoparticles of embodiments of aspects of the invention can be modified/optimized separately, and an analysis can be carried out using advanced methods of analysis, e.g., such as NMR or IR spectroscopy. These methods cannot be applied if particular cores are present.

As the surface is produced in a defined way and can be adequately analyzed, systematic optimization of surface properties becomes possible whereas with each state-of-the-art production method, in which a particle is treated as a whole, the surface is unknown and can only be optimized by trial and error.

The nanoparticles of embodiments of aspects of the invention are thus produced in several steps. The

iron-containing core is generally synthesized by means of a "single-pot" process, i.e. in the presence of a stabilizer (synthesis polymer). The stabilizer (synthesis polymer) is dissolved in water and mixed with the unimolecular iron compounds. The iron salts are converted into the preferred oxides by increasing the pH value.

Alternatively, the stabilizer solution can be alkalized and then mixed with the iron salts. The mixture is heated under reflux and neutralized, or vice versa. The crude substance is purified and the surplus or not firmly adsorbed/bound synthesis polymer is adjusted to an exact iron-to-stabilizer weight ratio by means of a desorption process. This purified and desorbed base substance consisting of an iron oxide core and (residual) synthesis polymer represents the basic structural unit of the (modular structured) nanoparticles. Sterilization by heat may follow as an option. The selected targeting polymer is adsorbed, either when required or for maintenance "in stock", by the basic structural unit, optionally with intermediate adsorption or co-adsorption of an adsorption mediator/enhancer. Other ingredients, e.g., pharmaceutical adjuvants or drugs may be added optionally.

Note that synthesis always takes place in the presence of a stabilizer according to the "single-pot" method for the following description of the iron-containing core.

Stoichiometric quantities of iron(II) and iron(III) salts are mixed as precursors to produce iron-containing cores. The quality of the resulting crystals is influenced by the salts used; according to the literature, salts of hydrochloric acid, i.e., ferrous and ferric chlorides, are mainly used. In general, however, all salts of strong acids including sulphates and nitrates may be used. When

these salts are used, it is difficult to ensure exact stoichiometry because iron(II) salts are highly sensitive to oxidation. It is advantageous here to use more complex salts, e.g., Mohr's salt, which is less sensitive to oxidation.

Surprisingly, it turned out that organic salts are superior to inorganic salts as the organic anions act as stabilizers or auxiliary stabilizers. Iron(II) gluconate or iron(III) citrate proved to be particularly suitable; but other organic anions, e.g., fumarates, tartrates, lactates, or salicylates may be used as well.

A synthesis variant that relies only on an iron(III) salt facilitates production without having to resort to highly oxidation-sensitive iron(II) salts and reduces the number of "foreign ions". This synthesis variant starts only from an iron(III) salt from which iron(II) salt is generated *in situ* during reaction only by means of a calculated amount of a reducing agent. Although, in general, it is possible to use all reducing agents that reduce iron(III) with stoichiometrical accuracy, hydroxylamine is preferred, as the reacted hydroxylamine quantitatively converts into laughing-gas and thus is easily, and completely, removed from the reaction mixture.

$$4\ Fe^{3+} + 2\ NH_2OH \text{ ---> } 4\ Fe^{2+} + N_2O + 4\ H^+ + H_2O$$

A disadvantage of previously described methods becomes obvious if one takes a closer look at the chemistry of iron salts. It is the goal of the precipitation step to convert iron(II) and iron(III) in stoichiometric composition into a crystal having a defined structure. The respective oxides are formed by increasing the pH value. If one considers that the iron(III) ions [pK_L $Fe(OH)_3$ 37[1]] the pK_L values are dependent on

concentration. The date refer to a solution of 10^{-2} mol/l form sparingly soluble hydroxides at a pH value of 2 while the iron(II) ions start to precipitate as hydroxides [pK_L $Fe(OH)_2$ 13.5] at pH 8, it becomes apparent that direct formation of the desired crystals hardly seems possible and that the reaction path must include a successive reaction of the hydroxides. It is possible, however, to shift the precipitation points of the iron compounds in relation to each other using appropriate complexing agents, thus achieving simultaneous precipitation and insertion at the various lattice sites

in the iron oxide crystal. The precipitation points of the iron compounds used can be controlled over a wide range by selecting an appropriate complexing agent.

Apart from "classical" substances according to Table 1, the above organic anions may be used as complexing agents. Complex salts, organic anion salts and inorganic salts of the iron(II) and iron(III) ions may be combined in whatever way may be desired.

Table 1: Selection of complexing and chelating agents for shifting precipitation points when pH values are increased during magnetite synthesis

Complexing agents (chelate)	iron II logK1*	iron III logK1*
CDTA trans-1,2-diamino-cyclohexan-N,N,N',N'-tetraacetic acid	16.27	28.05
EDTA ethylenediamine-tetraacetic acid	14.33	25.10
EGTA ethyleneglycol-O,O'-bis-(2-aminoethyl)-N,N,N',N'-tetraacetic acid	11.92	20.50
DTPA diethylenetriamine-pentaacetic acid	16.50	28.60
HEDTA N-(2-hydroxyethyl)-ethylenediamine-N,N',N'-triacetic acid	12.20	19.80
NTA nitrilotriacetic acid	8.84	15.87
TTHA triethylentetramin-N,N,N',N'',N''',N'''-hexaacetic acid	17.10	26.80

* K_1 is the absolute stability constant and is independent of pH. It refers to the deprotonated form of the chelating agent: $K_1 = [ML]/[M] \cdot [L]$, where [ML], [M] and [L] are the concentrations of chelate, metallic ion and chelating agent (ligand).

The chelates listed in Table 1 are only supposed to demonstrate the range of suitable compounds and should not be construed as restricted to these substances only.

In a synthesis variant, iron(II) hydroxide and iron(III) hydroxide are first produced separately. Surprisingly, the iron oxide crystals are successfully produced by combining the separately prepared hydroxide solutions; transformation and crystallization are accelerated when the combined solutions are heated.

Precipitation is an important step in the production of the iron-containing cores. The particular iron compounds are formed from low-molecular weight iron compounds by increasing the pH value; colloidal iron hydroxides may be an optional intermediate product during particle formation. Any substance that can raise the pH value of the dissolved acidic iron precursors is suitable for increasing the pH. Apart from soda lye, pH values are preferably increased using ammonia, either gaseous or as a salt, or alkaline amines and volatile buffers. It turned out, surprisingly, that the base used for precipitation affects the overall properties in such a way that "biological" effects become visible as, for example, differences in the distribution of the particles throughout body organs.

The concentration of the alkaline substance should be between 0.1 to 10 N; concentrated solutions of 1 to 4 N are preferred because particles having small core sizes preferably form when the pH increase takes place at a faster rate. Bases are added within 30 minutes, preferably within 30 seconds.

The iron compounds are precipitated onto the particles at a temperature range of 0 - 120 °C, with 50 - 80 °C being the preferred range. As a general rule, temperature can be

low when the iron oxide is formed directly, and should be high when formation involves hydroxides as an intermediate step. The product is neutralized after precipitation and, subsequently, the crude substance is refluxed, in particular, when hydroxides form as intermediate products; heating time should be between 0 minutes and 24 hours, preferably between 30 minutes and one hour. Neutralisation and refluxing may be carried out in reverse order.

Self-coloring of the substance is desirable for its use as a contrast medium (optical labeling substance) for visual detection in surgery.

The application for MR tomography requires high effectiveness that is determined by the magnetic properties of the nanoparticles. The iron oxides magnetite and maghemite seem to be particularly appropriate when the nanoparticles are used as an MR contrast material, as they show high saturation magnetization at the field strengths applied in clinical MR tomography. The special magnetic properties are determined by the crystal structure of the particular iron cores. But, surprisingly, foreign ions can be inserted in this crystal core, with a magnetite-like crystal structure still being arrived at. This doping with non-ferruginous metallic ions may generally be carried out in two ways. On the one hand, iron(II) and/or iron(III) ions are replaced at their lattice sites by other paramagnetic metallic ions while, on the other, diamagnetic ions can be used for substitution. It should be kept in mind for better comprehension that magnetization in the magnetite crystal stems from iron(II) ions only, as iron(III) ions occupy parallel/antiparallel lattice positions so that their individual magnetic vectors are neutralized. The net amount of magnetization can be increased by using ions that have a higher magnetic moment than iron, or by changing the equilibrium of

parallel/antiparallel lattice site occupation by iron(III) ions using para- or diamagnetic ions. If substitution involves paramagnetic metals with high magnetic moments, e.g., gadolinium, an increase may be yielded that equals
the difference in magnetic moments when compared to the iron replaced. If iron(III) is substituted by diamagnetic metals, the no-longer compensated moment of an iron(III) ion can contribute to the overall moment. As a variant, dia- and paramagnetic ions can be inserted in the magnetite-like crystal lattice together. Doping with non-iron ions is carried out by partial substitution of the low-molecular iron-containing parent compounds during synthesis.

The general method for producing the iron-containing cores is to synthesize a magnetite-like crystal lattice. Iron(II) and iron(III) ions are used for this purpose at ratios ranging from 1:1 to 1:20. Synthesis is achieved most easily using an exactly stoichiometric ratio of 1:2. Iron(II)-to-iron(III) ratios can be maintained during synthesis by means of a reducing agent. Iron(II) and iron(III) ions can be replaced by other metallic ions up to the equivalent of 25% of the total iron content

(weight). Besides paramagnetic ions, e.g., gadolinium or manganese, diamagnetic ions, e.g., lithium, magnesium, or calcium, or a mixture of para- and diamagnetic ions, may be used. Magnetite or magnetite-like structures are the preferred crystal structures. This so-called spinel or inverse-spinel crystal can be formed as a secondary product, for example, if the production first yields hydroxides, or the magnetite crystal is converted into other crystals, as, for example, from magnetite into maghemite by oxidation. The special quality of the nanoparticles used as contrast materials for MRT requires *superparamagnetic* properties. Superparamagnetism only occurs in solid matter; thus another requirement is that

the crystals have the properties of solids, i.e. that they are particular crystals or, at least, polyatomic clusters. Minimum iron content should be 50 iron atoms (or metal atoms) per crystal. The size of the iron-containing cores can be controlled by variation during synthesis throughout a wide range (from 1 to 30 nm), but synthesis of smaller cores with diameters of less than 15 nm and a minimum of 90% of particles within a range of 0.7 x mean < mean < 1.3 x mean (mean being the mean diameter determined using electron microscopy) is preferred.

It is one of the specific advantages of the production method of embodiments of aspects of the invention that it offers great flexibility in the selection of synthesis polymers; the term "polymer" is not to be taken literally, as both low-molecular weight substances and mixtures of low- and polymolecular weight substances can be used for producing iron-containing cores. Particularly preferred is the use of low-molecular and polymolecular substances that contain negative charge carriers in their molecule. The following are preferred: carboxylates or analogues, phosphates (or other P-containing groups) and sulphates (or other S-containing groups). These derivatives may simply carry one single functional group or contain several functional groups. The theory upon which this is based assumes that affinity to the surface of the iron-containing core is due to interaction of the positive iron oxide surface and the negative charge in the synthesis polymer. If the synthesis polymer contains several of these groups, interaction is particularly distinct ("multi-side attachment"). As there is a great number of suitable substances, they cannot all be listed here. Some classes of substances that are specially suitable for stabilization during synthesis are:

Low-molecular weight substances, e.g., carboxy-polyalcohols, polycarboxypolyalcohols, polycarboxy-alcohols, carboxyalcohols, alcohols, monosugars, oligosugars, and synthesis polymers, e.g., polyethylene glycol, polypropylene glycol and mixtures (block and copolymers), polyacrylic acid, polyvinyl alcohol, polylactic acid (polylactide and polylactide glycide), and natural or, specifically, partially-synthetic or chemically-and/or enzymatically-modified natural polymers, e.g., dextrans and its derivatives, arabinic acid, glycosaminoglycan and synthetic analogues, starch and its derivatives as well as gelatin derivatives.

It is particularly preferable to use low-molecular weight derivatives of dextran that contain negative charge carriers. (Mono)carboxydextran can serve as an example here; its manufacture is described, for example, in Bremner et al. [Bremner, I.; Cox, JSG; Moss, GF; Carbohydrate Research 11; 77-84; 1969], and another preferred example is the use of polycarboxydextran, which is produced by an ether bond between 6-bromohexanoic acid and the hydroxy groups of the dextran. [Noguchi, A.; Takahashi, T; Yamaguchi, T.; Kitamura, Y.; Takakura, T.; Hashida, M.; Sezaki, H.; Bioconjugate chemistry 3; 132-137; 1992]. The poly-carboxydextran is able, due to its many negative charges, to interact with the surface of the iron oxide through "multi-side attachment".

The quantity of synthesis polymer required for stabilization during production is 0.5 to 20 times the total weight of the metallic ions contained in the batch; its overall percentage in the reaction mixture is selected to ensure that the viscosity still allows thorough mixing of the batch when polymeric synthesis polymers are used (< 50% g/V). The weight of the synthesis polymer used

should preferably exceed the total weight of the metallic ions by 3 to 15 times.

After the crude substance has been produced, the synthesis polymer component in the batch is reduced by means of a desorption process. Chromatographic procedures, a magnetic separation method, dialysis, centrifugation or ultrafiltration, or other appropriate methods can be employed for desorption. Desorption can be carried out at increased temperatures in conjunction with one of the desorption processes. Another way to influence the extent of desorption is the use of desorbing substances, e.g., buffer solutions or tensides.
After desorbing the crude substance, a stable, physically-optimal solution/suspension is obtained which represents the basic structural unit for the manufacture of specific nanoparticles. The basic structural unit consists of an iron-containing core and the (residual) synthesis polymer. The quantity of residual synthesis polymer is between 0.01 and 1, depending on the ratio adjusted by the desorption process. The range between 0.25 and 0.75 is preferred because the best compromise between stability and adsorbability of the basic structural unit is achieved in this range. The overall size (hydrodynamic diameter) of the basic structural unit varies depending on the size of the iron-containing core and the synthesis polymer used and is thus smaller than 100 nm, preferably smaller than 50 nm. It is preferred to produce basic structural units having an overall diameter no greater than five times the core diameter.

The basic structural unit and targeting polymer are combined to yield the final nanoparticle. The adsorbed targeting polymer forms a secondary coat around the synthesis polymer/iron-containing core unit, thus being the surface of the system which mainly determines, besides

the particular nature of the particle, the *in-vivo* behaviour. The special advantage of this production method is that virtually every substance that can be adsorbed by the basic structural unit can be used to control the biological behaviour of the nanoparticle of embodiments of aspects of the invention. The targeting polymers are not exposed to the strains of synthesis, so that sensitive substances and substances that could not be used up to now can function as supporting molecules for controlling biological behaviour.

The following are examples of suitable targeting polymers:

Natural oligo- and polysaccharides, e.g., dextran with molecular weights of less than 100,000 Da, mixtures of various dextrans, dextrans of different origin, specially purified dextran (FP = free pyrogene quality), fucoidan, arabinogalactan, chondroitin and its sulfates, dermatan, heparin, heparitin, hyaluronic acid, keratan, polygalacturonic acid, polyglucuronic acid, polymannuronic acid, inulin, polylactose, polylactosamine, polyinosinic acid, polysucrose, amylose, amylopectin, glycogen, glucan, nigeran, pullulan, irisin, asparagosin, sinistrin, tricitin, critesin, graminin, sitosin, lichenin, isolichenan, galactan, galactocaolose, luteose, mannans, mannocarolose, pustulan, laminarin, xanthene, xylan and copolymers, araboxylan, arabinogalactan, araban, laevans (fructosans), teichinic acid, blood group polysaccharides, guaran, carubin, alfalfa, glucomannans, galactoglucomannans, phosphomannans, fucans, pectins, cyclo-dextrins, alginic acid, tragacanth and other gums, chitin, chitosan, agar, furcellaran, carrageen, cellulose, celluronic acid or arabinic acid. Additionally, chemically and/or enzymatically produced derivatives of the listed substances and the low-molecular weight decomposition products of polymolecular compounds are claimed. Optionally, these substances or derivatives can be

substituted by any other substance. Polyamino- and pseudopolyamino acids are suited as well.

Synthetic oligo- and polymers, e.g., polyethylene glycol, polypropylene glycol, polyoxyethylene ether, polyanethol sulphonic acid, polyethylene imine, polymaleimide, polyvinyl alcohol, polyvinyl chloride, polyvinyl acetate, polyvinyl pyrrolidone, polyvinyl sulphate, polyacrylic acid, polymethacrylic acid, polyactide, polyactide glycide.

Monosugars to oligosugars and related substances, e.g., aldo- and ketotrioses to aldo- and ketoheptoses, ketooctoses and ketononoses, anhydrosugars, monocarboxylic acids and derivatives containing 5 or 6 carbon atoms in their main chain, cyclites, amino and diamino sugars, desoxy sugars, aminodesoxy sugars and amino sugar carboxylic acids, aminocyclites, phosphor-containing derivatives of mono- to oligomers.

Monomer or oligomercarbohydrates or derivatives having antitumoral properties (higher plants, fungi, lichens and bacteria), e.g., lipopolysaccharides, or containing one or more of the following structures: ß-2,6-fructan, ß-1,3-glucan, mannoglucan, mannan, glucomannan, ß-1,3/1,6-glucans, ß-1,6-glucan, ß-1,3/1,4-glucan, arabinoxylan, hemicellulose, ß-1,4-xylan, arabinoglucan, arabinogalactan, arabinofucoglucan, α-1,6/1,3-glucan, α-1,5-arabinan, α-1,6-glucan, ß-2,1/2,6-fructan, ß-2,1-fructan .

An important prerequisite for the effect of antitumoral polysaccharides is solubility in water, which is guaranteed with the ß-1,3/1,6-glucans by branches at position 6. Solubility of polysaccharides that are insoluble in water can be improved by introducing

hydrophile and well-hydrated groups. Amino, acetyl, carboxymethyl or sulfate groups may be used, among others, e.g., methyl and ethyl, as substituents.

Tensides and surface-active substances, e.g., niotensides, alkyl glucosides, glucamides, alkyl maltosides, mono- and polydisperse polyoxyethylene, quaternary ammonium salts, bile acids, alkyl sulphates, betaines, CHAP derivatives.

As an example, and to illustrate the great number of control options and thus the advantages of the modular system, molecules for controlling *in-vivo* behavior (specificity) may also be cell fragments, cells, bacteria fragments, substances from the large group of lectins, hormones and mediator substances, proteins and neoproteins, peptides and polypeptides, antibodies, antibody fragments or the "molecular recognition units", of integrins (ELAM, LECAM, VCAM, etc.) or receptor-specific substances (e.g. Lewis-X, Sialyl-Lewis-X, etc.), or the great number of blood/plasma/serum components and opsonins, the group of oligonucleotides and synthetic oligonucleotides, DNA and RNA or their derivatives or fragments or analogues (PNA) and homologues, from the group of lipopolysaccharides, lipoproteins, glycerol esters, cholesterols and esters, or metabolites and antimetabolites, cytostatic agents, medical substances, conjugates of medical substances, chemotherapeutical substances and cytostatic agents.

Chemical and/or enzymatically produced derivatives or decomposition products may be used as targeting polymers in addition to, or instead of, the above substances.

The derivatives or "native" targeting polymers may include additional functional groups. These functional groups can

be located at one or both ends or at any other position in the basic targeting molecule. The functional groups can be the same, or combinations of different groups. Preferred among the derivatives themselves as well as the functional groups are those which contain N, S, O or P atoms, acid or analogues, hydroxy, ether or ester groups.

The exact composition of the nanoparticles of embodiments of aspects of the invention depends on the requirements of the indication. Targeting polymers can be individual substances or any combination of targeting polymers, e.g., synthetic and non-synthetic, low molecular and polymolecular, derivatized and non-derivatized.

A special variant of manufacture is the use of the same polymer as the synthesis and targeting polymer. This means that the targeting polymer is the same as the polymer used for synthesis, as the synthesis polymer that is present after synthesis will, as has been described, no longer be identical with the polymer used for synthesis. The targeting polymer is the same as the stated synthesis polymer, but it was not exposed to the crucial drastic conditions that occur during synthesis and has therefore maintained its "physiological" state.

Targeting polymer quantities in the final nanoparticle solution of embodiments of aspects of the invention can be varied throughout a wide range. In, general, they may vary between 0.5 to 50 times the overall weight of the metallic ions contained; however, 1 to 25 times that weight is the preferred quantity.

Adsorption mediators/enhancers are all substances that improve or facilitate adsorption of the targeting polymer or the mixture of target polymers by the surface of the iron-containing core or iron-containing core and primary coat. In general, adsorption mediators/enhancers must have bifunctional properties: while one molecular part has an

affinity for the basic structural unit, another molecular part, which may, however, be identical with that first functional part, causes affinity for the targeting molecule. Suitable substances are substances having two functional groups, or a hydrophobic and a hydrophilic molecular part. Peptides that have an affinity for the iron core, or for the iron core plus primary coat, are preferred adsorption mediators/enhancers. Such peptides can be selected from peptide libraries using advanced biochemical methods. Preferred are peptides containing the RRTVKHHVN or RRSRHH or RSKRGR sequence or parts thereof in their molecule [one-letter code of amino acids; see e.g. Stryer, Biochemistry; Freeman and Comp.; New York; 1988].

Another advantage of using peptides as adsorption mediators/enhancers is that the molecular part which is not required for affinity can be optionally coupled covalently with the targeting polymer or polymers, which makes affinity to the targeting polymer an optional property. The quantity of adsorption mediator required depends on the substances used (intensity of adsorption mediation) and on the properties of the targeting polymer or polymers; the total amount is less than, or equal to, the overall weight of metallic ions contained in the core.

Pharmaceutical additives or adjuvants that may be contained in nanoparticle solutions can be divided into five classes according to their function: preserving agents, pH stabilizers, antioxidants, isotonizing additives, peptisators and solutizers. Other adjuvants can be medically tolerable solvents, e.g., sugar solutions, plasma expanders, electrolyte solutions, physiological saline or water for injection, as well as parenterally applicable oily "solvents".

Examples of pharmaceuticals or drugs that may be contained in nanoparticle solutions of embodiments of aspects of the invention can be grouped as follows: antiallergic agents, antianaphylactic or prophylactic agents, vasodilators or vasoconstrictors, substances that influence the blood flow, substances that influence nanoparticle metabolism, substances that influence the pharmacokinetics of the nanoparticles, substances that change the iron balance, substances from the group of enzyme inductors and inhibitors, or general mediators and antimediators. Among the medical substances for therapeutic uses the main interest is in those coming from the groups of cytostatic agents, chemotherapeutic agents, hormones and antidiabetic agents.

Pharmaceuticals and drugs can be added to the nanoparticle solutions as optional components or can be coupled to the targeting polymers; the conjugate of polymer and medical substance is then used as the targeting polymer.

The "physiological" distribution of the nanoparticles cannot only be changed by pharmaceuticals that influence "physiological" factors, e.g., the blood flow, lymph flow and lymph production or the like; *in-vivo* distribution can also be changed by simple physiotherapeutic measures. Movement that can be "applied" directly by taking a walk or practising on an ergometer and, as its counterpart, immobility, as found, for example, with indoor patients and/or application under anaesthesia and the like, which result in a completely different distribution behavior and pattern. Furthermore, heat input should be mentioned here, which can be accomplished by simple use of infrared light, or whole-body or partial baths. A hyperthermia facility as used in many clinics for purposeful heat input for adjuvant tumor treatment is particularly preferred. Intentional local heating improves "selectivity" in accompanying physiotherapeutic measures.

The great flexibility of the modular production design allows a free combination of targeting polymer(s), adsorption mediator(s), pharmaceutical adjuvants and pharmaceuticals as well as application of various nanoparticle compositions along with physiotherapeutic measures.

The nanoparticles of embodiments of aspects of the invention or nanoparticle solutions of embodiments of aspects of the invention may be composed of many different ingredients, so that only a general statement can be made about an exact composition that depends on a specific application:

Table 2: Composition of nanoparticles of embodiments of aspects of the invention (percentage/quantities)

		relative portion (weight)	
basic structural unit	iron	= 1	total = 1
	doping with other metals	≤ 0.25	
	synthesis polymer(s)		0.01-1
	adsorption mediator		≤ 1
	targeting polymer(s)		0.5 - 50
	pharmaceuticals	as required	
	pharmaceutical adjuvants	as required	

The overall diameter of the nanoparticle of embodiments of aspects of the invention including all additives (measured with dynamic laser light scattering DLS) is no greater than ten times the diameter of the iron-containing core (measured using transmission electron microscopy; TEM). Preference is given to those

combinations in which the diameter of the basic structural unit (core + primary coat) is increased only to a minor extent by the targeting polymer or combination of targeting polymers plus optional adjuvants. The DLS-measured diameter may exceed the diameter of the basic structural unit by a maximum of 20%.

Nanoparticles of embodiments of aspects of the invention of unprecedented flexibility and quality that are suitable for applications requiring high biological specificity and high physical quality (particle size, magnetic properties) can be produced by combining optimum physical properties of the basic structural units with a multitude of potential targeting polymers. The modular design and the many combinations it permits result in a wide range of potential applications.

As the nanoparticles of embodiments of aspects of the invention combine high physical quality with excellent targetability by flexible adjustment (modular design) of the targeting polymer (secondary coat) to the respective problem, they are applicable for many special indications, e.g., MR lymphography after intravenous or local interstitial administration, tumor visualization, visualization of functions or malfunctions, of plaque (atherosclerosis imaging), thrombi and vascular occlusions, MR angiography, perfusion imaging, infarct visualization, visualization of endothelial damages, receptor imaging, visualization of blood-brain barrier integrity etc., as well as for differential diagnosis, in particular, for distinguishing tumors/metastases from hyperplastic tissue.

The particles are also suitable for the most varied *in-vitro* diagnostic applications due to their extraordinary production flexibility. For example, they may be used as specific carriers used in magnetic separation examinations for EIAs (enzyme-immunoassays). Selective depletion of

specific factors from the blood (*ex-vivo*) detoxification of the blood) is a combination of *in-vitro* methods with a therapeutic approach.

The nanoparticles of embodiments of aspects of the invention show distinct self-colouring. When combined with a targeting polymer that results in a particularly high concentration in lymphatic nodes, these particles are excellently suited as intraoperative labelling substances for lymph node staining. As lymphatic nodes are often surgically removed together with tumours, pre-administration of nanoparticles of embodiments of aspects of the invention makes it much easier for the operating surgeon to identify these nodes, which may be rather small, in the surrounding tissue. The nanoparticles of embodiments of aspects of the invention have a particularly wide time window for this purpose and can be applied from 60 min. to more than 24 hours prior to the operation.

Apart from lymph node visualization, intratumoral application or application in the tumor periphery facilitates staining of the tumor periphery, which improves distinction of the tumor from the surrounding tissue; in addition, particles in the tumor area are carried off via the same lymph vessels through which the tumor cells will spread metastatically. Thus the particles stain the lymph vessels or nodes that are preferred for metastatic spread.

In addition to their use as intravenously-applied contrast material, the nanoparticles of embodiments of aspects of the invention can be applied locally. Local application may be advantageous in the case of, e.g., a

diagnosis where findings after intravenous administration aroused suspicion or doubt.

Another field of application for the nanoparticles of embodiments of aspects of the invention is their use as a reinforcing substance in *in-vivo* diagnostics based on highly sensitive methods of measurement (SQUID) to determine magnetization or magnetic fields/flux densities. Development of highly sensitive methods of measurement in this field have facilitated *in-vivo* tracing of magnetic particles, so that magnetic particles, similar to radioactive substances used in scintigraphy, can be used for the diagnosis of malfunctions and lesions.

The particles may be used as vehicles for medical substances in the field of therapeutics. The specificity of the nanoparticles is used for the transport of medical substances to their place of action. The medical substances may be incorporated in the iron-containing core or chemically bonded to the synthesis polymer and/or the targeting polymer. Adsorption of conjugates of polymers and medical substances, or bonding of medical substances to adsorption mediators/enhancers, can be viewed as an alternative. Thus, for example, specific peptide sequences can be produced that show high affinity for iron oxide surfaces.

A possible indication could be accumulation of high concentrations of low-molecular chemotherapeutic agents in phagocytizing cells as therapeutically required, for example, for many diseases involving microorganisms that persist in RES cells. In all therapeutic approaches, systems of nanoparticles of embodiments of aspects of the invention and medical substances can be selectively accumulated in their target area using external magnetic fields. For treating very special problems, there is the option of implanting small magnets for local control in the target area, e.g., a tumour area.

