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(54) Diagnostic kit for blood pool imaging and process for its preparation.

(57) This invention relates to a method for efficiently labelling red blood cells, which permits the imaging of blood pools in the intact animal or human patient, accomplished by injection of a saline solution of an alkaline earth metal salt of glucoheptonic acid and a non-toxic stannous salt followed by injection with a solution of sodium pertechnetate Tc 99m to tag the red blood cells.

The invention also relates to the kit used in the method of our invention, consisting of a single vial containing a lyophilized mixture of 25 mg. of calcium glucoheptonate and 3 mg. of stannous chloride dihydrate in 2 ml. of saline solution.

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TITLE MODIFIED

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TITLE OF THE INVENTION

Red Blood Cell Labelling Kit

BACKGROUND OF THE INVENTION

5 One of the methods employed in the past
to image the blood pool for diagnostic purposes
involves the intravenous administration of $99m$ Tc
serum albumin which is available commercially and is
a sterile pyrogen-free solution of albumin labelled
10 with Technetium $99m$ having an activity of greater
than 100 microcuries/ml.

In utilizing this method of blood pool
imaging, it is important to predose the patient
immediately prior to the attempted visualization of
15 the blood pool, since the $99m$ Tc serum albumin is
very rapidly lost from the blood stream by exchange
in the kidney.

Another method used in the past for imag-
ing blood pools is the in vitro labelling of red
20 blood cells (outside the host animal) followed by
rejection of the cells into the vascular system
of the selected animal. By this method, it is pos-
sible to visualize both heart blood pools plus major
peripheral vessels up to 3 hours after injection of
25 the labelled cells. As can be seen, this is a com-
plicated procedure, and therefore is a more time-
consuming and expensive method.

Still another procedure for imaging blood involves the injection of stannous pyrophosphate followed by injection of ^{99m}Tc -pertechnetate. This technique is reported as successful in producing satisfactory imaging of blood pools.

5 In accordance with the present invention, there is provided a diagnostic kit suitable for the radioactive labelling of red blood cells in vivo, thus making possible the imaging of blood pools within the circulatory system of the patient being examined. As an integral part of the
10 diagnostic kit of the present invention, there is also provided a novel chemical composition comprising a lyophilized mixture of a glucoheptonate salt and a pharmaceutically acceptable stannous salt in the ratio of 25 parts by weight of glucoheptonate salt to 3 parts by weight of stannous salt
15 measured as calcium glucoheptonate and stannous chloride dihydrate or approximately 22.9 parts of glucoheptonate ion to 1.32 parts of tin calculated as available stannous ion.

An important feature of the present invention is the provision of an individual diagnostic kit containing a non-
20 toxic stannous salt capable of supplying an amount of stannous ion equivalent to no more than 4.0 mg and preferably at least 2.5 mg of stannous chloride dihydrate. Less than 2.5 mg of the stannous chloride does not provide sufficient stannous ion to effect a satisfactory degree of labelling of
25 red blood cells. Because of the known toxicity of stannous salts, no more than 4 mg of stannous chloride dihydrate or a stannous salt containing an equivalent amount of stannous ion should be incorporated in an individual dosage kit.

The present invention also includes the process for the preparation of the novel chemical composition and the diagnostic method for imaging blood pools in patients suspected of having abnormalities in the circulatory system.

In accordance with the process of the present invention, a sterile solution of a non-toxic, pharmaceutically acceptable salt of glucoheptonic acid, e.g., calcium glucoheptonate, is mixed with a non-toxic stannous salt in a ratio of 25 parts by weight of glucoheptonate salt to 3 parts by weight of stannous salt, calculated as calcium glucoheptonate and stannous chloride dihydrate.

The solution is adjusted to a neutral pH 6-8, subdivided, and lyophilized to produce individual vials containing a dry, sterile mixture comprising 25 mg. calcium glucoheptonate and 3 mg. stannous chloride dihydrate.

