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[54] **LABELLED SULFATED AMYLOPECTINS AND METHOD OF DETERMINING ABNORMAL GASTROINTESTINAL MUCOSA**

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[63] Continuation-in-part of Ser. No. 52,707, July 6, 1970, abandoned.

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[58] **Field of Search** ..... 424/1, 7, 9, 180; 250/106 T, 71.5 S; 260/209.5; 23/203 B

[56] **References Cited**

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[57] **ABSTRACT**

Described herein are dye and radionuclide labelled anionic polysaccharide compositions, useful as diagnostic aids in the qualitative and quantitative detection in mammals of abnormal mucosa, particularly of the gastrointestinal variety, a process for their preparation and a method for their use. Preferred embodiments are the compositions of: sodium amylosulfate combined with technetium-99m and sodium amylosulfate combined with methylene blue.

16 Claims, No Drawings

LABELLED SULFATED AMYLOPECTINS AND METHOD OF DETERMINING ABNORMAL GASTROINTESTINAL MUCOSA

This application for Letters Patent is a continuation-in-part of Applicant's prior copending application, Ser. No. 52,707 filed July 6, 1970, now abandoned.

The detection and accurate measurement of abnormal mucosa (i.e. mucous membrane), in mammals, especially humans, can be an invaluable aid to the medical practitioner in the diagnosis of a great number of disorders associated with the presence of such abnormal mucosa. Illustrative of such disorders are gastric and duodenal ulcers, carcinoma, benign lesions comprehending those with diffuse mucosal changes frequently interpreted as gastritis, oesophagitis, pre-ulcer, ulcerated colitis and similar pathological conditions. In the past, the most common method employed for the diagnosis of ulcers and stomach carcinoma was barium radiology including the familiar barium meal followed by X-ray examination. Unfortunately, the barium method suffers from several drawbacks including the well known dangers of X-ray examination, and inaccuracy and inconvenience in usage. For example, the barium method is not susceptible of visualizing ulcers unless gross changes have occured in the mucosa and the ulcerated area is susceptible of planar visualization. Consequently, it is difficult to detect gastric ulcers because it is frequently impossible to assess the dimensional aspects of the depth and angles of the abnormal area upon barium radiological examination. Conversely, in certain subjects it has been found that barium radiology has shown abnormally deep rugal folds, particularly on the greater curvature of the body of the stomach, supposedly indicative of gastric ulcers, whereas the presence of ulceration could be confidently excluded from the areas suspected upon appropriate fibroscopic examination, particularly utilizing as a diagnostic aid the labelled compositions of this invention. In the case of X-ray examination of the type employed in the barium meal method, i.e. where the radiological source of energy is outside the subject, in contradistinction to the source being within the subject as hereafter described, there is the ever present danger of excess radiation which can be harmful to both the radiological technician and the subject, being particularly acute in the case of pregnant patients.

The object of this invention is to provide a safe, simplified, highly accurate, and convenient method for the detection in mammals of abnormal mucosa, particularly of the gastrointestinal variety, as well as a labelled composition suitable for use in this method. According to this method, a labelled polysaccharide composition, which binds strongly and differentially to mucosa, is administered to the mammal and timely subsequent examination of the mammal by a suitable visualizing technique provides definitive detection and quantification of abnormal mucosa present in the tested area.

Polysaccharides suitable for the formulation of the compositions of this invention are anionic polysaccharides having a high charge density, conveniently of the order of 0.97×10^{-6} to $0.50 \times 10^{-6}\ \overline{Z}_{OH}/(A°)^3$ (negative charge per Angstrom^3) which characteristically bind differentially to mucosa and include sulfated and phosphorylated polysaccharides having a molecular weight of at least 1,000,000. Among such sulfated polysaccharides are sulfated starch, sulfated mequite gum, sulfated psyllium seed, sulfated amylopectin, sulfated cellulose, sulfated amylose and sulfated hyaluronic acid. Among the sulfated polysaccharides having specifically desirable application in this invention are those containing one to two sulfate groups per monosaccharide unit, for example per glucose unit in the case of starch, starch fractions, and particularly corn and potato amylopectin. It has been observed that polysaccharides lacking a high charge density lack the ability of the polysaccharide component of the instant compositions to differentially and strongly bind to mucosa, particularly without causing deleterious side effects to the subject, for example degraded carrageenin. Particularly preferred sulfated polysaccharides for use in this invention are the alkali metal, and especially sodium, salts of sulfated potato starch amylopectin, said alkali metal salts possessing about 1–1.8 sulfate groups per glucose unit and characterized also by a molecular weight of about $1–30 \times 10^7$. (The expression "molecular weight" as used in this application refers to weight average molecular weight.) A very preferred polysaccharide is the sodium salt of sulfated potato starch amylopectin possessing substantially 1.6 sulfate groups per glucose unit and characterized by a molecular weight of about 6.3×10^7, which material is known by the non-proprietary name "sodium amylosulfate". The preparation of the aforementioned alkali metal salts of sulfated potato starch amylopectin and in particular sodium amylosulfate, are described in U.S. Pat. No. 3,271,388, issued Sept. 6, 1966. The latter sulfated polysaccharides are preferred components of the labelled compositions of this invention because of their superior binding properties which promote not only their uniform binding with the labelling agent, but also their differential adhesion to mucosa, which most importantly enables the resultant labelled compositions of this invention to be characterized by this same advantageous binding property permitting optimal physiological and diagnostic results. Physiologically, for example, the labelled composition of this invention is not only safe, in view of its low toxicity and negligible absorption from the gastrointestinal tract, but simultaneously provides therapeutic treatment for the abnormal area as well as amazingly accurate visualization of the abnormality.