Apart from using nanoparticles of embodiments of aspects of the invention as vehicles for the purposeful transport of medical substances to specified tissues, types of pharmaceuticals may be produced that are characterized by a modified release of active agents. Release of the active agent can be controlled using biologically-decomposable conjugates or by inserting the medical substance in various components of the nanoparticle of embodiments of aspects of the invention that have a different biodegradability. A potential indication is the use of nanoparticles of embodiments of aspects of the invention as a repository for administering hormones.

It is also conceivable to use the nanoparticles of embodiments of aspects of the invention in novel therapeutic systems in which the magnetic properties of the nanoparticles of embodiments of aspects of the invention induce and control the release of the medical substance via a "magnetic switch" that may also be operated from outside. An important field of application is the development of therapeutic systems for the controlled release of an antidiabetic agent in the treatment of pancreatic diabetes.

Medical substances suitable for direct transport to their place of action by nanoparticles of embodiments of aspects of the invention are, first of all, chemotherapeutic agents and cytostatic agents. Also, antimicrobial therapy frequently requires purposeful transport of the medical substance to its place of action (e.g., tuberculosis, microorganisms that persist in macrophages). Medical substances suitable for release by the magnetic properties of the nanoparticles of embodiments of aspects of the invention are, in particular, antimicrobiotic agents, hormones, antidiabetic agents, cytostatic agents, and chemotherapeutic agents.

The nanoparticles of embodiments of aspects of the invention may be employed over and beyond their indirect therapeutic use as medical substances themselves, e.g., as absorbers in hyperthermia or Mossbauer nuclear absorption therapy, or, if doped appropriately with boron or gadolinium, in neutron capture therapy. Another application is medical radiation therapy for which the nanoparticles of embodiments of aspects of the invention are doped with radioactive elements either in their core or by the basic structural unit adsorbing suitable molecules with isotopes or molecules.

A preferred application of the nanoparticles of embodiments of aspects of the invention in radiation therapy is, for example, one in which the nanoparticles either contain an "autoradiator" through the radioactive ^{55}Fe isotope or one in which the nanoparticles of embodiments of aspects of the invention contain an isotope that can be induced to become a radiating isotope by external "activation". For example, the core may contain ^{157}Gd that is externally activated by neutrons.

Another application of the nanoparticles of embodiments of aspects of the invention in radiation therapy results from the fact that their core, synthetic or targeting polymer, or adsorption mediator can be modified to contain an autoradiator, e.g., ^{123}I or ^{125}I. Alternatively, the nanoparticles of embodiments of aspects of the invention may contain an isotope that is converted into a radiating isotope by external triggering. An example is the labelling of the targeting polymer with iodine and external activation of the iodine-K edge using monoenergetic X-ray radiation.

The nanoparticles of embodiments of aspects of the invention can also be used for removing bacteria, viruses, endo- and exotoxins from the vasal space, with

inactivation being brought about either by interaction with the nanoparticles themselves or by their interaction with the RES to identify conjugates/adsorbates and subsequent intracellular inactivation.

(e) DESCRIPTION OF THE FIGURES

In the accompanying drawings,

Fig. 1: Sectional structure of the nanoparticles of embodiments of aspects of the invention with iron-containing core, primary coat (synthesis polymer) and secondary coat (targeting polymer);

Fig. 2: General diagram showing synthesis of the nanoparticles of embodiments of aspects of the invention;

Fig. 3: FTIR spectrum of monocarboxydextran and its parent compound dextran 4;

Fig. 4: FTIR spectrum of polycarboxydextran and its parent compounds dextran T10 and 6-bromohexanoic acid;

Fig. 5: MR tomograms of agarose-embedded lymphatic nodes of rats;

Fig. 6: Quantitative evaluation (from Fig. 5) of relative signal intensities for SE 2000/15 in various lymphatic nodes of the rat;

Fig. 7: Quantitative evaluation (from Fig. 5) of relative signal intensities for GE 135/15/15° in various lymphatic nodes of the rat;

Fig. 8: Frontal pre- and post-contrast MR tomograms of the pelvic region of the rabbit in the proton-density weighted spin echo sequence (SE 2000/15);

Fig. 9: Frontal pre- and post-contrast MR tomograms of the pelvic region of the rabbit in the proton-density weighted spin echo sequence (SE 2000/15);

Fig. 10: Relative signal intensities for SE 2000/15 in various lymphatic nodes of the rabbit 24 h p.i.;

Fig. 11: Frontal pre- and post-contrast MR tomograms of the pelvic region of the rabbit in the T2*-weighted gradient echo sequence (GE 135/15/15°);

Fig. 12: Frontal pre- and post-contrast MR tomograms of the pelvic region of the rabbit in the T2*-weighted gradient echo sequence (GE 135/15/15°);

Fig. 13: Modified batch vs. original substance: relative signal intensities for GE 135/15/15° in various lymphatic nodes of the rabbit 24 h p.i.;

Fig. 14: Ex-vivo MR tomograms (GE sequence) of agarose-embedded lymphatic nodes of the rabbit;

Fig. 15: Relative signal intensities for GE 135/15/15° in various lymphatic nodes of the rabbit 24 h p.i.;

Fig. 16: Relative signal intensities for SE 2000/15 in various lymphatic nodes of the rat as a function of doses applied 24 h after injection of the nanoparticles of embodiments of aspects of the invention;

Fig. 17: Relative signal intensities as a function of the dose for GE 135/15/15° in various lymphatic nodes of the rat 24 h after application of the nanoparticles of embodiments of aspects of the invention;

Fig. 18: Relative signal intensities for SE 2000/15 in various lymphatic nodes of the rat as a function of time after application (reference substance);

Fig. 19: Relative signal intensities for SE 2000/15 in various lymphatic nodes of the rat as a function of time after application of specific nanoparticles of embodiments of aspects of the invention

Fig. 20: Relative signal intensities for GE 135/15/15° in various lymphatic nodes of the rat as a function of time after application (reference substance);

Fig. 21: Relative signal intensities for GE 135/15/15° in various lymphatic nodes of the rat as a function of time after application of specific nanoparticles of embodiments of aspects of the invention;

Fig. 22: Effect on accumulation in lymphatic nodes caused by the input of heat;

Fig. 23 : Transversal dynamics study of the rat's abdomen using a T1-weighted SE sequence (TR: 200 ms, TE: 10 ms) after bolus injection of the specific nanoparticles according to Example D2 (dose: 20 µmol Fe/kg);

Fig. 24: Comparison of relative signal intensities for SE TR/TE 200ms/10ms in the venous vessel and the liver parenchyma for the specific nanoparticles according to Example D2 and the unspecific reference substance according to Example C2;

Fig. 25: Coronary MIPS (maximum-intensity projections) of 3D flash tomograms (TR: 40ms, TE: 6 ms, FA 60°)

for the specific nanoparticles according to Example D2 and the reference substance C2;

Fig. 26: Nanoparticles of embodiments of aspects of the invention as "intraoperative" labelling substances for visual detection of lymphatic nodes (general view);

Fig. 27: Nanoparticles of embodiments of aspects of the invention as "intraoperative" labelling substances for visual detection of lymphatic nodes (detailed view);

Fig. 28: Demonstration of metastases in lymphatic nodes by visual detection in metastatic lymphatic nodes in rabbit;

Fig. 29: Cell tomogram of specific nanoparticles of embodiments of aspects of the invention (with transferrin) compared with the unspecific reference (nanoparticles without transferrin);

Fig. 30: *Ex-vivo* MR tomographic diagram of atherosclerotic plaques of the aorta of a rabbit with modification D7 (dose 200 μmol Fe/kg; aorta resection 5 h p.i.);

Fig. 31: Histological detection of iron in the atherosclerotic membrane of a rabbit's aorta with Prussian blue staining;

Fig. 32: Histochemical detection (Prussian blue staining) of accumulated nanoparticles of embodiments of aspects of the invention according to Example E6 in the aorta of a Watanabe rabbit;

Fig. 33: Transversal T1-weighted spin echo dynamics study (TR: 300 ms, TE: 15 ms) of the tumoral signal behaviour after bolus injection of nanoparticles of embodiments of aspects of the invention according to Example D2 (200 μmol Fe/kg);

Fig. 34: Curve of relative signal intensity (accumulation) in the tumour; and

Fig. 35: Time-dependent transversal proton-density weighted (SE 2000/15) tomograms after application of the nanoparticles of embodiments of aspects of the invention according to Example D2 (200 μmol Fe/kg).

Fig. 1 is a sectional view of the nanoparticles of embodiments of aspects of the invention. Iron-containing core (3) + primary coat (2) (basic structural unit) may be combined with the targeting polymer (1) (secondary coat) at any time. The basic structural unit (2) determines physical quality while the targeting polymer (1) (secondary coat) forms the surface of the nanoparticle and determines its *in-vivo* behaviour.

As an option, the adsorptive bonding of the primary coat (2) and the secondary coat (1) can be improved, or facilitated, by adsorption mediators. The option of additional adsorption of pharmaceutic adjuvants or drugs is not shown here.

Fig. 2 is a general overview of the method to produce the nanoparticles of embodiments of aspects of the invention. The great number of possible variants, e.g., to produce the "crude substances", are not shown in this Fig., but it can be seen that the nanoparticles of embodiments of aspects of the invention have a modular design and are

design and are "completed" by combining various structural units or blocks. The essential characteristic is that the basic structural unit with the primary coat and the targeting polymer are produced separately.

(f) AT LEAST ONE MODE FOR CARRYING OUT THE INVENTION

Examples of Production and Application

A: Production of Synthesis Polymers

A1 Synthesis of (mono)carboxydextran (CDx)

100 g of dextran 4 (Serva, Germany) are dissolved in 500 ml water and heated to 60 °C. 55 ml of 10 N soda lye is added while being stirred. The solution is (partly) neutralized to pH 8 after a reaction time of 5 hours. The brown solution is then purified on a mixed-bed ion exchanger (AMBERLITE™ IRA-400 and IR-120). The fractions having acidic properties are pooled and concentrated in a vacuum in the rotary evaporator at 40 °C. Then they are freeze-dried.

Table 3: Analytic data:

	Dimension	Result	Method
molecular weight	g/mol	ca. 2,000	size exclusion chromatography (SEC)
acid content (%)	%	7.3 ± 0.4	potentiometric titration
carboxylic acid - pKs		3.6 - 3.8	potentiometric titration
optical rotation (water)	(degree)	+ 156.9 ° ± 9.9 °	polarimeter with circular scale (Zeiss)
yield	%	59	anthrone method
ultimate analysis			
water	%	0.70	
carbon	%	41.25	
hydrogen	%	6.30	

Fig. 3 is a FTIR spectrum in KBr of synthesis polymer according to Example A1 (mono-carboxydextran). The spectrum of dextran 4 (Serva), the parent compound, has been inserted for comparison.

The FTIF spectrum (potassium bromide) of the modified dextran (= carboxydextran) is thus shown in Fig. 3.

A2 Synthesis of polycarboxydextran (P-CDx)

10 g of dextran T10 (Pharmacia, Germany) are weighed into a 250 ml two-neck flask and mixed with 100 ml of 4 N NaOH. One neck of the flask is equipped with a reflux condenser, and the solution is heated to ca. 80 °C. 30 g of 6-bromohexanoic acid (Aldrich, Germany) are added in portions via the second neck with constant stirring (magnetic stirrer). The neck is plugged after the substance has been added and the reaction mixture kept agitated for another 3 hours. After the reaction, the batch is neutralized under a fume hood using 6 N HCl and then reduced by preliminary concentration using a rotary film evaporator (60 °C, vacuum). Separation of the unconverted reactant, or cleaning of the modified carboxydextran, is carried out by precipitation with ethanol. The white precipitate is washed, redissolved in double distilled water, and finally filtered through an 0.22 µm filter (Schleicher and Schüll, Germany) and lyophilized.

Table 4: Analytic data

	Dimension	Result	Method
molecular weight	g/mol	ca. 12.000	size exclusion chromatography (SEC)
acid content (%)	%	19.86 ± 1.2	potentiometric titration
carboxylic acid - pKs		4.4 - 4.8	potentiometric titration
optical rotation (water)	(degrees)	+ 109.3 ° ± 6.9 °	polarimeter with circular scale (Zeiss)
yield	%	95	anthrone method
ultimate analysis			
water	%	2.3	
carbon	%	49.7	
hydrogen	%	5.4	

Fig. 4 is a FTIR spectrum in KBr of synthesis polymer according to Example A2 (poly-carboxydextran). The spectra of dextran T10 (Pharmacia) and 6-bromo-hexanoic acid (Sigma), the parent compounds, were inserted for comparison.

The FTIR spectrum of the polycarboxydextran is shown in Fig. 4.

B: Production of the Crude Substances

B1 Production from CDx using ammonia gas

5.0 g of monocarboxydextran (CDx, Example A1) having a molecular weight of 2000 Da are dissolved in 17.5 ml of double distilled water. The solution is degasified by blowing in nitrogen. 6.7 ml of 1-molar iron(III) chloride hexahydrate solution are prepared in a test tube and degasified using nitrogen. 648 mg of iron(II) chloride tetrahydrate are added to the iron(III) solution and dissolved in the nitrogen stream. The polymer solution is heated to 75 °C, and the iron solution added (with exposure to nitrogen gas). The heated reaction mixture is adjusted to alkaline by the quick introduction of ammonia from a gas cylinder while mixing thoroughly. Then the reaction solution is refluxed for 1 hour. It is subsequently heated for another 10 minutes in the open flask to sweep out the unconverted ammonia. It is centrifuged at 2500 g for 30 min. after cooling, and the filtrate is evaporated down to 7 ml using a rotary evaporator; the pH value is checked and neutralized, if required. After the concentration is determined, the solution is adjusted to a 1-molar iron concentration with double distilled water and filtered using a 0.22 µm filter. The solution can be sterilized in an autoclave (method A121).

Table 5: Analytic data:

	Dimension	Result	Comment
Content			
yield (iron)	%	87	
content (iron)	mol/l	1	ICP atomic emission spectroscopy
iron(II)/total iron	%	9.8	phenanthroline method
polymer (C-dextran)	mg/ml	500	anthrone method
polymer/iron:	(g/g)	9:1	
Dimensions			
core diameter	nm ± SD	3.8 ± 0.8	electron microscope (TEM)
overall diameter	nm ± SD nm	9.9 ± 6.1 11.1	laser light scattering (DLS) exclusion chromatography
relaxivity and susceptibility			
susceptibility	EMU/g	64	magnetic balance
T1 relaxivity	1 $mmol^{-1}$ s^{-1}	54	minispec pc 120
T2 relaxivity	1 $mmol^{-1}$ s^{-1}	24	minispec pc 120
Half-life in the blood (200 µmol/kg body wt, rat, n=5)	min	88 94 101	(T1)[1] (T2)[2] (Fe)[3]

Notes 1 to 3: Determination of half-life in the blood

- Half-life of clearance from the blood

A catheter of 50.5 cm length filled with heparinized sodium chloride solution (0.2 ml) is implanted in the common carotid artery of the etherized experimental animals (rat, ca. 200 g) and pushed forward about 1.5 cm to the heart. The free end of the catheter was led out and fixed with histoacrylate.

The test substance is applied i.v. via the caudal vein (ca. 1 ml/min.) about an hour after the end of the operation. Blood samples were taken at various times according to the expected elimination rates of the test substances when the animals were awake. At the end of the test the animals were killed under ether anaesthesia by draining the blood from the caval vein.

- Half-life of the T_1 and T_2 effects

The blood samples are centrifuged at 2900 rpm (1000 g) for 15 min. 0.250 ml of the supernatant liquid are drawn off. The samples are filled to 2.0 ml with double distilled water and the mixture then thermostated at 40 °C.

Decreasing blood concentration is determined by measuring the $T_{1,2}$ relaxation times with a pc120 relaxometer (Bruker, Germany). The measurement was carried out either with a 180°-90°-IR-(inversion recovery) sequence (T_1) or a CPMG sequence (T_2).

The results were analyzed based on a pharmacokinetic two-compartment model; the data were calculated using the TOPFIT™ pharmacokinetic computer program by protracting the concentrations over time in terms of reciprocal $T_{1,2}$ times (relaxation rates) minus the blank reading. TOPFIT™ first calculates the slope of the

straight line by linear regression from the floating point notation of "concentration" and time, and then the effective half-live from the values obtained.

• Half-life over iron content

800 µl of the solutions used for determining relaxation time were withdrawn by pipette, dissolved with concentrated nitric acid, and filled to 10.0 ml with double distilled water. The iron content is then quantified using atomic emission spectroscopy (AES). The results are converted into blood concentrations and analyzed by means of a concentration-time diagram using TOPFIT, while taking into account the relevant dilution factors.

B2: Production of P-CDx with NaOH and Fe(III) citrate and Fe(II) gluconate

5.0 g of polycarboxydextran (Example A2) with a molecular weight of 12000 Da are dissolved in 17.5 ml of double distilled water. The solution is degasified by blowing in nitrogen. 6.7 ml of 1-molar iron(III) citrate monohydrate solution are prepared in a test tube and degasified using nitrogen. 1.635 g of iron(II) gluconate trihydrate are added to the iron(III) solution and dissolved in the nitrogen stream. The polymer solution is heated to 75 °C, and the iron solution added (with exposure to nitrogen gas). Ca. 12 ml of 3 N soda lye are added to the heated reaction mixture within 30 seconds while mixing thoroughly. Then the reaction solution is neutralized with 6 N hydrochloric acid and refluxed for 1 hour. It is subsequently heated for another 10 minutes in the open flask to sweep out the unconverted ammonia. It is centrifuged at 2500 g for 30 min. after cooling, and the filtrate evaporated down to 7 ml using a rotary evaporator; the pH value is checked and neutralized, if required. After the concentration is determined, the solution is adjusted to a 1-molar iron concentration with double distilled water and filtered using the 0.22 µm filter. The solution can be sterilized in an autoclave (method A121).

Table 6: Analytic data:

	Dimension	Result	Comment
Content			
yield (iron)	%	84	
content (iron)	mol/l	1	ICP atomic emission spectroscopy
Fe(II)/total Fe	%	11.4	phenanthroline method
polymer (P-dextran)	mg/ml	505*	anthrone method
polymer/iron:	(g/g)	9:1	
Dimensions			
core diameter	nm ± SD	4.1 ± 1.3	electron microscope (TEM)
overall diameter	nm ± SD nm	20.4 ± 8.4 ca. 27	laser light scattering (DLS) size exclusion chromatography (SEC)
Relaxivity and Susceptibility			
susceptibility	EMU/g	77	magnetic balance
T1 relaxivity	l $mmol^{-1}$ s^{-1}	24	minispec pc 120
T2 relaxivity	l $mmol^{-1}$ s^{-1}	64	minispec pc 120
Half-life in the blood (200 µmol/kg body wt, rat, n=5)	min	68 64 59	(T1) (T2) (Fe) see notes for example B1

* P-CDx content is calculated from glucose equivalents multiplied by 1.64 (100 mg P-CDx = 61 mg of glucose equivalents)

B3: Production from CDx with Fe(III) NTA and ammonium hydroxide

5.0 g (mono)carboxydextran (CDx, Example A1) with a molecular weight of 2000 Da are dissolved in 35 ml of double distilled water. The solution is degasified by blowing in nitrogen. Concentrated ammonium hydroxide solution (32%) is added to the reaction mixture while heating and mixing thoroughly until the pH value is adjusted to 11. 6.85 ml of 1-molar iron(III) solution are prepared in a test tube, mixed with an equimolar quantity of NTA, and degasified using nitrogen. 667 mg of iron(II) chloride tetrahydrate are added to the iron(III) solution and dissolved in the nitrogen stream. The iron solution is added to the alkaline polymer solution within 20 seconds. Then the reaction solution is neutralized with 6 N hydrochloric acid and refluxed for 1 hour. It is centrifuged at 2500 g for 30 min. after cooling, the filtrate is evaporated down to 6 ml using the rotary evaporator, and the pH value is measured. After determining the concentration, the solution is adjusted to a 1-molar iron concentration with double distilled water and filtered using a 0.22 µm filter. The solution can be sterilized in an autoclave (method A121).

Table 7: Analytic data:

	Dimension	Result	Comment
Content			
yield (iron)	%	69	
content (iron)	mol/l	1	ICP atomic emission spectroscopy
iron-II/ total iron	%	7.1	phenanthroline method
polymer (P-dextran)	mg/ml	421	anthrone method
polymer/iron:	(g/g)	8:1	
dimensions			
core diameter	nm ± SD	5.5 ± 2.3	electron microscope (TEM)
overall diameter	nm ± SD nm	24.4 ± 8.4 ca. 31	laser light scattering (DLS) size exclusion chromatography (SEC)
Relaxivity and Susceptibility			
susceptibility	EMU/g	96	magnetic balance
T1 relaxivity	1 $mmol^{-1}$ s^{-1}	33	minispec pc 120
T2 relaxivity	1 $mmol^{-1}$ s^{-1}	148	minispec pc 120
Half-life in the blood (200 µmol/kg body wt, rat, n=5)	min	59 54 58	(T1) (T2) (Fe) see notes for example B1

B4: Production from P-CDx with iron(III) and reducing agent, soda lye

5.0 g of polycarboxydextran (Example A2) with a molecular weight of 12000 Da are dissolved in 17.5 ml of double distilled water. The solution is degasified by blowing in nitrogen. 10 ml of 1-molar iron(III) chloride hexahydrate solution are added to the polymer solution, then degasification using nitrogen is continued. The polymer solution is heated to ca. 75 °C, and 113.6 mg of hydroxylamine HCl are added under nitrogen gas. 12 ml of 3 N soda lye are added to the heated reaction mixture within 30 seconds while mixing thoroughly. Then the reaction solution is neutralized with ca. 6 N hydrochloric acid and refluxed for about 1 hour. It is centrifuged at 2500 g for 30 min. after cooling, and the filtrate is evaporated down to 7 ml using the rotary evaporator; the pH value is checked. After determining the concentration, the solution is adjusted to a 1-molar iron concentration with double distilled water and filtered using a 0.22 µm filter. The solution can be sterilized in an autoclave (method A121).

Table 8: Analytic data:

	Dimension	Result	Comment
Content			
yield (iron)	%	84	
content (iron)	mol/l	1	ICP atomic emission spectroscopy
iron-II/ total iron	%	5.4	phenanthroline method
polymer (P-dextran)	mg/ml	515	anthrone method
polymer/iron:	(g/g)	9:1	
Dimensions			
core diameter	nm ± SD	4.5 ± 1.4	electron microscope (TEM)
overall diameter	nm ± SD nm	21.4 ± 5.4 ca. 24	laser light scattering (DLS) size exclusion chromatography (SEC)
Relaxivity and Susceptibility			
susceptibility	EMU/g	88	magnetic balance
T1 relaxivity	1 $mmol^{-1}$ s^{-1}	28	minispec pc 120
T2 relaxivity	1 $mmol^{-1}$ s^{-1}	138	minispec pc 120
Half-life in the blood (200 µmol/kg body wt, rat, n=5)	min	57 55 51	(T1) (T2) (Fe) see notes example B1

B5: Production with a mixture ofdextran 4 and dextran 15

5.0 g of a 1:1 mixture of dextran 4 and dextran 15 (both Serva, Germany) having a molecular weight of 4,000 - 6,000 Da and 15,000 - 20,000 Da, respectively, are dissolved in 20 ml of double distilled water. The colorless polymer solution is adjusted to a pH value of 12 using 3 N soda lye, refluxed for 1 hour, and neutralized with 6 N HCl. The dark reddish brown solution is degasified by blowing in nitrogen. 6.7 ml of 1-molar iron(III) chloride hexahydrate solution are prepared in a test tube and degasified using nitrogen. 648 mg of iron(II) chloride tetrahydrate are added to the iron(III) solution and dissolved in the nitrogen stream. The polymer solution is heated to 75 °C, and the iron solution added (while exposed to nitrogen gas). 11.5 ml of 3 N soda lye are added to the heated reaction mixture within 30 seconds while mixing thoroughly. Then the reaction solution is refluxed for 1 hour. It is centrifuged at 2500 g for 30 min. after cooling, and the filtrate is evaporated down to 8 ml using a rotary evaporator; the pH value is checked. After determining the concentration, the solution is adjusted to a 1-molar iron concentration with double distilled water and filtered using a 0.22 µm filter. The solution can be sterilized in an autoclave according to method A121.

Table 9: Analytic data:

	Dimension	Result	Comment
Content			
yield (iron)	%	91	
content (iron)	mol/l	1	ICP atomic emission spectroscopy
iron-II/total iron	%	12.8	phenanthroline method
polymer (P-dextran)	mg/ml	570	anthrone method
polymer/iron:	(g/g)	10:1	
Dimensions			
core diameter	nm ± SD	4.0 ± 1.1	electron microscope (TEM)
overall diameter	nm ± SD	18.1 ± 3.4	laser light scattering (DLS)
	nm	ca. 21	size exclusion chromatography (SEC)
Relaxivity and Susceptibility			
susceptibility	EMU/g	68	magnetic balance
T1 relaxivity	l $mmol^{-1}$ s^{-1}	21	minispec pc 120
T2 relaxivity	l $mmol^{-1}$ s^{-1}	78	minispec pc 120
Half-life in the blood (200 µmol/kg body wt, rat, n=5)	min	61 59 67	(T1) (T2) (Fe) see notes example B1

C: Production of the Basic Substances

C1: Dialysis against water

5 ml of the solution according to Example B1 are filled in a VISKING™ dialysis tube (Serva, Germany) and dialyzed five times, each time for 6 hours, against 1 l of fresh double distilled water. The retentate is adjusted to an iron concentration of 200 mmol/l by dilution with double distilled water and filled in portions of 5 ml through 0.22 μm filters (cellulose acetate. ROTRAND™ , Fa. Schleicher & Schüll, Germany) into sterile 10 ml vials. The desorbed solution can be sterilized in an autoclave.

C2: Dialysis against 20 mMol sodium citrate

5 ml of the solution according to Example B1 are filled in a VISKING™ dialysis tube and dialyzed five times, each time for 6 hours, against 1 l of fresh sodium lactate solution (20 mmol/l, pH 7). Dialysis is repeated twice, each time for 5 hours, against 1l of fresh double distilled water. The retentate is adjusted to an iron concentration of 200 mmol/l by dilution with double distilled water and filled in portions of 5 ml through 0.22 μm filters into sterile 10 ml vials.

C3: Ultrafiltration with AMICON™

5 ml of the solution according to Example B1 are pipetted into a preparative ultrafiltration device and filled to the 15 ml mark with double distilled water (CENTRIPREP™ 100, Cut off 100 kDa, Fa. AMICON™ Germany) and ultrafiltered for 1 h at 1000 g. The filtrate is then discarded and the container of the retentate filled to the 15 ml mark with fresh double distilled water, and ultrafiltered again. This procedure is repeated twice. The

retentate is adjusted to an iron concentration of 200 mmol/l by dilution with double distilled water and filled in portions of 5 ml through 0.22 µm filters (cellulose acetate) into sterile 10 ml vials.

C4: Chromatographic separation

5 ml of the solution according to Example B1 are filled in a 10 ml SUPERLOOP™ (Fa. Pharmacia) on a S400HR sephacryl column (100 x 5 cm) and eluted in 50 mM citric acid/250 mM mannite at a flow rate of 300 ml/hour. The fraction from 450 ml to 840 ml is collected and concentrated to ca 50 ml using a rotary evaporator at 60 °C in a vacuum. The concentrate is dialyzed three times for 6 hours against double distilled water, concentrated again in the rotary evaporator and adjusted to a concentration of 200 mmol iron/l after determination of the iron content. The solution is filled in portions of 5 ml through 0.22 µm cellulose acetate filters into sterile 10 ml vials. The desorbed solution can be sterilized in an autoclave.

Table 10: analytical data of the base compounds according to Example C1-C4

Example	Yield (Fe)	Polymer : Iron [= 200 mmol/l] (g/g)
C1	96 %	0.501 ± 0.025
C2	94 %	0.300 ± 0.020
C3	89 %	0.610 ± 0.041
C4	67 %	0.143 ± 0.030

The physico-chemical data regarding the magnetic properties and size parameters correspond to the values of the parent compound (Example B1).

