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AUSTRALIA

PATENTS ACT 1990

NOTICE OF ENTITLEMENT

We, **The Regents of the University of California,** the applicant/Nominated Person in respect of Application No. 74753/91 state the following:-

The Nominated Person is entitled to the grant of the patent because the Nominated Person derives title to the invention from the inventors by assignment.

The Nominated Person is entitled to claim priority from the application listed in the declaration under Article 8 of the PCT because the Nominated Person is the assignee of the applicants in respect of the application listed in the declaration under Article 8 of the PCT, and because that application was the first application made in a Convention country in respect of the invention.

DATED this EIGHTEENTH day of NOVEMBER 1992

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a member of the firm of DAVIES COLLISON CAVE for and on behalf of the applicant(s)

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(57) Claim

1. A method of detecting blood flow abnormality or variation in a human or non-human body, said method comprising the steps of: administering into the systemic vasculature of a said body a contrast enhancing amount of an intravascular paramagnetic metal containing magnetic resonance imaging contrast agent, subjecting said body to a magnetic resonance imaging procedure capable of generating from magnetic resonance signals from said body a series of temporally spaced images of at least a part of said body into which said agent passes, said procedure being a fast imaging procedure having an image acquisition time of less than 5 seconds; detecting temporal variations in said signals or images; and from said temporal variations identifying regions of abnormal or modified blood flow in said body and providing a quantitative indication of the degree of blood flow abnormality or modification therein.

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(57) Abstract

The invention provides a method of detecting blood flow abnormality or variation in a human or non-human body, said method comprising administering into the systemic vasculature of a said body a contrast enhancing amount of an intravascular paramagnetic metal containing magnetic resonance imaging contrast agent subjecting said body to a magnetic resonance imaging procedure capable of generating from magnetic resonance signals from said body a series of temporally spaced images of at least a part of said body into which said agent passes and detecting temporal variations in said signals or images whereby to identify regions of abnormal or modified blood flow in said body and to indicate the degree of blood flow abnormality or modification therein.

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IMPROVEMENTS IN AND RELATING TO MAGNETIC RESONANCE IMAGING

This invention relates to improvements in and relating to magnetic resonance imaging, in particular imaging of phenomena associated with blood flow variations and abnormalities.

Magnetic resonance imaging (MRI) has been used successfully to study blood flow *in vivo*. Moreover Villringer et al. Magnetic Resonance in Medicine 6:164-174 (1988), Cacheris et al. Society of Magnetic Resonance in Medicine, 7th Annual Meeting, San Francisco, 1988, (SMRM 1988) Works in Progress, page 149, Belliveau et al. SMRM 1988, Book of Abstracts, page 222 and Moseley et al. SMRM 1989, Book of Abstracts, page 43 have proposed the use of certain paramagnetic lanthanide chelates as magnetic susceptibility, that is T_2* shortening, MRI contrast agents for studies of cerebral blood flow and perfusion.

Unlike many previous imaging procedures, T_2 or T_2*-weighted MRI using magnetic susceptibility (MS) contrast agents (hereinafter MS imaging) enabled blood perfusion deficits, e.g. cerebral ischemias, to be visualized rapidly as the MR signal intensity was reduced in the regions of normal perfusion due to the effect of the contrast agent, with ischemic tissue being revealed by its retention of signal intensity.

Blood perfusion deficits are associated with several serious and often life-threatening conditions. Rapid identification and location of such deficits is highly desirable in order that the appropriate corrective action, be it therapeutic or surgical, may be taken promptly. Thus in the case of cerebral ischemia, any delay in post ischemic recirculation and reoxygenation of brain tissue reduces neuronal survivability.

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MS imaging therefore represents a major improvement over routine T_2 or T_2*-weighted imaging in the absence of MS contrast agents, since in the routine procedures ischemias or infarcts only become detectable 2 to 3 hours after the event, e.g. a stroke, which gave rise to the perfusion deficit. However, while determination of the existence and location of a perfusion deficit is important, it is also desirable to be able to detect the degree or severity, and if possible the onset and duration of blood flow abnormalities or variations, in a quantifiable manner. We now propose that this be done using a modified MS imaging procedure.

Viewed from one aspect the invention provides a method of detecting blood flow abnormality or variation in a human or non-human, especially mammalian, body, said method comprising administering into the systemic vasculature of a said body a contrast enhancing amount of an intravascular paramagnetic metal, e.g. transition metal or lanthanide, containing magnetic resonance imaging contrast agent, subjecting said body to a magnetic resonance imaging procedure capable of generating from magnetic resonance signals from said body a series of temporally spaced images of at least a part of said body into which said agent passes, and detecting temporal variations in said signals or images whereby to identify regions of abnormal or modified blood flow in said body and to indicate the degree of blood flow abnormality or modification therein.

Thus the method of the invention provides a quantitative and temporal determination of local perfusion variations, e.g. deficits or increases, which may arise from, for example, stroke, microsurgery or administration of blood flow modifying pharmaceuticals.

The method of the present invention is preferably carried out using spin-echo techniques. Alternatively

and also preferably the method may be carried out using a so-called fast or ultra fast imaging technique in order to enable a series of T_2* dependent images to be generated with as short as possible a time interval between successive images. For this reason, techniques capable of generating images with time intervals of less than 5 seconds, especially less than 0.5 seconds and more especially less than 100 milliseconds, are particularly preferred. Thus, in general, techniques such as spin echo, gradient echo, TurboFLASH, and most especially the various varieties of echo planar imaging (EPI), are particularly suitable for use in accordance with the method of the invention.

In the method of the invention, an indication of the degree of blood flow abnormality or modification for a given voxel may readily be determined by comparison of the MR signal intensity for that voxel with a reference value, e.g. the signal intensity for similar tissue with normal blood flow. The reference intensity values may be predetermined or may be selected as the MR signal intensity values for voxels of normal tissue in the same image. In the case of cerebral ischaemias, signal intensity values from the normally perfused gray matter and white matter of the brain may be used to provide reference values for the affected tissue. As is discussed in further detail below, the location and spatial extent of the blood flow abnormalities, and the location and spatial extent of the regions having the most severe blood flow abnormalities detected in this way according to the method of the invention, correspond closely to the same extents and locations as determined using conventional non-MRI techniques such as histopathologic tissue-staining and quantitative autoradiography.

In one particularly preferred embodiment of the invention, temporally spaced images are generated following repeated administrations of the MS contrast

agent, e.g. at intervals of no less than 15-30 minutes, whereby to detect the time of onset and thereafter to monitor the development of the blood flow abnormality or modification, e.g. to identify the extent and location of reperfusable tissue and the degree of success of reperfusion, or to identify tissue for which surgical intervention is required before reperfusion is possible.

