United States Patent Office 2,912,309
Patented Nov. 10, 1959

2,912,309

INDICATOR FOR DETECTING GLUCOSE

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No Drawing. Application February 6, 1956
Serial No. 563,810

6 Claims. (Cl. 23—253)

This invention relates to the detection of glucose and more particularly to improved glucose-testing compositions and devices.

While the compositions and test devices of the present invention are useful in the determination of the glucose content of a wide variety of materials, one of the most important applications is in the detection of glucose in body fluids such as urine, particularly. The determination of glucose in urine is, of course, of importance not only to diabetic patients who must control their sugar input, but is essentially involved in those situations where large numbers of people are screened to determine the incidence of diabetes among them. A simple, rapid, convenient and reliable test for detecting glucose in urine, particularly, in situations such as the foregoing, would be of tremendous importance as an aid in the detection of this disease.

There are a number of tests, techniques and methods already known which can be, and in fact are being, used to measure or estimate the amount of glucose in urine. The more widely used of the conventional procedures are based on the use of alkaline copper solutions which are heated with the materials being tested whereby to precipitate cuprous oxide when a reducing sugar is present.

The older methods have the disadvantage that their use has required a certain amount of skill and familiarity with the use of measuring equipment such as pipettes and the like, and the use of liquid reagents some of which, especially the alkaline ones, are dangerous to handle and inconvenient to transport easily.

This invention is directed to improvements in compositions (and test devices containing such compositions) for detecting glucose in various materials including body fluids, particularly urine, which are, in use, simple, economical, rapid, convenient and reliable, which do not require the use of external or in fact any heat source, lend themselves particularly well to use when "vast screening" of people for diabetes detection is employed, and which are free of many of the disadvantages which characterize prior glucose-testing devices and compositions.

In practicing this invention, I first prepare a composition containing hereinafter described enzymes, an indicator whose color is affected by hydrogen peroxide in the presence of one of these enzymes, a protein material which functions as a stabilizer (and in certain embodiments of this invention as a means for securing the active ingredients to a carrier). In certain situations a dye to make color reading easier may also be added; fillers and buffer compositions may also be included.

The enzymes used in practicing this invention are glucose aerodehydrogenase sometimes known as glucose oxidase which is capable of converting glucose to gluconic acid in the presence of atmospheric oxygen and at the same time forming hydrogen peroxide, and an enzyme (or a material containing such an enzyme) commonly known as peroxidase, although the term catalase may (while commonly used for a different type of action on hydrogen peroxide) in some instances be applied to this

enzyme, which is capable of oxidizing certain substances such as oxidizable dyes when it is present together with such dyes and hydrogen peroxide. Among the materials which contain an enzyme of the latter type are, for example, blood and the like. In some instances a buffer composition may be included, as above mentioned, in order to keep the pH of the reactants at the site of reaction within a predetermined range.

A composition such as above described may be made into a suspension or a solution and used to impregnate a bibulous material like paper, wood, fibre, or the like having any desired shape or size; such a product after drying (though drying is not essential) will undergo a distinct color change when contacted with glucose-containing material, e.g. urine.

Alternatively, this composition may be applied to splinters, sticks or strips made of, for example, wood, fibre, paper, glass, metal, or plastic using gelatin or similar adhesive material for effecting adhesion. Such "sticks" will turn color when moistened with a glucose-containing fluid.

Or such a composition may be formed into a tablet and used by applying the fluid to be tested to the tablet e.g. placing a drop or two of suspect urine on the face of the tablet, and observing the color change if any which takes place.

The following examples will serve to document a number of specific embodiments in my invention and illustrate its flexibility. These have been chosen as illustrative of my invention and it will, of course, be apparent to those skilled in the art that various modifications may be made without departing from the spirit and scope of my invention.

Example I

A mixture was prepared containing:

5 mg. peroxidase
200 mg. glucose oxidase
200 mg. o-tolidine dihydrochloride
100 mg. gelatin

The foregoing was made into an aqueous suspension or solution of about 20 ml. in volume and was used to impregnate paper, wood, and fiber in a variety of forms, sizes and shapes such as strips or "sticks." These test articles as made, or after drying, when moistened with a liquid containing glucose or moistened with water and touched to a solid containing glucose, turned blue.

Example II

A mixture was prepared containing:

5 mg. peroxidase
200 mg. glucose oxidase
200 mg. o-tolidine dihydrochloride
1600 mg. boric acid

The foregoing was ground into a fine powder. Into this powder were then dipped "sticks" generally of the size and shape of match sticks, or strips made variously, of wood fiber, paper cardboard, glass, metal, and plastic, coated with a layer of gelatin deposited from a 33% solution of gelatin. Test devices made according to this technique will turn blue when moistened with a liquid containing glucose, such as a positive diabetic urine.

Example III

100 mg. of the powder produced in accordance with Example II, and 0.5 ml. of a 33% solution of gelatin was used to coat sticks or strips as those described in Example II, and used to detect glucose in liquids as above indicated.

One of the characterizing features of the present inven-

tion lies in the fact that the glucose oxidase-peroxidase-indicator composition used, and which under some conditions is unstable, is now, in accordance with my invention, made relatively stable to such destructive influences as are brought about by heat, light and air.

In Example III it is seen that the protein component which functions as the stabilizer has been incorporated into the active ingredient mixture, so that the concentrated gelatin solution which is used not only supplies the stabilizing protein, but serves simultaneously as an adhesive to cause the active ingredients to adhere to the "stick" or "strip." Another one of the characterizing features of my invention lies in the fact that there is provided thereby a match-like product, having incorporated therein (i.e. onto a "stick" or "strip") the active ingredients of a glucose oxidase-peroxidase-indicator system for the detection of glucose.