D: Solutions for Application

D1 Dextran T10

5.0 ml of solution according to Example C1 at a concentration of 200 mmol Fe/l (corresponding to 56 mg of total iron content) are prepared in a 10 ml vial. 33.6 mg of dextran T10 as the targeting polymer are dissolved in 6.0 ml of distilled water and 5.0 ml of this solution are added to the iron oxide solution in aseptic conditions using a syringe with a filter attachment (0.22 µm). The polymer-to-iron weight quotient is 1 (residual synthesis polymer = 28 mg + targeting polymer = 28 mg).

The preparation now contains 10 ml of a 100 mmolar (iron) solution that is immediately suitable for application in intravenous MR lymphography.

D2: Dextran FP1: Production from 2 solutions

5.0 ml of solution according to Example C1 at a concentration of 200 mmol Fe/l (corresponding to 56 mg of total iron content) are prepared in a 10 ml vial. 302.4 mg of dextran FP1 as the targeting polymer are dissolved in 6.0 ml of distilled water, 5.0 ml of which are added to the iron oxide solution in aseptic conditions using a syringe with a filter attachment (0.22 µm). The polymer-to-iron weight quotient is 5 (residual synthesis polymer = 28 mg + targeting polymer = 252 mg).

The preparation now contains 10 ml of a 100 mmolar (iron) solution that is immediately suitable for application in intravenous MR lymphography.

D3: Dextran FP1 as lyophilisate

5.0 ml of solution according to Example C1 at a concentration of 200 mmol Fe/l (corresponding to 56 mg of total iron content) are prepared in a 10 ml vial. 302.4 mg of dextran FP1 as the targeting polymer are dissolved in 6.0 ml of distilled water, 5.0 ml of which are added to the iron oxide solution in aseptic conditions using a syringe with a filter attachment (0.22 µm). The polymer-to-iron weight quotient is 5 (residual synthesis polymer = 28 mg + targeting polymer = 252 mg).

The solution is lyophilized in the injection bottle, and the bottle is plugged.

The solution for application is prepared by adding 10 ml of physiological saline; the bottle now contains 10 ml of a 100 mmolar (iron) solution that is immediately suitable for application in intravenous MR lymphography.

D4: Dextran FP1

252 mg of dextran FP1 as the targeting polymer are weighed-in in a 5 ml injection bottle and filled with 5.0 ml of the solution according to Example C1 at a concentration of 200 mmol Fe/l (corresponding to 56 mg of total iron content), and the flask is plugged. The dextran FP1 is dissolved by turning the injection bottle. The polymer-to-iron weight quotient is 5 (residual synthesis polymer = 28 mg + targeting polymer = 252 mg).

The preparation now contains 5 ml of 200 mmolar (iron) solution that is immediately suitable for application in intravenous MR lymphography.

D5: Laminarin

5.0 ml of solution according to Example C1 at a concentration of 200 mmol Fe/l (corresponding to 56 mg of total iron content) are prepared in a 10 ml vial. 33.6 mg of laminarin as the targeting polymer are dissolved in 6.0 ml of distilled water, 5.0 ml of which are added to the iron oxide solution in aseptic conditions using a syringe with a filter attachment (0.22 µm). The polymer-to-iron weight quotient is 1 (residual synthesis polymer = 28 mg + targeting polymer = 28 mg).

The preparation now contains 10 ml of a 100 mmolar (iron) solution that is immediately suitable for application in intravenous MR lymphography.

D6: with transferrin

5.0 ml of solution according to Example C1 at a concentration of 200 mmol Fe/l (corresponding to 56 mg of total iron content) are prepared in a 10 ml vial. 33.6 mg of human Fe_2 transferrin as the targeting polymer are dissolved in 6.0 ml of distilled water, 5.0 ml of which are added to the iron oxide solution in aseptic conditions using a syringe with a filter attachment (0.22 µm). The polymer-to-iron weight quotient is 1 (residual synthesis polymer = 28 mg + targeting polymer = 28 mg).

The preparation now contains 10 ml of a 100 mmolar (iron) solution that is suitable for application as a specific contrast medium for visualizing proliferating cells (tumors).

D7: with endothelin agonist

5.0 ml of solution according to Example C1 at a concentration of 200 mmol Fe/l (corresponding to 56 mg of total iron content) are prepared in a 10 ml vial. 33.6 mg of an endothelin-receptor-specific heptapeptide [cys-his-leu-asp-ile-ile-trp] as the targeting polymer are dissolved in 6.0 ml of double distilled water, 5.0 ml of which are added to the iron oxide solution in aseptic conditions using a syringe with a filter attachment (0.22 µm). The polymer-to-iron weight quotient is 1 (residual synthesis polymer = 28 mg + targeting polymer = 28 mg).

The preparation now contains 10 ml of a 100 mmolar (iron) solution that is suitable for application in intravenous MR plaque imaging (atherosclerosis imaging).

E: Applications

Application Example E1

- MR lymphography in the rat

Objective: Comparison of relative signal intensity in various lymphatic nodes/groups of lymphatic nodes between the parent compound (synthesis polymer = targeting polymer) and a modification produced according to the desorption-adsorption-method (synthesis polymer ≠ targeting polymer) in the rat.

Substance:	Specific nanoparticles (Example D5); comparison = basic structural unit according to Example C1 (= D5 without targeting polymer)
Dosage:	100 µmol Fe/kg body weight (body wt)
Times:	24 h p. i. (post injectionem)

MR method:

Device:	Siemens Magnetom 1.5 T MR whole-body MR scanner with extremity coil
MR parameters:	Field of view (FOV) = 150 mm, matrix = 256x256; slice thickness = 3 mm section orientation = frontal
Sequence 1:	Proton-density-weighted spin echo sequence (SE) with TR = 2000 ms and TE = 15 ms
Sequence 2:	T2-weighted gradient echo sequence (GE) with TR = 135 ms and TE = 15 ms; FA = 15°

Ex-vivo model:

•Accumulation in the lymphatic nodes of rats and rabbits

An *ex-vivo* agar phantom was used to examine the accumulation and distribution of substances in various lymphatic nodes/groups of lymphatic nodes. This *ex-vivo* model has the advantage that accumulation in various central and peripheral lymphatic nodes or lymph node groups can be assessed even for small experimental animals (mouse, rat, rabbit); it also makes it possible to draw conclusion about distribution homogeneity; signal interference can be quantified.

The nanoparticle solution of embodiments of aspects of the invention is injected (bolus) in the experimental animals via the caudal (mouse, rat) or the ear vein (rabbit). The animals are sacrificed after 24 hours, and various lymph nodes or lymph node groups are prepared (popliteal, iliac, axillary, mandibular, inguinal lymph nodes). The lymph nodes are then placed in an agar phantom and kept refrigerated until the MR measurement is carried out (max. 24 hours).

•Manufacture of the *ex-vivo* agar phantom

10 g of microbiological agar-agar are suspended in 500 ml of double distilled water to which 0.5 ml of magnevist (0.5 mol/l gadolinium DTPA dimeglumin) has been added for a homogeneous signal background of the MR tomogram. The suspension is boiled up, then cooled to 80 °C and kept at this temperature. Half of the agar solution is poured into a plastic dish to form a layer having a thickness of 0.5 to 1 cm. After allowing the solution to cool down, the specimens are

arranged on the agar layer (according to left/right body half, or "in physiological order" from top to bottom) and fixed with a little agar solution (Pasteur pipette). Finally, a second layer of agar solution is poured over the tissue samples. The phantom is measured within 24 hours and kept refrigerated until the measurement has been performed.

The animals that were not injected with nanoparticles are taken along for reference and the tissues prepared identically, or a respective phantom is produced.

Apart from visual inspection, relative signal reduction in the individual tissues is now quantified according to:

$$\text{relative signal intensity} = \frac{\text{signal intensity}_{\text{with nanoparticle}}}{\text{signal intensity}_{\text{without nanoparticle}}}$$

The tissue samples are carefully removed from the agar solution after the measurement, decomposed in concentrated hydrochloric acid and their iron content quantified using ICP AES (inductively coupled plasma atomic emission spectroscopy). The blank values (without contrast medium) were determined from adequately treated control animals without any application of nanoparticles and taken into account when the iron content of the samples was determined.

Results:

Fig. 5: Depicts MR tomograms of agarose-embedded lymphatic nodes of rats; resection carried out 24 h after application of the reference substance (Example C2, left) or modified substance according to Example D2 (right); dose 100 μmol Fe/kg in each case;

Fig. 6: Depicts a bar chart showing a modified chart vs. original substance: Quantitative evaluation (from Fig. 5) of relative signal intensities for SE 2000/15 in various lymphatic nodes of the rat 24 h after the application of magnetite (100 μmol Fe/kg);

Fig. 7: Depicts a bar chart showing modified charge vs. original substance: Quantitative evaluation (from Fig. 5) of relative signal intensities for GE 135/15/15° in various lymphatic nodes of the rat 24 h after the application of magnetite (100 μmol Fe/kg).

An analysis of interference with the relative lymphonodal signal intensity of the specific nanoparticles (Fig 6 = SE; Fig. 7 = GE) demonstrates clearly that the modified substance is accumulated more homogeneously in the lymph nodes than the original substance. Lymphonodal signal reduction of mandibular, axillary, iliac, popliteal lymph nodes as well as the mean accumulation throughout all the lymph node groups caused by the modified batch with a secondary coat of dextran FP1 differs significantly (t-test, $p < 0.05$) from the unmodified parent compound (Figs. 6 and 7).

The superiority of the specific nanoparticles of embodiments of aspects of the invention is impressively illustrated by a look at the "blackening" (Fig. 5) of the individual lymph nodes in Figure 5. The homogeneous distribution throughout all the lymph nodes examined is particularly remarkable.

Application Example E2

- MR lymphography in the rabbit

Objective: comparison of relative signal intensity in various lymphatic nodes/groups of lymphatic nodes between the parent compound (synthesis polymer = targeting polymer) and a modification produced according to the desorption-adsorption method (synthesis polymer ≠ targeting polymer) in the rabbit.

Substance: Specific nanoparticles of embodiments of aspects of the invention (Example D2); comparison = basic structural unit according to Example C2 (=D2 without targeting polymer)

Dosage: 150 μmol Fe/kg body weight (body wt)

Times: 24 h p. i (post injectionem)

MR method: MR tomography (SE and GE methods) (see Application Example E1)

In-vivo model: rabbit with induced lymph node hyperplasia

Ex-vivo model: agarose phantom

Result:

Fig. 8: Depicts frontal pre- and post-contrast MR tomograms of the pelvic region of the rabbit in the proton-density-weighted spin echo sequence (SE 2000/15). (Left: pre-contrast; right: specific substance D2 (150 μmol Fe/kg)).

Fig. 9: Depicts frontal pre- and post-contrast MR tomograms of the pelvic region of the rabbit in the proton-density-weighted spin echo sequence (SE 2000/15). (Left: pre-contrast; right: parent compound C2 (150 μmol Fe/kg)).

Fig. 10: Depicts a bar graph showing specific nanoparticles of embodiments of aspects of the invention vs. unspecified particles: Relative signal intensities for SE 2000/15 in various lymphatic nodes of the rabbit 24 h p. i. (150 μmol Fe/kg, n=3). (Quantitative evaluation according to Figs. 8 and 9).

Fig. 11: Depicts frontal pre- and post-contrast MR tomograms of the pelvic region of the rabbit in the T2*-weighted gradient echo sequence (GE 135/15/15°). (Left: pre-contrast; right: specific substance D2 (150 μmol Fe/kg)).

Fig. 12: Depicts frontal pre- and post-contrast MR tomograms of the pelvic region of the rabbit in the T2*-weighted gradient echo sequence (GE 135/15/15°). (Left: pre-contrast; right: parent compound C2 (150 μmol Fe/kg)).

Fig. 13: Depicts a bar graph showing specific nanoparticles of embodiments of aspects of

the invention vs. unspecified particles: relative signal intensities for GE 135/15/15° in various lymphatic nodes of the rabbit 24 h p.i. (150 μmol Fe/kg, n=3). (Quantitative evaluation according to Figs. 11 and 12).

Fig. 14: Depicts *ex-vivo* MR tomograms (GE sequence) of agarose-embedded lymphatic nodes of the rabbit; dose: 150 μmol Fe/kg; left: unspecific reference particles; right: specific nanoparticles.

Fig. 15: Depicts a bar graph of specific nanoparticles of embodiments of aspects of the invention vs. unspecified particles: Relative signal intensities for GE 135/15/15° in various lymphatic nodes of the rabbit 24 h p.i. (150 μmol Fe/kg, n=3).

An analysis of interference with the relative lymphonodal signal intensity of the specific nanoparticles of embodiments of aspects of the invention (Figs. 8, 9 = SE; Figs. 11, 12 = GE) demonstrates clearly that the modified substance is accumulated more homogeneously in the lymph nodes than the original substance. Lymphonodal signal reduction (GE sequence) of subiliac, iliac, popliteal lymph nodes as well as the mean accumulation throughout all the lymph node groups caused by the FP1-modified nanoparticles differs significantly (paired t-test, $p < 0.05$) from the unmodified reference particles (Fig. 13). The more homogeneous interlymphonodal signal interference is clearly visible in the MR tomography images of agarose-embedded lymph nodes (Fig. 14); similar to what could be observed with rats, the reference , substance shows strong signal reduction which is limited, however, to the mesenterial lymphatic nodes.

Application Example E3

- Dependence of lymphonodal accumulation in the rat on dosage

Objective: Comparison of relative signal intensity in various lymphatic nodes/groups of lymphatic nodes between the parent compound (synthesis polymer = targeting polymer) and a modification produced according to the desorption-adsorption-method (synthesis polymer ≠ targeting polymer) as a function of the applied dose.

Substance: Specific nanoparticles of embodiments of aspects of the invention (Example D2); comparison = basic structural unit according to Example C2 (=D2 without targeting polymer)

Dosage: 50-200 µmol Fe/kg body weight (body wt)(n=3 per dose)

Times: 24 h p. i. (post injectionem)

MR method: MR tomography (SE and GE methods) (see Application Example E1)

Ex-vivo model: agarose phantom (see Application Example E1)

Result:

Fig. 16: Depicts a bar graph showing specific nanoparticles of embodiments of aspects of

the invention according to Example D2 vs. unspecified particles: Relative signal intensities as a function of doses applied for SE 2000/15 in various lymphatic nodes of the rat 24 h after i.v. injection of the particles.

Fig. 17: Depicts a bar graph showing specific nanoparticles of embodiments of aspects of the invention according to Example D2 vs. unspecified particles: Relative signal intensities as a function of doses applied for GE 135/15/15° in various lymphatic nodes of the rat 24 h after contrast media injection.

Significantly improved signal reduction ($p > 0.05$) with the parent compound modified according to Example D2 to provide nanoparticles of embodiments of aspects of the invention is found for all lymph node groups except the mesenterial and inguinal lymph nodes at half the dose (200 μmol Fe/kg (C2) vs. 100 μmol Fe/kg (D2). These clear differences are also evident when a look is taken at the mean signal interference over all the lymph node stations (see Table 11).

Table 11: Mean relative signal intensities and standard deviation over all lymph node stations as a function of substance and dose applied.

Sample	Dose	Mean relative signal intensity
C2	100 µmol Fe/kg	0.85 ± 0.13
D2	100 µmol Fe/kg	0.45 ± 0.23
C2	200 µmol Fe/kg	0.49 ± 0.17
D2	200 µmol Fe/kg	0.35 ± 0.15

Application Example E4

- Time-dependence of lymphonodal accumulation in the rat

Objective: Comparison of relative signal intensity in various lymphatic nodes/groups of lymphatic nodés between the parent compound (synthesis polymer = targeting polymer) and a modification produced according to the desorption-adsorption-method (synthesis polymer ≠ targeting polymer) as a function of time after application.

Substance: Specific nanoparticles of embodiments of aspects of the invention (Example D2); comparison = basic structural unit according to Example C2 (=D2 without targeting polymer)

Dosage: 200 µmol Fe/kg body weight(n=3 / timepoint)

Times: 4 - 168 h p. i. (post injectionem)

MR method: MR tomography (SE and GE methods) (see Application Example E1)

Ex-vivo model: agarose phantom (see Application Example E1)

Result:

Fig. 18: Depicts a bar graph of reference substance according to Example C2: Relative signal intensities for SE 2000/15 in various lymphatic nodes of the rat as a function of time after application.

Fig. 19: Depicts a bar graph of specific nanoparticles of embodiments of aspects of the invention according to Example D2: Relative signal intensities for SE 2000/15 in various lymphatic nodes of the rat as a function of time after application.

Fig. 20: Depicts a bar graph of reference substance according to Example C2: Relative signal intensities for GE 135/15/15° in various lymphatic nodes of the rat as a function of time after application.

Fig. 21: Depicts a bar graph of specific nanoparticles of embodiments of aspects of the invention according to Example D2: Relative signal intensities for GE 135/15/15° in various lymphatic nodes of the rat as a function of time after application.

The time-dependent MR tomographic studies on lymphonodal signal reduction after intravenous application of the substances clearly show that the non-specific parent substance causes poorer signal reduction in the lymph nodes than the specific modification of nanoparticles of embodiments of aspects of the invention according to Example D2.

Application Example E5

- Combination with physiotherapeutic measures: temperature

Objective: Comparison of relative signal intensity in various lymphatic nodes/groups of lymphatic nodes as a function of body temperature regulated by a thermobath.

Substance: Specific nanoparticles (Example D2);

Dosage: 100 µmol Fe/kg body weight (n=7 / group)

Times: 24 h p. i. (post injectionem)

Method: MR tomography (SE and GE methods)

Hyperthermic model:

- **Application of heat**

To study the influence of heat on the accumulation of the contrast medium in various lymph node groups, the rats were anaesthetized for 3 - 4 h, and then placed partially in a water bath for 2 h. The left side of the rats' body lay on a heater plate and the right side of their body lay on a synthetic insulating plate of the same height that was not heated. This arrangement caused a difference in temperature of the water on the left and right side of the rats' bodies. Water temperature under the left shoulder of the rats initially was 41.0 - 41.5 °C, reaching a constant value of 41.5 - 42.0 °C after 30 min. Water temperature under the left shoulder of the rats initially was 37.0 - 37.5 °C, reaching a constant value of 37.5 - 38.0 °C after 30 min. After a period of 30 minutes in the water bath, the rats were i.v. injected (bolus) with a dose of

100 µmol Fe/kg body wt of nanoparticles. After remaining in the water bath at constant temperatures for another 1.5 h, the rats are put back into their cage, the lymph nodes are prepared 24 h after the injection and examined using MR tomography.

Fig. 22: Depicts the influence on accumulation in the lymphatic nodes exerted by purposeful application of heat. The popliteal lymph nodes can only be guessed as being the bright spots in the pre-contrast picture on the left. The figure on the right impressively demonstrates the influence of heat treatment. The left side of the anaesthetized rat lay on an insulating synthetic plate and had a normal body temperature while the right side was heated in a water bath to 41.5 - 42.0°C. The "cold" side shows little or no accumulation, while the heated side shows high and homogeneous intralymphonodal accumulation of nanoparticles. (Nanoparticles of embodiments of aspects of the invention according to Example D2; 100 μmol/kg body wt; 24 hp.i.; GE 135/15/15°)

The *in-vivo* tomogram (Fig. 22) clearly shows the effects of heat treatment. While the cold, unheated left side of the rat's body shows no visible accumulation in the popliteal lymph nodes, the heated right side shows a high and homogeneous signal reduction (accumulation) in the popliteal lymph nodes examined.

The rats were anaesthetized and put into the thermal bath to show the effects of heating particularly clearly. Anaesthesia causes a standstill of peripheral muscular activity, diminished lymphatic flux and reduced vascular permeability. As a result, virtually no accumulation of nanoparticles of embodiments of aspects of the invention can be detected without heating.

Application Example E1

- MR angiography

Substance: Specific nanoparticles (Example D2)

Animals: rat (see Example of Application E1)

Dosage: 20 µmol Fe/kg i.v.

Time: 0-2 h p. i.

MR equipment:

Device: Siemens Magnetom 1.5 T MR
whole-body tomograph with extremity coil

MR parameters: transversal dynamic study using a T1-weighted SE sequence (TR: 200 ms, TE: 10 ms), FOV 170 mm, matrix 256x256; SD: 3 mm;

coronary MIPS from 3D flash (TR: 40 (60) ms, TE: 6 ms, FA 60 (40) °) and 3D FISP sequence (TR: 40 ms, TE: 7 ms, FA 35 °) FOV 240 mm, matrix 256x256; SD: 17 mm;

MR evaluation: signal intensities in user-defined regions of interest in vessels (caval vein), the liver, fat and muscles. The signal intensities are standardized with respect to the background

Results:

Fig. 23: Depicts a transversal dynamic study of the rat's abdomen using a T1-weighted SE sequence (TR: 200 ms, TE: 10 ms) after bolus injection of the specific nanoparticles of embodiments of aspects of the invention according to Example D2 (dose: 20 μmol Fe/kg); clear signal enhancement (1 min. p.i.) in the intrahepatic vessels and the caval vein).

Fig. 24: Depicts a distribution graph of relative signal intensity as ordinate and like p.i (min) as abscissa showing a comparison of relative signal intensities for SE/TR/TE 200 ms/10 ms in the venous vessel and the liver parenchyma for the specific nanoparticles of embodiments of aspects of the invention according to Example D2 and the unspecific reference substance according to Example C2; dose 20 μmol Fe/kg.

Fig. 25: Depicts coronary MIPS (maximum-intensity projections) of 3D FLASH tomograms (TR:40 ms, TE: 6 ms, FA 60°); comparison of the specific nanoparticles of embodiments of aspects of the invention according to Example D2 (left) and the reference substance C2 (right); dose 20 μmol Fe/kg.

Figures 23 and 25 clearly show the advantages of the specific nanoparticles of embodiments of aspects of the invention (according to Example D2) as compared with the parent substance according to Example C2. The summary graph of the signal's time history, (Fig. 24) in the caval vein or in the liver parenchyma demonstrates the excellent properties of the specific nanoparticles of embodiments of aspects of the invention for use as

contrast media in MR angiography. Enhancement is three times higher than with the reference substance, and the brightening effect lasts for a long time and is very constant (diagnostic time window > 60 min.).

Application Example E7

- Visualization of lymph nodes in the healthy rat and the tumor-carrying rabbit

Objective: Proof of suitability of the nanoparticles according to the invention for use as a visual labeling substance in surgical medicine.

Substance:	specific nanoparticles (Example D2)
Animals:	rat, SPF Han-Wistar; ca. 150 g Russian rabbit (Chbb: HM, Thomae GmbH) with an implanted VX2 tumor (tumor bank of Deutsches Krebsforschungszentrum, Heidelberg); ca. 2.6 kg. The tumor was implanted by injecting 3 x 10^6 living tumor cells in the caudolateral femoral muscles. Uptake takes place 20 days after implantation.
Dosage:	rat: intravenous injection of 500 µmol Fe/kg body weight rabbit: interstitial application of 20 µmol per paw
Times:	rat: 1, 4 and 24 h p. i. rabbit: 12 h p. i.

Results:

Fig. 26: Depicts the use of nanoparticles of embodiments of aspects of the invention as "intraoperative" labelling substances for visual detection of lymphatic nodes (general view).

Fig. 27: Depicts the use of nanoparticles of embodiments of aspects of the invention as "intraoperative" labelling substances for visual detection of lymphatic nodes (detailed view).

Fig. 28: Depicts a demonstration of metastases in lymphatic nodes in the rabbit. The metastases can be identified as bright recesses in the lymphatic nodes that are otherwise shown in dark colouring.

The images of the rats (Figs. 26 and 27) show that a great number of the most varied lymph nodes/lymph node groups can be stained by a single intravenous application of the nanoparticle solution of embodiments of aspects of the invention. The lymphatic nodes are clearly distinguishable from the surrounding tissue and can thus be easily detected for removal, if required, by the operating surgeon.

The studies of VX2 tumour-carrying rabbits demonstrate that lymph nodes in the tributary area are homogeneously stained by the specific nanoparticles of embodiments of aspects of the invention after interstitial application, and that small metastases can be distinguished visually as bright recesses in the darkly stained healthy lymphonodal tissue (Fig. 28).

Application Example E8

- Cell experiment to prove the specificity of the nanoparticles

Objective: Proof of the specific cellular uptake (receptor-mediated endocytosis) of nanoparticles having a secondary coat of transferrin (targeting polymer)

Substance: specific nanoparticles (Example D6)

Comparison: basic substance according to Example C1 (D6 without transferrin)

Concentration: 0.5 mmol Fe/l medium

Times: 18 h incubation at 37 °C; 5 % CO_2 - 95 % air

Cell culture:

- Uptake by human myeloma cells

Human myeloma cells (ATCC CRL 9068; cell line NCI 929) are cultivated at a concentration of at least 1×10^6 cells/ml in RPMI 1640 10% FCS (fetal calf serum) and 0.05 mmol/L of 2-mercaptoethanol (37°C, 5% carbon dioxide; 225 cm^2 culture flasks).
When the cells have reached a concentration of ca. 1.5×10^6 cells/ml they are centrifuged and resuspended in fresh medium.
The cells are incubated with the nanoparticles at a concentration of 0.5 mmol/l (calculated in terms of iron) for 18 hours.

The cells are pelleted, and washed twice with PBS. Then the cell number is determined in an aliquot (Neubauer counting chamber). The cell pellet is

dissolved by heating in 500 µl conc. nitric acid/ 100 µl hydrogen peroxide and filled to a volume of 5.0 ml. Then the iron concentration is determined using atomic emission spectroscopy (AES, detection limit 0.1 ppm).

Results:

Fig. 29: Depicts a bar graph of a cell tomogram of specific nanoparticles of embodiments of aspects of the invention (with transferrin) compared with the unspecific reference (nanoparticles without transferrin). The NCI cells (human myeloma cell line) accumulate nearly twice as many specific particles as reference particles.

The specific nanoparticles of embodiments of aspects of the invention are clearly taken up to a greater extent by the NCI 929 myeloma cells. The advantages of the specific nanoparticle design of embodiments of aspects of the invention are demonstrated by the fact that 50% fewer nanoparticles without a targeting polymer are taken up.

Application Example E9

- Atherosclerosis imaging in the Watanabe rabbit (plaque visualization)

Objective: Visualization of atherosclerotic plaques in the rabbit using nanoparticles to which a peptide with an affinity for plaque was applied according to the desorption-adsorption method (secondary coat, targeting polymer).

Substance:	specific nanoparticles (Example D7)
Dosage:	200 µmol Fe/kg body weight (body wt)
Time:	5 h p. i. (post injection)
MR method:	
Device:	Siemens MAGNETOM™ 1.5 T MR whole-body tomograph with extremity coil
MR parameters:	Field of view (FOV) = 150 mm, matrix = 256x256; slice thickness = 3 mm orientation of sections = frontal
Sequence 1:	Proton-density-weighted spin echo sequence (SE) with TR = 2000 ms and TE = 15 ms
Sequence 2:	T2-weighted gradient echo sequence (GE) with TR = 135 ms and TE = 15 ms; FA = 15°

Ex-vivo model: agarose phantom (see Application Example E1)

The aorta was excised, carefully cut open and rinsed with cold PBS solution to remove unbound nanoparticles or those nanoparticles which were not taken up. Then the aorta is bisected, poured in the agarose phantom and examined using MR tomography.

Histology: Prussian blue staining

Results:

Fig. 30: Depicts an *ex-vivo* MR tomographic diagram of atherosclerotic plaques of the aorta of a rabbit with modification D7 (dose 200 μmol Fe/kg; aorta resection 5 h p.i.); left: proton-density-weighted spin echo sequence; right: T2*-weighted gradient echo sequence.

Fig. 31: Depicts the histological detection of iron in the atherosclerotic membrane of a rabbit's aorta with Prussian blue staining. A comparison with the MR tomogram (GE 135/15/15°) shows that the histologically-detected iron is located at sites that show a clear signal reduction in the image due to accumulation of the specific nanoparticles of embodiments of aspects of the invention. The aorta was resected 5 h after intravenous administration of 200 μmol Fe/kg of the specific particles according to D7.