The choice of an appropriate labelling agent for use in the instant composition is dependent upon numerous factors which affect the diagnostic effectiveness and physiological suitability of the labelling agent and the ultimate composition. Moreover, each of these critical factors must be coordinated with the selection of polysaccharide and visualizing instrument which is to be employed. Consequently, considering the magnitude and complexity of choices available, the safe, simplified, highly accurate and convenient labelled composition provided by this invention is most unexpected against the background of known materials which have been employed for such purposes previously. Throughout this application the expression "diagnostically effective" as applied to the labelling agent of the instant composition shall refer to that combination of properties, discussed with particularity hereinafter with respect to both the dye and the radionuclide labelling agents, which enable the agent, as well as the composition in which it is incorporated, to permit definitive detection and quantification of mucosal abnormalities by facilitating a satisfactory visualization of the abnormality by the imaging instrument employed. The expression "physiologically suitable" as applied to the label-

ling agent of the instant composition refers to those properties discussed with particularity hereinafter with respect to both the dye and radionuclide labelling agents, which enable the agent, as well as the composition in which it is incorporated to produce a diagnostically effective response while assuring patient and technician safety. It has been found that in view of their respective properties, certain radionuclides and certain dyes can meet the stringent requirements of "diagnostically effective" and "physiologically suitable" and consequently constitute the appropriate labelling agents of the instant compositions.

Specifically, in the selection of an appropriate dye for use in this invention from the standpoint of diagnostic effectiveness, one must ascertain that they exhibit strong light absorption and a capacity to provide the desired contrast. Suitably such properties are present in materials having at least 1/10 the light absorption as does methylene blue. As a measure of its absorption, it is noted that methylene blue has a molecular extinction coefficient of 80,000 at 658 millimicrons in water. In assessing physiological suitability, appropriate dyes are those which have been found to substantially lack toxicity in mammals at diagnostic doses, i.e. doses sufficient to permit visualization of the abnormality upon endoscopic examination, and which chemically combine with the selected polysaccharide to form a uniformly stained composition. Such dyes include basic aniline dyes, including azins (e.g., phenosafranine, methylene violet, diethylphenosafranine, etc.), pyronins (e.g., acridine red 3B, pyronin B, etc.), oxazins (e.g., brilliant cresyl blue, cresyl fast violet, nile blue [sulfate]), basic azo dyes (e.g., Janus green, indazole blue, Bismark brown, etc.), triphenyl- and diphenylnaphthylmethanes (e.g., malachite green, pararosaniline chloride, pararonsaniline acetate, methyl violet, ethyl green, Victoria blue, etc.), phthalocyanines (e.g., alcian blue, luxol fast blue, etc.), and preferably the thiazins (e.g., thionin, azure A, B and C, methylene green, toluidine blue and especially methylene blue). Other polysaccharide staining dyes can also be used including fluorochrome stains such as the fluoresceins, fat stains such as sudan black B, the tetrazoles (e.g., blue tetrazolium), and food dyes such as F. D. and C. No. 1 (food and drug color No. 1). As indicated, the thiazins are the preferred dyes for the dye labelling component of the instant diagnostic compositions, with methylene blue being most preferred, in view of their suitable extinction coefficient, which is both strong as well as providing the desired contrast, and their substantial lack of toxicity. A very preferred embodiment of this invention comprises the labelled composition containing preferably no greater than 2 percent, but conveniently within the range of about 0.01 to 5.0 percent, by weight of methylene blue and preferably no less than 90 percent by weight of sodium amylosulfate. Said very preferred embodiment is especially advantageous as a diagnostic reagent in comparison with methylene blue used alone because of the former's superior binding qualities. That is, the intensity of color in the stained area is greater and persists longer than methylene blue when employed as a stain apart from the instant composition.

