

UNITED STATES PATENT OFFICE

2,171,961

BLOOD-SUGAR TEST

William B. Fortune, Indianapolis, Ind., assignor
to Eli Lilly and Company, Indianapolis, Ind.,
a corporation of Indiana

No Drawing. Application August 19, 1938,
Serial No. 225,718

11 Claims. (Cl. 23-230)

It is the object of my invention to provide a simple, rapid, and convenient test for blood sugar; and one that can readily be made by a physician in a sick-room without elaborate equipment, with a very small quantity of blood, and even if only tap water is available.

The ordinary procedure for testing for sugar in the blood is one that requires technical knowledge and skill; and it also requires the use of a rather large quantity of blood, as of the order of 5 cc. obtained from the vein of the patient's arm. While this can be done in hospitals, even there it involves technical difficulties as well as inconvenience to the patients; and it is practically impossible in the ordinary home sick-room.

My invention permits the test to be made readily by unskilled persons, with scarcely more than a drop of blood such as may readily be obtained from the patient's finger or ear-lobe incidentally to other blood tests (as for red-corpuscle count or for hemoglobin determination), with any kind of water that may be available. For instance, a physician may readily determine by my test whether a patient found unconscious on the street is suffering from the effect of 'Insulin' shock (hypoglycemia) or is in a diabetic coma (hyperglycemia), and adjust his treatment accordingly so that it is definitely in the right direction instead of in the wrong direction.

In accordance with my invention I proceed generally as follows:

I dilute approximately one drop of the patient's blood (about 0.1 cc.) many times, desirably about 80 to 100 times, with any available water, such as tap water. I precipitate the proteins present in this diluted blood, desirably with the diluted blood acidulated to a pH at least as low as pH 4.5, as by adding "sodium hexametaphosphate," which is available on the market under the trade-mark 'Calgon.' The acidulating agent is most conveniently oxalic acid, because it is available in a dry powder which can be mixed with dry sodium hexametaphosphate without reacting with it; although I desirably also mix in several volumes of inert material, most conveniently talc, to make more certain that there will be no reaction between the oxalic acid and the sodium hexametaphosphate until the combined reagent is mixed with the diluted blood, and also to promote better precipitation of the proteins.

When the protein precipitation is completed, the precipitate is removed, as by filtration; and

the clear filtrate, containing any sugar which was in the blood, is further treated.

In that treatment, that clear filtrate is first made alkaline to a pH approximating pH 10.7 ± 0.15 . This may conveniently be done by adding sodium carbonate in excess. This alkalized filtrate is treated, conveniently simultaneously with the alkalization, with sodium sulfite and with either formaldehyde or a substance which yields formaldehyde on heating, such for instance as trioxymethylene, to produce a reaction in which formose is produced in an amount proportional to the original sugar (glucose) present in the blood; and is also treated, simultaneously or after the preceding reactions, with a definite quantity of 2,4-dinitro-1-naphthol-7-sulfonic acid, which produces a coloration which by comparison with a color chart will indicate directly the quantity of blood sugar. For that comparison with the color chart, of course, it is necessary that fixed quantities of certain of the reagents be used; although those quantities may be varied if different color charts are used in accordance with those variations.

I produce the reactions which yield the formaldehyde in one instance (by decomposition of trioxymethylene) and the red coloration in the other instance (by reaction of formose with the 2,4-dinitro-1-naphthol-7-sulfonic acid) by the application of heat, and desirably with soap powder and talc present both to prevent too violent boiling and to provide a background for the coloration obtained. The sodium sulfite is provided to destroy any excess formaldehyde added or produced.

This test for blood sugar permits the necessary reagents to be put up in a convenient form, which may be carried in a small pocket-size kit with all the necessary paraphernalia for conducting the test. While the proportions may of course vary, a convenient set of proportions for the different things used is as follows:

A. Precipitation capsules are prepared which contain all the necessary ingredients for obtaining the protein precipitation. Each precipitation capsule conveniently includes the following substances and amounts:

Sodium hexametaphosphate	1/4 grain or 16.2 mg.
Oxalic acid, anhydrous	1/4 grain 16.2 mg.
Talc, powdered	2 grains 129.6 mg.