Thus the method of the invention may be used to characterize quantitatively the regional microcirculation of the brain before and after acute arterial occlusion and differentiate between regions with normal blood flow, reduced blood flow and no blood flow. With the different distributions of MS contrast media in occluded and reperfused cerebral tissues, the method may also be used to document reperfusion of ischemic tissue. Moreover, with the use of MS contrast media in the method of the invention, distinction can be made between central cores of necrosis and the surrounding penumbrae of salvageable tissue, i.e. between irreversibly and reversibly injured brain tissue. Using ultrafast imaging techniques in the method of the present invention, the kinetics of the distribution of the contrast medium into tissue and of the wash-out of the contrast medium from the tissue can be followed so as to provide a diagnostic "signature" which could be used to distinguish between normal, ischemic, infarcted and reperfused tissue and to characterize the type of ischemic event and to identify tissues at risk from ischemia.

In one embodiment of the method of the invention, using a fast imaging procedure, the determination of the location and severity of ischaemia is effected by determining the time dependence of the MR signal intensity for the voxels in the seconds following administration of the contrast agent, and generating an image where voxel image intensity value is dependent on the time post-administration at which MR signal

intensity for that voxel is lowest. Normal tissue reaches minimum MR signal intensity sooner than ischaemic tissue, and the resulting image thus enables the spatial extent and local severity of blood flow abnormality to be visualized. Alternatively, a similar image may be generated by making the voxel image intensity value dependent on the time taken before voxel MR signal intensity reattains a pre-selected control value, e.g. its pre-injection value or a percentage of that value (for example 80%).

The contrast agent used according to the method of the invention should be an intravascular contrast agent, that is to say one which is substantially retained within the systemic vasculature at least until it has passed through the body region or organ of particular interest. Generally, therefore, blood pooling, particulate and hydrophilic contrast agents or contrast agents possessing more than one of these properties are of particular interest.

Besides its obvious application in terms of identifying and giving an indication of the severity of cerebral or cardiac ischemias or infarcts, the method of the present invention has a broad range of possible diagnostic and evaluative applications of which the following list names but a few:

Assessment of cerebral perfusion in brain dysfunction associated with acute severe symptomatic hyponatremia;

Evaluation of new therapies (for example thrombolytic therapies and clot removal, calcium channel blockers, anti-inflammatory agents, angioplasty, etc) in the treatment of cerebral vasospasm;

Assessment of cerebral perfusion following induced subarachnoid haemorrhage;

Assessment of different degrees of ischemia in large tissue masses;

Study of the relationship between blood ammonia, lactate, pH and cerebral perfusion in cerebral ischemia associated with acute liver failure (this has

implications for the treatment of Alzheimer's disease);
Localisation and assessment of thrombus and plaque;
Evaluation of new therapies for stroke (for example t-PA, aspirin antiphospholipids/lupus anticoagulants, antiphospholipid antibodies, etc);
Evaluation of risk factors for stroke (for example elevated serum lipids, etc);
Assessment of the impact of induced brain hypothermia on cerebral perfusion during neurosurgery for stroke;
Assessment of the effects of ageing on cerebral perfusion including the study of the etiology of lacunar infarcts;
Assessment of the effects of cocaine, amphetamine and ethanol on cerebral perfusion in mildly and severely ischemic brain;
Definition of the "therapeutic window" in reversible focal ischemia for heparin, vasodilators, antihypertensives and calcium antagonists; and
Monitoring of other induced vasodilator effects.

The method of the invention may, in one application, be for detecting and quantitatively evaluating the severity of ischemias in a human or non-human, especially mammalian, body, such method comprising administering into the systemic vasculature of said body a contrast enhancing amount of an intravascular paramagnetic metal containing magnetic susceptibility magnetic resonance imaging contrast agent, subjecting said body to a magnetic resonance imaging procedure capable of generating from magnetic resonance signals from said body a series of temporally spaced images of at least a part of said body into which said agent passes, and detecting temporal variations in said signals or images whereby to detect ischemic tissue and to provide a quantitative indication of the degree of blood perfusion deficit therein.

The present invention may, in another application, be for monitoring the vasodilatory or vasocontractory effects of a physiologically active substance administered to a human or non-human animal body, for example a calcium antagonist, such method comprising administering said substance into said body, administering into the system vasculature of said body a

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contrast enhancing amount of an intravascular paramagnetic metal containing magnetic susceptibility magnetic resonance imaging contrast agent, subjecting said body to a magnetic resonance imaging procedure capable of generating from magnetic resonance signals from said body a series of temporally spaced images of at least a part of said body into which said agent passes, and detecting temporal variations in said signals or images whereby to monitor the vasoconstriction or vasodilation induced by said substance.

The present invention may, in a still further application, be for monitoring surgically induced blood perfusion variations, either before or during surgery, such method comprising administering a contrast enhancing amount of an intravascular paramagnetic metal containing magnetic susceptibility magnetic resonance imaging contrast agent into the systemic vasculature of a human or animal body which is undergoing or has undergone surgery, in particular microsurgery on said vasculature, subjecting said body to a magnetic resonance imaging procedure capable of generating from magnetic resonance signals from said body a series of temporally spaced images of at least a part of said body into which said agent passes, and detecting temporal variations in said signals or images whereby to identify regions of surgically induced variations in blood perfusion.

Viewed from a still further aspect the invention provides the use of a MS contrast agent for the

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manufacture of a contrast medium for use in the methods of the invention.

The magnetic susceptibility, T_2*-reducing effect, of MS contrast agents is to a large degree dependant on the magnitude of the magnetic moment of the magnetic species within the contrast agent - the higher the magnetic moment the stronger the effect. Indeed the effect is approximately proportional to the square of the magnetic moment making the effect of Dy(III) about 1.95 times larger than that of Gd(III). In general paramagnetic metal species having magnetic moments of ≥ 4 BM will be preferred. The contrast agents particularly preferred for use in the method of the present invention are those containing paramagnetic lanthanide ions, especially high spin lanthanides such as ions of Dy, Gd, Eu, Yb and Ho, in particular Dy(III).

In order that they may be administered at effective but non-toxic doses, such paramagnetic metals will generally be administered in the form of ionic or much more preferably non-ionic, complexes, especially chelate complexes optionally bound to larger carrier molecules which may be selected to manifest greater residence times in plasma, or to enhance the blood pooling nature of the contrast agent or to reduce the osmolality of the contrast medium by increasing the number of paramagnetic centres per contrast agent molecule (or molecular ion).