The choice of an appropriate radionuclide for use as an instant labelling agent is likewise dependent upon its ability to be diagnostically effective and physiologically suitable. With respect to a radionuclide, the term "physiologically suitable" comprehends the properties of: (1) short half life, (2) essentially pure gamma emission and no substantial emission of beta-particles, and (3) chemical compatibility with the selected polysaccharide such that negligible absorption of the resultant composition occurs from the gastrointestinal tract. The importance of a short half life, that is one no greater than 60 days and more preferably of considerably low magnitude such as 2–24 hours, stems from the fact that the radiation dose to the patient should be an intense burst of high energy in order to maximize effectiveness and safety, since high energy radiation is better tolerated by mammals than low energy radiation. The use of an essentially pure gamma emittor provides an additional safety factor because it has been observed that radiation damage is frequently the result of the absorption of beta emissions, rather than gamma emissions. Generally gamma energy of 0.03–2 Mev is desirable for an optimal and safe result. Among those characteristics maximizing the diagnostic effectiveness of a radionuclide labelling agent is its chemical ability to bind irreversibly to the polysaccharide carrier. Such property is essential to an effective diagnostic aid since less than essentially quantitative and irreversible combination would provide: (1) an aggregate of label in the composition which would not permit accurate visualization of the abnormality, or (2) loosely bound or unbound radionuclide or nuclide labelled composition which would result in the loss of the material throughout the body of the mammal being studied and, perhaps, necessitate the administration of an unusually high dosage of of labelled composition in order to achieve any useful visual result. For obvious reasons of safety an unusually high radiation dosage is to be avoided. A significant factor which must be considered in the selection of a diagnostically effective radionuclide for use in the instant labelled composition is its ability to be visualized on existing instrumentation. Many types of radiation imaging equipment provide varying tolerances of isotope energy, most commonly in the range of 20–2000 Kev, though this wide range is not necessarily available on any one instrument currently marketed. Among possible radionuclides for use in this invention are iodine-131, iodine-125, iodine-123, indium-111, indium-113m, iodine-132, indium-114m, ytterbium-169, gallium-67, dysprosium-157, mercury-203, mercury-197, gold-198, bismuth-204, chromium-51 and most preferred, in view of its short half life, ideal lack of beta emission and ability to be imaged on both rectilinear scanners and scintillation cameras, is technetium-99m. Additional radionuclides, which because of unusually long half life, failure to be adequately imaged on present detectors, etc. are less desirable, though nevertheless of potential usefulness as advances in radiation imaging instrumentation may provide better energy resolution enabling use of lower dosages of radionuclides, include selenium-75, gallium-68, cobalt-57, samarium-153, and lutetium-177.

The dye labelled compositions can be manufactured by mixing the dye with the selected polysaccharide in a suitable medium followed, if desired, by dialysis of the resulting product to enhance its purity. Alternatively other conventional methods of staining carbohydrate materials may be employed.

The radionuclide labelled compositions of this invention can be prepared by methods analagous to those used for the labelling of proteins, such as albumin, and

fats such as triolein, as well as other radiolabelled carbohydrate materials known in the art. As an illustration of such a procedure the preferred polysaccharide material of this invention, sodium amylosulfate, was combined with ^{125}I-sodium iodide in a suitable solvent such as acetone, in the optional presence of an oxidizing agent, such as (N-chloro-p-toluenesulfonamido) sodium. After stirring and cooling, the resultant composition is optionally dialyzed against water to provide greater purity of the ^{125}I-sodium anylosulfate composition product thereof. The technetium-99m labelled compositions of this invention are most conveniently prepared by utilization of a reducing agent such as sodium borohydride, ascorbic acid, a source of stannous ion, illustratively stannous chloride, or most preferably a source of ferrous ion, such as ferric chloride and ascorbic acid or ferrous chloride. The most preferred procedure entails the addition of a few drops of hydrochloric acid to a saline solution of ^{99m}Tc-sodium pertechnetate, followed by the addition of a reducing agent such as ferric chloride coupled with ascorbic acid. The mixture is thereafter permitted to stand for several minutes, after which time upon adjustment of pH, the desired sulfated polysaccharide, such as sodium amylosulfate, is added to the reaction mixture, and the pH suitably adjusted to provide the desired technetium-99m labelled sodium amylosulfate. Any unbound technetium-99m may be removed by dialysis or by passing a solution of the product through an ion exchange or gel filtration column.

As indicated above, the selection of radionuclide labelling agent for incorporation in the composition of this invention is dependent upon the visualizing instrument which is to be used for the detection of abnormal mucosa. Described hereinafter are several types of radiation imaging devices currently in use, each of which can be used with a least one or more of the instant radionuclide labelled compositions. Specifically, the technetium-99m labelled compositions of this invention are detectable on all of the instruments discussed hereinafter and consequently these radionuclide labelled polysaccharides are particularly preferred embodiments of this invention. Two basic types of radiation imaging devices are rectilinear instruments or scanners, whose detecting heads move back and forth over the scanning area; and scintillation cameras, which are characterized by their ability to produce a unitary image of the object under study at any given moment.

With respect to the rectilinear scanner, the concentration of radionuclide in the target organ is outlined by a corresponding pattern of dots. Various black and white printout systems are often employed. However, systems in which the color of the printout changes with the count rate may be of particular value when scanning areas of gradual change in concentration. A typical rectilinear scanner is Nuclear-Chicago Corporation's PHO/DOT Isotope Scanner, which automatically produces a display of the location and concentration of isotopelabelled compositions within selected organs or areas of the body. Data is recorded on X-ray film by a photorecording system (photoscans) and is also printed on paper by a dot recording system (dotscans). The PHO/DOT has the ability to visualize isotopes within the energy range of 20–2000 Kev.