In utilizing the kit of the present invention for imaging the blood pools of a patient to diagnose abnormalities in the cardiovascular system, a vial containing the dry, sterile mixture of calcium glucoheptonate and stannous chloride dihydrate is reconstituted by mixing with 2-8 ml. of a USP saline solution. The reconstituted solution is then used for injection of the patient to be examined. After a period of 30 minutes, a second injection of 2-8 ml. of a sterile saline solution of sodium pertechnetate is made. Following the injection of sodium pertechnetate, it is possible, after waiting from 30 seconds to 2 minutes, to image the blood pools in the patient being examined. This "in vivo" labeling of the red blood cells is exceptionally stable and approximately 95% of the radioactivity is retained

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by the red blood cells for at least 6 hours follow-
ing injection. This simple procedure avoids the
instability of the human serum albumin/^{99m}Tc injec-
tion of the prior art as well as the expense and
5 inconvenience of the in vitro labeling of the red
blood cells noted as an alternate prior art method.

EXAMPLE 1Blood Pool Imaging Kit

A solution is prepared under sterile conditions with 25 g. of calcium glucoheptonate in sterile, pyrogen-free water which has been purged with nitrogen. The solution of calcium glucoheptonate is stored and purged under nitrogen. In a separate container, 3 g. of stannous chloride dihydrate is dissolved in 1 ml. of hydrochloric acid, and the resulting solution diluted with nitrogen-purged, sterile water to a volume of 10 ml. The stannous chloride dihydrate solution is then added to the calcium glucoheptonate with stirring and flushing with nitrogen. The solution is mixed thoroughly, and the pH is adjusted to neutrality with a solution of sterile 1N sodium hydroxide solution. The volume is then adjusted to 2000 ml. with sterile, nitrogen-purged water and subdivided into vials, each containing 2 ml. of the solution. The vials are then lyophilized and sealed under nitrogen. Each vial contains 25 mg. of calcium glucoheptonate and 3 mg. of stannous chloride dihydrate.

EXAMPLE 2Method of Using Blood Pool Imaging Kit

A solution of sodium chloride for injection USP (2 ml.) is added to a vial containing a lyophilized mixture of 3 mg. of stannous chloride dihydrate and 25.0 mg. calcium glucoheptonate. The resulting solution is used for the intravenous injection of patients for the purpose of imaging the blood pools for diagnostic purposes. The amount of solution used is based on the weight of the patient and sufficient volume is used so that 30 mcg./kg.

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of stannous ion, measured as stannous chloride di-
hydrate, is injected. It is recommended that no
more than the contents of one vial be administered
to any patient. After waiting a period of 30 min-
5 utes, a sterile saline solution of sodium pertechn-
netate-Tc99m (2-20 mCi) is injected. This tags the
red blood cells and permits imaging of the blood
pools of the patient being examined almost immedi-
ately (from 30 seconds to 2 minutes).

10

CLAIMS

- 1 A diagnostic kit used for blood pool imaging comprising
a sterile sealed vial containing a lyophilized mixture of a
non-toxic pharmaceutically acceptable salt of glucoheptonic
acid and a non-toxic pharmaceutically acceptable stannous
5 salt in a ratio of 22.9 parts by weight of glucoheptonate
ion to 1.32 parts of stannous ion, provided that the
individual dose of stannous ion does not exceed 4 mg,
calculated as stannous chloride dihydrate.
- 2 A kit according to claim 1 in which the salt of
10 glucoheptonic acid is calcium glucoheptonate and the
stannous salt is stannous chloride dihydrate.
- 3 A kit according to claim 2 in which the lyophilized
mixture comprises from 2.5-4 mg of stannous chloride
dihydrate and 25 mg of calcium glucoheptonate.
- 15 4 A kit according to claim 3 that comprises a lyophilized
mixture of 3 mg of stannous chloride dihydrate and 25 mg of
calcium glucoheptonate.
- 5 A process for the preparation of a blood pool
imaging kit that comprises preparing a sterile aqueous
20 solution of a water-soluble non-toxic pharmaceutically
acceptable salt of glucoheptonic acid and a non-toxic
pharmaceutically acceptable stannous salt, subdividing the
said solution into individual dose vials under nitrogen,
and lyophilizing and sealing the said vials under nitrogen,
25 in which the individual dose of stannous ion does not
exceed 4 mg, calculated as stannous chloride dihydrate,

and there is 22.9 parts by weight of glucoheptonate ion to 1.32 parts of stannous ion.