While gelatin is a preferred proteinaceous material for producing the products of the present invention, other substances, including other proteins may be substituted therefor as well, as, for example, dog plasma, dog serum, dried beef serum, bovine alubmin, and egg albumin. Casein and soluble starch (Merck) are also useful though generally to a more limited extent. And in general other soluble proteins, as well as plasma and serum can be used with some effectiveness as stabilizing agents to produce a stability effect similar though ordinarily to a lesser degree than that obtained by the use of gelatin.

Examples of substitutes that may be used for (preferably with) the gelatin specified, for example, in Example II are such products as the plastic spray known as "Spraint," soluble starch (Merck), bovine albumin, gum ghatti, rubber cement, egg albumin, casein, starch glycolate, plaster of Paris, Glyptal (household glue), pectin, varnish such as that known commercially as "Cenco" Label Varnish, potato starch and Canada balsam.

It is also found that the gelatin in Example III may also be substituted (preferably only in part) by such products as the plastic spray illustrated by the commercial product known as (Spraint), soluble starch (Merck), bovine albumin, gum ghatti, rubber cement, egg albumin, casein, starch glycolate, plaster of Paris, and potato starch. Generally speaking, it appears that any common adhesive material which does not contain glucose would be suitable for this purpose, and particularly so if used in combination with gelatin or other proteins.

While peroxidase in the foregoing formulations of the examples provides an effective working composition, blood may be substituted for the peroxidase although generally it does not appear to be as effective as peroxidase, under most conditions.

With respect to the boric acid used in Examples II and III this can be substituted by any other filler which does not contain glucose and whose other properties are not inimical to the functioning of the composition, such as, for example, talc, starch, sodium citrate-citric acid mixtures, titanium oxide, silica gel and the like.

Buffers can be used when and if desired in the foregoing compositions to obtain a predetermined pH.

It is generally desirable to have as an additive in the composition a dye such as a red, violet, or orange dye; other colors could probably be used, provided that they will function to cover up, or mask, the discolorations of the "strip" or "stick" which is brought about by air, heat or light.

The "sticks" or "strips" mentioned in Examples II and III have been successfully made from aluminum foil, from such diverse materials as pipe cleaners, aluminum wire, paper clips, polystyrene, polyethylene, wood, paper, cardboard, plastic and rayon; almost any inert material could be used more or less effectively.

The components of the compositions described hereinbefore are, of course, widely variable as those skilled in the art will appreciate. For example, illustratively Example II contains high amounts of all ingredients. It has been found, however, that the peroxidase content can be decreased to $\frac{1}{10}$ of that figure, and that with optimum amounts of other ingredients, it could be decreased even further. It (the peroxidase content) has also been increased four fold, and insofar as applicant is aware there is no upper limit within reason, other than as may be set by the cost of the material. The glucose oxidase content has been decreased to $\frac{1}{36}$ of the indicated amount on that example, using optimum amounts of other ingredients, while a five-fold increase of the glucose oxidase content appears to inhibit the reaction when high amounts of other ingredients are used. A decrease to $\frac{1}{36}$ and a three-fold increase of the indicator o-tolidine dihydrochloride can be effected with optimum concentrations of other ingredients.

With respect to variations in the amounts of components present in Examples II and III the active ingredients have been decreased to as little as $\frac{1}{10}$ with such modified formulations still providing a good reaction; and increases up to ten-fold of all of the ingredients are also feasible as far as applicant is aware.

The particular glucose oxidase which was used had an activity of about 2600 units per gram, a unit being by definition that quantity of enzyme which will cause a rate of oxygen uptake of 10 cu. mm. of oxygen at 30° C. by a solution of glucose contained in a Warburg flask. The peroxidase used was obtained from horseradish and its activity was about the same order as that of the hemoglobin of blood.

This invention provides a means for stabilizing glucose oxidase-peroxidase indicator systems used for glucose determination, which compositions under certain conditions are apt to be unstable to the action of heat, light, and air. My invention also provides a means for preparing improved test "sticks" or "strips" or similar devices for testing glucose by contacting such a "strip" or "stick" with glucose-containing material, by incorporating into the active ingredients of the composition, a protein such as a gelatin solution, which not only imparts stability but also serves, simultaneously, to cause these active ingredients to adhere to the "stick" or to the "strip."

I claim:

1. A composition for detecting glucose which comprises glucose oxidase, peroxidase, an indicator which is oxidized by hydrogen peroxide in the presence of peroxidase and undergoes a color reaction during such oxidation, and a soluble protein.

2. A test indicator for detecting glucose which comprises a self-supporting carrier containing thereon a composition comprising glucose oxidase, peroxidase, an indicator which is oxidized by hydrogen peroxide in the presence of peroxidase and undergoes a color reaction during such oxidation, and a soluble protein.

3. The test indicator of claim 2, wherein the protein is a member selected from the group consisting of plasma, serum, albumin and gelatin.

4. The test indicator of claim 2, wherein the carrier is a bibulous material.

5. A test indicator for detecting glucose which comprises a self-supporting carrier containing thereon the dry solids deposited from a solution of a composition comprising glucose oxidase, peroxidase, an indicator which is oxidized by hydrogen peroxide in the presence of peroxidase and undergoes a color reaction during such oxidation, and a soluble protein.

6. A test indicator for detecting glucose which comprises a self-supporting carrier having adhesively secured thereto a composition comprising a powdered mixture of glucose oxidase, peroxidase, an indicator which is oxidized by hydrogen peroxide in the presence of peroxidase, and a soluble protein.

References Cited in the file of this patent

Keilin et al.: Biochemical J., vol. 42 (1948), pp. 230–238.