A test strip for the detection of ketone bodies, comprising an absorbing material, a heavy metal salt and a nitroprusside salt. The test strip is useful in the diagnosis of various diseases such as diabetes, disorders in the digestive organs, renal insufficiency, uremia, self-poisoning or malignant carcinoma.
This invention relates to a test strip for the detection of ketone bodies and a composition therefor. More particularly, the present invention relates to a test strip for the detection of ketone bodies, comprising an absorbing material, a heavy metal salt and a nitroprusside salt, and to a composition therefor comprising a heavy metal salt and a nitroprusside salt.

It is known that ketosis takes place owing to insufficient intake of sugars or disorder of sugar availability in the living bodies. In patients with ketosis, ketone bodies such as acetoacetic acid, acetic acid, acetone or $\beta$-hydroxybutyric acid increase in the blood, and they are excreted mainly as acetoacetic acid in the urine. The ketosis indicates the presence of various diseases such as diabetes, disorders in the digestive organs, renal insufficiency, uremia, self-poisoning, malignant carcinoma, etc. Thus, detection of ketone bodies in the body fluids such as urine, serum or plasma can provide a diagnosis of such diseases.

Hitherto, a number of methods for the detection of ketone bodies have been employed in the art. For example, a test strip comprising a nitroprusside salt and an alkali substance is known for the detection of ketone bodies. However, since nitroprusside salts are very unstable to an alkali, such known method which is carried out under alkaline conditions is not a good test method for the detection of ketone bodies. Further, the test strips hitherto employed in the art have drawbacks in that their production is complicated and their sensitivity to ketone bodies is not high. As a result of investigations to overcome such problems, the present inventors have discovered that, without addition of a substance to keep the system at an alkaline pH, use of a nitroprusside salt in combination with a heavy metal salt provides easily a stable test strip having a good sensitivity and specificity to ketone bodies, particularly to acetoacetic acid. The present invention is based on these discoveries.

The test strips and the compositions for the detection of ketone bodies in accordance with the present invention can be prepared in the following manner.

A nitroprusside salt and a heavy metal salt are dissolved in a suitable solvent to give a solution. Then, an absorbing material is dipped in the solution and dried in the air or at a temperature of from room temperature to about 50°C, to give a test strip for the detection of ketone bodies. The solution itself may be used in the detection of ketone bodies, but a test strip prepared as above can be more conveniently used in view of the preservation, stability and handling, rather than the solution itself.

The heavy metal salt can be inorganic salts of a heavy metal of which specific gravity is approximately more than 5. Examples of the heavy metal are nickel, copper, cobalt, manganese, chromium and zinc, and typical examples of such heavy metal salt are nickel chloride, ferric chloride, ferrous chloride, ferric ammonium sulfate, ferrous sulfate, cuprous chloride, cupric chloride, cobalt dichloride and cobaltic chloride. Among them, the most preferred heavy metal salt is nickel chloride. The metal salts can be used usually at a concentration of 1-20%, preferably 5-15%.

As the nitroprusside salt, it is preferred to use a nitroprusside inorganic salt such as alkali metal salts (e.g. sodium salt, potassium salt). The nitroprusside salt can be usually used at a concentration of 1-20%, especially 5-15%.

Examples of the solvent in preparing an impregnating solution containing the heavy metal salt and the nitroprusside salt are water, organic solvents (e.g. dimethylformamide, dimethylsulfoxide, methanol, ethanol) and a mixture thereof.

The absorbing material to be dipped in a solution containing the nitroprusside salt and the heavy metal salt can be a sheet of paper, a piece of cloth and a stick of porous wood. Examples of the kind of paper are filter paper, blotting paper, adsorbent paper (e.g. silica gel paper, alumina paper), ion-exchange cellulose paper (e.g. phosphonomethyl cellulose paper, sulfoethyl cellulose paper, phospho cellulose paper, carboxymethyl cellulose paper, guanidoethyl cellulose paper, diethylaminoethyl cellulose paper, aminooethyl cellulose paper, "Ectecta" cellulose paper which is an epichlorhydrin, triethanolamine reacted cellulose ion exchange paper, as noted U.S. Pat. No. 3,598,704, p-aminobenzyl cellulose paper, polyethylenimine cellulose paper), ion-exchange Sephadex paper and ion-exchange resin paper. The shape of the absorbing material is not particularly limited, though it is usually used in a strip form. Among such absorbing materials, ion-exchange cellulose papers, particularly diethylaminoethyl cellulose paper, can be preferably employed.

The solution itself containing the necessary ingredients may be used to detect ketone bodies by adding it to a specimen of body fluids such as urine, plasma or serum, whereby detectable color change results. However, from the point of view of handling, stability, and preservation, the composition is advantageously used in the form of a solid preparation, rather than the impregnating solution itself. Solid preparations such as tablets, granules, pills or powders can be prepared in a conventional manner. In the production of such preparations, suitable additives (e.g. excipient, disintegrating agent, dispersing agent, stabilizer, binder) not affecting the color reaction may be added without changing the basis of the present invention. The color change is detectable only by dissolving such solid preparation in the body fluids containing the ketone bodies. Also, the color reaction may be affected in such a manner that a tablet containing a heavy metal salt and a tablet containing a nitroprusside salt, normally separately packed, are combined in a specimen of body fluids when used. Usually, the test strip of the invention is used by dipping it in a test sample or by impregnating a test sample into the test strip, whereby detectable color change results. When plasma or serum is used as a test sample, the same application is possible using the test strip of the invention. The test strip of the invention may be, if desired, used in a more convenient form such as, for example, being held on a plastic sheet.