B. Color tablets are prepared which contain all the necessary ingredients for obtaining the formose proportional to the glucose present, and

for getting the desired coloration. Each color tablet conveniently includes the following substances:

Sodium carbonate, mono-			
5	hydrate	3.4 grains or	220 mg.
	Trioxymethylene	0.46 grain	30 mg.
	Sodium sulfite, dried	1.9 grains	120 mg.
	2,4-dinitro-1-naphthol-7-		
	sulfonic acid	0.46 grain	30 mg.
10	Soap powder, U. S. P.	0.23 grain	15 mg.
	Talc, powdered	0.69 grain	45 mg.

C. Combustion tablets are prepared, for timed burning. Each combustion tablet contains:

15	Methenamine	2.6 grains	168.5 mg.
----	-------------	------------	-----------

Since the kit includes two tablets, B and C, it is desirable as a safeguard against confusion to have them of different colors; but that necessitates no special coloring step, because the natural colors of the ingredients used make the color tablets B a light orange color and the combustion tablets C white.

In using these three prepared things, A, B, and C, I proceed as follows:

25 0.1 cc. of a patient's blood is dropped into a test tube and diluted to 8 cc., with any available water, such as tap water. The tube may conveniently be provided with a mark to indicate the 8 cc. level.

30 To this 8 cc. of diluted blood I add the contents of one precipitation capsule A, by emptying such contents into that test tube. The whole is desirably shaken vigorously, although complete reaction promptly occurs. The precipitate formed is removed by filtration. The filtration is conveniently made into a second test tube, until 4 cc. have been received in that second test tube; after which the rest of the filtrate and the precipitate may be discarded.

40 To this 4 cc. of the filtrate in this second test tube, I add one color tablet B; and mount the second test tube in a clip with one C tablet in a holder below it; and ignite that combustion tablet C so that the bottom of the test tube is in the flame from it. If this is done in the absence of air currents, the heat developed is just sufficient to heat the contents of the second tube to boiling; and boiling over is prevented by the presence of the soap powder and the talc. Within a couple of minutes after boiling has occurred, the reaction is complete.

Thereupon the contents of the second test tube are diluted to a fixed value, in this case 8 cc.; and the resultant diluted solution is shaken vigorously and compared in color with the colors on the prepared color chart. The colors on the color chart range from a light yellow to a fairly deep red, and indicate blood-sugar values marked on the chart and ranging from zero (at the light-yellow end) to 350 mg. per 100 cc. of blood (at the red end).

60 If the color is a deeper red than the red end of the color chart, the test is repeated using one-half the former quantity of blood, or 0.05 cc.; in which case the numerical value indicated by the final reading on the color chart must be multiplied by 2.

70 If the color is fairly well toward the light-yellow end of the color chart, it is then desirable to repeat the test, using two or four times the original quantity of blood; in which case the numerical value indicated by the final reading on the color chart must be divided by 2 or by 4 respectively.

I claim as my invention:

1. A process for testing for blood sugar, which consists in diluting many times a small quantity of the blood to be tested, precipitating proteins present in the diluted blood with that diluted blood acidulated to an acidity at least as great as pH 4.5, and removing the precipitated proteins, alkalizing the liquid remaining to approximately pH 10.7, reacting the alkalized liquid with formaldehyde in the presence of sodium sulfite to produce formose in an amount proportional to the original sugar present in the blood, and adding a definite quantity of 2,4-dinitro-1-naphthol-7-sulfonic acid to produce a reaction with the formose which yields a coloration which by comparison with a color chart indicates the quantity of blood sugar in the blood.