The scintillation camera, commonly known as the Anger camera, is described in its basic embodiment in U.S. Pat. No. 3,011,057. Such camera devices have the feature of viewing at least a substantial portion of the object under study at any one time, thus significantly reducing examination time in comparison to rectilinear scanners. A typical scintillation camera, Nuclear-Chicago Corporation's PHO/GAMMA Scintillation Camera, includes an image or detector head containing a sodium iodide thallium activated scintillation crystal and a matrix of photomultiplier tubes; interchangeable collimators (multiaperture or pinhole); and image data computation, display and control modules. The distribution of radionuclides is often imaged on Polaroid film (scintiphotos). The PHO/GAMMA instrument has the ability to visualize isotopes within the energy range of 50–680 Kev.

Very recent improvements of the scintillation camera are those having tomographic imaging capabilities. These involve various additions to the basic Anger camera system such as those described in Canadian Pat. Nos. 872386 and 872387, in German Offenlegungsschrift No. 2011164 and in an article by G. Muehllehner, "A Tomographic Scintillation Camera", *Physics in Medicine and Biology*, Vol. 16., No. 1, pp. 87–96 (1971). Nuclear-Chicago Corporation's PHO/GAMMA Tomocamera^{tm} Accessory System comprises the conventional PHO/GAMMA Scintillation Camera, a rotating slanted hole collimator assembly, a floating-top radiographic table and an associated operating control module, and has the ability to image data from four distinct parallel enatomical planes in a single display.

The instant compositions can be combined with pharmaceutically acceptable carriers suitable for the administration of such a diagnostic aid. Obviously, the chemical properties of the particular labelling agent chosen for use in the composition will, in practice, limit the type of carriers with which it can be combined. For example, the instant technetium-99m composition, because of the short half life of technetium-99m, can most conveniently be used as a liquid of relatively few ingredients, since compounding time for complicated formulations would reduce the usefulness and practicality of the end product. The term "pharmaceutically acceptable carrier" as used herein in reference to the compositions of this invention means a solid or a liquid composed of a single substance or a number of substances which may be solids, liquids or a combination of solids and liquids each of which is less toxic than an equal weight of the active ingredient present in the composition when measured in the same mammalian host using the same method and conditions of administration. The concentration of the active ingredient in the composition is not critical, but for economy of preparation, when a dye is the labelling component, the concentration should be at least 0.5 percent by weight and preferably 1–80 percent. An illustration of a suitable radionuclide composition is that containing 0.5 mg to 10 mg. of sodium amylosulfate per kilogram weight of the mammalian patient species (1.7mg./kg. to 5mg./kg. being the more preferred range) and 0.1 to 10 mCi "milliCuries" of a radionuclide such as technetium-99m (more preferably in the amount of 2 milliCuries). These compositions can be administered either orally or parenterally. For oral administration, tablets, lozenges, capsules, dragees, pills and powders are suitable, while aqueous and non-aqueous solutions or suspensions are appropriate for both oral (e.g. in the case of radionuclide labelling agents via a stomach tube)

and parenteral (e.g. rectal) administration. Acceptable pharmaceutical carriers are exemplified by chewable tablets, sugars such as lactose or sucrose, starches such as corn starch or potato starch, cellulose derivatives such as sodium carboxymethyl cellulose, ethyl cellulose, methyl cellulose or cellulose acetate phthalate, talc, calcium phosphates such as dicalcium phosphate or tricalcium phosphate, sodium sulfate, calcium sulfate, polyvinyl alcohol, acacia, stearic acid, alkaline earth metal stearates such as magnesium stearate, vegetable oils such as peanut oil, cottonseed oil, sesame oil, olive oil, corn oil, and oil of theobroma, water, agar, alginic acid, benzyl alcohol, isotonic saline and phosphate buffer solution as well as other non-toxic compatible substances used in pharmaceutical formulations. Some typical formulations as well as the method of preparation thereof, are presented below.

Formulation I

Liquid Composition Containing 2% Methylene Blue: Sodium Amylosulfate

Ingredient	Amount (Conc. 250 mg/5 ml.)	Amount (Conc. 500 mg/5 ml.)
Composition of methylene blue with sodium amylosulfate (in a ratio of preferably 2:100, respectively)	250 mg.	500 mg.
Distilled Water	5 ml.	5 ml.

To the composition of methylene blue with sodium amylosulfate was added slowly, with stirring, 100 ml. of distilled water. To this slurry was added, with stirring, sufficient distilled water to bring the mixture to the volume of 1 liter and stirring was continued until solution was complete. The solution was then poured into amber bottles of the desired quantity, for example, 5 milliliter portions, sampled and assayed.

