

1

2,990,253

DIAGNOSTIC COMPOSITION

Robert R. Smeby, Elkhart, Ind., assignor to Miles Laboratories, Inc., Elkhart, Ind., a corporation of Indiana
No Drawing. Filed May 21, 1959, Ser. No. 814,666
3 Claims. (Cl. 23-253)

This invention relates to an improved diagnostic composition and to a method for its preparation. Particularly, this invention is concerned with a diagnostic test which is useful for the qualitative detection and quantitative determination of ketone bodies in body fluids, especially acetoacetic acid (acetoacetate) in urine, and wherein the reagent composition is incorporated upon a bibulous carrier.

In the metabolism of fat, acetone bodies or ketone bodies are regarded as normal intermediate compounds which are subsequently oxidized to carbon dioxide and water. The ketone bodies include acetone, acetoacetic acid (beta-ketobutyric acid or diacetic acid) and beta-hydroxybutyric acid. Under normal circumstances no significant quantity of these ketone substances appears in the urine. However, if there is an excessive metabolism of fat either in the presence or absence of abnormal carbohydrate metabolism, the intermediate ketone bodies accumulate in the blood and are excreted in the urine in variable amounts. In diabetes mellitus such an excessive fat metabolism occurs and many of the symptoms of the disease can be ascribed to the toxic effects of the ketone bodies. The medical practitioner is well aware of the usefulness of tests for ketone bodies in the urine in diabetes mellitus. These bodies occur in the urine in other well recognized disturbances of metabolism, and in such cases it is also important to carry out tests for these substances.

A variety of reagents and techniques have been used or proposed in the past for the detection of ketone bodies in urine. A number of such reagents and techniques have involved the use of a water soluble nitroprusside as a reactive ingredient or agent. In one particular reagent formulation the nitroprusside reaction is carried out in the presence of ammonia in order to develop particular colorations (see United States Patent No. 2,186,902 to Fortune). An improvement over the Fortune type formulation is disclosed in United States Patent No. 2,509,140, issued May 23, 1950, to Alfred H. Free and assigned to the assignee of the present application. This patent discloses formulations for the detection of ketone bodies in urine which contain water soluble nitroprusside, an aliphatic amino acid and an alkaline material. It was found, according to the patent, that when the soluble nitroprusside is present in alkaline solution with an aliphatic amino acid (e.g. glycine), a diagnostic composition is provided which is particularly adapted for the detection of ketone bodies in urine without evolution of ammonia.

An improvement of this test composition is described and claimed in U.S. Patent No. 2,577,978, issued December 11, 1951, to Nicholls and Fonner and assigned to the assignee of the present application. It was discovered by these patentees that incorporation of lactose or similar sugars into the diagnostic composition of U.S. Patent No. 2,509,140, greatly enhanced the utility and reliability of the diagnostic composition.

The above-mentioned patents have contributed greatly to the art of diagnosing for ketonuria and the advances made have been worthwhile. Commercial diagnostics made in accordance with the disclosures thereof have aided the physician and clinician in the diagnosis and control of ketonuria.

Prior art, in summary, then teaches the use of a water soluble nitroprusside, an aliphatic amino acid and an alka-

2

line phosphate buffer as essential ingredients of a test for ketone bodies. The test device itself comprises the reagent composition in the form of a tablet.

However, from a commercial point of view embodiment of an invention of a diagnostic composition in the form of bibulous strips or "sticks" is highly preferred since this provides for diagnosticians simple "dip and read" tests. The advantages of a diagnostic in this form are obvious: ease and simplicity of test procedure, absence of cumbersome equipment, ease of disposal of test device, rapidity of test procedure, etc.

But if an aqueous solution of the above basic constituents were used to impregnate a bibulous strip, no practical diagnostic product would be obtained. Sodium nitroprusside is very unstable in an aqueous, alkaline medium, i.e., most of it is destroyed so that no perceptible reaction with acetoacetic acid (acetoacetate) is obtained with such a strip. Attempts to place the aqueous alkaline material separately on the sticks or strips failed when upon exposure to air the reagent composition rapidly deteriorated.

As mentioned above, sodium nitroprusside is very unstable in an aqueous, alkaline medium, but it is a medium of this type that is required to achieve the desired reaction between ketone (sodium acetoacetate) and sodium nitroprusside. Until now, it has thus not been possible to achieve a soluble, alkaline impregnating mixture to prepare test strips which will preserve the sodium nitroprusside required for the chromogenic reaction.