Fig. 32: Depicts the histochemical detection (Prussian blue staining) of accumulated nanoparticles of embodiments of aspects of the invention according to Example E6 in the aorta of a Watanabe rabbit. The upper part of the figure gives a general view of the prepared aorta on the agar, the lower part illustrates the good correlation of the iron staining (blue granules) and the visually detectable plaques in the aortic arch, which is changed to a particularly great extent.

due to nanoparticle accumulation. The findings of the MR tomogram correlate with the plaques that are clearly visible. The most extensive plaques are located in the aortic arch, which is confirmed by the MR tomogram and the histological view; smaller plaques are also well-detectable both in the MR tomogram and the histological picture.

Application Example E10

- Accumulation in tumours studied in tumour-carrying mice

Objective: Proof was to be provided that nanoparticles of embodiments of aspects of the invention can accumulate in tumours. The tests are to show, on the one hand, that the particles are suitable vehicles for chemotherapeutic agents, and on the other, that the nanoparticles of embodiments of aspects of the invention can help to check whether the therapeutic agents have reached their desired place of action, i.e., the tumour, so that this is a combination of diagnostic and therapeutic applications.

Substance: specific nanoparticles of embodiments of aspects of the invention (Example D2)

Animals: Swiss nude mice with an implanted tumor (n = 5/dose)
(LS 174T, s.c.: application 10 days prior to the experiment)

Anaesthesia: Rompun/Ketavet (1:1), ca. 0.5 ml per kg body weight i.m.

Dosage: 200 µmol Fe/kg body weight (body wt)

Times: 0-120 minutes and 12 or 24 hours after application

MR method:

Device: Siemens MAGNETOM™ 1.5 T MR
whole-body tomograph with extremity coil

MR parameters: Field of view (FOV) = 150 mm, matrix = 256x256; slice thickness = 3 mm
orientation of sections = frontal

Sequence 1: Proton-density-weighted spin echo sequence (SE) with TR = 2000 ms and TE = 15 ms

Sequence 2: Dynamic study: SE sequence with TR/TE = 300 ms/15 ms

5 MR evaluation: signal intensities in user-defined regions of interest in tumor, muscle, fat and background. The relative signal intensities in the various tissues are standardized and refer to the signal intensity in fat.

Results:

Fig. 33: Depicts transversal T1-weighted spin echo dynamics study (TR: 300 ms, TE: 15 ms) of the tumoral signal behaviour after bolus injection of nanoparticles of embodiments of aspects of the invention according to Example D2 (200 μmol Fe/kg). The tomograms show a slow and time-dependent increase in signal enhancement (accumulation) in the tumour with increasingly clear demarcation of spatial requirement.

Fig. 34: Depicts a curve of relative signal intensity (accumulation) in the tumour, in a graph where relative intensity is ordinate and time p.1 (min) is abscissa. The time history of the signal (enhancement) for a dose of 200 μmol/kg body wt illustrates the strong enhancement that increases over time (increasing accumulation) in the tumour (SE 2000/15).

Fig. 35: Depicts a time-dependent transversal proton-density-weighted (SE 2000/15) tomograms after application of the nanoparticles of embodiments of aspects of the invention according to Example D2 (200 μmol Fe/kg).

Increasing accumulation of the nanoparticles in the tumour in conjunction with a linear increase in signal enhancement over time were found in the T1-weighted and proton-density-weighted spin echo sequence (Figs. 33; 35). 35 to 40% enhancement were observed until 135 min. after injection, which permits a clear distinction of the tumour from the healthy tissue and confirms the accumulation of nanoparticles of embodiments of aspects of the invention. Unlike the observations made here, it

was found in angiographic studies that an enhancement in the tumour caused only by perfusion will have disappeared completely after a maximum of 30 min. (p.i.).

CLAIMS

1. A nanoparticle compound for use in diagnosis and therapy, said nanoparticle compound comprising:

an iron-containing core comprising iron material which is selected from the group consisting of magnetite and maghemite;

a synthesis polymer coating said iron-containing core, said synthesis polymer being selected from the group consisting of dextrans and derivatives of dextrans; and

a targeting polymer which is non-covalently bonded to, and envelopes, said synthesis polymer to form a second coating, wherein said targeting polymer is not exposed to synthesis conditions.

2. The nanoparticle compound according to claim 1, wherein said iron-containing core comprises 0.1 to 25% weight of non-iron metallic ions.

3. The nanoparticle compound according to claim 2, wherein said non-iron metallic ions are selected from the group consisting of paramagnetic ions and diamagnetic ions.

4. The nanoparticle compound according to claim 1, claim 2 or claim 3, wherein said iron-containing core and said synthesis polymer coating have a diameter less than 100 nanometres.

5. The nanoparticle compound according to any one of claims 1 to 4, wherein said nanoparticle compound has a hydrodynamic diameter of less than 10 times the diameter of the iron-containing core.

6. The nanoparticle compound according to any one of claims 2 to 5, wherein said targeting polymer has a

weight that is between 0.5 times to 50 times the weight of said non-iron metallic ions.

7. The nanoparticle compound according to any one of claims 1 to 6, further comprising an optical absorption-permitting peptide substance which is selected from the group consisting of RRTVKHHVN, RRSRHH and RSKRGR.

8. The nanoparticle compound according to any one of claims 1 to 7, further comprising a pharmaceutically-active compound.

9. The nanoparticle compound according to any one of claims 1 to 8, wherein said targeting polymer is selected from the group consisting of dextran, dextran derivatives, laminarin, transferrin, and endothelin-receptor-specific heptapeptide.

10. A nanoparticle compound for use in diagnosis and therapy, said nanoparticle compound comprising:

an iron containing core comprising:

an iron compound which is selected from the group consisting of magnetite and maghemite, and

a plurality of non-iron metallic ions;

a synthesis polymer coating said iron-containing core, said synthesis polymer having a weight ratio of 0.1 to 1.0 to the iron compound; and

a targeting polymer which is non-covalently bonded to, and envelopes, said synthesis polymer to form a second coating, wherein said targeting polymer is not subject to synthesis conditions and has a weight that is between 0.5 times to 50 times the weight of said non-iron metallic ions.

11. The nanoparticle compound according to claim 10, wherein said targeting polymer is selected from the group consisting of dextran, dextran derivatives, laminarin,

transferrin, and endothelin-receptor-specific heptapeptide.

12. The nanoparticle compound according to claim 10 or 11, wherein said synthesis polymer has a molecular weight smaller than 100,000 Da.

13. The nanoparticle compound according to claim 10, claim 11 or claim 12, wherein said synthesis polymer is selected from the group consisting of dextran and derivatives of dextran.

14. A method for the fabrication of a nanoparticle compound for use in diagnosis and therapy, said method comprising:

mixing an iron-containing compound with a synthesis polymer in the presence of a base to create a first mixture, said synthesis polymer being selected from the group consisting of dextran and derivatives of dextran;

subjecting said first mixture to desorption to create a second mixture with an iron-containing core and a synthesis polymer coating, said second mixture having a polymer-to-iron weight ratio of 0.1:1; and

non-covalently bonding a targeting polymer to said second mixture to create a nanoparticle compound comprising said iron-containing core surrounded by said synthesis polymer coating which is surrounded and enveloped by said targeting polymer coating, wherein said targeting polymer coating is not exposed to synthesis conditions.

15. The method according to claim 14, further comprising mixing an iron(II) salt and an iron (III) salt to produce said iron-containing compound with the ratio of divalent to trivalent iron being between 1:1 and 1:20.

16. The method according to claim 14 or claim 15, further comprising reacting an iron(III) salt mixture with a reducing agent to produce the iron-containing compound while selecting the quantity of reducing agent that generates an iron(II) to iron(III) ratio between 1:1 and 1:20.

17. The method according to claim 14, claim 15 or claim 16, which further comprises selecting said base to be a 0.1:10N base which is used to precipitate said iron compounds.

18. The method according to any one of claims 14 to 17, which further comprises selecting said base from the group consisting of ammonia gas, ammonia salt, an amine, an amine derivative and a volatile buffer.

19. The method according to any one of claims 14 to 18, which further comprises selecting said iron-containing core to comprise 0.1 to 25% weight of non-iron metallic ions.

20. The method according to claim 19, which further comprises selecting said non-iron metallic ions from the group consisting of paramagnetic ions and diamagnetic ions.

21. The method according to any one of claims 14 to 20, which further comprises selecting said iron-containing core and said synthesis polymer coating to have a diameter less than 100 nanometres.

22. The method according to any one of claims 14 to 21, which further comprises selecting said nanoparticle compound to have a hydrodynamic diameter of less than 10 times the diameter of the iron-containing core.

23. The method according to any one of claims 19 to 22, which further comprises selecting said targeting polymer to have a weight that is between 0.5 times to 50 times the weight of said non-iron metallic ions.

24. The method according to any one of claims 14 to 23, further comprising adding an optical absorption permitting peptide substance which is selected from the group consisting of RRTVKHHVN, RRSRHH and RSKRGR, prior to adding said targeting polymer.

25. The method according to any one of claims 14 to 24, further comprising adding a pharmaceutically-active compound prior to adding said targeting polymer.

26. The method according to any one of claims 14 to 25, which further comprises selecting said targeting polymer from the group consisting of dextran, dextran derivatives, laminarin, transferrin, and endothelin-receptor-specific heptapeptide.

27. The method according to any one of claims 14 to 26, which further comprises removing most of said synthesis polymer during desorption.

28. The use, in diagnosis and therapy, of a nanoparticle as claimed in any one of claims 1 to 13.

29. The use, in imaging atherosclerosis, of a nanoparticle as claimed in any one of claims 1 to 13.

30. The use for the manufacture of a composition for diagnosis and therapy, of a nanoparticle as claimed in any one of claims 1 to 13.

31. The use for the manufacturing of a diagnostic composition for imaging atherosclerosis, of a nanoparticle as claimed in any one of claims 1 to 13.

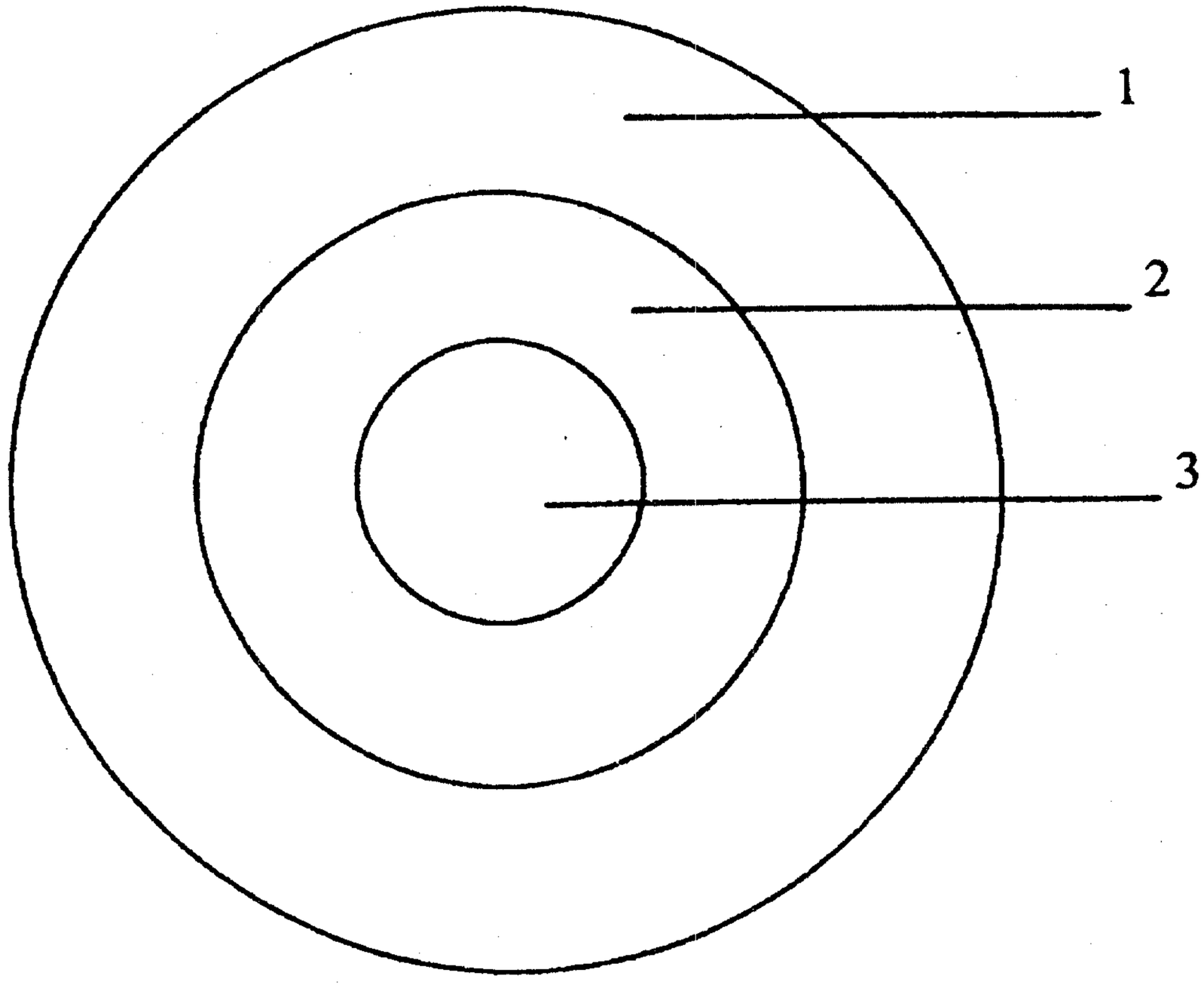


FIG. 1

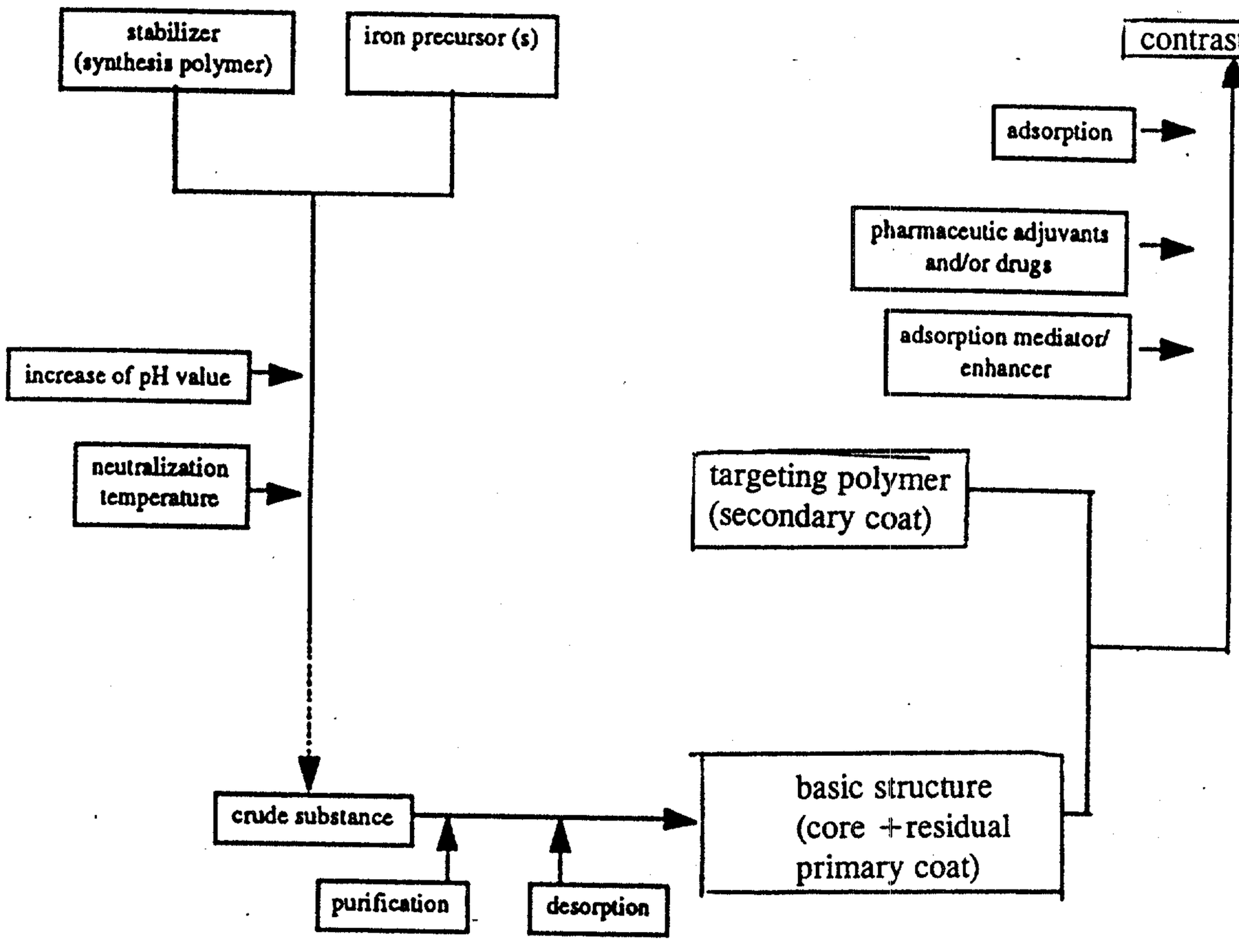

stabilizer
(synthesis polymer)
iron precursor (s)
increase of pH value
neutralization
temperature
crude substance
purification
desorption
basic structure
(core +residual
primary coat)
targeting polymer
(secondary coat)
contrast agent
adsorption
pharmaceutic adjuvants
and/or drugs
adsorption mediator/
enhancer

FIG. 2

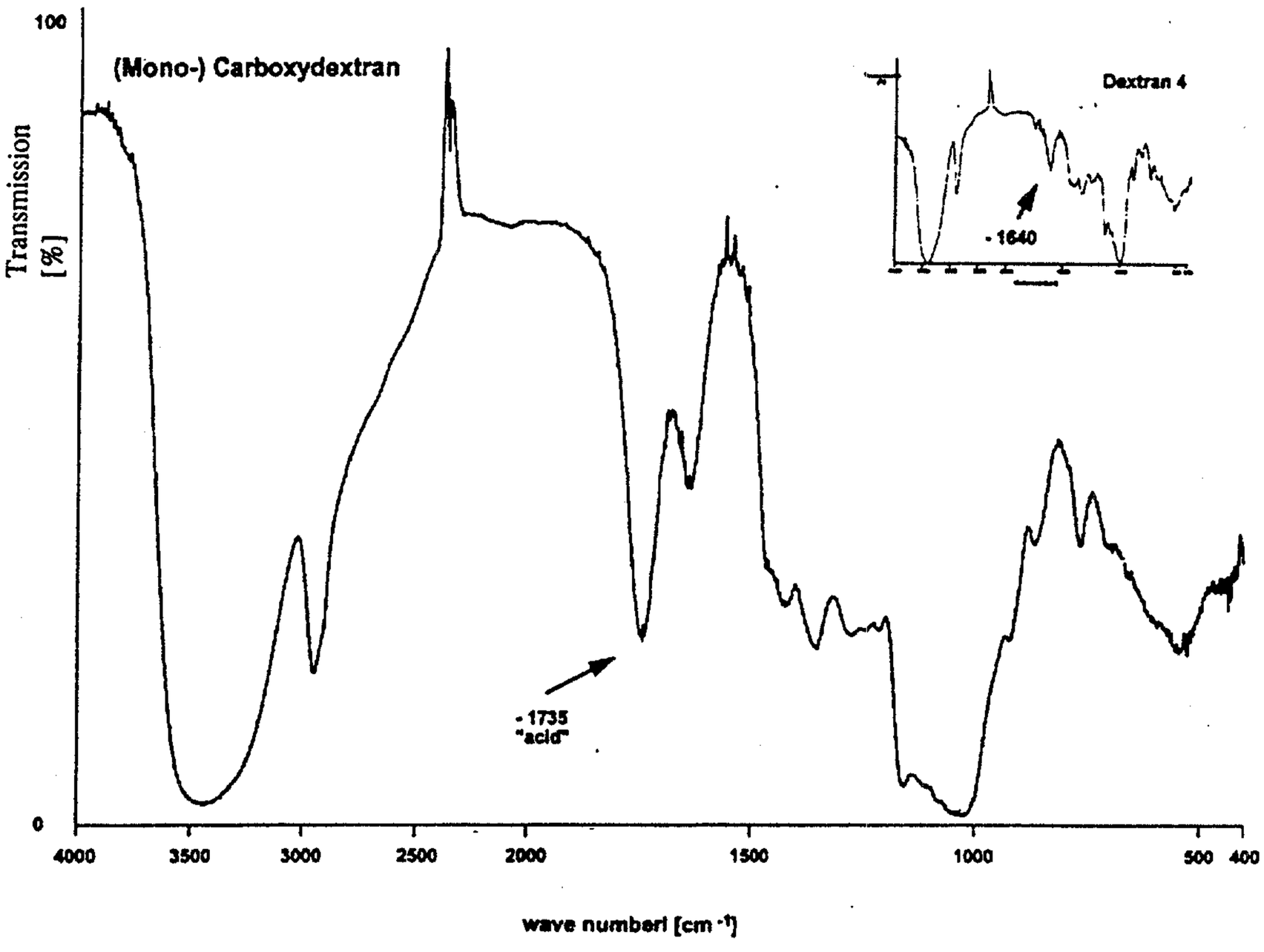

100
(Mono-) Carboxydextran
Transmission [%]
- 1735 "acid"
0
4000
3500
3000
2500
2000
1500
1000
500
400
wave number [cm -1]
Dextran 4
- 1640

FIG. 3

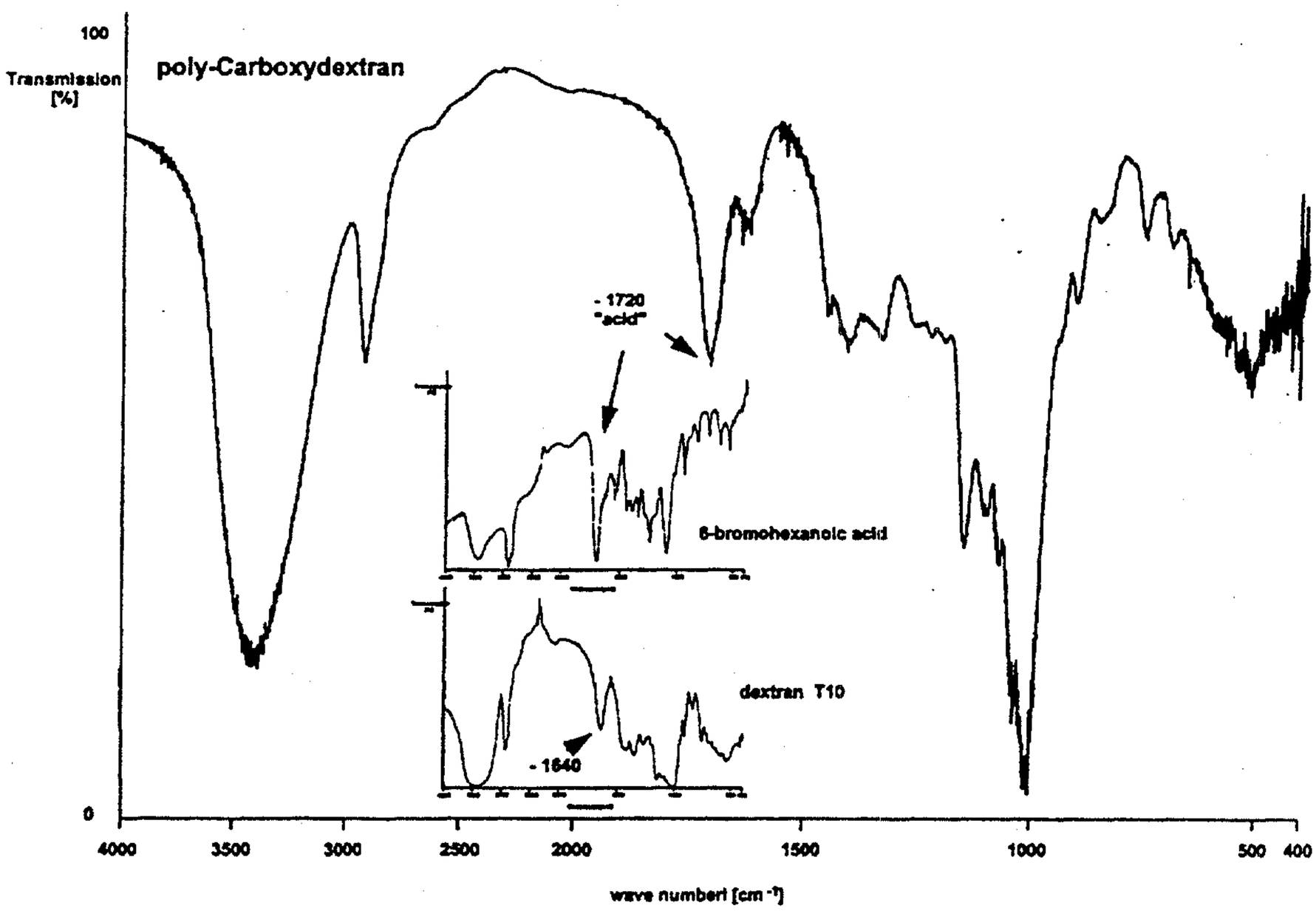

100
Transmission [%]
poly-Carboxydextran
~ 1720 "acid"
6-bromohexanoic acid
dextran T10
~ 1640
0
4000
3500
3000
2500
2000
1500
1000
500
400
wave number [cm -1]

FIG. 4

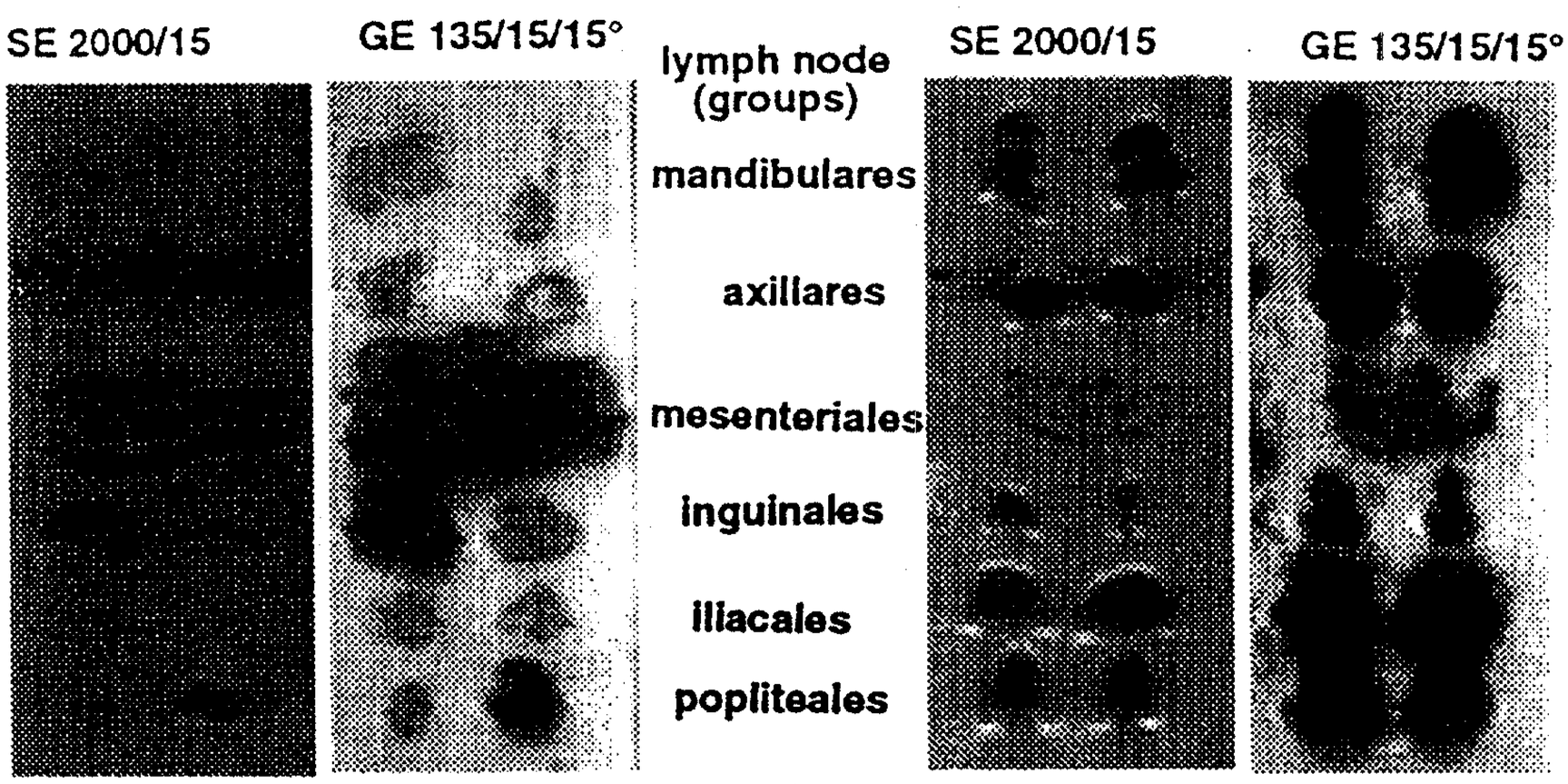

SE 2000/15
GE 135/15/15°
lymph node (groups)
SE 2000/15
GE 135/15/15°
mandibulares
axillares
mesenteriales
inguinales
iliacales
popliteales
unspecific particle
specific particle