Formulation II

Chewable Tablet Composition Containing 2% Methylene Blue: Sodium Amylosulfate

Ingredient	Amount Per Tablet	Amount Per Batch of 1000
Composition of methylene blue with sodium amylosulfate (in a ratio of preferably 2:100, respectively)	500 mg.	500 g.
Dextrose USP, anhydrous	1.66 mg.	1660 g.
Polyvinylpyrrolidone	67.4 mg.	67.4 g.
Hydrogenated cottonseed oil	22.6 mg.	22.6 g.
Flavor (e.g. Florasynth entrapped black cherry flavor)	1.76 mg.	1.76 g.

500 Grams of the composition of sodium amylosulfate with 2 percent methylene blue (powder form) was blended with 1660 grams of dextrose, and 67.4 grams polyvinylpyrrolidone in a large bowl for 5 minutes. That mixture was thereafter comminuted at high speed, remixed, granulated with 300 ml. of ethanol, and dried for 4 hours at 60°C. After oscillation through a No. 12 screen, followed by the addition of 22.6 grams of hydrogenated cottonseed oil and 1.76 grams of Florasynth entrapped black cherry flavor, the mixture was blended for 5 minutes and a sample was taken for assaying. Thereafter it was compressed into tablets of the appropriate size.

The method of this invention for the detecting in mammals of abnormal mucosa, particularly of the gastrointestinal variety, comprises the administration of a diagnostic dose of the instant novel diagnostic composition. The term "diagnostic dose" is defined as the amount of active ingredient that will adequately adhere to the mucosa and effect labelling thereof sufficient to permit detection upon examination, endoscopically in the case of dye labelling agents and by radiation imaging devices in the instance of radionuclide labelling agents. This dose will vary with the particular labelling agent chosen, the type and location of the particular disorder being diagnosed, the route of administration, the particular polysaccharide used, and the subject's physical characteristics, e.g. body weight and physiological state such as hypersecretory. A typical diagnostic dosage for a subject of about 22 to about 136 kg. body weight for the composition of sodium amylosulfate with up to 2 percent methylene blue is 100 to 500 milligrams, administered about ¼ hour prior to endoscopic examination for gastric ulcer. For a subject of identical weight and disorder, 2 milliCuries of techetium-99m combined with 250 mg. of sodium amylosulfate, administered 1–2 hours prior to examination with a radiation imaging device, would be a typical diagnostic dosage.

Application of the instant method to the diagnosis of abnormal gastric mucosa, particularly ulcers, has been found to be especially effective when the preferred dye labelled composition of this invention, i.e. sodium amylosulfate with no greater than 2 percent by weight methylene blue, is administered to the patient 15 minutes prior to endoscopic examination employing a gastroscope (i.e. a hollow tubular instrument designed to pass into the stomach by way of the mouth and esophagus and fitted with optical and lighting equipment that permits visual inspection of the stomach) to which a camera suitable for color photography has been affixed. As will be apparent to those skilled in endoscopic identification, the light source for the gastroscope may be visible or ultraviolet lighting dependent upon the particular dye employed in the composition. In a very preferred embodiment of this invention, methylene blue, which may be viewed by visible light, serves as the dye.

In one clinical illustration of this very preferred method and diagnostic aid of this invention, a human subject, previously known to possess an ulcer as diagnosed by barium radiology, was administered 5 ml. of

an aqueous solution containing 250 mg. of the composition comprising 2 percent methylene blue with sodium amylosulfate. Fifteen minutes later, gastroscopic examination of the patient accompanied by color photography of the area in question revealed the ulcer. The visualization of the ulcer, both to the naked eye and in the color photographs, was marked by sharpness of color, namely bright blue, at the edges of the ulcer crater, while the crater was differentially deprived of such color. Moreover, the shape and character of the ulcer as well as an indication of depth was apparent upon inspection. Thus, in contract and preference to X-ray pictures produced via the barium meal method, wherein the crater itself is often poorly defined or distorted, the shape and character of the ulcer or other abnormality in the mucosa is easily detected and quantified. Hence the instant diagnostic composition and method are particularly useful in detecting aberations of gastric mucosa since the characteristics of the stomach (its asymmetry, flabbiness and variability in shape and motility) make it difficult to examine by radiological techniques employing a source of radioenergy ouside the subject, such as the barium meal method. Most effective and desirable results are obtained when the radioenergy source is placed within the subject, as with the instant compositions wherein the labelling agent is a radionuclide, most preferably technetium-99m, as illustrated in Examples 3 to 11 described hereinafter.

The following examples are given for the purpose of illustrating the preparation of the instant diagnostic composition according to the present invention. It will be understood that the invention is not to be construed as limited in spirit or in scope by the details contained therein, as many modifications in materials and methods will be apparent from this disclosure to those skilled in the art. In the following examples, temperatures are given in degrees Centigrade (°C.)