A wide range of suitable chelants, polychelants, and macromolecule bound chelants for paramagnetic metal ions has been proposed in the patent literature over the last decade and in this respect particular regard may be had to US-A-4647447 (Gries), US-A-4687659 (Quay), US-A-4639365 (Sherry), EP-A-186947 (Nycomed), EP-A-299795 (Nycomed), WO-A-89/06979 (Nycomed), EP-A-331616 (Schering), EP-A-292689 (Squibb), EP-A-232751 (Squibb), EP-A-230893 (Bracco), EP-A-255471 (Schering), EP-A-277088 (Schering), EP-A-287465 (Guerbet), WO-A-85/05554 (Amersham) and the documents

referred to therein, the disclosures of all of which are incorporated herein by reference.

Particularly suitable chelants for the formation of paramagnetic metal chelate MS contrast agents for use in the method of the present invention include the following:
N,N,N',N",N"-diethylenetriaminepentaacetic acid (DTPA), 6-carboxymethyl-3,9-bis(methylcarbamoyl-methyl)-3,6,9-triazaundecanedioic acid (DTPA-BMA), 6-carboxymethyl-3,9-bis(morpholinocarbonylmethyl)-3,6,9-triazaundecanedioic acid (DTPA-BMO), 1,4,7,10-tetraazacyclododecane-N,N',N",N"'-tetraacetic acid (DOTA), 1,4,7,10-tetraazacyclododecane-N,N',N"-triacetic acid (DO3A), 1-(2-hydroxypropyl)-1,4,7,10-tetraazacyclododecane-N,N',N",N"-triacetic acid (HP-DO3A), 1-oxa-4,7,10-triazacyclododecane-N,N',N"-triacetic acid (OTTA), polylysine-bound DTPA and DTPA derivatives or DO3A and DO3A derivatives or DOTA and DOTA derivatives (eg. DTPA-polylysine, DO3A-polylysine and DOTA-polysine), soluble dextran-bound DTPA and DTPA derivatives having with a total molecular weight ≥ 40KD, preferably in the range 60-100KD (DTPA-dextran).

Particularly suitable paramagnetic metal ions for chelation by such chelates are ions of metals of atomic numbers 21 to 29,42,44 and 57 to 71, especially 57 to 71, more especially Cr, V, Mn, Fe, Co, Pr, Nd, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb and Ln, in particular Cr(III), Cr(II), V(II), Mn(III), Mn(II), Fe(III), Fe(II) and Co(II) and especially Gd(III), Tb(III), Dy(III), Ho(III), Er(III), Tm(III) and Yb(III) especially Dy(III), Ho(III) and Er(III).

All paramagnetic ions have both T_1 and T_2 reducing effects on the surrounding non-zero spin nuclei and as the effect on MR signal intensity of these two effects is generally opposed in unweighted images, T_1 reduction leads to image intensity increases whereas T_2 reduction leads to image intensity losses. Thus for the purposes

of the present invention it is particularly preferred to use paramagnetic metals which have relatively poor T_1-relaxivity in order to maximize the MR effect of the contrast agents in T_2* or T_2 weighted MR imaging. Thus Dy(III) or even Yb(III) would generally be used in preference to Gd(III).

In order to perform the method of the invention with as high as possible a safety factor, the ratio between the dose of the contrast agent and its LD_{50}, it is particularly preferred to use non-ionic or low osmolality chelates, i.e. chelates which carry no overall ionic charge, such as Dy DTPA-BMA for example, or where the complex has an overall ionic charge to paramagnetic metal centre ratio of 1.5 or less.

Furthermore, to ensure that the contrast agent remains wholly or essentially within the blood vessels during passage through the body region of interest, the contrast agent will as mentioned above preferably be hydrophilic and retained in the vasculature for a sufficiently long time to permit effective imaging.

Examples of suitable blood-pooling agents include the inert soluble macromolecule-bound chelates of the type described by Nycomed in EP-A-186947 and WO-A-89/06979. Binding the chelant to a macromolecule, e.g. a polysaccharide such as dextran or derivatives thereof, to produce a soluble macromolecular chelant having a molecular weight above the kidney threshold, about 40KD, ensures relatively long term retention of the contrast agent within the systemic vasculature.

Examples of suitable hydrophilic contrast agents include linear, branched or macrocyclic polyamino-carboxylic acid chelates of paramagnetic metal ions, especially chelates of chelants in which carboxylic acid groupings are replaced by hydrophilic derivatives thereof, such as amides, esters or hydroxamates, or in which the chelant backbone is substituted by hydrophilic groupings such as for example hydroxyalkyl or

alkoxyalkyl groups. Chelants of this type are disclosed for example in US-A-4687658 (Quay), US-A-4687659 (Quay), EP-A-299795 (Nycomed) and EP-A-130934 (Schering).

Particular mention however must be made of the Dy(III), Ho(III) and Er(III) chelates of DTPA-BMA, DTPA-BMO, and DO3A and HP-DO3A.

The dosages of the contrast agent used according to the method of the present invention will vary according to the precise nature of the contrast agent used. Preferably however the dosage should be kept as low as is consistent with still achieving an image intensity reduction in T_2*-weighted imaging. Thus for Dy(III) based chelates, for example, dosages of Dy of 0.05 to 0.5 mmol/kg bodyweight, and especially 0.08 to 0.3 mmol/kg, are particularly preferred. In this way not only are toxicity-related problems minimized but the sensitivity of the imaging method towards the detection of ischemia of varying degrees of severity is increased. At higher dosages the signal suppression by the MS contrast agent may be unduly abrupt and intense, making regions with relatively minor perfusion deficits appear to have the characteristics of relatively normal blood flow. For most MS contrast agents the appropriate dosage will generally lie in the range 0.02 to 3 mmol paramagnetic metal/kg bodyweight, especially 0.05 to 1.5 mmol/kg, particularly 0.08 to 0.5, more especially 0.1 to 0.4 mmol/kg. It is well within the skill of the average practitioner in this field to determine the optimum dosage for any particular MS contrast agent by relatively routine experiment, either in vivo or in vitro.

Where the contrast agent is ionic, such as is the case with Dy DTPA, it will conveniently be used in the form of a salt with a physiologically acceptable counterion, for example an ammonium, substituted ammonium, alkali metal or alkaline earth metal cation or an anion deriving from an inorganic or organic acid. In

this regard, meglumine salts are particularly preferred.

Contrast agents may be formulated with conventional pharmaceutical or veterinary aids, for example stabilizers, antioxidants, osmolality adjusting agents, buffers, pH adjusting agents, etc., and may be in a form suitable for injection or infusion directly or after dispersion in or dilution with a physiologically acceptable carrier medium, e.g. water for injections. Thus the contrast agents may be formulated in conventional administration forms such as powders, solutions, suspensions, dispersions etc., however solutions, suspensions and dispersions in physiologically acceptable carrier media will generally be preferred.