The test strip of the invention is sensitive to ketone bodies, particularly to acetoacetic acid in the urine. Since characteristic color reaction takes place depending on the concentration of the ketone bodies to be detected, semi-quantitative detection for such ketone bodies is possible. It is possible to detect acetoacetic acid in an amount of more than 1 mg/100 ml in the urine with the test strip of the invention. Other ketone bodies such as acetone or $\beta$-hydroxybutyric acid are generally not detectable with the present test strip. In general, color tone of the test strip depends on the kind of the heavy metal salts used. For example, when nickel
salts or cobalt salts; iron salts; and copper salts are employed, the test strip turns purple; orange to brown; and blue to purple, respectively, in the presence of acetoacetic acid. This various color tones seem to be due to the formation of specific colored chelate among the nitroprusside salt, acetoacetic acid and heavy metal salt. It is considered that this chelate formation contributes much to the stability of the nitroprusside salt, thus giving a distinct and uniform color with strong intensity. When an acidic urine sample is tested, it is preferred to use a test strip containing additionally tris-(hydroxymethyl)-aminomethane having a buffer action.

The following working examples will be given only to illustrate the embodiments of the present invention, and it is to be construed that the technical scope of the invention is not limited by the examples given, many variations of which are possible without departing from the concept of the present invention.

EXAMPLE 1

To a 5% aqueous solution (10 ml) of nickel chloride was added a 5% dimethylformamide solution (3 ml) of sodium nitroprusside in water, thus giving a turbid pale yellowish solution. A piece (5 × 6 cm) of diethylami-
noethyl cellulose paper DE-81 (Whatman Co.) was dipped in the solution for one minute and then the strip was dried at room temperature in a dark place. Then, the strip was cut into narrower pieces (1 × 0.8 cm) and the strips were each held on a basic body such as a plastic sheet. The test strips thus obtained were dipped independently in urine samples containing acetoacetic acid of 1, 5, 10, 20, 50, and 100 mg/100 ml, respectively. The test strip turned reddish purple with strong intensity within one minute, depending on the concentration of acetoacetic acid of more than 1 mg/100 ml. When the test strip was dipped in a test sample wherein acetoacetic acid was not contained, the strip did not change color and it retained its original color (pale yellow).

The test strip did not react with ascorbic acid or thiol compounds, but it turned greenish blue in the presence of pyruvic acid and pale brown in the presence of α-keto glutaric acid.

EXAMPLE 2

A 5% dimethylformamide solution (3 ml) of sodium nitroprusside in water was added to a 5% aqueous solution (10 ml) of ferric chloride to give a pale yellowish solution. A piece (5 × 5.5 cm) of silica gel paper M3F 8860 (Carl Chleicher & Chull Co.) was dipped in this solution for one minute, dried in the air, cut into pieces (1 × 1 cm) and held on a plastic sheet to give the desired strips. The sensitivity of the test strip to acetoacetic acid was the same with that of the test strip obtained in EXAMPLE 1.

EXAMPLE 3

Using a 10% aqueous ferrous chloride, ferric sulfate, cuprous chloride or cobalt dichloride in place of 5% aqueous nickel chloride, the procedure of EXAMPLE 1 was followed to give corresponding test strips.

The sensitivity of the test strips obtained above was the same with that of the test strip obtained in EXAMPLE 1.

EXAMPLE 4

Using a filter paper (Toyo filter paper No. 131) or a diethylaminoethyl cellulose paper SG-81 (Whatman Co.), the same procedure as described in EXAMPLE 1 was carried out to give corresponding test strips.

EXAMPLE 5

Using a 5% nickel chloride solution (10 ml) prepared with 0.3M tris-(hydroxymethyl)-aminomethane solution (pH 6.8), the same procedure as described in EXAMPLE 1 was carried out to give the desired test strip.

What we claim is:

1. A test strip for the detection of ketone bodies, comprising an absorbing material, a heavy metal salt and a nitroprusside salt.

2. A test strip for the detection of ketone bodies according to claim 1, wherein the heavy metal salt is an inorganic salt of a metal selected from the group consisting of iron, nickel, copper, cobalt, manganese, chromium and zinc, and the nitroprusside salt is a nitroprusside alkali metal salt.

3. A test strip for the detection of ketone bodies according to claim 1, wherein the heavy metal salt is a member selected from the group consisting of nickel chloride, ferric chloride, ferrous chloride, ferric ammonium sulfate, ferrous sulfate, cuprous chloride, cupric chloride, cobalt chloride and cobaltic chloride, and the nitroprusside salt is a member selected from the group consisting of sodium nitroprusside and potassium nitroprusside.

4. A composition for the detection of ketone bodies, comprising a heavy metal salt and a nitroprusside salt.

5. A composition for the detection of ketone bodies according to claim 4, wherein the heavy metal salt is an inorganic salt of a metal selected from the group consisting of iron, nickel, copper, cobalt, manganese, chromium and zinc, and the nitroprusside salt is a nitroprusside alkali metal salt.

6. A composition for the detection of ketone bodies according to claim 4, wherein the heavy metal salt is a member selected from the group consisting of nickel chloride, ferric chloride, ferrous chloride, ferric ammonium sulfate, ferrous sulfate, cuprous chloride, cupric chloride, cobalt chloride, and cobaltic chloride, and the nitroprusside salt is a member selected from the group consisting of sodium nitroprusside and potassium nitroprusside.

* * * * *