COMPOSITION FOR TESTING FOR GLUCOSE IN BIOLOGICAL FLUIDS

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2 Claims. (Cl. 252—408)

1 This invention relates to a composition and method for testing biological fluids to determine the presence of reducing substances therein, is designed primarily for determining the presence of and the amount of sugar in urine, and is in the nature of an improvement upon the composition and method described in copending application Serial No. 208,668, filed January 30, 1951, now Patent No. 2,608,833.

This application is a continuation in part of our copending application Serial No. 38,713, filed July 10, 1948, now abandoned, for Composition and Method for Testing Biological Fluids.

In the diagnosis and treatment of certain diseases it is essential to ascertain whether the urine or blood contains sugar or alcohol and the amount thereof. This is particularly true in the treatment for diabetes. No cure for that disease has been found but it can usually be controlled by the regulation of the diet or by the administration of insulin, or both. The character of the diet and/or the dosage of insulin are determined largely by the amount of sugar content in the urine, which may change from time to time. It is desirable therefore that the patient should be able to test his urine periodically to determine what if any change in treatment is necessary, such changes, of course, being made under the instructions from his physician. There are various materials and methods by which the urine may be so tested and some of these have been made available to diabetic patients in the form of equipment, or kits, intended for individual use in the home. Prior to the invention of the above identified application these tests had not been well adapted for use in the home but for the most part at least involved one or more objectionable characteristics. Usually the presence of sugar was indicated by a change in the color of the testing reagent and the amount of sugar was indicated by the particular shade of resulting color, and was determined by comparison with a color chart. Such a comparison by an untrained eye is apt to be inaccurate and the color chart sometimes fades to an extent which is unnoticed by the patient but seriously affects the determination. Some of the tests require the use of external heat, which not only produces highly objectionable odors, but is inconvenient and subjects the patient to danger of burns. Many such tests require considerable time for their completion. The materials required are usually expensive and in some instances lack stability and quickly deteriorate.

2 One object of the present invention is to provide such a test which can be easily and accurately effected by the ordinary patient with a minimum of equipment.

A further object of the invention is to provide such a test which does not require a comparison of colors; which is both qualitative and quantitative; which will not be materially affected by other reducing substances in the fluid being tested, and which can be effected quickly and at a low cost.

A further object is to provide such a test which can be supplied to the patient in a dry condition and prepared for use by the mere addition of water in the proper amount and can therefore be handled, carried or transported without danger of spilling or leakage.

A further object of the invention is to provide a reagent which is much less corrosive than are reagents heretofore used for such tests, and which can be easily protected against deterioration.

A further object of the invention is to provide a reagent which will react with the reducing substances in the fluid being tested to impart to the testing solution a color which will be the same regardless of the quantity of reducing substance in said fluid, and the time required for the completion of the reaction will accurately indicate the quantity of the reducing substance present in the fluid being tested.

A further object of the invention is to provide a method whereby such a reagent can be easily and accurately produced.

A further object of the invention is to provide a test unit suitable for a single test which can be easily carried by the patient and discarded after being used.

Other objects of the invention may appear as the method and apparatus are described in detail.

Our invention contemplates the provision of a dry reagent containing a color indicator, a stabilizing agent, a heat absorbing agent and, if desired, a color reinforcing agent. The reagent is in dry form and can be packed, shipped and stored for relatively long periods of time in this condition. When it is desired to use the reagent a relatively small quantity may be placed in solution and mixed with the biological fluid to be tested or, under certain circumstances, may be used in dry form.
The color indicator should be readily soluble in water, should impart definite color to even a relatively dilute solution and should react readily with a reducing agent to bring about a definite and noticeable change in color. For this purpose, we have found that the desired results are obtained where we employ as the color indicator, a manganate of an alkali metal such as lithium, sodium, potassium, rubidium and cesium. Potassium or sodium manganate are more readily available at an economic cost and we prefer to employ potassium manganate because it is anhydrous.

To stabilize the alkali metal manganate we employ a stabilizing agent which should be alkali in nature, should be readily soluble in water, should not react with the manganate or inhibit or interfere with the reaction of the manganate with the reducing agent. These functions are served by an alkali metal hydroxide. However, the alkali should preferably be in the form which is not too caustic to handle by inexperienced operators and which generates relatively less heat of solution while still serving to stabilize the alkali metal manganate when in powdered form. For this purpose, sodium aluminate and potassium aluminate serve very satisfactorily and we prefer to use sodium aluminate because it dissolves more readily.