The contrast agents may therefore be formulated for administration using physiologically acceptable carriers or excipients in a manner fully within the skill of the art. For example, the compounds, optionally with the addition of pharmaceutically acceptable excipients, may be suspended or dissolved in an aqueous medium, with the resulting solution or suspension then being sterilized. Suitable additives include, for example, physiologically biocompatible buffers (as for example DTPA or DTPA-bisamide (e.g. 6-carboxymethyl-3,9-bis(methylcarbamoylmethyl)-3,6,9-triazaundecanedioic acid)) or calcium chelate complexes (as for example calcium DTPA salts, calcium DTPA-bisamide salts or NaCaDTPA-bisamide) or, optionally, additions (e.g. 1 to 50 mole percent) of calcium or sodium salts (for example, calcium chloride, calcium ascorbate, calcium gluconate or calcium lactate and the like).

Parenterally administrable forms, e.g. intravenous solutions, should of course be sterile and free from physiologically unacceptable agents, and should have low osmolality to minimize irritation or other adverse effects upon administration and thus the contrast medium

should preferably be isotonic or slightly hypertonic. Suitable vehicles include aqueous vehicles customarily used for administering parenteral solutions such as Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, Lactated Ringer's Injection and other solutions such as are described in Remington's Pharmaceutical Sciences, 15th ed., Easton: Mack Publishing Co., pp. 1405-1412 and 1461-1487 (1975) and The National Formulary XIV, 14th ed. Washington: American Pharmaceutical Association (1975). The solutions can contain preservatives, antimicrobial agents, buffers and antioxidants conventionally used for parenteral solutions, excipients and other additives which are compatible with the contrast agents and which will not interfere with the manufacture, storage or use of products.

In the method of the present invention where the lanthanide has any significant T_1-reducing effect, which is especially the case where the paramagnetic metal is Gd rather than Dy, this T_1-reducing effect may also be utilised to increase the degree of certainty with which perfused regions are identified by generating corresponding T_1 weighted images and determining the signal ratio for each pixel or voxel between the two types of image. In this way tissue with very limited perfusion may perhaps be distinguished from tissue in which blood flow has ceased entirely. Where such a technique is used however it will be especially desirable to use the low toxicity, low osmolar forms of the paramagnetic complex in order to operate with as large a safety factor as possible. Thus for Gd it will generally be preferably to use GdHP-DO3A, GdDO3A, GdDTPA-BMA or GdDTPA-BMO rather than GdDOTA or GdDTPA salts.

Generally data manipulation forms a major part of the method of the invention since information regarding the severity of perfusion deficit may be

extracted from the rate at which signal intensity loss takes place for the region of interest following MS contrast agent administration (the less obstructed the blood flow the more rapidly the signal is lost) and the duration and magnitude of signal loss. Clearly comparison with data obtained for healthy tissue will enable a form of perfusion calibration to be made. Moreover indications of the blood volume affected may also be obtained by measurement of the area under the curve for a plot of pixel or voxel signal intensity loss over time for the duration of the MS contrast agent induced signal loss. The necessary data manipulation, including display of zones of reduced or enhanced perfusion optionally superimposed on a selected background image, e.g. the "native" image obtained in the absence of the MS contrast agent, can of course be performed by a computer, generally the same computer as is arranged to operate the MR imager and generate MR images from the detected MR signals.

The methods of the invention are particularly suited to the early detection of ischaemias as ischaemic events may in this way be detected significantly less than 1 hour after occurrence, as opposed to the 2-3 hours or more of conventional T_2 weighted MRI, so making it possible to take steps to reperfuse the affected tissue at an earlier stage or to treat it with a cerebroprotective pharmaceutical, and thus raising the chances of reducing permanent tissue damage and of increasing tissue survivability.

The method of the invention will now be described further by way of example with particular reference to certain non-limiting embodiments and to the accompanying drawings in which Figures 1 to 24 are images or diagrams of the cat brain before, during or after unilateral MCA occlusion.

Study 1

Young adult cats weighing 2.0 to 4.5 kg were anaesthetized with 30 mg/kg i.v. Nembutal. Polyethylene catheters were placed in the femoral artery and vein for blood pressure monitoring and drug administration. The right middle cerebral artery (MCA) was isolated via the transorbital approach and occluded just proximal to the origin of the lateral striate arteries with bipolar electrocautery followed by complete surgical transection. The dural incision and orbit were covered with saline moistened gauze and absorbable gelatin sponge.

A General Electric CSI (2 Tesla) unit, equipped with Acustar S-150 self-shielded gradient coils (±20 gauss/cm, 15 cm bore size) was used. MRI was performed with an 8.5 cm inner-diameter low-pass birdcage proton imaging coil. Successive multislice T_2-weighted coronal images were obtained for up to 12 hours following occlusion. Spin-echo T_2-weighted images (TR 2800, TE 80 and 160, 3 mm slices, 1 mm gap) were obtained with a field-of-view (FOV) of 80 mm in which two scans were averaged for each one of the 128 phase-encoding steps resulting in a total acquisition time of 12 minutes.

In order to evaluate the anatomic region of perfusion deficiency following MCA occlusion, cats were injected with a non-ionic T2*-shortening contrast agent, DyDTPA-BMA. The DyDTPA-BMA complex was prepared by refluxing an aqueous suspension containing stoichiometric amounts of dysprosium oxide and DTPA-BMA. The contrast agent was infused i.v. at doses of 0.25, 0.5 or 1.0 mmol/kg beginning at phase-encoding step #32 and finishing at step #60 (approximately 3 min) of T_2-weighted image acquisition. DyDTPA-BMA injections were given at different time points post MCA occlusion in individual cats. After injection, the magnetic susceptibility effect was quantified for up to 60 minutes in both ischemic and normal hemispheres by

comparing region-of-interest (ROI) intensity to pre-contrast T_2-weighted ROI intensities. ROI image analyses were carried out in the ischemic inferior parietal gyrus, caudate, putamen, and internal capsule, and compared with the corresponding uninjured contralateral regions. A signal intensity ratio was calculated as the ROI image intensity ratio of an abnormal, ischemic region over that of the normal, contralateral side. Results were expressed as the mean percentage change ± Standard Error of the Mean (X±S.E.M.)

At the conclusion of the MR protocol, 15 ml/kg of a 2% solution of 2,3,5-triphenyl tetrazolium chloride (TTC) was infused transcardially. The brain was removed from the cranium after 10-20 minutes, immersed in a 2% TTC solution for another 10-20 minutes, and then stored overnight in 10% buffered formalin in a light shielded container. The brain was sectioned coronally (2-3 mm slices) from 24-36 hours later and immediately examined for histologic evidence of ischemic damage as evidenced by pallor of TTC-staining.