FIG. 5

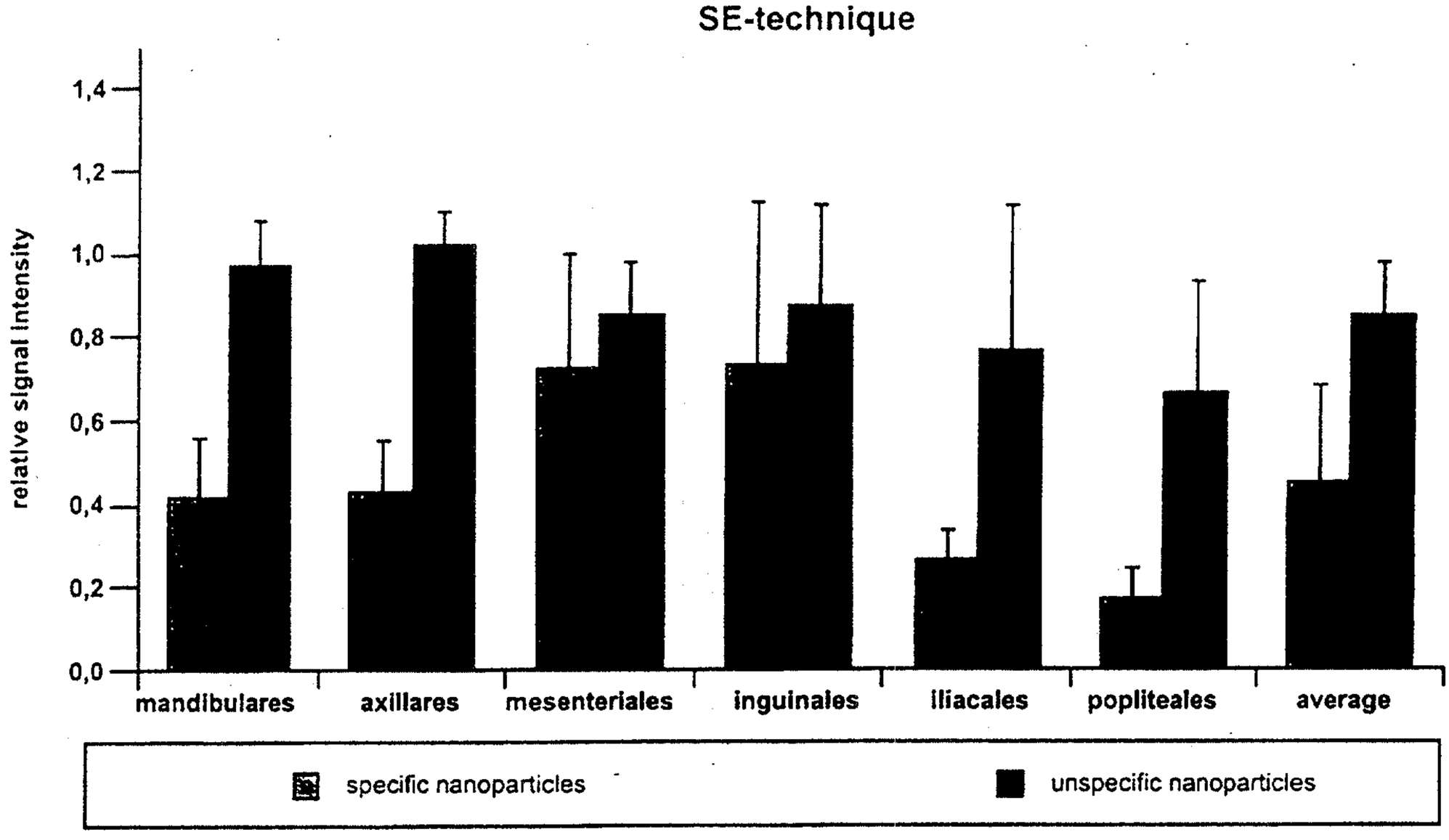

SE-technique
relative signal intensity
1,4
1,2
1,0
0,8
0,6
0,4
0,2
0,0
mandibulares
axillares
mesenteriales
inguinales
iliacales
popliteales
average
specific nanoparticles
unspecific nanoparticles

FIG. 6

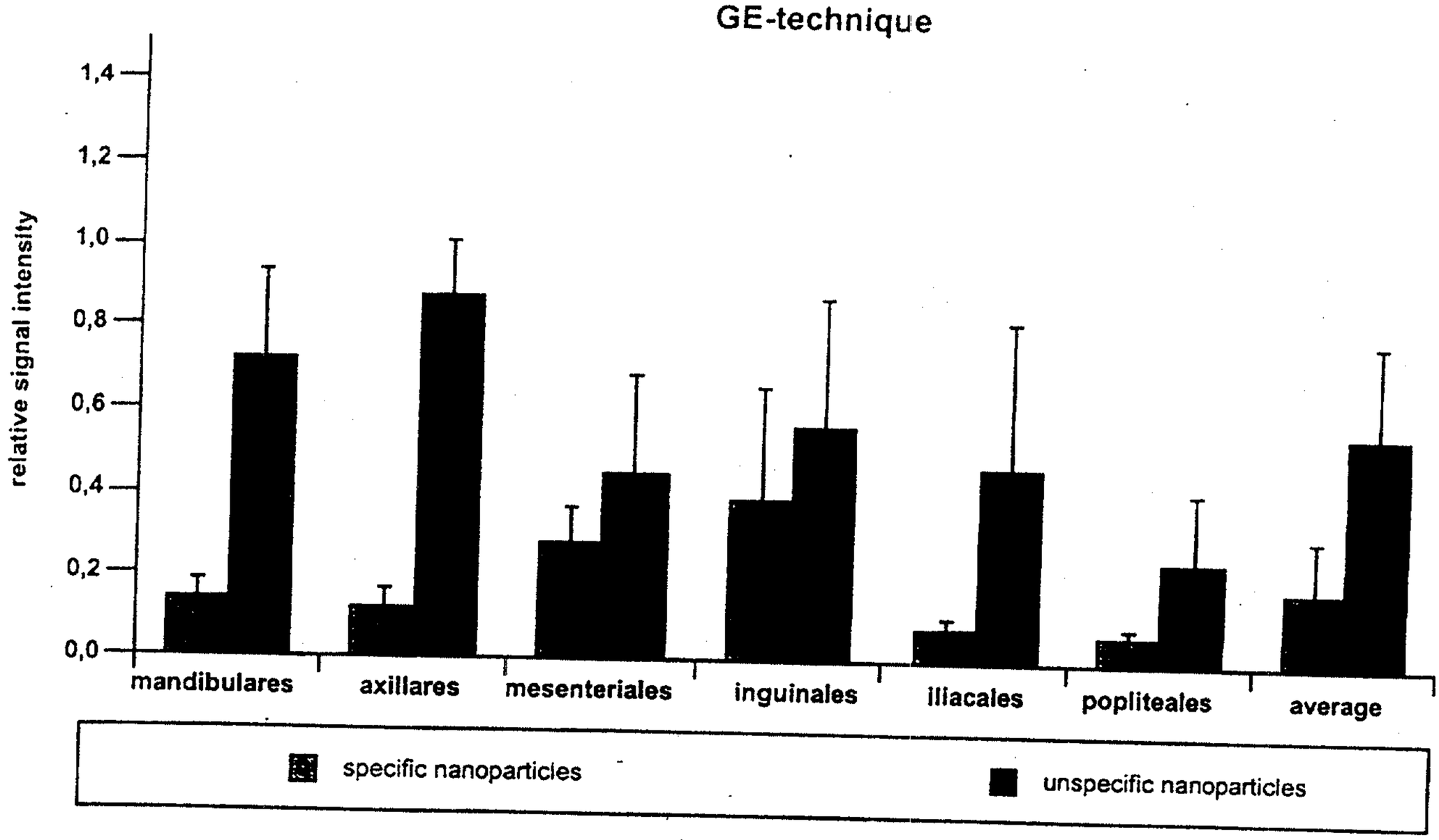

GE-technique
relative signal intensity
1,4
1,2
1,0
0,8
0,6
0,4
0,2
0,0
mandibulares
axillares
mesenteriales
inguinales
iliacales
popliteales
average
specific nanoparticles
unspecific nanoparticles

FIG. 7

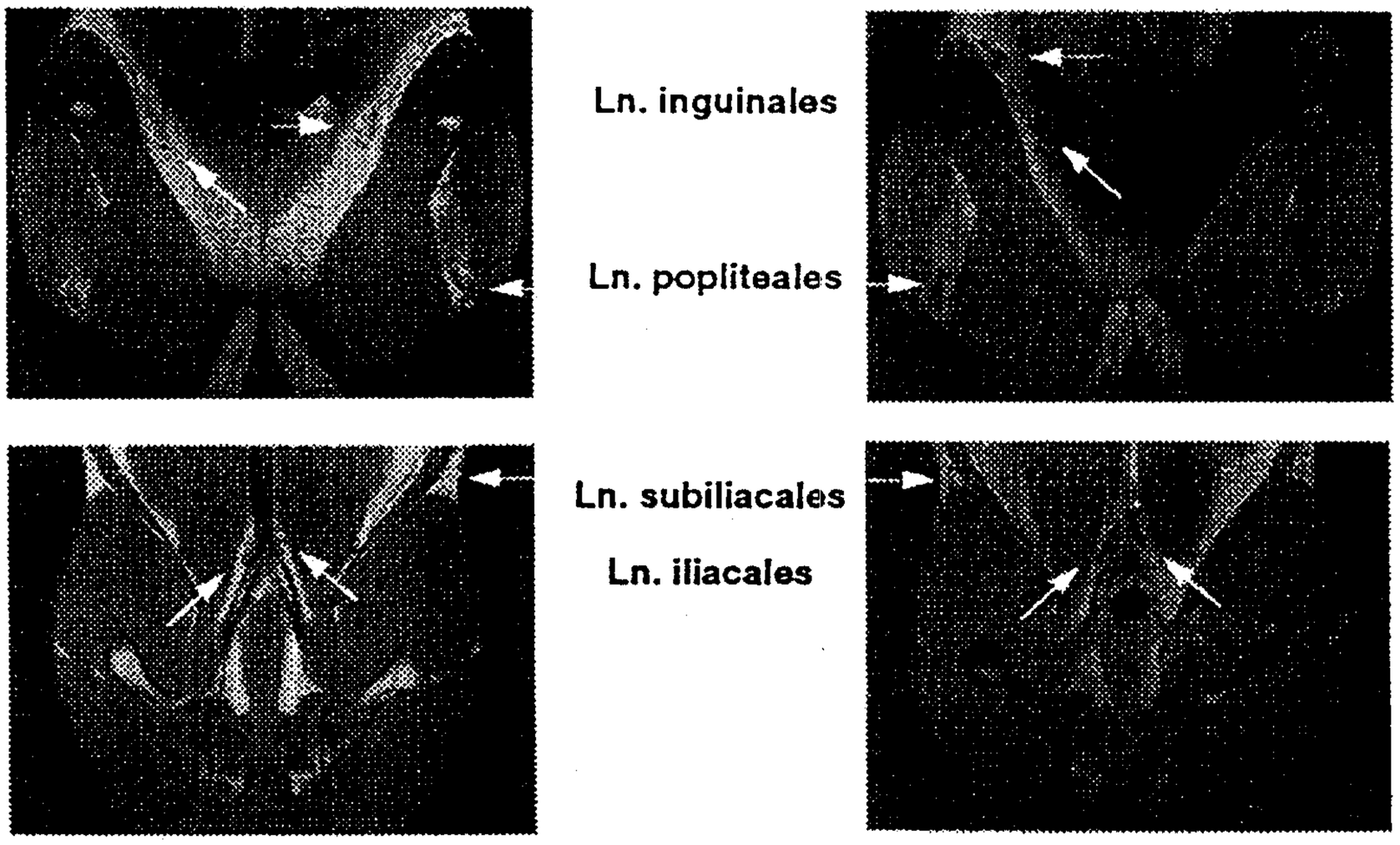

Ln. inguinales
Ln. popliteales
Ln. subiliacales
Ln. iliacales
prae
24 h p.i.

FIG. 8

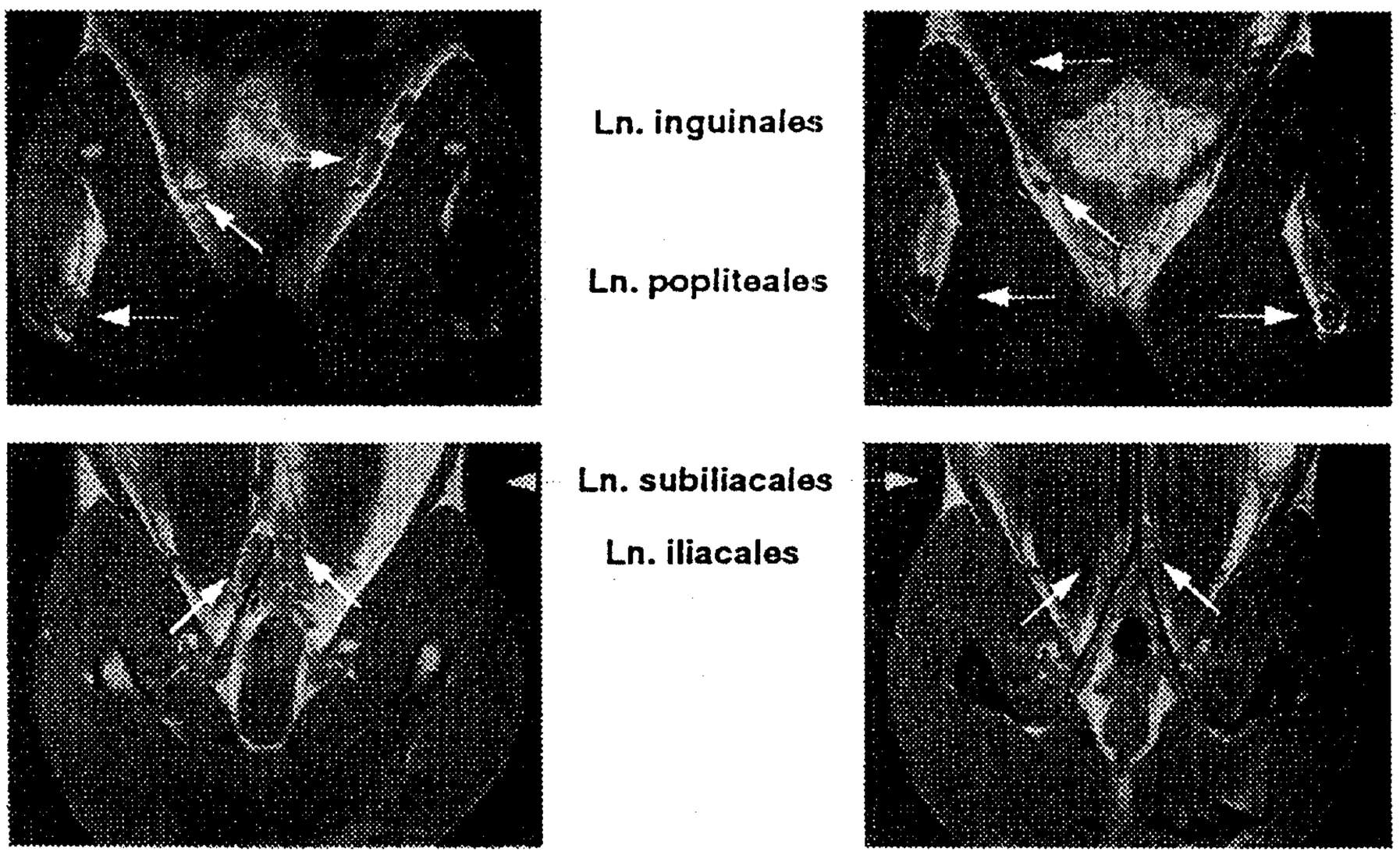

Ln. inguinales
Ln. popliteales
Ln. subiliacales
Ln. iliacales
prae
24 h p.i.

FIG. 9

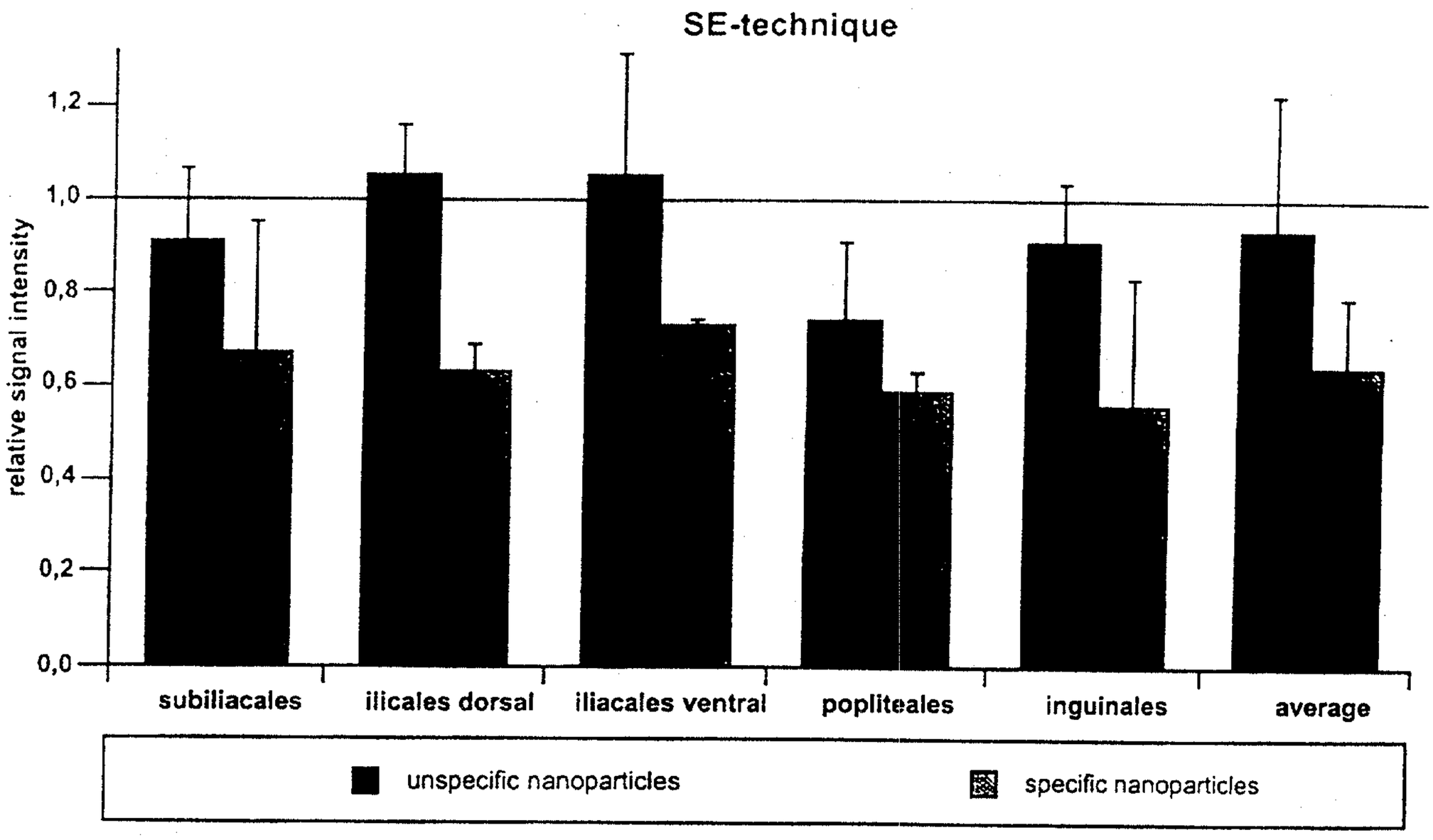

SE-technique
relative signal intensity
1,2
1,0
0,8
0,6
0,4
0,2
0,0
subiliacales
ilicales dorsal
iliacales ventral
popliteales
inguinales
average
unspecific nanoparticles
specific nanoparticles

FIG. 10

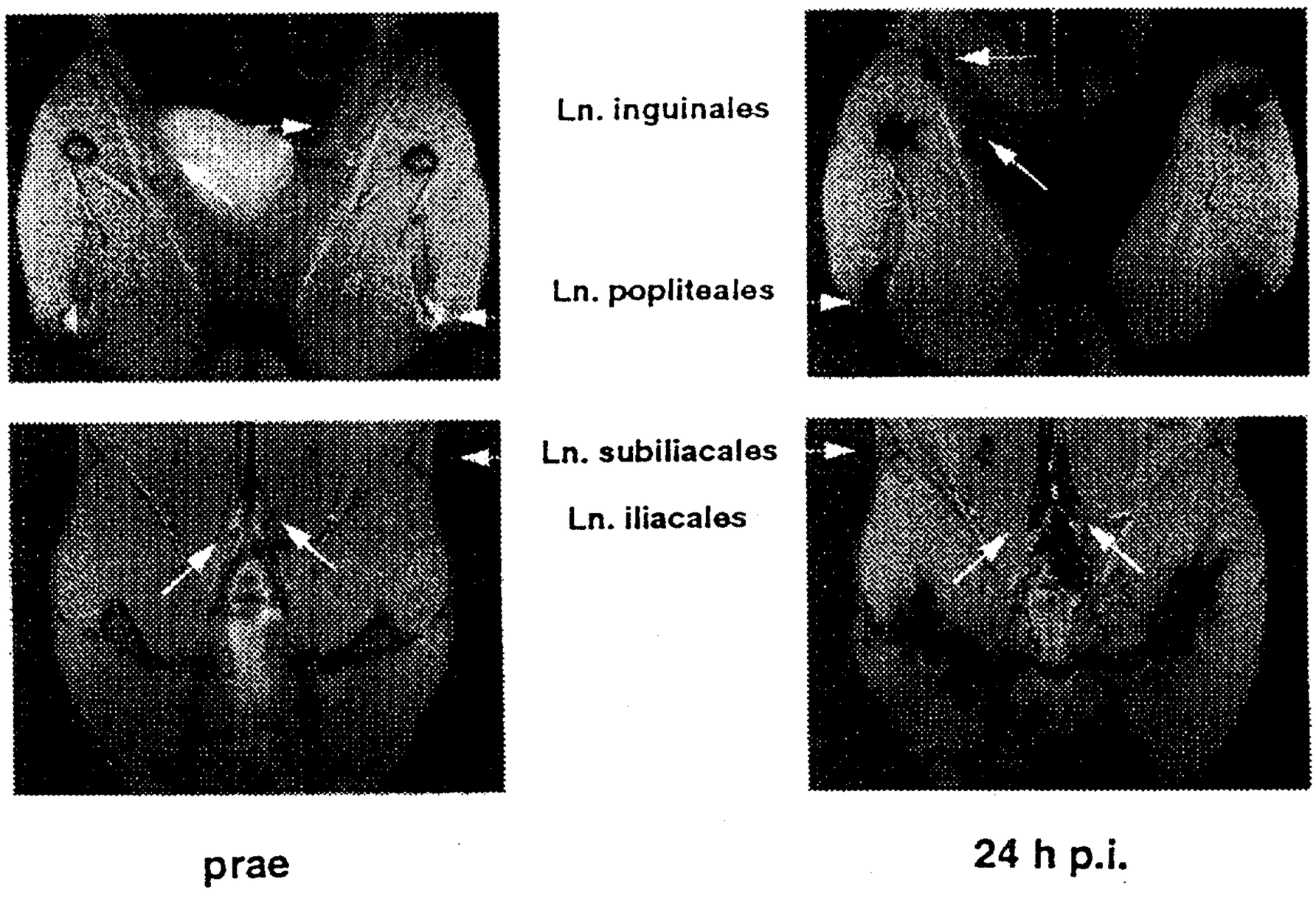

Ln. inguinales
Ln. popliteales
Ln. subiliacales
Ln. iliacales
prae
24 h p.i.

FIG. 11

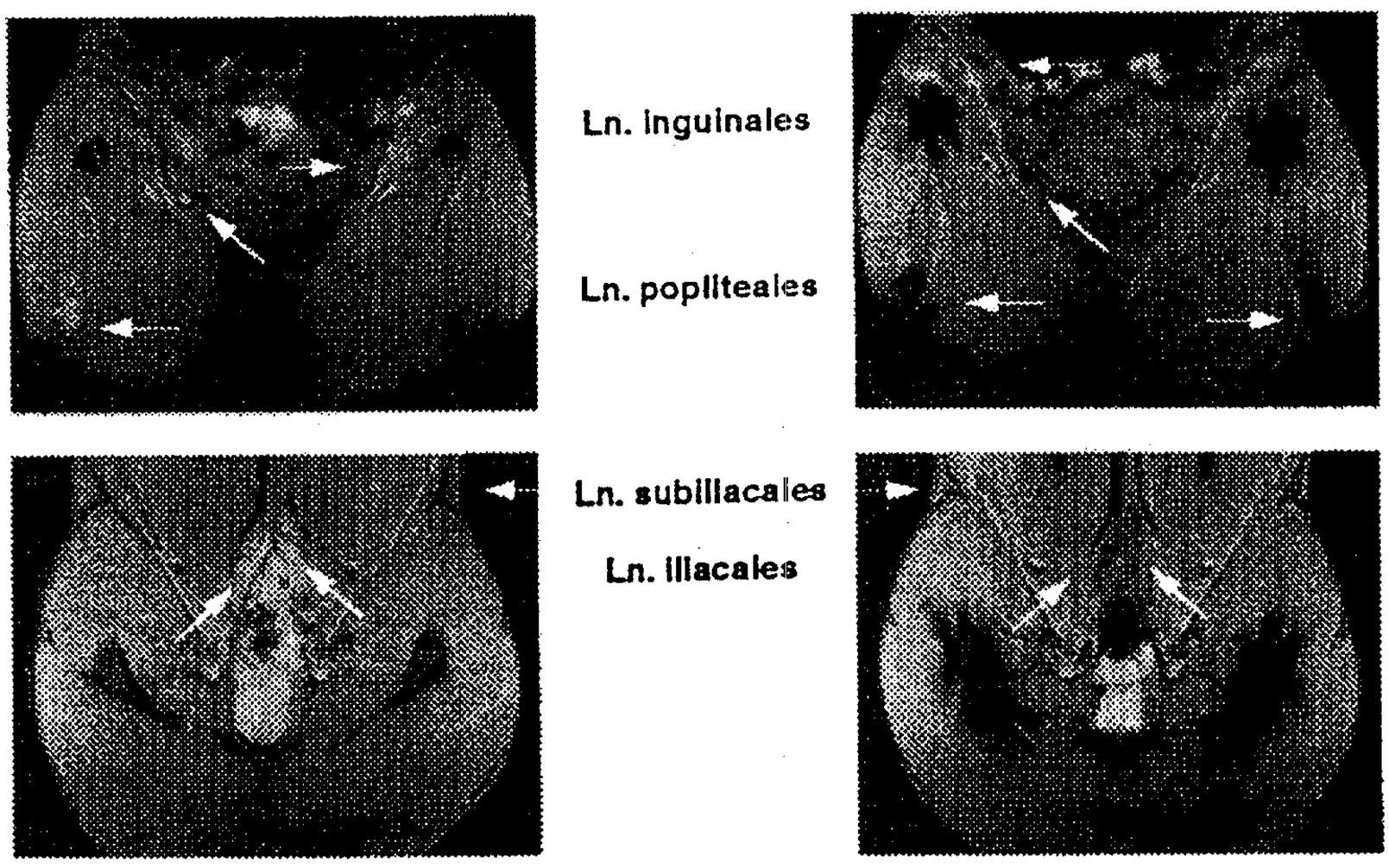

Ln. inguinales
Ln. popliteales
Ln. subiliacales
Ln. iliacales
prae
24 h p.i.