The heat absorbing agent should have a negative heat of solution. It should be readily soluble in water and should not react with the other ingredients or inhibit or interfere with the reaction between the alkali metal manganate and the reducing agent in the biological fluid. For this purpose we have found that sodium nitrate and potassium nitrate serve satisfactorily and optimum results are obtained from sodium nitrate.

As the color reinforcing agent, we employ an agent which is red in the presence of an alkali, which will not react with the other ingredients or inhibit or interfere with the reaction between the alkali metal manganate and the reducing agent in the biological fluid. For this purpose we may employ a small trace of iron oxide, phenolsulphonic red or bromocresol purple.

When our preparation is used for testing the presence or quantity of reducing agents such as glucose in a biological fluid it is preferably (but not necessarily) dissolved in water. The proportions of ingredients should be such that the alkali metal manganate in the aqueous solution is between approximately 0.4% and 4% by weight of the solution and the quantity of sodium or potassium aluminate is between approximately 2% and 6% by weight of the solution. This means that the caustic or alkali metal hydroxide in the aluminate should be roughly between 1% and 3% of the test solution. In order to obtain these desired proportions in the test solution the alkali metal manganate in the powdered preparation should be between approximately 0.66% and 20% of the potassium or sodium aluminate (i.e. between 1.3% and 40% of the alkali metal hydroxide in the aluminate).

The quantity of sodium or potassium nitrate may be varied, but it is desirable to use the maximum amount of nitrate up to the limits of its solubility. The quantity of sodium or potassium nitrate should be at least two times the quantity (by weight) of the sodium or potassium aluminate (i.e. approximately four times the caustic content of the aluminate). Optimum results are obtained by employing six or seven times the quantity (by weight) of nitrate than aluminate (i.e., approximately thirteen times the caustic content of the aluminate).

Only a trace of the color reinforcing agent should be employed. Thus, the quantity should be such that the desired result of a normally green coloring of the preparation in its original form results from the color of the alkali metal manganate. However, this will be sufficient to reinforce the color of the manganese dioxide which is precipitated when the manganate reacts with a reducing agent in the biological fluid.

The several components of the composition may be prepared in any desired manner and then thoroughly mixed together in finely divided form in the proportions indicated above. However, we have found that the reagent may be conveniently produced by preparing a strong solution of an alkali metal hydroxide, preferably sodium or potassium hydroxide, and aluminum hydroxide in water, thereafter heating the mixture to a fused mass and while the fused mass is still hot adding an alkali metal permanganate which is reduced to an alkali metal manganate. Thereafter, sodium or potassium nitrate and a trace of the color reinforcing agent or pigment is added so that the entire mass is finely comminuted and ground to a fine powder which is readily soluble in water. The materials are added in the proper quantities so as to produce a reagent having the ingredients in the proportions indicated above. Specific examples of reagents embodying our invention and the method of preparing them are as follows:

**Example 1**

Approximately 5 grams of sodium hydroxide are mixed with approximately 10 grams of aluminum hydroxide and approximately 5 cc. of water is added thereto. This concentrated solution is heated to form a fused mass of sodium aluminate and while the fused mass is hot there is added thereto approximately 0.3 gram of potassium permanganate which is reduced to potassium manganate in the mass. Potassium nitrate is added to the mixture and the entire mass is finely comminuted, thoroughly mixed, ground and passed over a fine powder. The reagent thus prepared is strongly oxidizing in character, is green in color and will readily dissolve in water and will react with a reducing substance in the fluid to be tested to change its color to a reddish brown.