Using a 0.5 mmol/kg dosage of DyDTPA-BMA, maximum signal intensity losses of 35% were observed in the gray matter of the normal non-occluded cerebral hemisphere during the first 15 minutes after injection. Signal intensity changes in white matter (internal capsule) in both the normal and ischemic hemispheres were smaller than in gray matter, presumably because of higher cerebral blood flow to gray matter. The resulting contrast-enhanced images had superior gray/white matter contrast than T_2-weighted spin-echo MR images without contrast. At 45 minutes after administration of DyDTPA-BMA, signal intensity had recovered to at least 90% of pre-contrast control values in all cerebral tissues. Increasing the dosage of DyDTPA-BMA from 0.5 to 1.0 mmol/kg produced only a minimal difference in immediate post-contrast signal intensity. Long TE times (160 msec) produced the

highest gray/white matter contrast after DyDTPA-BMA at each of the 3 doses tested. (In general in the method of the invention using higher TE values leads to a slight loss in signal to noise ratio but also to increased sensitivity to T_2* - induced proton dephasing and hence to the MS contrast agent).

Perfusion deficits resulting from occlusion of the MCA were detected as regions of signal hyperintensity of the occluded ischemic tissue compared to the normally perfused areas in the contralateral hemisphere. Relative hyperintensity was found in the occluded basal ganglia as early as 30 minutes post-occlusion for both the 1 mmol/kg and 0.5 mmol/kg dosages. Signal differences between ischemic and contralateral control tissues were observed for gray matter in the inferior parietal gyrus (42 ± 14%), and basal ganglia (26 ± 8%), and to a lesser extent, for the white matter in the internal capsule (5 ± 4%). By comparison, T_2-weighted MRI without contrast failed to demonstrate any significant signal differences prior to approximately 2-3 hours post MCA occlusion (see Table 1). As well, DyDTPA-BMA administration allowed detection of small developing infarcts that were not visible or were ambiguous on T_2-weighted images without contrast (see Table 2).

Table 1. Effect of DyDTPA-BMA administration on the time of detection of cerebral ischemic damage.

Dose DyDTPA-BMA (mmol/kg)	# Cats Tested	Onset of signal hypertensity (T_2-weighted image) Earlier	Same Time	Later
0.25	5	1	4	0
0.50	16	12	4	0
1.0	6	4	2	0

Table 2. Effect of DyDTPA-BMA administration on the definition of injury site (signal intensity ratio of injured tissue to corresponding contralateral control tissue) compared to pre-contrast T_2-weighted image.

Dose DyDTPA-BMA (mmol/kg)	# injections contrast	Signal intensity ratio (T_2 weighted image) Better	Same	Worse
0.25	5	4	1	0
0.50	23	17	6	0
1.0	16	14	2	0

Within 3-5 hours after MCA occlusion, T_2-weighted images also demonstrated tissue injury clearly, including increased mass-effect and hyperintensity (edema) throughout the MCA territory. The distribution of increased signal intensity correlated

well anatomically with regions of perfusion deficiency demonstrated with DyDTPA-BMA-enhanced MR imaging. A continuing close anatomic correspondence between areas of perfusion deficit and edematous regions was seen 9 hours and 11 hours post occlusion. In subsequent TTC-stained coronal sections, these areas were found to exhibit characteristics typical of ischemic tissue injury, such as pallor of staining, coagulation, necrosis, and glial proliferation.

These results confirm that MS contrast agent enhanced MRI can significantly advance the time of detection of cerebral ischemic insults. Evidence of stroke-induced perfusion deficits was observed in the MCA territory as early as 45 minutes post-occlusion using contrast-enhanced MRI, whereas T_2-weighted spin-echo images without contrast did not demonstrate increased signal intensity until 2-3 hours after occlusion.

Contrast in T_2-weighted spin-echo MRI can be produced by changes in the microscopic magnetic fields experienced by protons undergoing molecular diffusion. These field gradients cause spin dephasing and loss of spin echo signal intensity. Field gradients arise at the interface of two volumes with different magnetic susceptibilities and thus different induced magnetic fields.

The presence of paramagnetic chelates can alter the magnetic susceptibility of tissue. In the brain, since the chelates are confined to the intravascular space by the blood-brain barrier, a field gradient is induced between the capillary space and surrounding (perfused) tissue resulting in significant signal loss. These results show that this approach to MR contrast enhancement can be used to differentiate ischemic from normally perfused regions.

A further notable advantage of the method of the invention is its relative insensitivity to motion

compared to diffusion-weighted MR imaging. Given the relatively high safety index (LD_{50} i.v. administration in mice is 34 mmol/kg), the long duration of the magnetic susceptibility effect of DyDTPA-BMA and its negligible T_1-reducing effect makes this a particularly good MS contrast agent for use with T_2-weighted MRI.

The contrast-enhanced images suggested considerable regional heterogeneity in perfusion throughout the ischemic MCA territory. Post-contrast signal hyperintensity was observed earlier in the basal ganglia than the neocortex. This finding suggests that non-anastomosing end-arterial tissues, such as the caudate and putamen, are most susceptible to post-ischemia perfusion deficits, since no collateral circulation is available. In collaterally perfused areas such as neocortex, on the other hand, tissue injury may be mitigated somewhat by continued blood flow in the partially ischemic watershed regions. It seems likely that the method of the invention may be able to help identify normal and abnormal regional blood flow differences, and especially to differentiate reversibly ischemic penumbra from infarcted tissue based on the degree and duration of perfusion deficit to cerebral tissues.

In further studies of cerebral perfusion deficits on the cat MCA model described above, using echo planar imaging in conjunction with low, ie. 0.1 and 0.15 mmol/kg, dosages of DyDTPA-BMA, quantitative spatial and temporal assessment of stroke affected tissue may be made. This dosage reduction further increases both the potential sensitivity and the safety profile of the method of the invention.

The advantage of using echo planar MRI with a MS contrast agent is that images may be acquired continuously before, during and after contrast injection. This allows the time course of the contrast agent passage through a tissue to be monitored and to

obtain images at the maximum contrast dosage. Since the echo-planar pulse sequence does not require a 180° refocussing pulse, the T_2* effects of the MS contrast agent are accentuated.

Echo planar images on the GE CSI 2 Tesla were acquired in a sequential fashion. Sixteen images were obtained one each second or less, each image possessing a 66 msec acquisition time with a data matrix of 64 x 64 pixels over a 60 x 60 mm field-of-view. The slice thickness was 3 mm. The echo-planar sequence was that of a gradient-echo nature, with the time of echo (TE) value adjusted to maximise the T_2*-shortening contrast effect.

Study 2

The method of the invention is further illustrated by the images shown in Figures 1 to 11.

T_2-weighted spin-echo (TR/TE 2800/180 msec) images of the cat brain were acquired following a unilateral MCA occlusion of one hour duration and subsequent reperfusion of the occluded artery.

At 108, 160 and 320 minutes following arterial reopening 0.5 mmol/kg Dy DTPA-BMA was injected intravenously over 90 seconds between phase encoding-step steps 32 and 60 of 128 phase encoding image acquisitions.