6 A process according to claim 5 in which the
glucoheptonate salt is calcium glucoheptonate and
5 the stannous salt is stannous chloride dihydrate.

7 A process according to claim 6 in which the
individual dose vials contain from 2.5-4 mg of stannous
salt calculated as stannous chloride dihydrate and 25 mg
of calcium glucoheptonate.

10 8 A process according to claim 7 in which the
individual dose vials contain 3 mg of stannous salt,
calculated as stannous chloride dihydrate, and 25 mg
of calcium glucoheptonate.

9 A method of imaging blood pools in patients
15 suspected of having cardiovascular abnormalities which
comprises the steps of

- (1) intravenous injection of a solution comprising
a water soluble, non-toxic, pharmaceutically
acceptable salt of ketoglucoheptonic acid and a
20 water soluble non-toxic stannous salt in the
ratio set forth in claim 1, provided that the individual
dose of stannous ion does not exceed 4 mg, calculated
as stannous chloride dihydrate,
- (2) waiting a period of 30 minutes; and
- 25 (3) intravenous injection of a sterile saline solution
containing from 2-20 mCi of sodium pertechnetate-
Tc99m.

10 A method according to claim 9 in which the glucoheptonate salt used is calcium glucoheptonate and the stannous salt is stannous chloride dihydrate.



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PARTIAL EUROPEAN SEARCH REPORT

which under Rule 45 of the European Patent Convention shall be considered, for the purposes of subsequent proceedings, as the European search report

Application

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DOCUMENTS CONSIDERED TO BE RELEVANT		CLASSIFICATION OF APPLICATION (Int. Cl.)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim
X	<p>US - A - 4 027 005 (NORMAN ADLER et al)</p> <p>* Column 4, lines 38 to 50; column 6, lines 24 to 29; line 49 to column 7, line 10 *</p>	1,5
	<p>JOURNAL OF NUCLEAR MEDICINE, vol. 16, pages 357 to 367 (1975)</p> <p>* Pages 358, 359 "Materials and methods" *</p>	1,5
A	<p>JOURNAL OF NUCLEAR MEDICINE, vol. 17, pages 203 - 207 (1976)</p> <p>* Page 203, right-hand column, last paragraph *</p>	1
A	<p>JOURNAL OF NUCLEAR MEDICINE, vol. 16, page 875 to 878 (1975)</p> <p>* Page 875, left-hand column, last paragraph *</p>	1
A	<p>CHEMICAL ABSTRACTS, vol. 85, Ref 137277v (1976)</p> <p>* Abstract *</p>	1
INCOMPLETE SEARCH		TECHNICAL FIELDS SEARCHED (Int. Cl.)
<p>The Search Division considers that the present European patent application does not comply with the provisions of the European Patent Convention to such an extent that it is not possible to carry out a meaningful search into the state of the art on the basis of some of the claims.</p> <p>Claims searched completely:</p> <p>Claims searched incompletely:</p> <p>Claims not searched: 9,10 (Diagnostic method practised on the human or animal body.)</p> <p>Reason for the limitation of the search:</p>		<p>A 61 K 43/00</p> <p>A 61 K 43/00</p>
		CATEGORY OF CITED DOCUMENT.
		<p>X: particularly relevant</p> <p>A: technological backg</p> <p>O: non-written disclos</p> <p>P: intermediate docum</p> <p>T: theory or principle of the invention</p> <p>E: conflicting applicati</p> <p>D: document cited in t application</p> <p>L: citation for other re.</p>
		<p>&: member of the sam family, corresponding doc.</p>
Place of search	Date of completion of the search	Examiner
	12 02 1979	DRD

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DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int. Cl. 7)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
	<p>PHENOL ABSTRACTS, vol. 36, Ref 1150 (1977) * abstract *</p> <p>-----</p>	1	
			TECHNICAL FIELDS SEARCHED (Int. Cl. 7)