FIG. 12

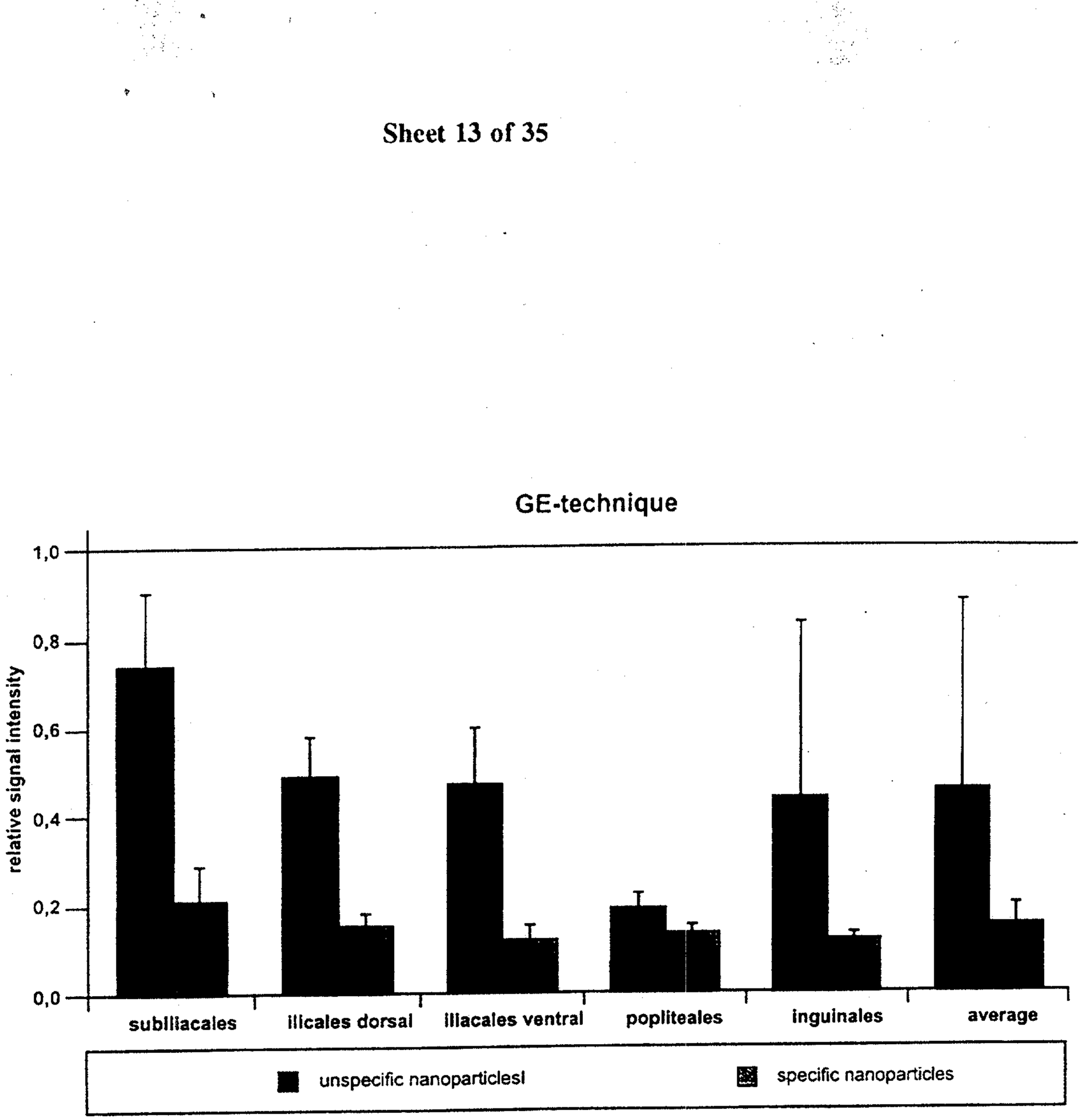
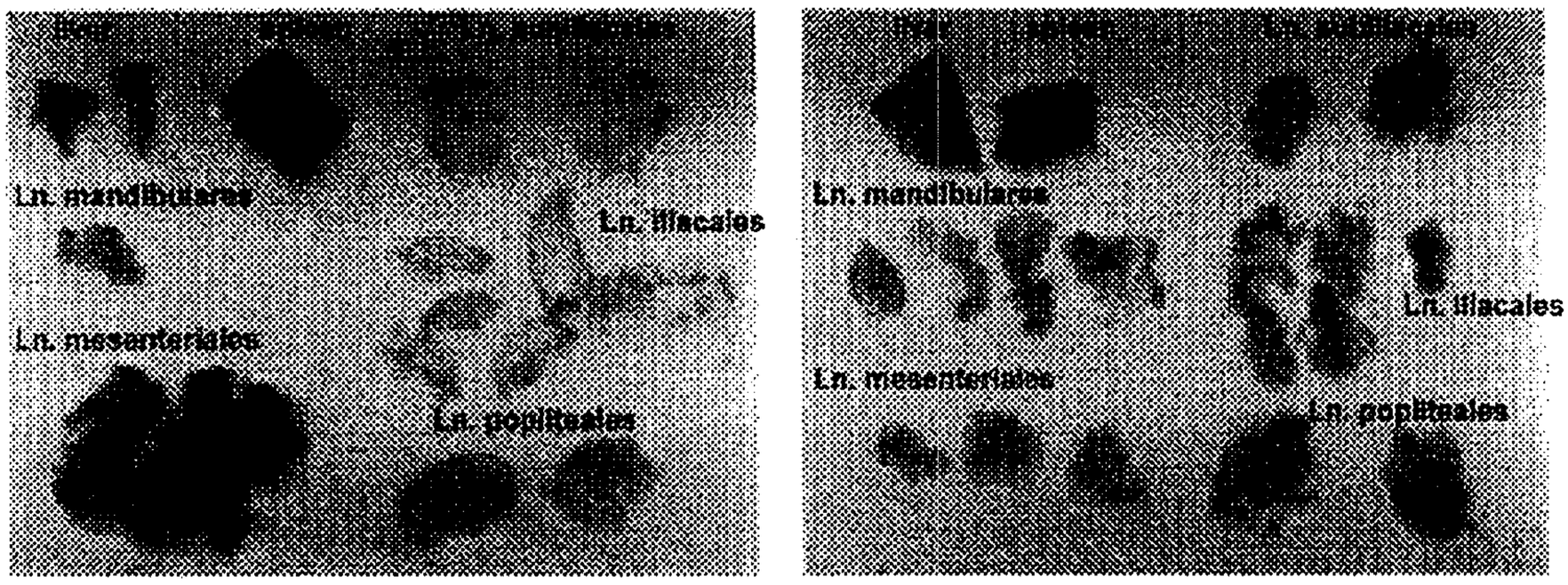

GE-technique
1,0
0,8
0,6
0,4
0,2
0,0
relative signal intensity
subiliacales
iliacales dorsal
iliacales ventral
popliteales
inguinales
average
unspecific nanoparticlesl
specific nanoparticles

FIG. 13



unspecific nanoparticles (C2)

specific nanoparticles (D2)

FIG. 14

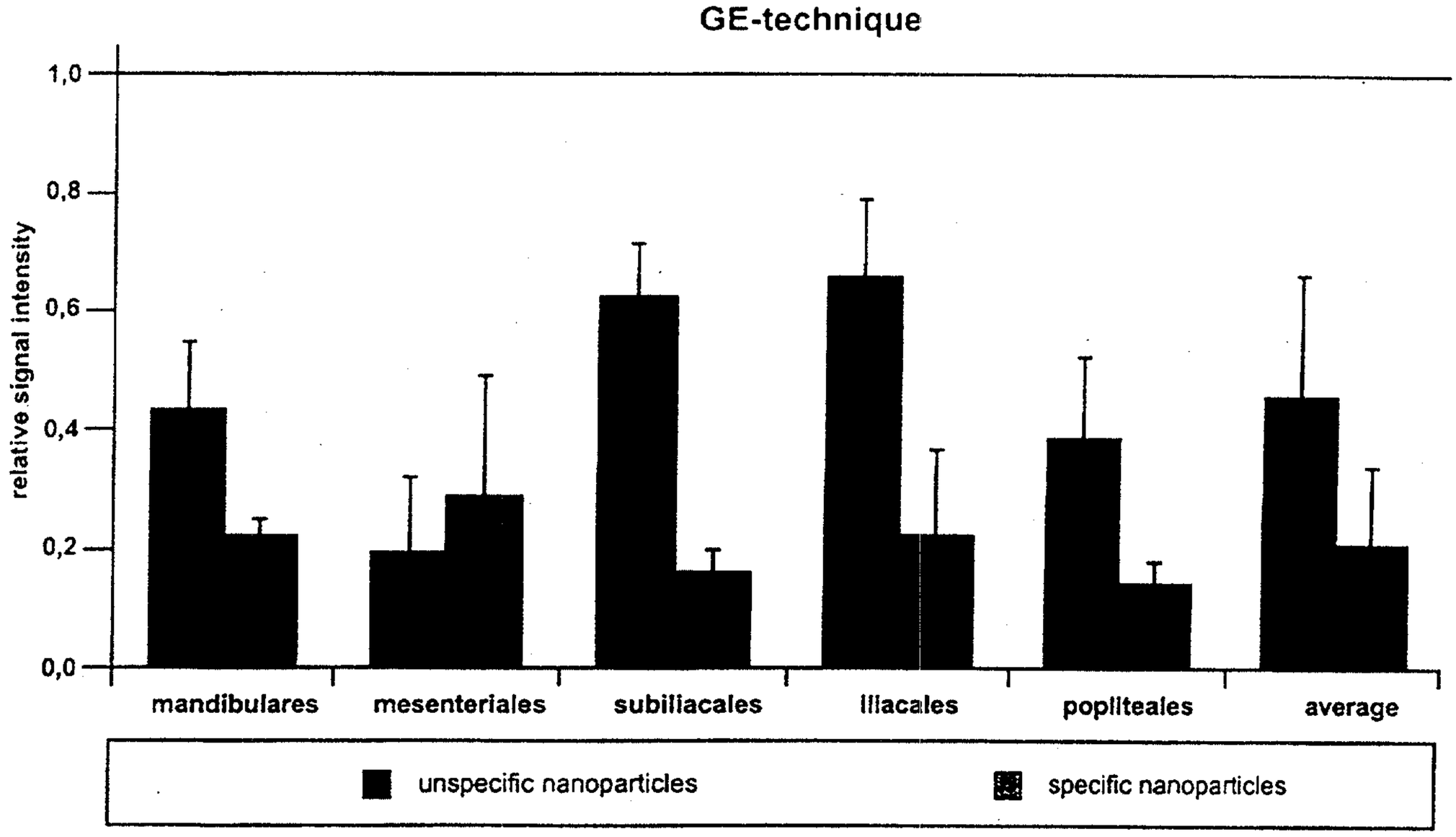

GE-technique
relative signal intensity
1,0
0,8
0,6
0,4
0,2
0,0
mandibulares
mesenteriales
subiliacales
iliacales
popliteales
average
unspecific nanoparticles
specific nanoparticles

FIG. 15

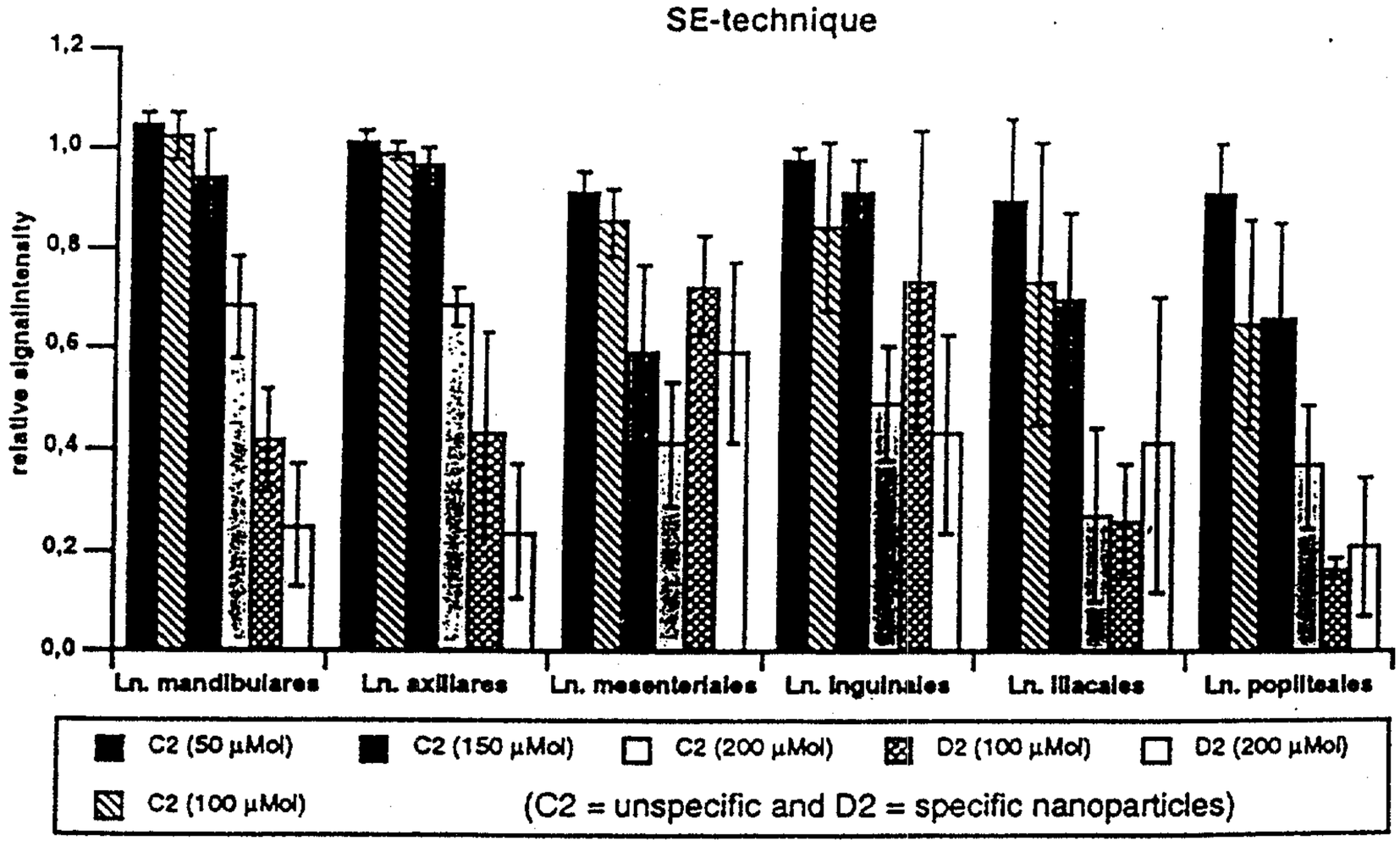

SE-technique
relative signalintensity
1,2
1,0
0,8
0,6
0,4
0,2
0,0
Ln. mandibulares
Ln. axillares
Ln. mesenteriales
Ln. inguinales
Ln. iliacales
Ln. popliteales
C2 (50 µMol)
C2 (100 µMol)
C2 (150 µMol)
C2 (200 µMol)
D2 (100 µMol)
D2 (200 µMol)
(C2 = unspecific and D2 = specific nanoparticles)

FIG. 16

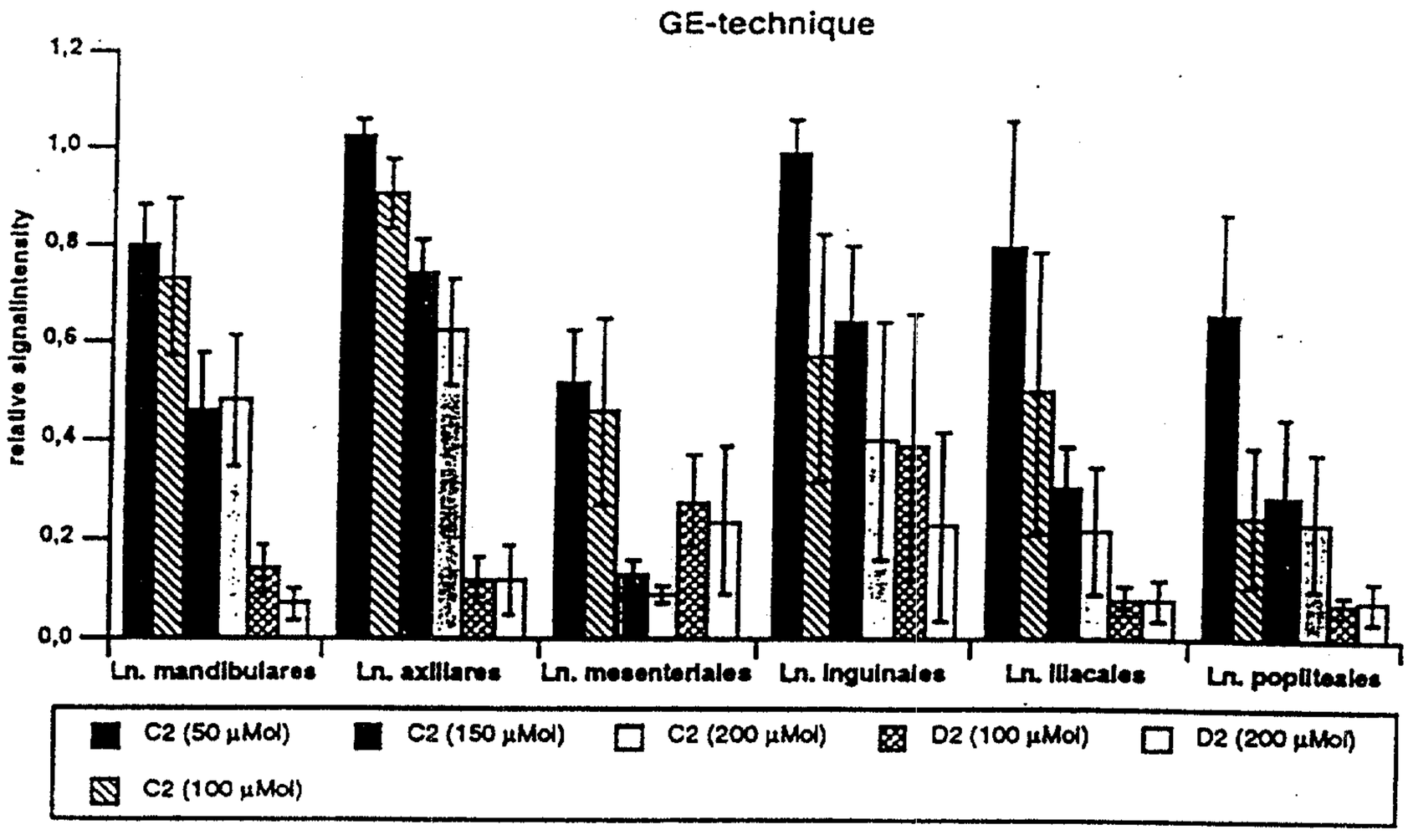

GE-technique
relative signalintensity
1,2
1,0
0,8
0,6
0,4
0,2
0,0
Ln. mandibulares
Ln. axillares
Ln. mesenteriales
Ln. inguinales
Ln. iliacales
Ln. popliteales
C2 (50 µMol)
C2 (100 µMol)
C2 (150 µMol)
C2 (200 µMol)
D2 (100 µMol)
D2 (200 µMol)

FIG. 17

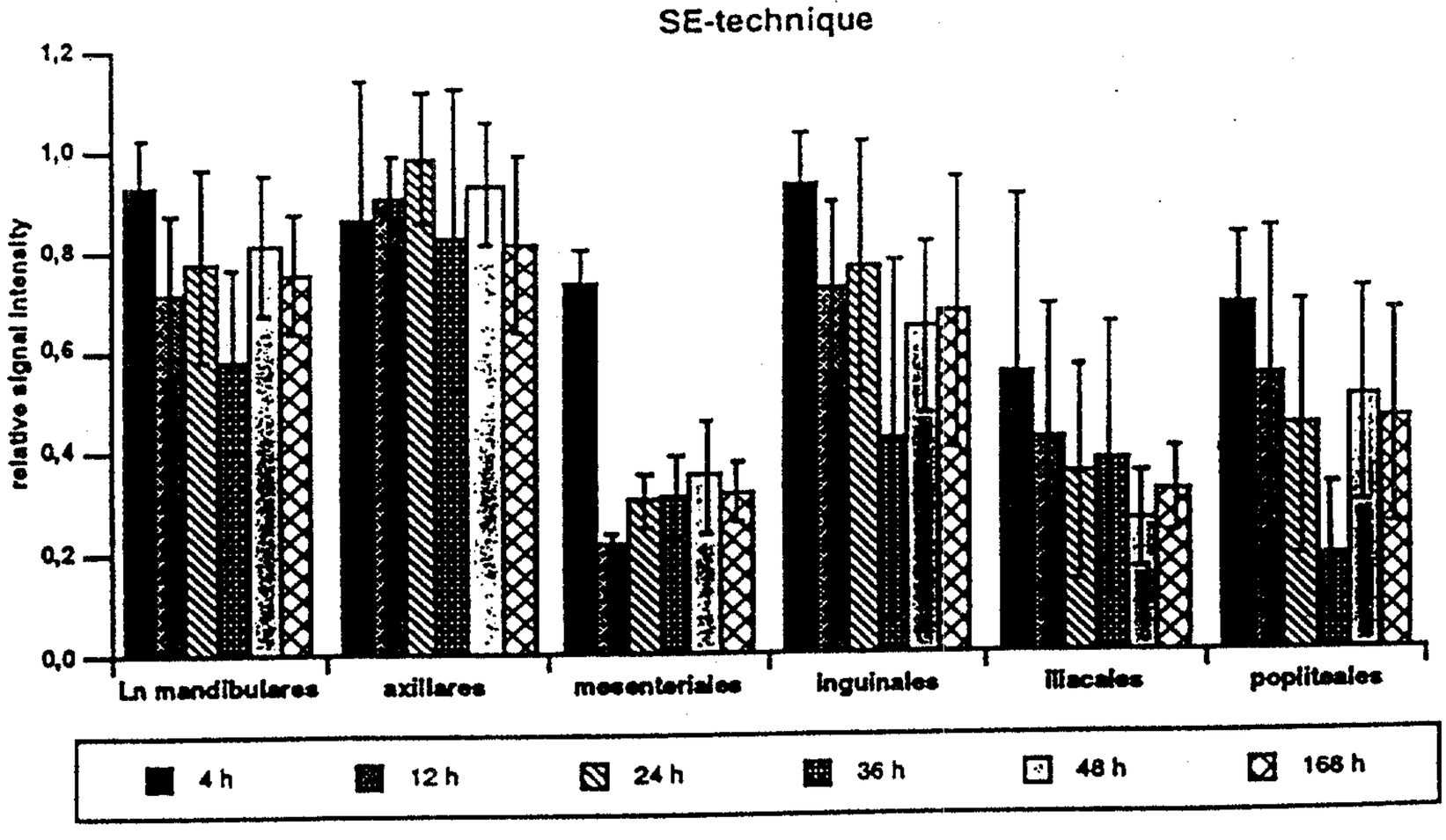

SE-technique
relative signal intensity
1,2
1,0
0,8
0,6
0,4
0,2
0,0
Ln mandibulares
axillares
mesenteriales
inguinales
iliacales
popliteales
4 h
12 h
24 h
36 h
48 h
168 h

FIG. 18

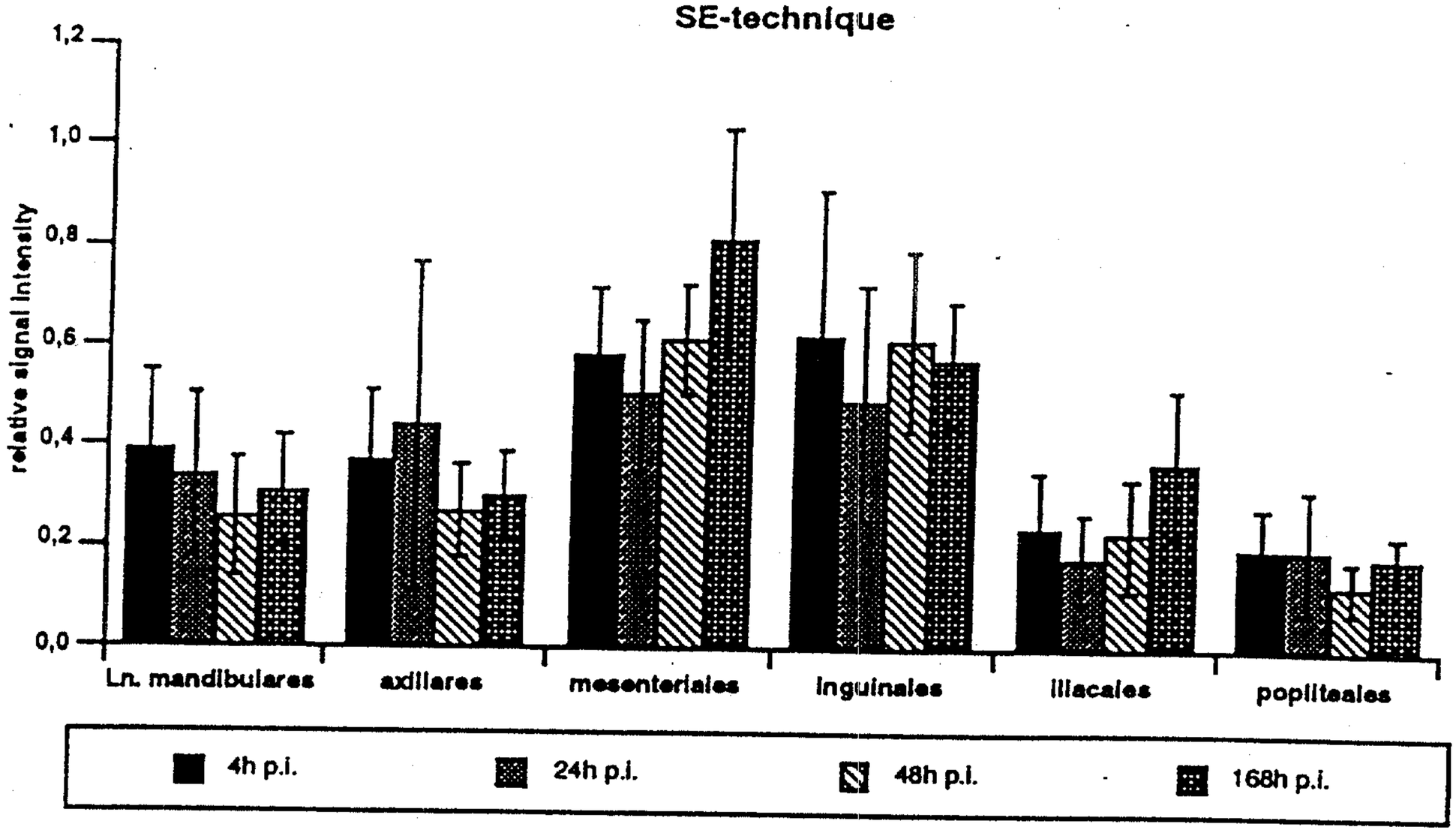

SE-technique
relative signal intensity
1,2
1,0
0,8
0,6
0,4
0,2
0,0
Ln. mandibulares
axillares
mesenteriales
inguinales
iliacales
popliteales
4h p.i.
24h p.i.
48h p.i.
168h p.i.