A section of the unaffected brain hemisphere was selected as a signal intensity reference (100%) and for each temporal image of the selected slice contours showing the degree of the hyperintensity of the affected hemisphere were plotted. The images at 108, 160 and 320 minutes post-occlusion are shown in Figures 1, 2 and 3. The corresponding contour maps of hyperintensity, i.e. blood perfusion deficit, are shown in Figures 4, 5 and 6 and, superimposed on the MR images, in Figures 7, 8 and 9.

The accuracy of this technique in identifying the location, extent and severity of ischaemic damage is demonstrated by the corresponding TTC-stained histopathologic sections shown in Figure 10 and, with superimposed staining contours, in Figure 11.

These images illustrate a failed reperfusion which led ultimately to severe and extensive brain damage, which was corroborated by the histopathologic results.

Study 3

The method of the invention is further illustrated by the images shown in Figures 12 to 18.

Echo planar (EP) images (65msec acquisition time) of the cat brain were recorded before, during and after a unilateral MCA occlusion, in each case with intravenous bolus injection of 0.25 mmol/kg DyDTPA-BMA between the first and second of sixteen sequential EP images.

A region of the unaffected brain hemisphere was selected as a signal intensity reference (100%) and for each temporal image of the selected slice the extent and the severity of perfusion deficit was plotted. The images before occlusion, during occlusion and after successful reperfusion are shown in Figures 12, 13 and 14. The area showing 20% or greater hyperintensity, indicative of the extent of the perfusion deficit, is shown in Figure 15 and a contour map of hyperintensity illustrating the areas of severe deficit is shown in Figure 16, in each case for the image acquired during occlusion as the pre- and post-occlusion images did not show hyperintense regions. Once again the area and severity of blood flow reduction during occlusion thus identified closely correlates with the corresponding TTC-stained histopathologic sections as shown in Figures 17 and 18.

Study 4

The method of the invention is further illustrated by the images shown in Figures 19 to 22.

The information on extent and severity of perfusion deficit available using the method of the invention was compared with that available using conventional techniques of autoradiography (using 99Tc-HMPAO) and TTC histopathology. Figures 19a, b and c, 20a, b and c, 21a, b and c and 22a, b and c show respectively a 99Tc-HMPAO autoradiograph, TTC-staining of a histopathologic section, a T_2-weighted MR image (without contrast agent) and a T_2-weighted MR image following iv administration of 0.25 mmol/kg DyDTPA/BMA. The (a) images are the images as recorded, the (b) images show contour maps of regions of modified intensity (the selected reference (100%) regions are also shown) and the (c) images superimpose the raw images (a) and the contour maps (b). The correlation between the information from the autoradiograph (Fig. 19), which is currently accepted as being particularly accurate and sensitive to cerebral blood volume determination and from the images obtained according to the invention (Fig. 22) is particularly good.

Study 5

The method of the invention is further illustrated by the images shown in Figures 23 and 24.

T_2-weighted spin-echo (TR/TE 2800/180 msec) images of the cat brain were recorded during unilateral MCA occlusion, more particularly at 128 minutes and 280 minutes after arterial occlusion occurred. 0.25 mmol/kg DyDTPA/BMA was injected intravenously over 45 seconds between phase encoding steps 32 and 60 of the 128 phase encoding-step acquisitions. Figures 23a, b and c and 24a, b and c show the recorded images at 128 and 280 minutes (the (a) images), the contour maps of hypertensity (the (b) images showing the reference areas

(100%) of the unaffected hemisphere) and the superpositions of the MR images and the contour maps (the (c) images). At 128 minutes seven different regions of perfusion deficiency were identified. This heterogenicity of the perfusion deficiency is to be expected early in the course of a cerebral ischaemia. At 280 minutes the increased levels of hyperintensity confirm worsening perfusion deficit in most brain regions but the heterogeneity of the hyperintensity suggests that some brain areas may still retain some blood flow.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:-

1. A method of detecting blood flow abnormality or variation in a human or non-human body, said method comprising the steps of: administering into the systemic vasculature of a said body a contrast enhancing amount of an intravascular paramagnetic metal containing magnetic resonance imaging contrast agent, subjecting said body to a magnetic resonance imaging procedure capable of generating from magnetic resonance signals from said body a series of temporally spaced images of at least a part of said body into which said agent passes, said procedure being a fast imaging procedure having an image acquisition time of less than 5 seconds; detecting temporal variations in said signals or images; and from said temporal variations identifying regions of abnormal or modified blood flow in said body and providing a quantitative indication of the degree of blood flow abnormality or modification therein.

2. A method according to claim 1 wherein said contrast agent comprises a physiologically tolerable complex of a paramagnetic lanthanide ion or a physiologically tolerable salt of such a chelate.

3. A method according to claim 2 wherein said contrast agent is a chelate complex of a metal ion selected from the paramagnetic ions of Yb, Tm, Dy, Ho, Er and Gd, or a physiologically tolerable salt thereof.

4. A method according to claim 3 wherein said contrast agent is a chelate complex of Dy(III) or a physiologically tolerable salt thereof.

5. A method according to any one of claims 1 to 4 wherein said contrast agent comprises a physiologically

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tolerable non-ionic paramagnetic lanthanide chelate complex.

6. A method according to any one of claims 2 to 5 wherein said chelate complex is a complex of a linear, branched or macrocyclic chelant selected from polyaminocarboxylic acid chelants and from chelants wherein one or more carboxylic acid groupings are replaced with an amide, ester or hydroxamate grouping.

7. A method according to claim 6 wherein said chelate complex is a complex of a chelant selected from the group consisting of DTPA, DTPA-BMA, DOTA, DO3A, DTPA-BMO and HP-DO3A.

8. A method according to claim 2 wherein said chelate complex is DyDTPA-BMA.

9. A method according to any one of claims 1 to 8 wherein said contrast agent is administered at a dosage of 0.02 to 3 mmol/kg bodyweight.

10. A method according to claim 9 wherein said contrast agent is administered at a dosage of 0.08 to 0.5 mmol/kg.

11. A method according to any one of claims 1 to 10 wherein said procedure is one having an image acquisition time of less than 0.5 seconds.

12. A method according to any one of claims 1 to 13 wherein said procedure is an echo planar imaging procedure.

13. A method according to any one of claims 1 to 12 wherein administration of said contrast agent is by bolus injection.

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14. A method according to any one of claims 1 to 10 comprising generating temporally spaced T_2* or T_2-weighted images.

15. A method according to claim 14 wherein said magnetic resonance imaging procedure is a spin-echo or gradient echo procedure.

16. A method according to either of claims 14 and 15 comprising generating and comparing T_1-weighted images or signals transformable thereto and T_2* or T_2-weighted images or signals transformable thereto whereby to identify body regions in which blood perfusion occurs.