**Example 2**

Approximately 10 grams of sodium hydroxide are mixed with approximately 10 grams of aluminum hydroxide and approximately 7 cc. of water is added thereto. This concentrated solution is heated to form a fused mass of sodium aluminate and while the fused mass is hot there is added thereto approximately 0.2 gram of potassium permanganate which is reduced to potassium manganate in the mixture. Thereafter approximately 100 grams of sodium nitrate, .1 gram of iron oxide, and .005 gram of phenol red are added to the mixture and the entire mass is finely comminuted, thoroughly mixed, ground and passed over a fine powder. The reagent thus prepared is strongly oxidizing in character, is green in color, the active agents will readily dissolve in water and will react with a reducing substance in the fluid to be tested to change its color to a reddish brown.
Example 3

Approximately 10 grams of potassium hydroxide are mixed with approximately 10 grams of aluminum hydroxide and approximately 7 cc of water is added thereto. This concentrated solution is heated to form a fused mass of potassium aluminate and while the fused mass is hot there is added thereto approximately .5 gram of potassium permanganate which is reduced to potassium manganate in the mixture. Thereafter, approximately 130 grams of sodium nitrate, .08 gram of iron oxide, and .005 gram of phenol red are added to the mixture and the entire mass is finely comminuted, thoroughly mixed and ground to a fine powder. The reagent thus prepared is strongly oxidizing in character, is green in color, the active agents will readily dissolve in water and will react with a reducing substance in the fluid to be tested to change its color to a reddish brown.

Example 4

Approximately 10 grams of sodium hydroxide are mixed with approximately 10 grams of aluminum hydroxide and approximately 7 cc of water is added thereto. This concentrated solution is heated to form a fused mass of sodium aluminate and while the fused mass is hot there is added thereto approximately 5 grams of sodium permanganate which is reduced to sodium manganate in the mixture. Thereafter, approximately 50 grams of potassium nitrate, 15 gram of iron oxide, and .003 gram of phenol red are added to the mixture and the entire mass is finely comminuted, thoroughly mixed and ground to a fine powder. The reagent thus prepared is strongly oxidizing in character, is green in color, the active agents will readily dissolve in water and will react with a reducing substance in the fluid to be tested to change its color to a reddish brown.

In the foregoing examples the permanganate of any of the other alkali metals or mixtures thereof may be substituted for the potassium permanganate and the sodium permanganate.

A reagent embodying our present invention and made in accordance with any of the foregoing examples is particularly useful in determining the presence of and the relative amount of sugar in urine. The method of testing may vary but we prefer to dissolve the reagent in water immediately prior to conducting the tests. A small quantity of the reagent is dissolved in water in an amount sufficient to form a solution of the desired strength and a small sample of urine or other fluid to be tested is introduced into the solution. The sugar which, in the form of glucose, is present in varying amounts in diabetic urine, is a strong reducing substance which reacts with the oxidizing agent to produce manganese dioxide, which is a precipitate reddish brown in color and changes the color of the solution from the original green to an easily distinguishable reddish brown. In making the tests the proportions of urine and reagent are determined in part by the speed with which the reaction is to be effected. While the speed of the reaction may be varied it is quickly completed but is not instantaneous and the time interval varies with the amount of sugar in the urine, the time required for completion of the reaction decreasing as the amount of sugar increases. Thus, the amount of sugar present in the urine is indicated by the time required for the reaction to take place, and the completion thereof is indicated by the change in color of the solution.

In making the test a predetermined amount of the reagent, such as a measured amount of powdered reagent or a tablet containing a known amount of the reagent, is placed in a clean, dry, transparent receptacle of a suitable size and shape, preferably a vial approximately 1 1/2 inches long and 1/4 inch in diameter. A measured amount of water is added to the reagent, which is completely dissolved. When this solution has been completed a predetermined quantity, or sample, of urine is added to the solution and the time for the solution to change from the green color to a reddish brown is noted.

The proportion of urine to reagent may vary in accordance with the character of the reagent or the desired speed of reaction. With a reagent having the composition as set forth in Example 1 it is preferable to place 0.125 gram of the reagent in the vial and add thereto one cubic centimeter, 15 drops, of water. The vial is preferably shaken to expedite solution. One drop of urine is then added to the solution and the time thereof is mixed therewith, as by shaking. The time which elapses between the introduction of the urine into the solution and the complete change of color is measured. When a complete change of color takes place in twenty seconds or less the rating is four plus. If a complete change of color requires more than eighty seconds the amount of sugar is not of material importance and the result is considered negative. The time intervals corresponding to the several readings are shown in the following table, which is furnished to the patient:

<table>
<thead>
<tr>
<th>Time</th>
<th>Quantity of Glucose Present</th>
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</thead>
<tbody>
<tr>
<td>0 to 20 sec.</td>
<td>4 plus</td>
</tr>
<tr>
<td>20 to 40 sec.</td>
<td>3 plus</td>
</tr>
<tr>
<td>40 to 60 sec.</td>
<td>2 plus</td>
</tr>
<tr>
<td>60 to 80 sec.</td>
<td>1 plus</td>
</tr>
<tr>
<td>Over 90 sec.</td>
<td>Negative</td>
</tr>
</tbody>
</table>

Should the test show a rating of four plus and it is desired to determine the actual percentage of sugar present, a second test may be made by diluting a sample of urine with an equal amount of water and using the same amount of reagent. The reaction is timed in the same manner as above and the result is multiplied by two.

While the reaction of the reagent on the sugar is sufficiently slow to permit proper timing it is nevertheless completed in less time than is required to oxidize other reducing substances contained in the urine and therefore such other substances do not interfere with the test.

The foregoing test for determining the presence and the quantity of sugar in urine contemplates using a reagent made in accordance with Example 1. It will be appreciated that reagents containing varying proportions of alkali metal manganate and alkali metal aluminate within the limits set forth above may also be employed and that the time of reaction will vary as the proportions of ingredients are varied. Thus, if the proportion of either the aluminate or the manganate is increased within the indicated proportions, the reaction time is increased and vice versa. The reaction times of reagents of varying formulae may be readily determined by simple tests with a given quantity of urine having known percentages of sugar. It will also be understood that the reaction time will be changed by varying the relative amount of urine used in the tests.

Thus, where a relatively larger quantity of urine is employed the reaction time will be speeded up.
When the test is to be performed with a dry reagent about 0.25 gram of powdered reagent is placed on a waterproof surface, such as a piece of glass, and one drop of urine is added to the powder. The same color change takes place as above if sugar is present and the change is almost instantaneous and too rapid in time for quantitative use.

To further simplify the use of the test by the patient the reagent may be supplied to the patient sealed in a transparent container, such as the vial above mentioned, there being in each vial the quantity of reagent required for a single test. When the test is to be made the patient opens the vial, as by removing a sealing closure, and adds the required water, in the amount above mentioned, and when the reagent is completely dissolved adds the sample of urine and times the reaction as above described. Such a test unit, or several such test units, can be easily carried by the patient either in his pocket or in a handbag so as to be available for use when needed. The only thing required other than the unit itself is a small liquid dropper.

From the foregoing, it will be appreciated that our test and our testing reagent is very simple in character. Its technique is easily acquired, no knowledge of chemical reactions is required and it is not necessary to compare various shades of colors with a color chart. Therefore, it is well suited for use by patients. Furthermore, the test requires very little equipment, no external heat and only one reagent. It is effected at room temperatures and produces no objectionable odors. Thus, it is convenient for use in privacy in the home or when traveling. Moreover, the cost per test is low.

While we have described our reagent and the preferred methods of producing and using the same we wish it to be understood that we do not desire to be limited to the details thereof as various modifications may occur to a person skilled in the art.

We claim:
1. A method of preparing a reagent for testing biological fluids which comprises mixing 5 grams of sodium hydroxide with 10 grams of aluminum hydroxide, adding thereto 5 cc. of water, heating the mixture to a fused mass to form sodium aluminate, adding to the fused mass while hot 0.3 gram of potassium permanganate, adding approximately 80 grams of sodium nitrate, and comminuting the entire mixture.
2. A dry reagent for determining the presence and proportion of glucose in biological fluids by a marked change of color of the reagent within predetermined periods of time and without the application of external heat comprising a dry alkali metal manganate and a dry soluble alkali stabilizer selected from the group consisting of sodium aluminate and potassium aluminate, the alkali metal manganate being in the proportion of between approximately 66% and 20% by weight of the alkali stabilizer.

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References Cited in the file of this patent

UNITED STATES PATENTS

<table>
<thead>
<tr>
<th>Number</th>
<th>Name</th>
<th>Date</th>
</tr>
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<tbody>
<tr>
<td>2,608,533</td>
<td>Carson</td>
<td>Aug. 26, 1952</td>
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