EXAMPLE 1

A 1 percent aqueous solution of sodium amylosulfate (i.e., the sodium salt of sulfated potato starch amylopectin, possessing substantially 1.6 sulfate groups per glucose unit, characterized by molecular weight of about 6.3×10^7 and having been prepared according to the method described in Example 4 of U.S. Pat. No. 3,271,388) was placed inside a dialysis bag, and the bag was then placed inside a container having a 0.5 percent solution of methylene blue (U.S.P.) in water. The ratio of methylene blue solid, in the outer compartment, to sodium amylosulfate solid, in the inner compartment, was 2/100. The two solutions were allowed to equilibrate for 24 hours, after which time practically all the methylene blue had diffused into the dialysis bag containing the sodium amylosulfate solution. At the end of this time, the dialysis bag was removed from the exhausted methylene blue outer solution and placed in a large container of hydrochloric acid at pH 2, where it was dialyzed for 24 hours. No detectable methylene blue was removed by 24 hour dialysis against the acid solution, proving the irreversible binding of methylene blue to sodium amylosulfate under acid conditions analogous to that of gastric juice. At the end of the acid dialysis period, the contents of the dialysis bag were neutralized to a pH of 7.4 with sodium hydroxide and lyophilized.

Alternatively, upon completion of the 24 hour equilibration described above, the dialysis bag containing the sodium amylosulfate-methylene blue solution was removed from the exhausted methylene blue outer solution and was adjusted to a pH of 7.8 with sodium hydroxide and the solution was thereafter lyophilized for 72 hours. The lyophilized material was first extracted with ethanol and then with redistilled acetone, followed by filtration. The resultant filtercake was dried in vacuo for 48 hours to provide a composition, identical with that prepared in the preceding paragraph containing 2 percent methylene blue with about 93 percent sodium amylosulfate, which composition is characterized by ultraviolet absorption maxima at about 246, 285 and 575 millimicrons. It will be observed that the methylene blue component individually displays ultraviolet absorption maxima at about 609 and 667 millimicrons. Thus by comparison with the maxima described above for the prepared composition, this metachromatic behavior is evidence that the composition consists of a unique composition of matter distinguishable from its component, methylene blue.

EXAMPLE 2

A 0.1 percent solution of methylene blue in water was pumped slowly, over a 48 hour period, at a rate of 0.1 ml./min., into a rapidly stirred 1.0 percent solution of the alkali metal salt of sulfated potato starch amylopectin possessing 1–1.8 sulfate groups per glucose unit and being characterized also by a molecular weight of $1–30 \times 10^7$ in water. After the solutions were combined, the dyed salt solution was dialyzed against acid, then against water, and finally lyophilized and purified as described in the above example to provide a composition containing 2 percent methylene blue and an alkali metal salt of sulfated potato starch amylopectin possessing 1–1.8 sulfate groups per glucose unit and being characterized also by a molecular weight of $1–30 \times 10^7$.

EXAMPLE 3

To a suspension of 503 mg. of sodium amylosulfate in 10 ml. of acetone was added a solution of 1 mCi of carrier-free ^{125}I-sodium iodide in 10 ml. of acetone. To that mixture was then added 10 μl. of an aqueous solution of Chloramine-T, i.e. (N-chloro-p-toluenesulfonamido)sodium [concentration = 2 mg./ml.], and the resultant mixture was stirred at 60°C. for about 2 hours, then allowed to cool to room temperature over a period of 1 hour. The mixture was diluted with approximately 50 ml. of water and then dialyzed against three 5.5 liter portions of water during a period of 4 days, using cellulose dialyzer tubing. The contents of the tubing were then lyophilized, affording approximately 0.45 g. of ^{125}I-sodium amylosulfate composition having a specific activity of 18.9 μCi/g.

A solution containing the ^{125}I-sodium amylosulfate product described above in 50 ml. of water was administered intragastrically to a 7.8 kg. female dog (Beagle). After waiting 5 minutes, the stomach region was visualized by means of a Nuclear-Chicago Corporation

PHO/DOT Isotope Scanner. The dot and photo scans demonstrated the binding effect of ^{125}I-sodium amylosulfate to the muscosa observed.

EXAMPLE 4

To a suspension of 503 mg. of sodium amylosulfate to 10 ml. of acetone was added a solution of 1 mCi of carrier-free ^{125}I-sodium iodide in 10 ml. of acetone. That mixture was stirred at 60°C. for about 2 hours, then allowed to cool to room temperature over a period of 1 hour. The resultant mixture was transferred to dialysis tubing with approximately 2 ml. of acetone and 60 ml. of water and then dialyzed against 5.5 liters of water for about 2 hours. The contents of the tubing were lyophilized. There were obtained approximately 409 mg. of ^{125}I-sodium amylosulfate composition having a specific activity of 18.0 μCi/g.