FIG. 19

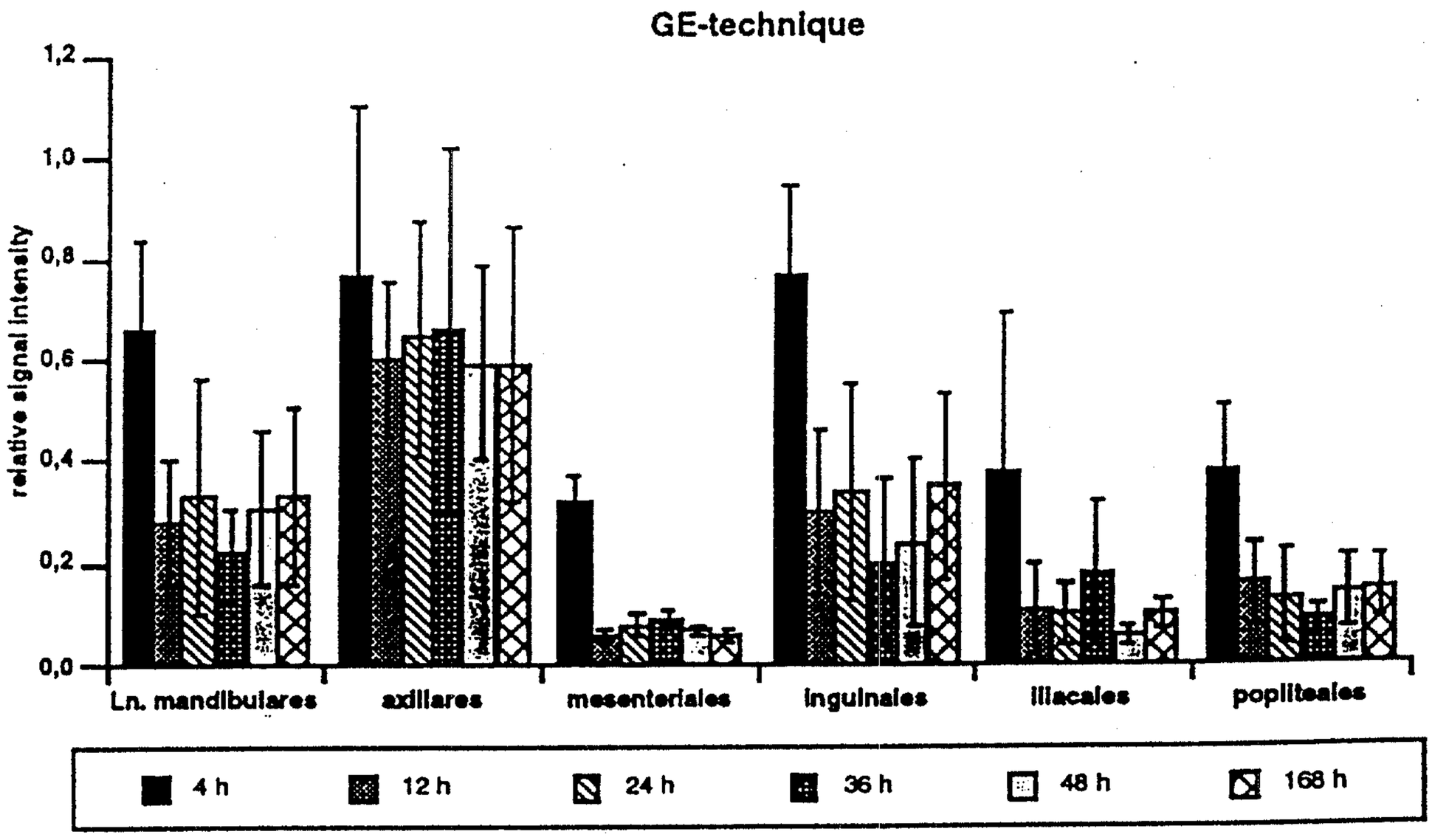

GE-technique
relative signal intensity
1,2
1,0
0,8
0,6
0,4
0,2
0,0
Ln. mandibulares
axillares
mesenteriales
inguinales
iliacales
popliteales
4 h
12 h
24 h
36 h
48 h
168 h

FIG. 20

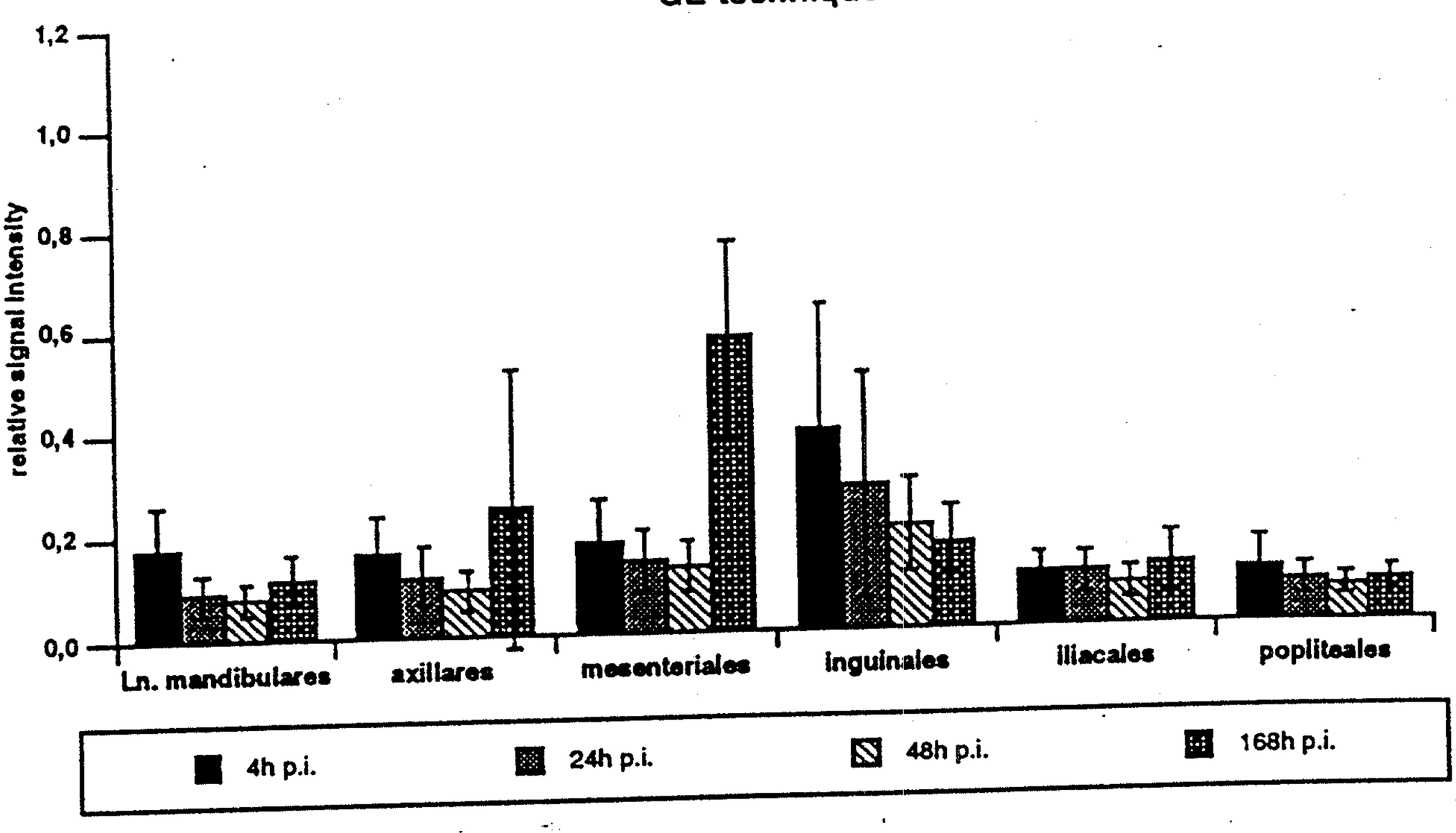

GE-technique
relative signal intensity
1,2
1,0
0,8
0,6
0,4
0,2
0,0
Ln. mandibulares
axillares
mesenteriales
inguinales
iliacales
popliteales
4h p.i.
24h p.i.
48h p.i.
168h p.i.

FIG. 21

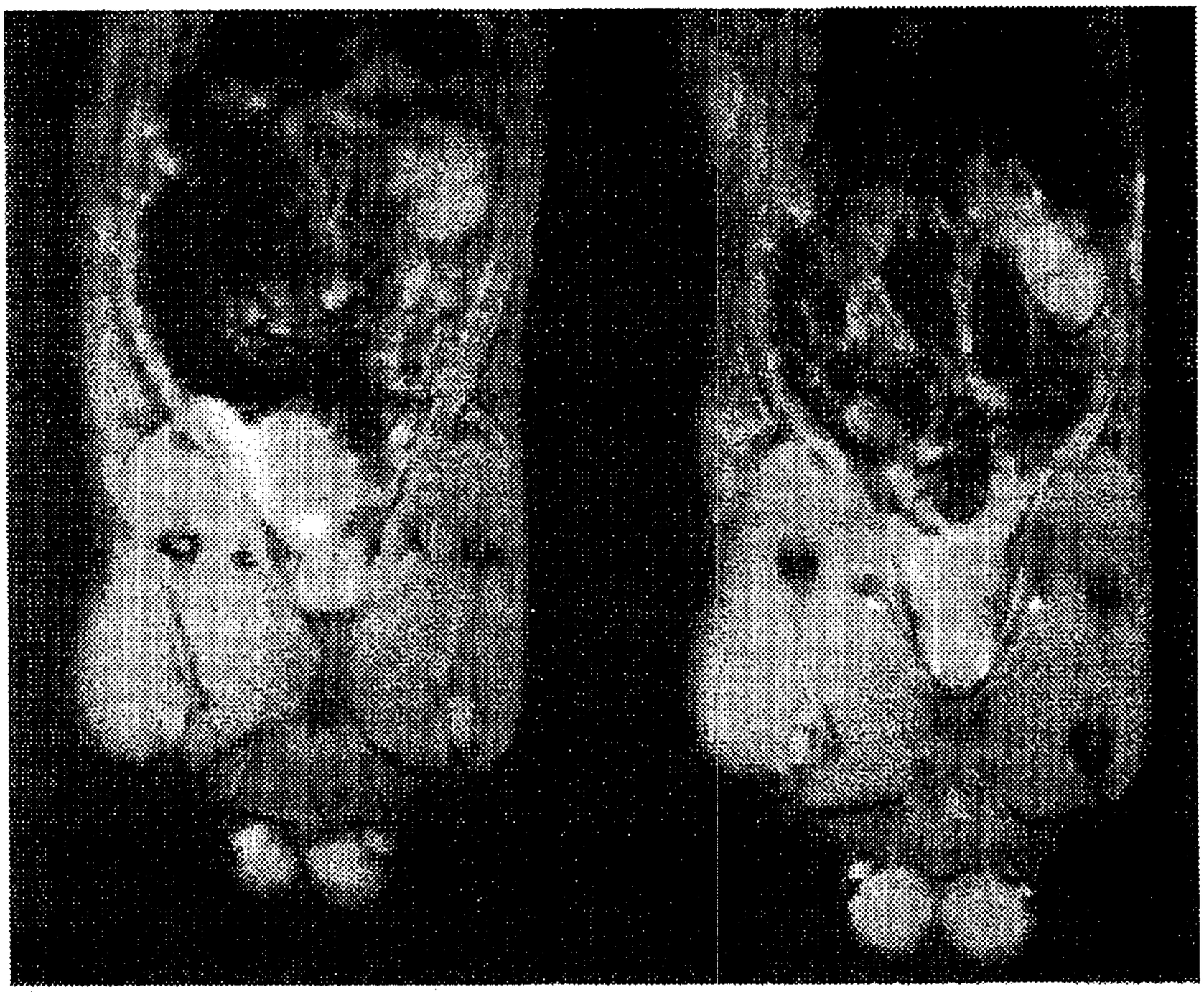


FIG. 22

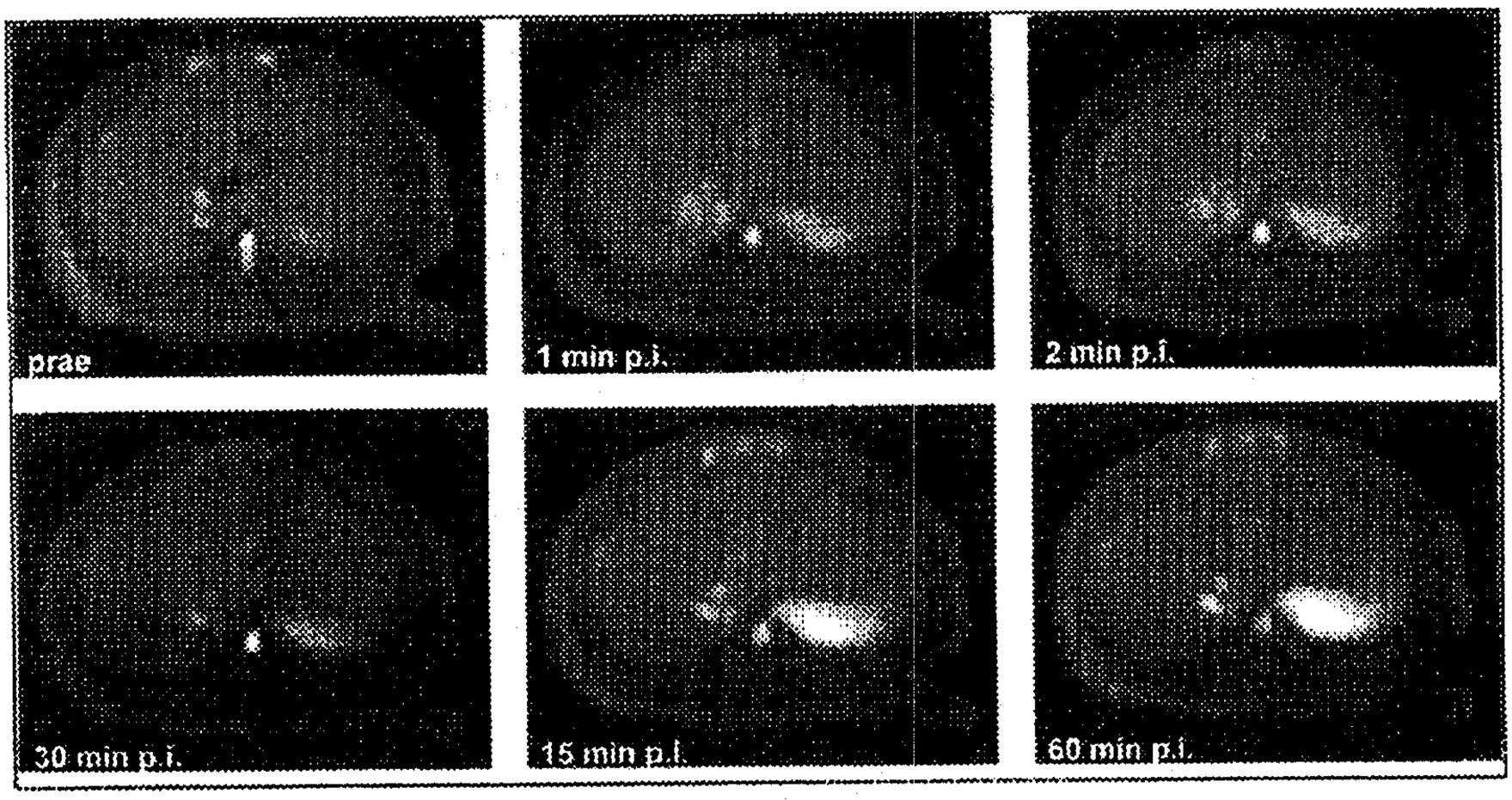


FIG. 23

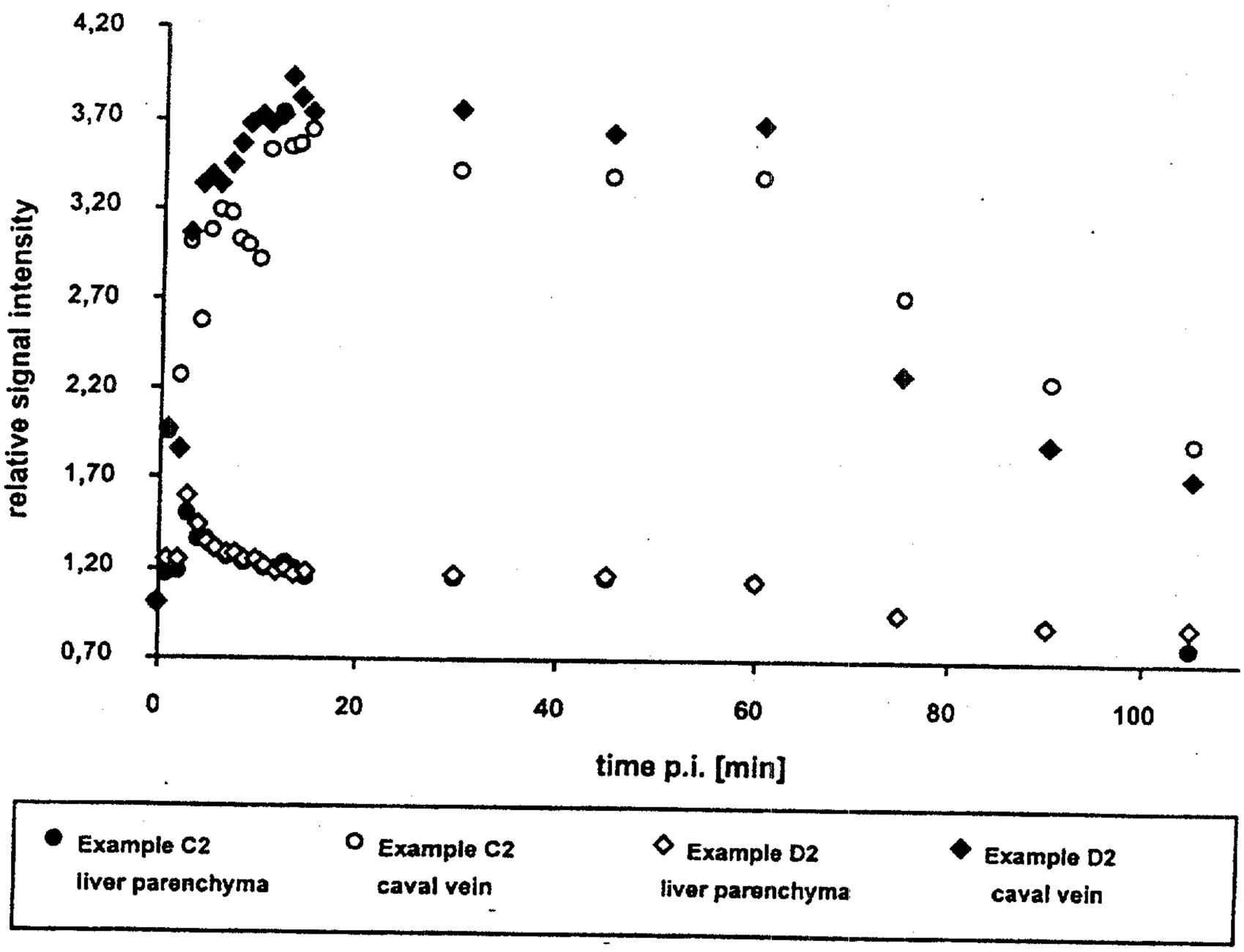

4,20
3,70
3,20
2,70
2,20
1,70
1,20
0,70
relative signal intensity
0
20
40
60
80
100
time p.i. [min]
Example C2 liver parenchyma
Example C2 caval vein
Example D2 liver parenchyma
Example D2 caval vein

FIG. 24

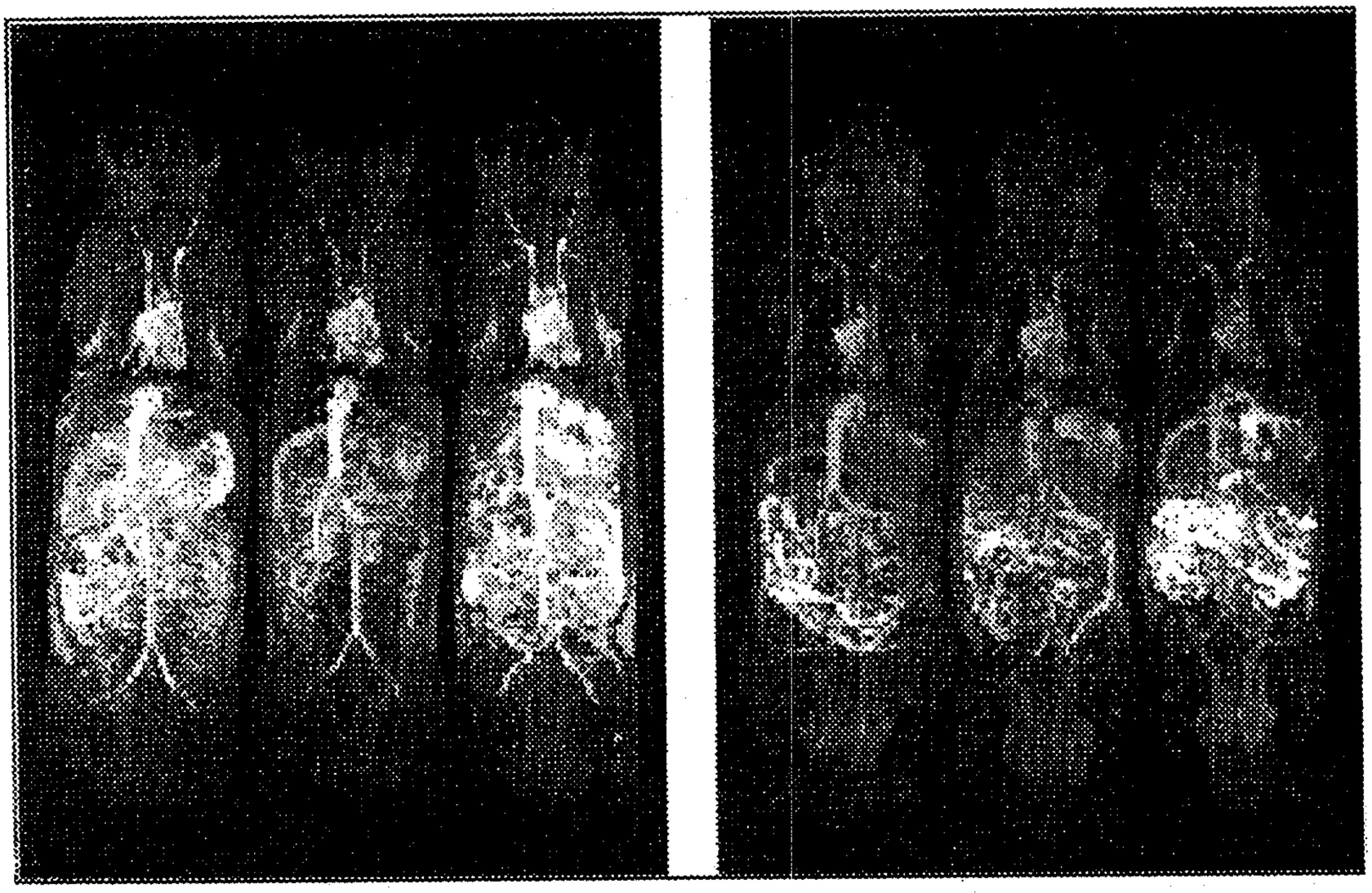


FIG. 25

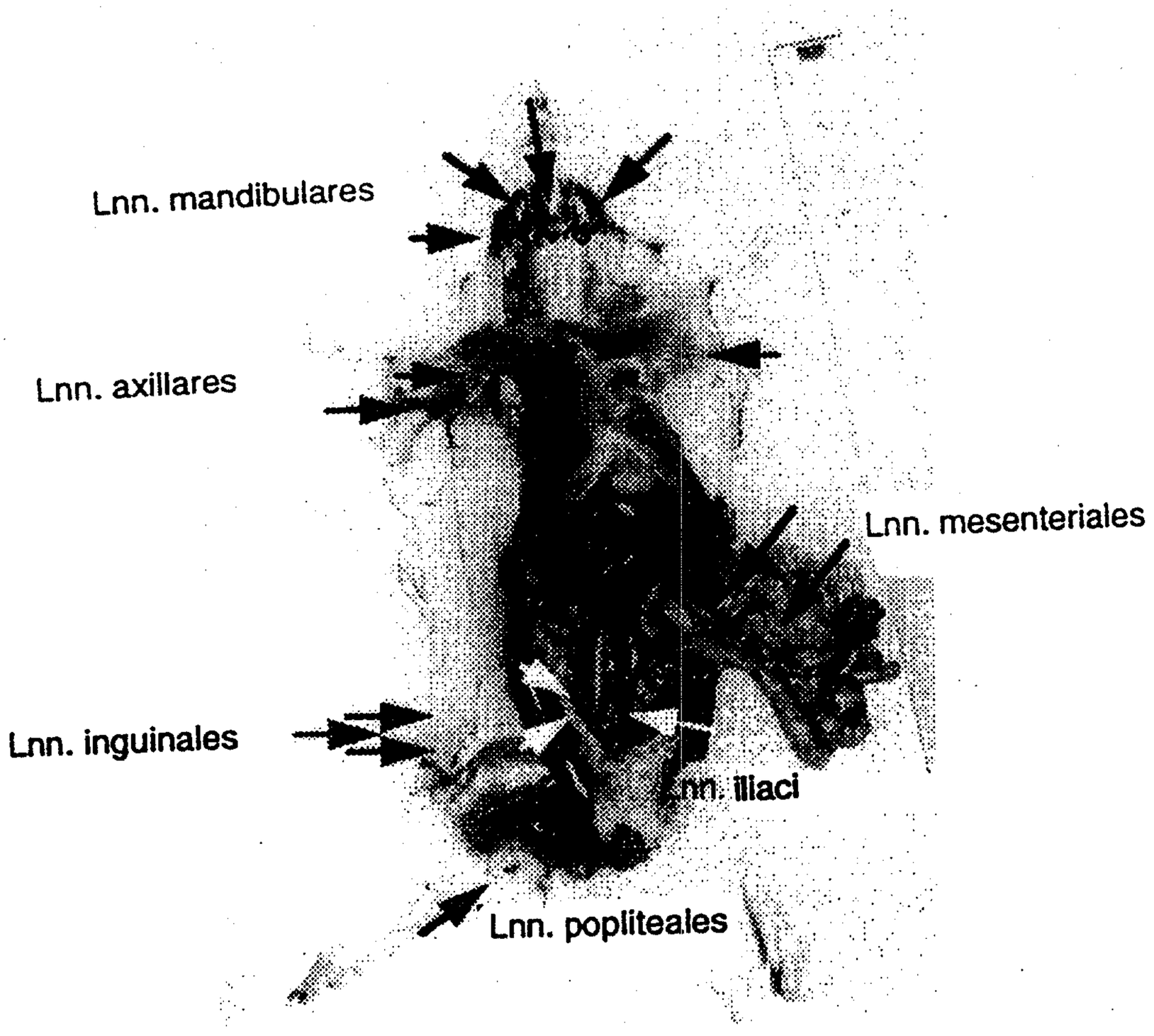

Lnn. mandibulares
Lnn. axillares
Lnn. mesenteriales
Lnn. inguinales
Lnn. iliaci
Lnn. popliteales