I have now discovered a process whereby the required alkaline nitroprusside is obtained on test strips without being prematurely exposed to the unfavorable medium of aqueous alkali.

More specifically, I have found that a ketone diagnostic in "stick" form which is not subject to instability can be prepared in a two-step procedure by first applying the sodium nitroprusside to the bibulous carriers in an acidic aqueous medium which will preserve this compound of precarious stability and, after drying, dipping these carriers, to achieve the necessary alkalinity, into a non-aqueous solution of organic bases, such as various amines or aminoalcohols or mixtures thereof.

In addition, I have also found that incorporation of such substances as sodium chloride and sodium alginate further helps to stabilize these compositions appreciably. Their function as stabilizers, however, is not yet fully understood.

A diagnostic composition according to the present invention then comprises as essential constituents sodium nitroprusside, glycine, mono- and disodium phosphate, sodium chloride or alginate and one or two amines or aminoalcohols and is thus patentably distinct from prior art compositions.

The invention will now be illustrated, but not limited, by the following examples:

EXAMPLE I

Formulation of the impregnating solutions

	Gm.
A. Glycine	10
Sodium nitroprusside	0.5
Sodium chloride	10
Monosodium phosphate-monohydrate	23.8
Disodium phosphate-anhydrous	1.25
Water, to make 100 ml.	
B. 1,3-diamino-2-propanol	10
2-amino-2-methyl-1,3-propanediol	20
Anhydrous ethanol, to make 100 ml.	

Preparation of impregnating solutions

Solution A.—Ten grams of glycine and 0.5 gm. of sodium nitroprusside were mixed together and 50 ml. of water added thereto. Similarly, 23.8 gm. of mono-

sodium phosphate-monohydrate and 1.25 gm. of disodium phosphate were mixed together and dissolved in enough water to make a total volume of 50 ml. The phosphate buffer solution (pH 4) was then immediately added to the glycine-nitroprusside solution. After the resulting solution cleared, 10 gm. of sodium chloride was added.

Solution B.—Ten grams of 1,3-diamino-2-propanol and 20 gm. of 2-amino-2-methyl-1,3-propanediol were mixed together and dissolved in enough anhydrous ethanol to give a total volume of 100 ml.

Preparation of reagent strips

Bibulous "sticks," that is, absorbent paper cut into narrow strips, were dipped into the impregnating solution A so that through the process of submersion and capillary attraction about $\frac{1}{4}$ to $\frac{1}{2}$ inch of the strip at one end was completely impregnated. These strips were then dried in a drying tunnel at a temperature ranging between 75° C. and 100° C. After drying, these strips were similarly dipped into solution B and dried at 45° C. for 10 minutes in a forced draft oven. The test portion of these strips is of an "off-white" color.

It is understood that other bibulous materials, such as small sticks of wood, etc. as well as other methods for applying the impregnating solutions to the test strips and for drying the thus-impregnated strips may also be employed.

EXAMPLE II

About 0.4 gm. of sodium alginate was dissolved in 50 ml. of water and added to a dry mixture of 20 g. of glycine and 0.5 gm. of sodium nitroprusside. The phosphate salts, mentioned above, were dissolved in enough water to yield 50 ml. of buffer solution, which was then added to the above solution to give a solution that corresponded to impregnating solution A of Example I. Preparation of impregnating solution B as well as the preparation of the test sticks were effected as described in Example I.

EXAMPLE III

Impregnating solution A was prepared according to the description given in Example II. Impregnating solution B was made as follows: 30 gm. of 2-(2-aminoethylamino)-ethanol was added to enough chloroform to make a 100 ml. solution. Strips that were dipped in solution A and after drying, dipped in solution B, were dried at 35° C. for 16 hours in a vacuum oven.

In addition to the organic bases listed above, I have found that the following amines and aminoalcohols, among others, may be utilized in the preparation of impregnating solution B: 2-(2-aminoethylamino)ethanol, 3,3'-diaminodipropylamine, diethanolamine, 1,3-diaminopropane, 1,4-diaminobutane, benzylamine, β -dimethylaminoethanol, triethanol amine, 3-amino-1-propanol, 2-amino-2-ethyl-1,3-propanediol, di-isopropanolamine. These amine compounds have been tested and found to be quite satisfactory. In general, however, I have found that secondary and tertiary amines are superior in performance when compared with primary amines.