17. A method according to any one of claims 1 to 16 being a method of detecting body regions of blood flow deficit.

18. A method according to claim 17 being a method of detecting ischemic regions.

19. A method according to claim 17 being a method of detecting body regions in which blood perfusion is surgically, thermally or chemically modified.

20. A method according to claim 1 wherein said contrast agent comprises a physiologically tolerable complex of a paramagnetic transition metal ion or a physiologically tolerable salt of such a chelate.

21. A method as claimed in claim 1 for detecting and quantitively evaluating the severity of ischemias in a human or non-human body, said method comprising the steps of: administering into the systemic vasculature of said body a contrast enhancing amount of an intravascular paramagnetic metal containing magnetic susceptibility magnetic resonance imaging contrast

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agent; subjecting said body to a magnetic resonance imaging procedure capable of generating from magnetic resonance signals from said body a series of temporally spaced images of at least a part of said body into which said agent passes, said procedure being a fast imaging procedure having an image acquisition time of less than 5 seconds; detecting temporal variations in said signals or images; and from said temporal variations detecting ischemic tissue and providing a quantatitive indication of the degree of blood perfusion deficit therein.

22. A method as claimed in claim 1 for monitoring the vasodilatory or vasocontractory effects of a physiologically active substance administered to a human or non-human animal body said method comprising administering said substance into said body, administering into the systemic vasculature of said body a contrast enhancing amount of an intravascular paramagnetic metal containing magnetic susceptibility magnetic resonance imaging contrast agent, subjecting said body to a magnetic resonance imaging procedure capable of generating from magnetic resonance signals from said body a series of temporally spaced images of at least a part of said body into which said agent passes, said procedure being a fast imaging procedure having an image acquisition time of less than 5 seconds, and detecting temporal variations in said signals or images whereby to monitor the vasoconstriction or vasodilation induced by said substance.

23. A method as claimed in claim 1 for monitoring surgically induced blood perfusion variations said method comprising administering a contrast enhancing amount of an intravascular paramagnetic metal containing mass magnetic susceptibility magnetic resonance imaging contrast agent into the systemic vasculature of a human or animal body which is undergoing or has undergone

surgery, subjecting said body to a magnetic resonance imaging procedure capable of generating from magnetic resonance signals from said body a series of temporally spaced images of at least a part of said body into which said agent passes, said procedure being a fast imaging procedure having an image acquisition time of less than 5 seconds, and detecting temporal variations in said signals or images whereby to identify regions of surgically induced variations in blood perfusion.

24. A method as claimed in any one of claims 1 to 23 wherein said contrast agent is administered as a contrast medium composition comprising DyDTPA-BMA and CaNaDTPA-BMA in a molar ratio of about 20:1.

Dated this 7th day of April, 1994
THE REGENTS OF THE UNIVERSITY OF CALIFORNIA
By its Patent Attorneys
Davies Collison Cave