EXAMPLE 5

501.5 Mg. of sodium amylosulfate, 5.1 ml. of water containing 1 mCi of carrier-free ^{131}I-sodium iodide, and an aqueous solution of 4.4×10^{-11} millimoles of Cloramine-T were combined and the required amount of water needed to bring the total amount of water to 50 ml. was added. The resulting mixture, containing ^{131}I-sodium amylosulfate, was administered intragastrically to a 7.8 kg. dog (Beagle) and the stomach region was visualized by means of a Nuclear-Chicago Corporation PHO/GAMMA Scintillation Camera. Scintiphotos were taken over a period of 2½ hours. Binding to the gastrointestional mucosa was observed throughout the length of the visualization period.

EXAMPLE 6

A solution of 3.69 mCi of carrier-free ^{99m}Tc-sodium pertechnetate in 2 ml. of saline (obtained by elution from a ^{99}Mo-^{99m}Tc generator) was acidified with 3 drops of 2 N aqueous hydrochloric acid solution. To that solution were added 10 mg. of ferric chloride hexahydrate, followed by 8 mg. of ascorbic acid. The acidity of the resulting solution was adjusted to approximately pH 5 by addition of 14 drops of 1N aqueous sodium hydroxide solution. 500 Mg. of sodium amylosulfate were then added and the mixture was stirred for about 5 minutes. At the end of that time, the mixture was passed through a small Sephadex chromatographic column in order to remove free technetium. The first 25 ml. of eluate were discarded and the next 50 ml. were collected. The latter eluate contained ^{99m}Tc-sodium amylosulfate with an activity, corrected to zero time, of 0.0426 mCi/ml. (total radioactivity = 2.1 mCi). 43 Ml. of that solution (radioactivity = 1.8 mCi) were administered to a 7.8 kg. dog (Beagle) for gastrointestinal visualization using a Nuclear-Chicago Corporation PHO/GAMMA Scintillation Camera. Scintiphotos were taken beginning at about 4 minutes after administration and continuing for 3½ hours. Binding to the gastrointestinal mucosa was observed throughout the length of the visualization period.

EXAMPLE 7

A solution of 3.4 mCi of carrier-free ^{99m}Tc-sodium pertechnetate in 2 ml. of saline (obtained by elution from a ^{99}Mo-^{99m}Tc generator) was stirred with 500 mg. of sodium amylosulfate in 50 ml. of water for 5 minutes. The resultant mixture was poured into dialysis tubing and dialyzed against three 5.5 liter portions of water during a period of about 5½ hours. There was thus obtained 25 ml. of a ^{99m}Tc-sodium amylosulfate composition having a total radioactivity, corrected to zero time, of about 0.12 mCi.

EXAMPLE 8

A solution of 3.4 mCi of carrier-free ^{99m}Tc-sodium pertechnetate in 2 ml. of saline (obtained by elution from a ^{99}Mo-^{99m}Tc generator) was stirred while adding 3 drops of 2 N aqueous hydrochloric acid solution. There were then added, with stirring, 10 mg. of ferric chloride, followed by 8.5 mg. of ascorbic acid. The acidity of the resulting solution was adjusted to pH 4.5–5.5 with 1 N aqueous sodium hydroxide solution. 503 Mg. of sodium amylosulfate were added and stirring was continued for about 30 minutes. The resultant solution was transferred to dialysis tubing and dialyzed against three 4 liter portions of water during a period of about 5½ hours. There were obtained 28 ml. of a ^{99m}Tc-sodium amylosulfate composition having a total radioactivity, corrected to zero time, of 1.24 mCi.

EXAMPLE 9

A solution of 3.86 mCi of carrier-free ^{99m}Tc-sodium pertechnetate in 2 ml. of saline (obtained by elution from a ^{99}Mo-^{99m}Tc generator) was acidified with 3 drops of 2 N aqueous hydrochloric acid solution. To that solution were added 17.5 mg. of ferric chloride, followed by 16 mg. of ascorbic acid. The acidity of the resulting solution was adjusted to pH 4.5–5.5 by the addition of approximately 14 drops of 1 N aqueous sodium hydroxide solution. 500 Mg. of sodium amylosulfate were then added and the mixture was stirred for about 1 hour. At the end of that time, the mixture was passed through a small Sephadex column. The first 20–30 ml. eluted from the column were discarded. The next 50 ml. which were eluted were collected. There were thus obtained 50 ml. of a ^{99m}Tc-sodium amylosulfate composition having a total radioactivity, corrected to zero time, of 3.36 mCi.

EXAMPLE 10

A solution of 3.86 mCi of carrier-free ^{99m}Tc-sodium pertechnetate in 2 ml. of saline (obtained by elution from a ^{99}Mo-^{99m}Tc generator) was acidified with 3 drops of 2 N aqueous hydrochloric acid solution. To that solution were added 17.5 mg. of ferric chloride, followed by 16 mg. of ascorbic acid. The acidity of the resulting solution was adjusted to pH 4.5–5.5 by the addition of approximately 14 drops of 1 N aqueous sodium hydroxide solution. 500 Mg. of the sodium salt of sulfated potato starch amylopectin possessing 1.8 sulfate groups per glucose unit and being characterized also by a molecular weight of 12.5×10^7 were then added and the mixture was stirred for about 1 hour. At the end of that time, the mixture was passed through a small Sephadex column. The first 25 ml. eluted from the column were discarded. The next 50 ml. which were eluted were collected. There were thus obtained 50 ml. of a composition containing the sodium salt of sulfated potato starch amylopectin possessing 1.8 sulfate groups per glucose unit and being characterized by a molecular weight of 12.5×10^7, labelled with technetium-99m.