2. A process for testing for blood sugar, which consists in diluting many times a small quantity of the blood to be tested, precipitating proteins present in the diluted blood, and removing the precipitated proteins, alkalizing the liquid remaining to approximately pH 10.7, reacting the alkalized liquid with formaldehyde in the presence of sodium sulfite to produce formose in an amount proportional to the original sugar present in the blood, and adding a definite quantity of 2,4-dinitro-1-naphthol-7-sulfonic acid to produce a reaction with the formose which yields a coloration which by comparison with a color chart indicates the quantity of blood sugar in the blood.

3. A process for testing for blood sugar as set forth in claim 1, in which the acidity for the precipitation step is obtained with oxalic acid.

4. A process for testing for blood sugar as set forth in claim 1, in which the formaldehyde is obtained in situ by the decomposition of trioxymethylene.

5. A process for testing for blood sugar, which consists in diluting many times a small quantity of the blood to be tested, adding a definite quantity of a fixed-proportion mixture containing sodium hexametaphosphate and oxalic acid and talc and removing the precipitate formed, then adding to the remaining liquid a definite quantity of a fixed-proportion mixture containing sodium carbonate, trioxymethylene, sodium sulfite, 2,4-dinitro-1-naphthol-7-sulfonic acid, soap powder, and talc, and heating the mixture to boiling by the burning of a combustion tablet which gives timed burning, to produce in the mixture which results when the reaction is complete a coloration which by comparison with a color chart indicates the quantity of blood sugar in the blood.

6. A process for testing for blood sugar, which consists in diluting many times a small quantity of the blood to be tested, adding a definite quantity of a fixed-proportion mixture containing sodium hexametaphosphate and oxalic acid and removing the precipitate formed, then adding to the remaining liquid a definite quantity of a fixed-proportion mixture containing sodium carbonate, trioxymethylene, sodium sulfite, and 2,4-dinitro-1-naphthol-7-sulfonic acid, and heating the mixture to boiling by the burning of a combustion tablet which gives timed burning, to produce in the mixture which results when the reaction is complete a coloration which by comparison with a color chart indicates the quantity of blood sugar in the blood.

7. A process for testing for blood sugar, which consists in diluting many times a small quantity of the blood to be tested, adding a definite quantity of a fixed-proportion mixture containing sodium hexametaphosphate and oxalic acid and

5 talc and removing the precipitate formed, then
adding to the remaining liquid a definite quan-
tity of a fixed-proportion mixture containing so-
dium carbonate, trioxymethylene, sodium sulfite,
2,4-dinitro-1-naphthol-7-sulfonic acid, soap pow-
der, and talc, and heating the mixture to boiling,
to produce in the mixture which results when the
reaction is complete a coloration which by com-
parison with a color chart indicates the quantity
10 of blood sugar in the blood.

15 8. A process for testing for blood sugar, which
consists in diluting many times a small quantity
of the blood to be tested, adding a definite quan-
tity of a fixed-proportion mixture containing so-
dium hexametaphosphate and oxalic acid and
removing the precipitate formed, then adding to
the remaining liquid a definite quantity of a fixed-
proportion mixture containing sodium carbonate,
trioxymethylene, sodium sulfite, and 2,4-dinitro-
20 1-naphthol-7-sulfonic acid, and heating the mix-

ture to boiling, to produce in the mixture which
results when the reaction is complete a colora-
tion which by comparison with a color chart indi-
cates the quantity of blood sugar in the blood.

9. A sugar-test reaction unit, which reaction
unit contains an alkalinizing agent, a substance
which yields formaldehyde on heating, sodium
sulfite, and 2,4-dinitro-1-naphthol-7-sulfonic
acid.

10. A sugar-test reaction unit, which reaction
unit contains a definite amount of a fixed-pro-
portion mixture of sodium carbonate, trioxymethylene, sodium sulfite, and 2,4-dinitro-1-naphthol-7-sulfonic acid.

11. A sugar-test reaction unit, which reaction
unit contains a definite amount of a fixed-pro-
portion mixture of sodium carbonate, trioxymethylene, sodium sulfite, 2,4-dinitro-1-naphthol-7-sulfonic acid, soap powder, and talc.

WILLIAM B. FORTUNE. 20