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FIG. 7



FIG. 8



FIG. 4

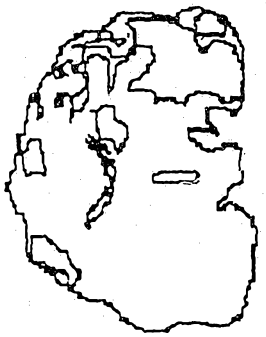


FIG. 5

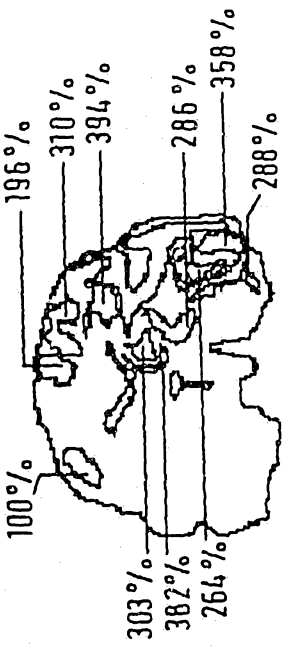


FIG. 1



FIG. 2



FIG. 9



FIG. 11



FIG. 6



FIG. 10



FIG. 3



FIG. 14

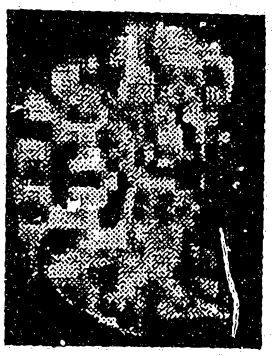


FIG. 16B

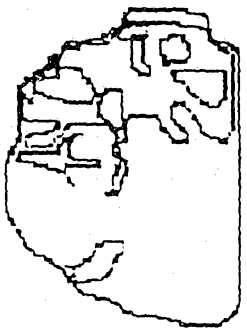


FIG. 16A

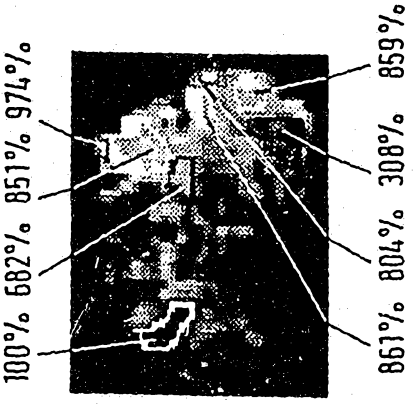


FIG. 12

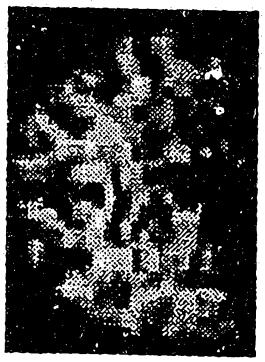


FIG. 15



FIG. 13



FIG. 17

FIG. 18

FIG. 19A

FIG. 19B

FIG. 19C

SUBSTITUTE SHEET

FIG. 20C

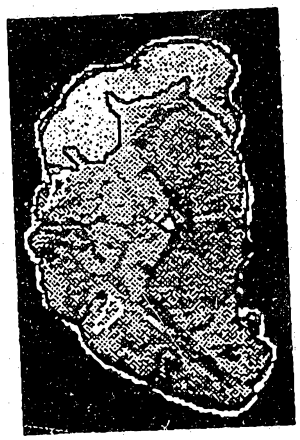


FIG. 21C



FIG. 20B

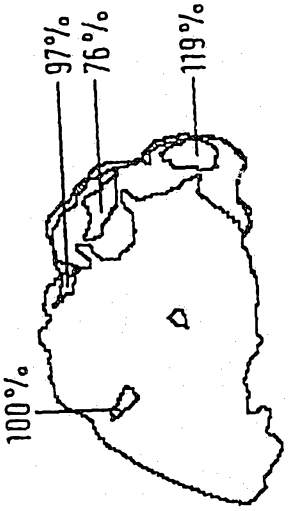


FIG. 21B

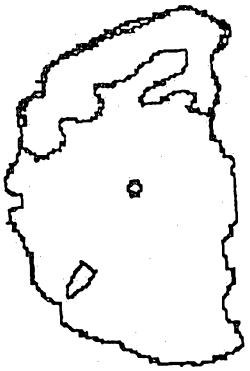


FIG. 20A



FIG. 21A

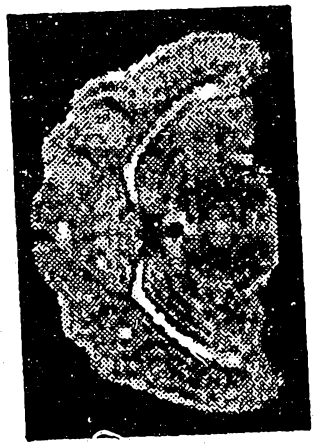


FIG. 22C



FIG. 22B

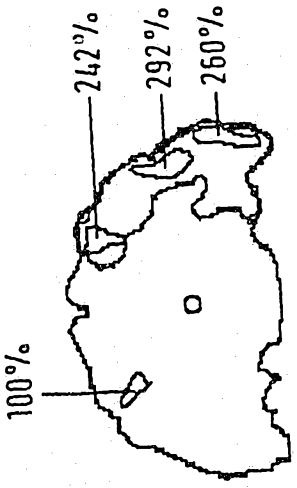


FIG. 22A



FIG. 23C

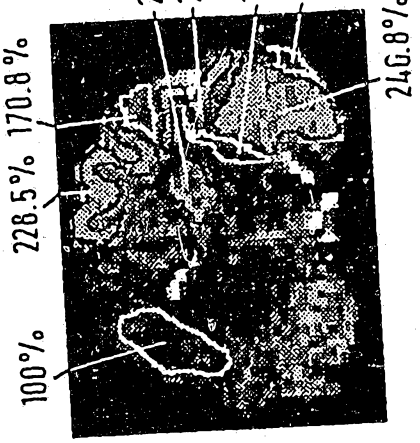


FIG. 23B



FIG. 23A



FIG. 24A

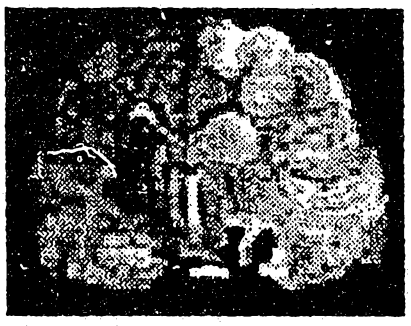


FIG. 24B

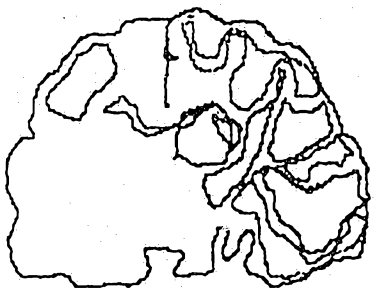
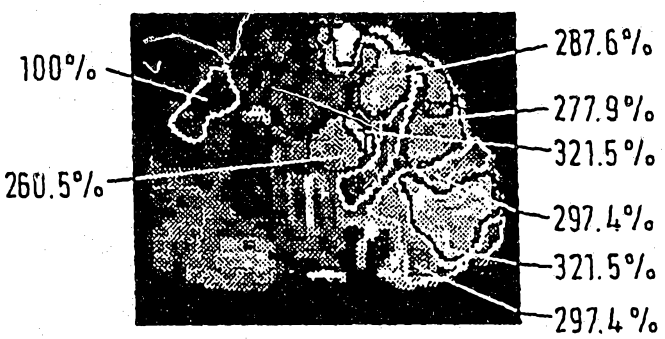


FIG. 24C



INTERNATIONAL SEARCH REPORT

International Application No PCT/EP 91/00443

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

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

IPC[5]: G 01 R 33/48, A 61 K 49/00

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC[5]	G 01 R, A 61 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X	Society of Magnetic Resonance in Medicine, 8th Annual Meeting, August 1989 Amsterdam, NL, Book of Abstracts, vol. 1, page 43, M.E. Moseley et al.: "Early detection of perfusion deficits induced by regional cerebral ischemia in cats: T2-weighted magnetic susceptibility MRI using a non-ionic dysprosium contrast agent", page 43, see the whole article --	1-10,15,18, 19,21,23
X	Society of Magnetic Resonance in Medicine, 7th Annual Meeting, August 1988 San Francisco, US, Book of Abstracts, vol. 1, page 222 J.W. Belliveau et al.: "Real-time proton susceptibility-contrast imaging of cerebral physiology", page 222, see the whole article	1-4,6,7,9-12,18
A	cited in the application --	19-21,24,26

./.

[10] Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

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

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

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

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
15th May 1991	12. 07. 91
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	Nuria TORIBIO

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, ** with indication, where appropriate, of the relevant passages	Relevant to Claim No.
X	Society of Magnetic Resonance in Medicine, 7th Annual Meeting, August 1988, San Francisco, US, Works in Progress, page 149, W. Cacheris et al.: "Magnetic susceptibility imaging with a nonionic contrast agent", page 149, see the whole article	1-9,18,20,24
A	cited in the application	19
	--	
A	Magnetic Resonance in Medicine, vol. 6, no. 2, February 1988, Academic Press, Inc. (Duluth, Mn, US) A. Villringer et al.: "Dynamic imaging with lanthanide chelates in normal brain: contrast due to magnetic susceptibility effects", pages 164-174, see pages 164-169; figures 1-3 cited in the application	1-7,9-13,15, 16,18-21
	--	
A	WO, A, 8909625 (J.R.M. COCKBAIN) 19 October 1989 see page 4, line 4 - page 9, line 27	1-8,26
	--	
A	Magnetic Resonance in Medicine, vol. 10, no. 2, May 1989, Academic Press, Inc. (Duluth, Mn, US) J.R. Ordidge et al.: "Snapshot imaging at 0.5 T using echo-planar techniques", pages 227-240, see pages 227-229, chapters "Introduction" and "Imaging Strategies"; figure 1	11-14

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. [X] OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. [X] Claim numbers .....22, because they relate to subject matter not required to be searched by this Authority, namely:

see PCT-Rule 39.1(iv): Methods for treatman of the human or animal body by surgery or therapy, as well as diagnostic methods.

2. [ ] Claim numbers....., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claim numbers....., because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. [ ] OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. [ ] As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

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

[ ] No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. EP 9100443 SA 45339

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 05/07/91
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A- 8909625	19-10-89	AU-A- 3359889	03-11-89
		EP-A- 0414700	06-03-91