EXAMPLE 11

A solution of 3.69 mCi of carrier-free ^{99m}Tc-sodium

pertechnetate in 2 ml. of saline (obtained by elution from a ^{99}Mo-^{99m}Tc generator) was acidified with 3 drops of 2 N aqueous hydrochloric acid solution. To that solution were added 10 mg. of ferric chloride, followed by 8 mg. of ascorbic acid. The acidity of the resulting solution was adjusted to approximately pH 5 by the addition of 14 drops of 1 N aqueous sodium hydroxide solution. 500 Mg. of the alkali metal salt of sulfated potato starch amylopectin possessing 1–1.8 sulfate groups per glucose unit and being characterized also by a molecular weight of 1–30 × 10^7 were then added and the mixture was stirred for about 5 minutes. At the end of that time, the mixture was passed through a small Sephadex column. The first 25 ml. of eluate were discarded and the next 50 ml. were collected. The latter eluate contained the aforementioned alkali metal salt of sulfated potato starch amylopectin, labelled with technetium-99m.

What is claimed is:

1. A composition comprising an alkali metal salt of sulfated potato starch amylopectin, which is characterized by a molecular weight of about 1–30 × 10^7 and a sulfate content of about 1–1.8 sulfate groups per glucose unit, chemically combined with a diagnostically effective, physiologically suitable labelling agent.

2. The composition according to claim **1** wherein the alkali metal salt is the sodium salt possessing about 1.6 sulfate groups per glucose unit and characterized also by a molecular weight of about 6.3 × 10^7.

3. A composition according to claim **1** wherein the alkali metal salt is the sodium salt possessing about 1.8 sulfate groups per glucose unit and characterized also by a molecular weight of about 12.5 × 10^7.

4. The composition according to claim **1** wherein the diagnostically effective, physiologically suitable labelling agent is a thiazin dye.

5. The composition according to claim **1** wherein the diagnostically effective, physiologically suitable labelling agent is methylene blue.

6. A composition according to claim **1** comprising an alkali metal salt of sulfated potato starch amylopectin, which is characterized by a molecular weight of 1–30 × 10^7 and a sulfate content of 1–1.8 sulfate groups per glucose unit chemically combined with 0.01 to 5 percent by weight of methylene blue.

7. A composition according to claim **1** wherein the diagnostically effective, physiologically suitable labelling agent is a radionuclide.

8. A composition according to claim **1** wherein the diagnostically effective, physiologically suitable labelling agent is iodine-125.

9. The composition according to claim **1** wherein the diagnostically effective, physiologically suitable labelling agent is iodine-131.

10. The composition according to claim **1** wherein the diagnostically effective, physiologically suitable labelling agent is technetium-99m.

11. A composition comprising the sodium salt of sulfated potato starch amylopectin, which is characterized by a molecular weight of about 6.3 × 10^7 and a sulfate content of about 1.6 sulfate groups per glucose unit, chemically combined with technetium-99m.

12. A method of detecting abnormal gastrointestinal mucosa which comprises administering to a mammal a diagnostic dose of a composition comprising a sulfated polysaccharide, which differentially binds to the gastrointestinal mucosa, and which is characterized by a molecular weight of at least one million and a sulfate content of substantially 1–2 sulfate groups per monosaccharide unit, chemically combined with a diagnostically effective, physiologically suitable labelling agent, followed by examination of the mucosa in order to determine those portions containing bound labelled sulfated polysaccharide.

13. The method according to claim **12** wherein the polysaccharide is an alkali metal salt of sulfated potato starch amylopectin, said salt possessing about 1–1.8 sulfate groups per glucose unit and characterized also by a molecular weight of about 1–30 × 10^7.

14. The method according to claim **12** wherein the diagnostically effective, physiologically suitable labelling agent is methylene blue, which is visualized upon endoscopic examination of the mucosa in order to determine those portions containing bound labelled sulfated polysaccharide.

15. The method according to claim **12** wherein the diagnostically effective, physiologically suitable labelling agent is technetium-99m and the examination is conducted with a radiation imaging device in order to determine those portions containing bound labelled sulfated polysaccharide.

16. The method of detecting abnormal gastrointestinal mucosa in mammals which comprises administering to the mammal a diagnostic dose of a composition comprising the sodium salt of a sulfated potato starch amylopectin possessing 1.8 sulfate groups per glucose unit and characterized also by a molecular weight of 6.3 × 10^7, chemically combined with the diagnostically effective, physiologically suitable labelling agent, technetium-99m, followed by examination with a radiation imaging device in order to determine those portions containing bound labelled sulfated polysaccharide.

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