The impregnating solutions described above illustrate optimum contents of the various ingredients. I have found that considerable variations in amounts of certain of the essential components are permissible. For example, 5 to 20 gm. of glycine, $\frac{1}{4}$ to 1 gm. of sodium nitroprusside and 10 to 20 gm. of sodium chloride may be employed.

Procedure of testing

In use, an impregnated strip made as described above, is dipped into the liquid specimen to be tested. When contacted with urine containing ketone bodies, a test strip will give a positive reaction in about 15 to 30 seconds evidenced by various shades of purple color as follows:

With urines containing 0.1% or more of ketone bodies

an intense purple color develops while with urine containing as little as 0.015% or less of ketone bodies, the whitish color of the impregnated strip changes to a distinctly light purple color. With urines having a content from 0.015% to 0.1% of ketone bodies the purple colors which develop are of intermediate intensities: the urine having the higher ketone content produces the brighter color. When dipped in urine containing no ketone bodies, the strips undergo no color change. A simple color chart based on this phenomenon may be conveniently prepared for use in testing for ketone bodies in urine.

Extensive stability tests have been conducted which have shown that test strips impregnated with solutions described in the above examples are strikingly stable. For example, after exposure of such test strips for 24 hours to a temperature of 50° C. and a relative humidity of 50%, no substantial loss in reactivity was perceptible. Contacted with urine containing 0.04% of acetoacetate, test strips thus exposed still gave a positive reaction within 30 seconds.

In summary, this invention pertains to a diagnostic test for the detection of ketone bodies in body fluids, especially acetoacetic acid (acetoacetate) in urine, consisting of a bibulous strip or stick that has been impregnated in a two-step procedure, first, by an aqueous solution comprising sodium nitroprusside, glycine, monosodium phosphate-monohydrate, disodium phosphate and sodium chloride, and secondly, by an impregnating solution comprising preferably a secondary or tertiary amine or aminoalcohol or a mixture thereof in anhydrous ethanol or chloroform. This procedure makes it possible to apply the sodium nitroprusside, which is very unstable in an aqueous alkaline medium, to the paper strips, and yet obtain the necessary alkalinity which is necessary for its reaction with ketone bodies.

What is claimed is:

1. A diagnostic composition for the detection of ketone bodies in body fluids which comprises a bibulous carrier impregnated with a solution comprising sodium nitroprusside, disodium phosphate, monosodium phosphate, glycine, a compound selected from the group consisting of sodium chloride and sodium alginate and compounds selected from the group consisting of 1,3-diamino-2-propanol, 2-amino-2-methyl-1,3-propanediol, 2-(2-aminoethylamino)ethanol, 3,3'-diaminodipropylamine, diethanolamine, 1,3-diaminopropane and 1,4-diaminobutane.

2. A diagnostic composition for the detection of ketone bodies in body fluids which comprises a bibulous carrier impregnated with a solution comprising sodium nitroprusside, disodium phosphate, monosodium phosphate, glycine, sodium chloride, 1,3-diamino-2-propanol and 2-amino-2-methyl-1,3-propanediol.

3. A test indicator for detecting ketone bodies in body fluids comprising a bibulous material which contains therein the dried residue resulting from depositions on said material, each followed by drying, of two solutions, one, aqueous, comprising 0.25 to 1 part of sodium nitroprusside, 5 to 20 parts of glycine, about 1.25 parts of disodium phosphate, about 24 parts of monosodium phosphate, a compound selected from the group consisting of sodium chloride, 10 to 20 parts thereof, and sodium alginate, 0.1 to 1 part thereof, and the other, ethanolic, comprising 10 to 30 parts of compounds selected from the group consisting of 1,3-diamino-2-propanol, 2-amino-2-methyl-1,3-propanediol, 2-(2-aminoethylamino)ethanol, 3,3'-diamino-dipropylamine, diethanolamine, 1,3-diaminopropane and 1,4-diaminobutane.

References Cited in the file of this patent

UNITED STATES PATENTS

2,577,978	Nichols	Dec. 11, 1951
2,734,802	Closs	June 14, 1952
2,897,058	Galat	July 28, 1959