FIG.26

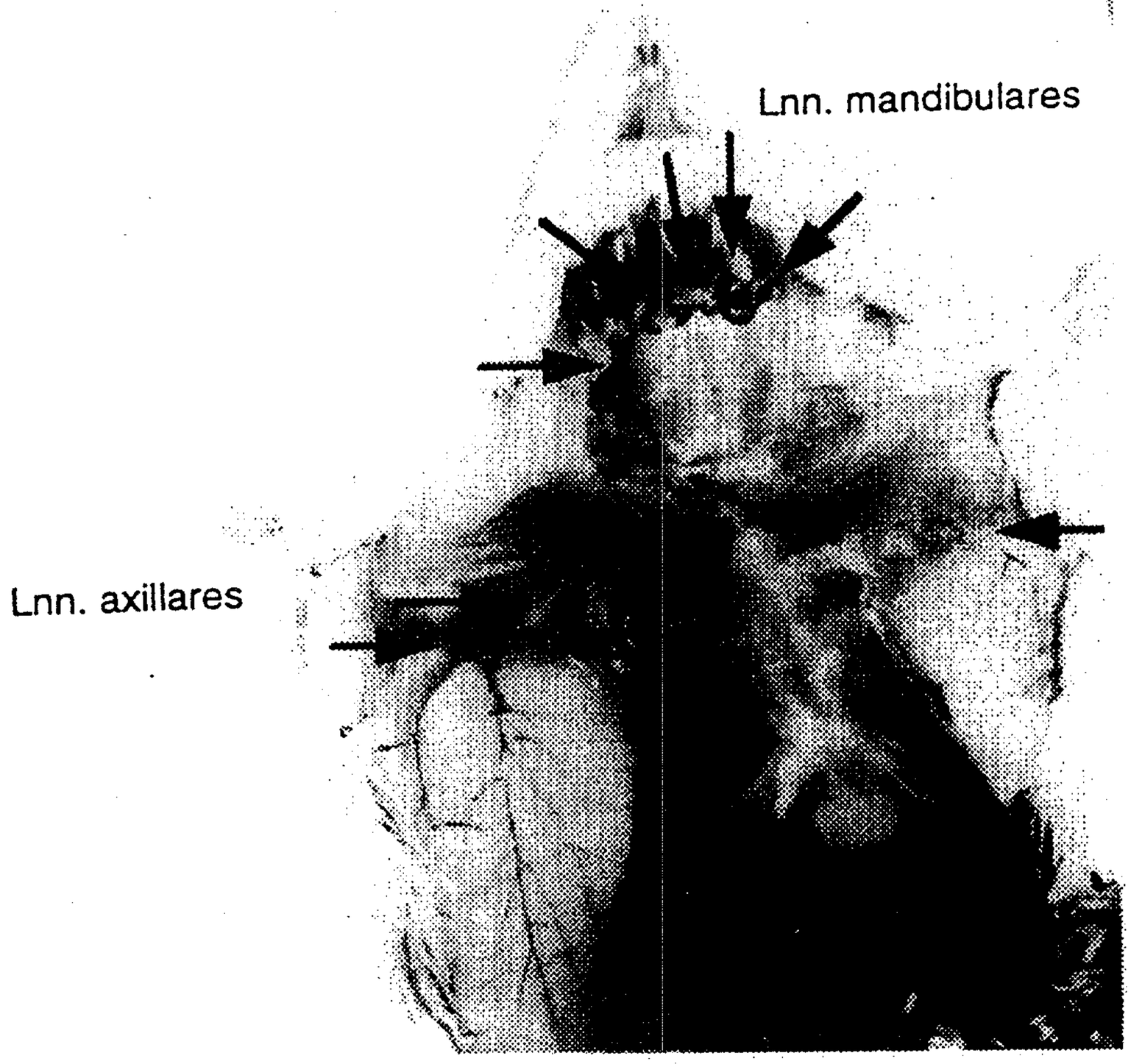


FIG. 27

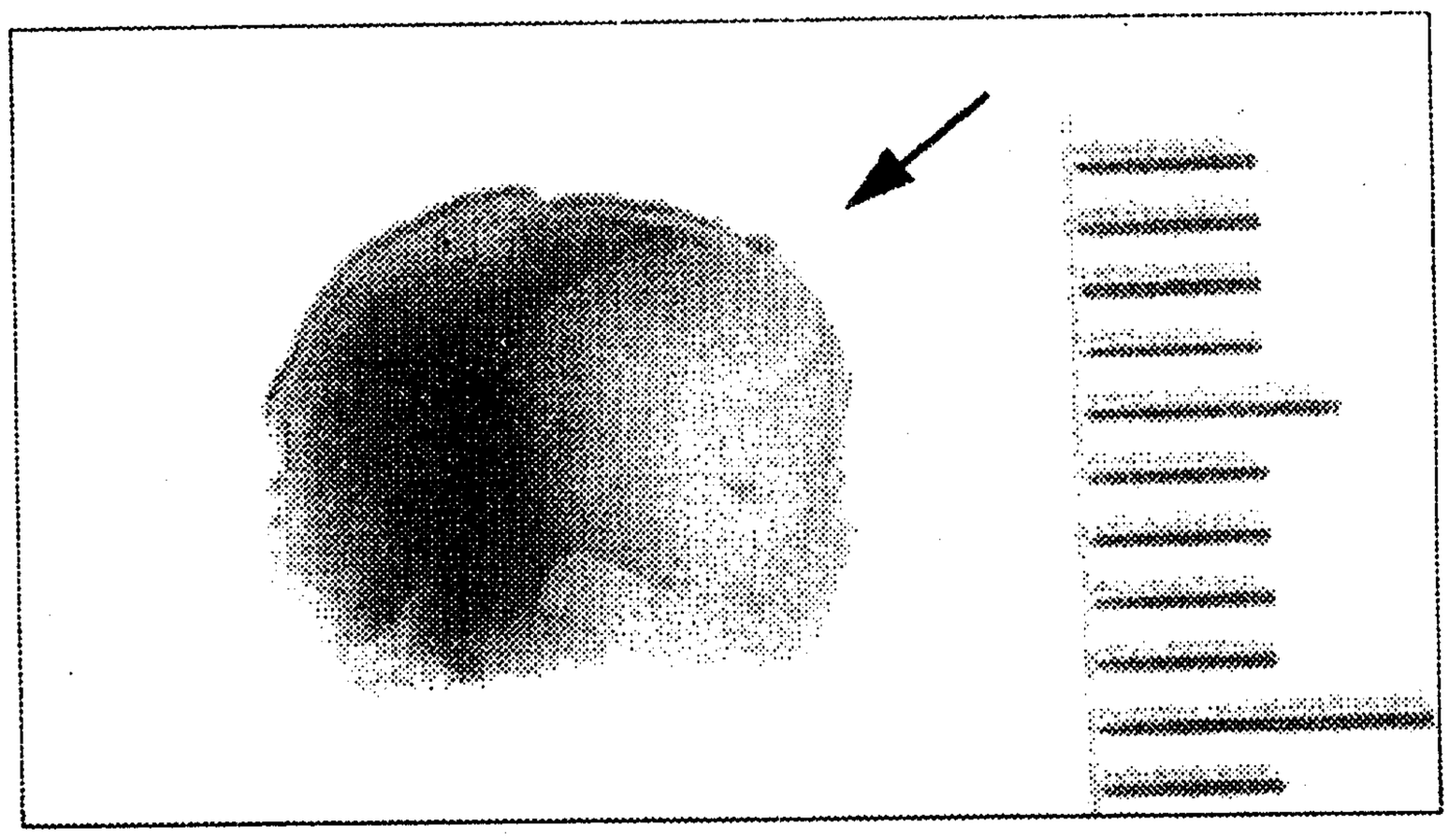


FIG. 28

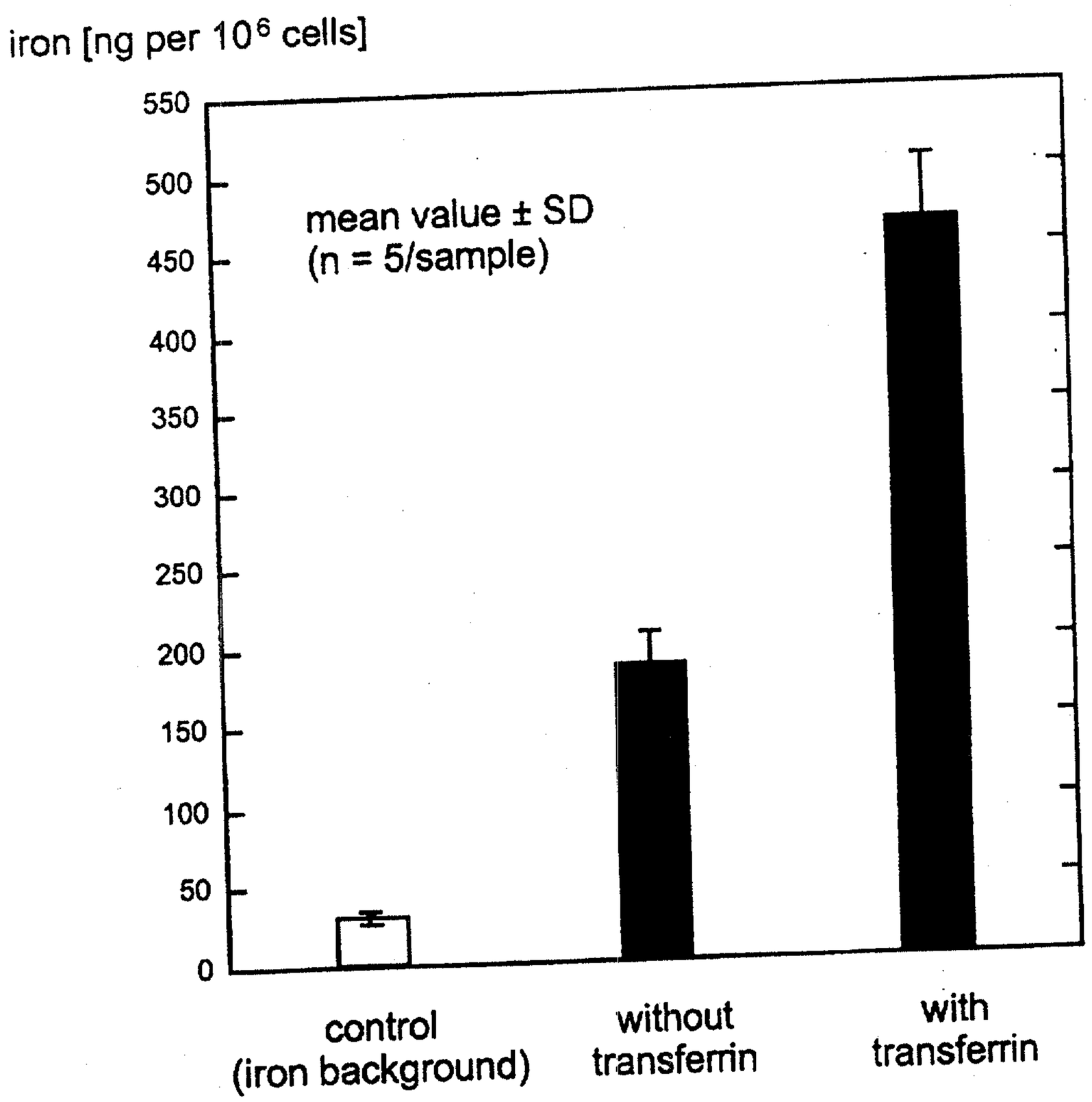

iron [ng per 10^6 cells]
550
500
450
400
350
300
250
200
150
100
50
0
mean value ± SD
(n = 5/sample)
control
(iron background)
without
transferrin
with
transferrin

FIG. 29

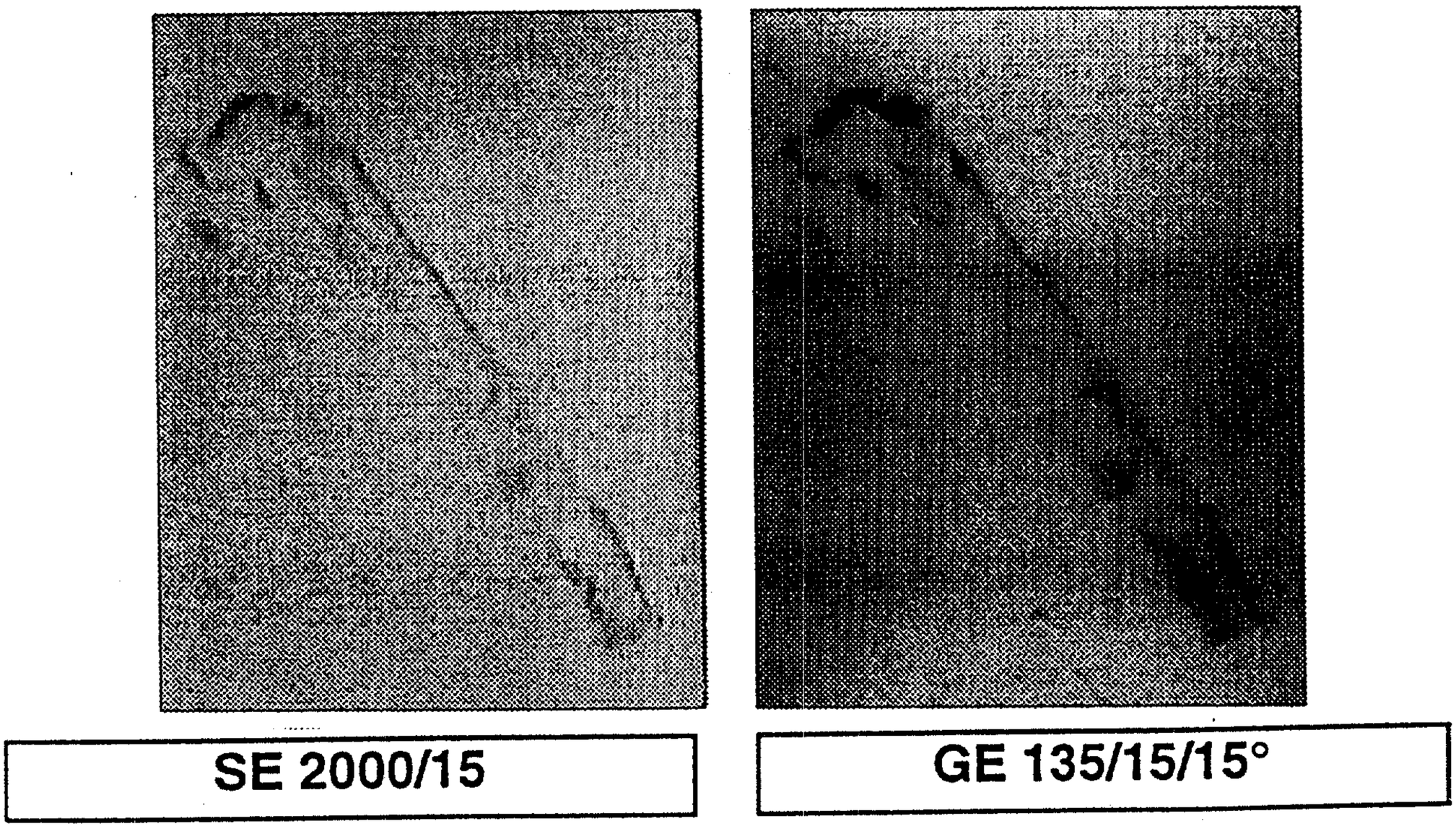


FIG. 30

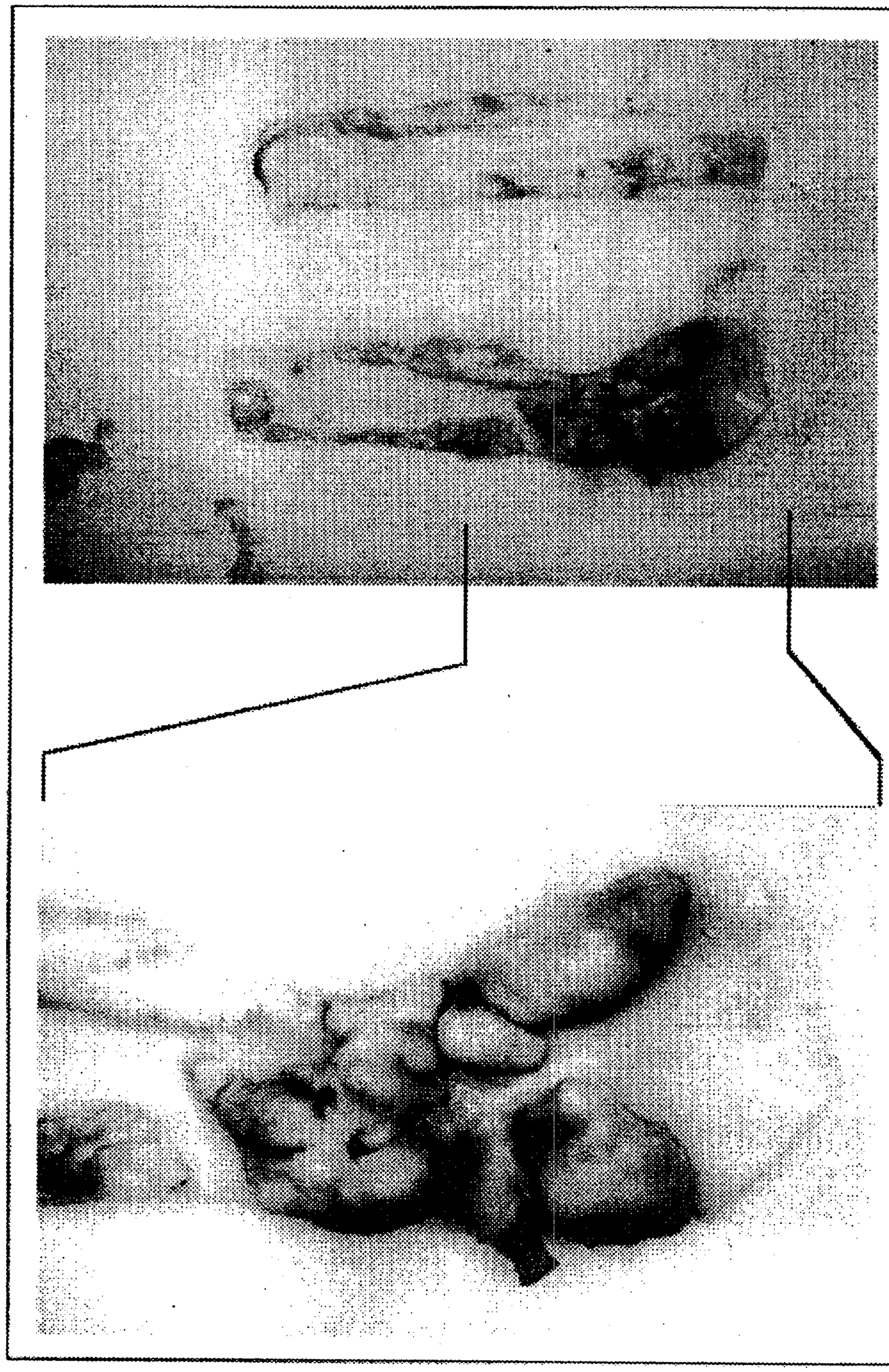


FIG. 31

FIG. 32

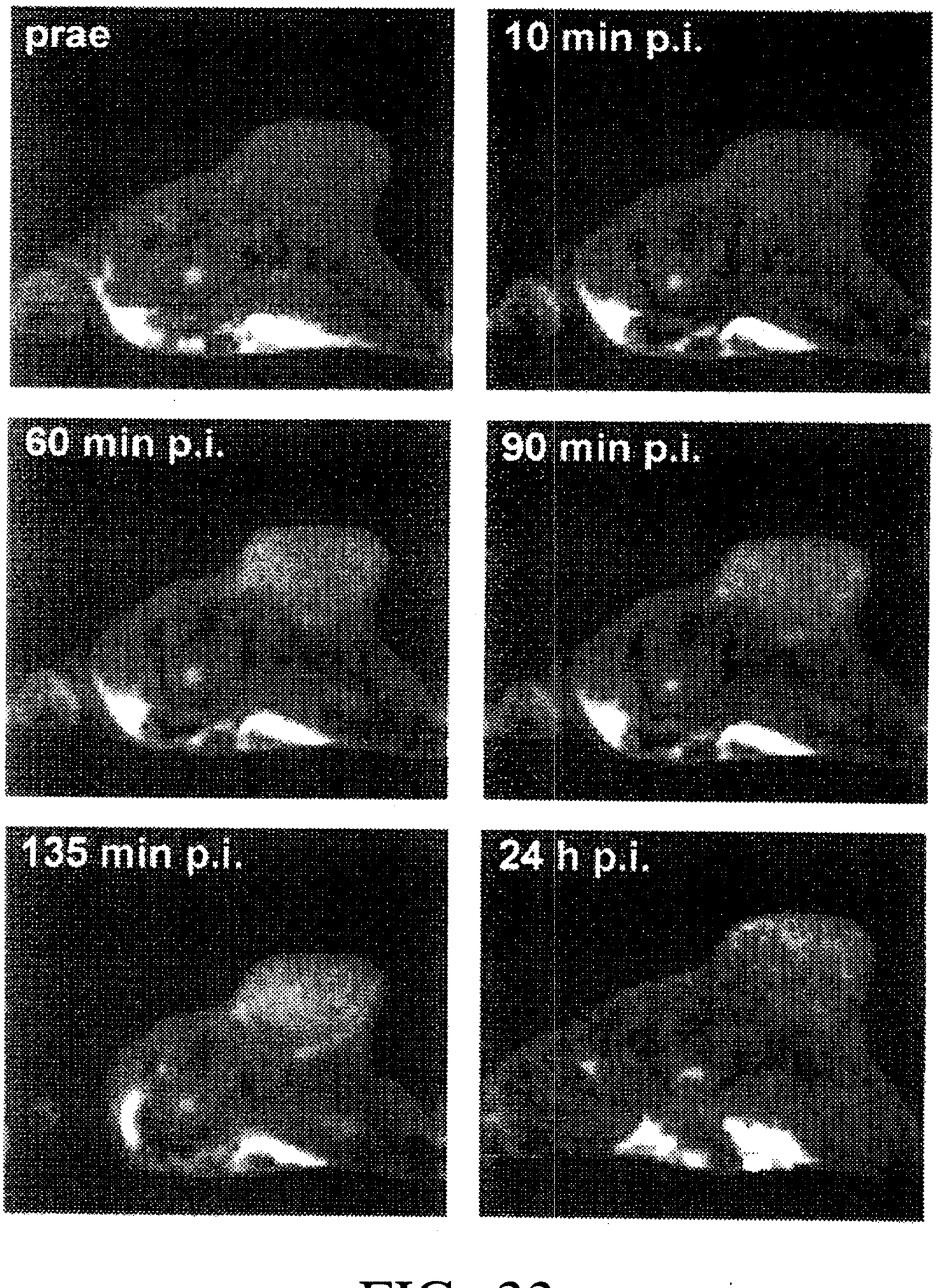


FIG. 33

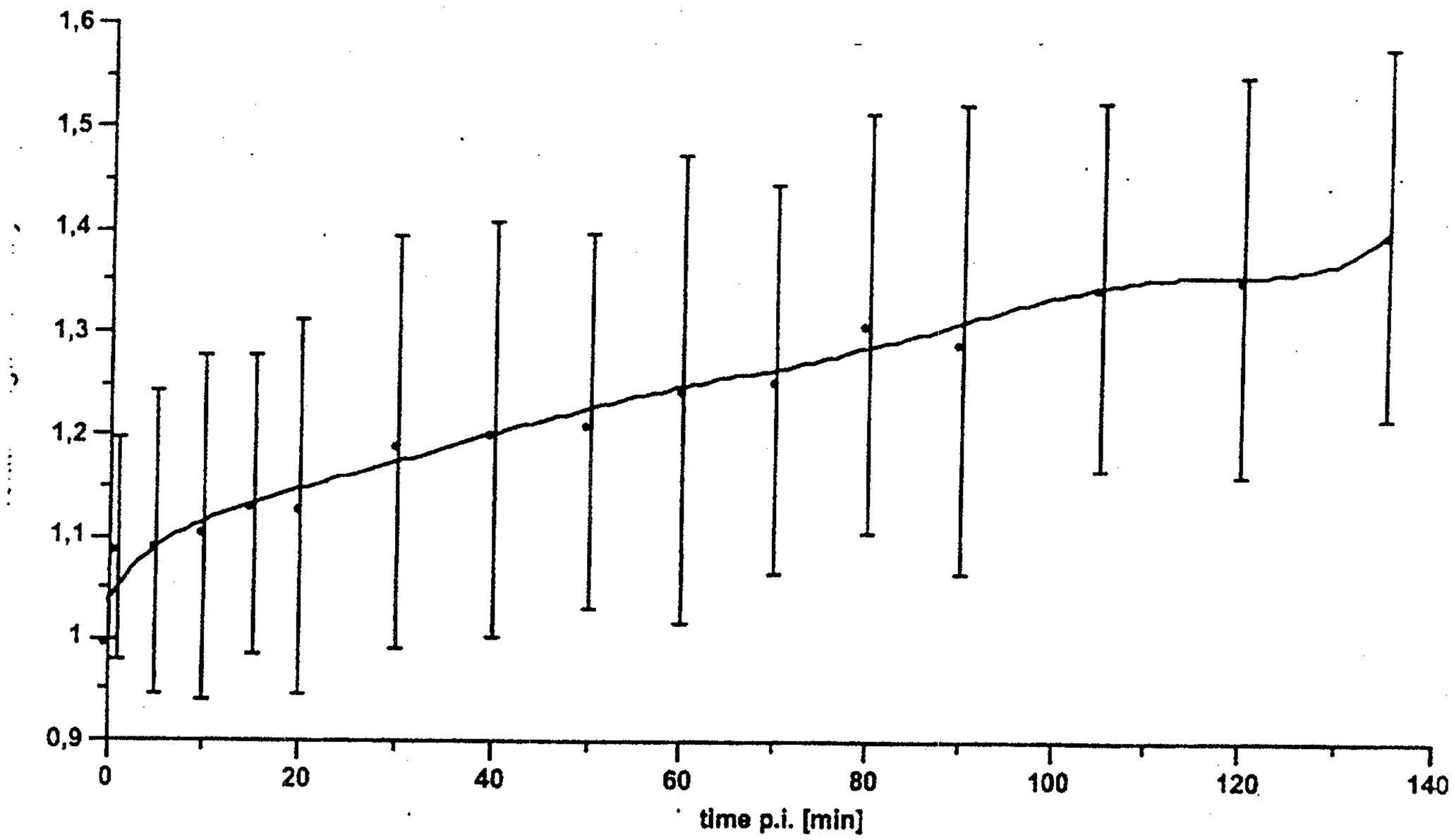


FIG. 34

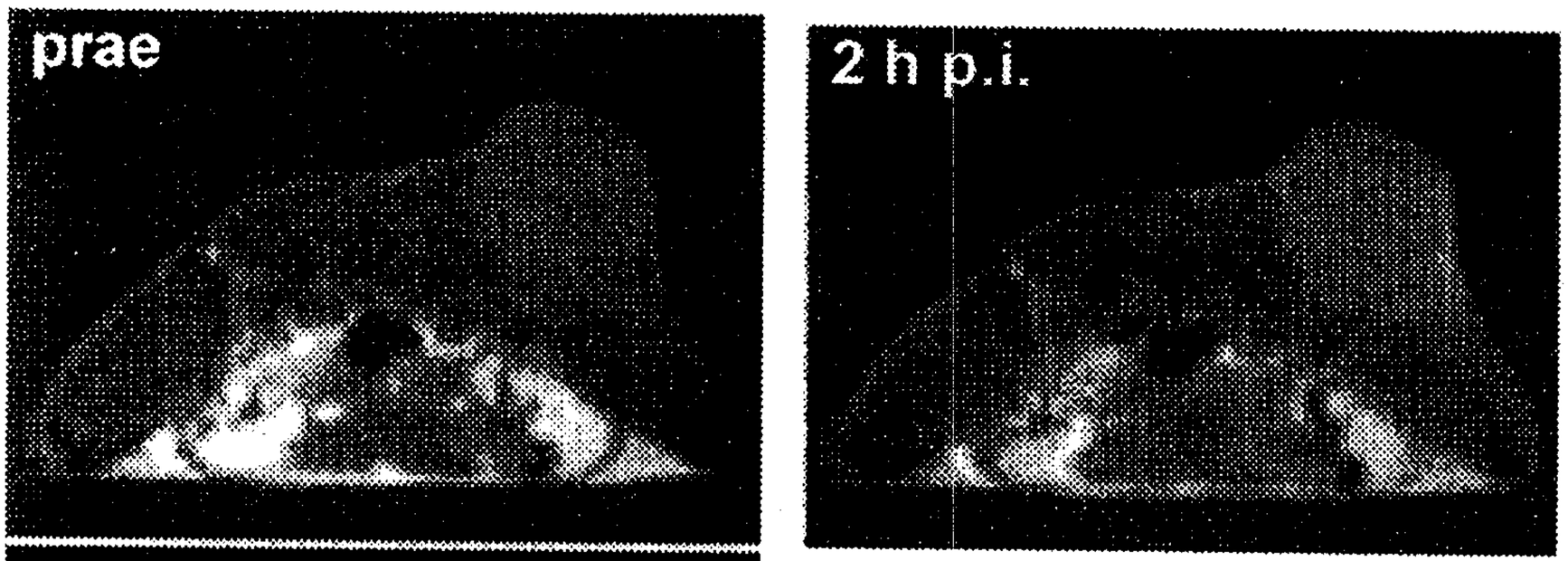


FIG. 35