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PROCESS FOR THE DETERMINATION OF BLOOD GLUCOSE

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This invention relates to the determination of the content in whole blood of a blood component. It relates typically to improvements in the determination of the glucose content of blood when utilizing a chromogenetic enzymatic reagent responsive to the presence of glucose.

One of the things that is extensively tested for in the medical diagnostic field is the glucose content of blood. Determination of the glucose level of blood is important, for example, in connection with the diagnosis and treatment of diabetes. Largely because of convenience both as regards the diagnostician and the patient, it is common practice to resort to the testing of urine as providing an indication of the diabetic patient's metabolic state. One of the types of reagents used in testing for glucose is an enzymatic reagent that is chromogenetically responsive to the presence of glucose. Glucose in the presence of the enzyme glucose oxidase becomes converted to gluconic acid and hydrogen peroxide. When the enzyme peroxidase also is present, the hydrogen peroxide which is formed becomes converted to oxygen and water. Since one atom of oxygen is liberated for each molecule of hydrogen peroxide formed by conversion of glucose to gluconic acid and hydrogen peroxide, the oxygen thus produced may be utilized for modifying the color of a chromogenetic hydrogen donor by reaction therewith to form water and the oxidized form of the donor molecule. A color change thus effected indicates the presence of glucose in the aqueous solution to be tested and the extent of color change or development is indicative of the amount of glucose in the original solution.

The above described enzymatic reaction for determining the presence of glucose in an aqueous solution has previously been used for determining the glucose content of urine. In one procedure for doing so the reagent is caused to be taken up by an absorbent paper, and the paper with the reagent absorbed thereby has been dried and cut into pieces. The test is performed by contacting a piece of the test paper with the solution to be tested, e.g., urine, and noting any color change that develops after a predetermined interval of time. A standard chart showing different intensities of coloration may be prepared which can be used for comparison so that values of glucose concentration may be had directly from the chart. While tests for determining the glucose content of urine have their place, blood glucose levels usually are considered to offer a more meaningful indication of the metabolic state of a diabetic patient.

It has heretofore been known to take a quantity of blood and obtain a non-protein-free filtrate therefrom. For example, this may be done by mixing a quantity of blood with a protein coagulant and then obtaining a filtrate from the resulting coagulum by filtration or by use of a centrifugal machine. If desired, the resulting filtrate may be subjected to tests including tests using reagents chromogenetically responsive to the presence of glucose. However, this method of testing for the presence of glucose has the disadvantage of requiring the removal from the patient of a quantity of blood sufficiently great to permit the production of a substantial quantity of filtrate by passage through a filtering medium or by discharge from a centrifugal machine. Moreover, the test is one requiring laboratory technique and personnel and is not

suitable for making an immediate test in a doctor's office or for the performance of tests by the patient himself.

It is an object of this invention to provide a method of testing for determining the glucose content of blood using a reagent, and more especially an enzymatic reagent, that is chromogenetically responsive to the presence of glucose, which method is simple to carry out and may be effectively carried out using a very small quantity of blood such as a single drop.

According to this invention, a protein-free filtrate is obtained by the lateral capillary spread from a small quantity of coagulum obtained by the coagulation of the protein content in a blood sample, when the coagulum is deposited on a sheet of absorbent paper. White filter paper such as that commonly used in laboratories is extremely well suited for use according to this invention. The technique thus provided is such that all that is required is a single drop of blood. Moreover, the testing procedure is so simple that it can readily be carried out on the spot in a physician's office or even by the patient himself. At the same time a more accurate indication of the blood level of glucose is obtainable than ordinarily is obtainable by the testing of urine for its glucose content.

In typical practice of this invention a drop of blood is obtained by the familiar finger-prick technique. The drop of blood thus obtained is then mixed with the deproteinizing agent and a drop of the resulting coagulum is applied to a piece of filter paper. Upon so doing the coagulated protein, including the hemoglobin and the colored constituents of the blood plasma, remain localized at the site of application, whereas the colorless deproteinized solution diffuses laterally with respect to the site of application of the coagulum, thereby wetting a substantial area of the paper with the substantially colorless filtrate. A portion of the area of the paper which has become wet with the colorless filtrate thereby is made available for the observation of its response to a reagent chromogenetically responsive to the presence of glucose. The reagent may be applied to the area which has become wet with the filtrate as by addition from a dropper. Alternatively, the paper itself may be one which has been pretreated with the chromogenetically responsive testing reagent. In either case the test area is examined after a predetermined time interval has elapsed and the developed color, if any, is compared with a set of known standards of color intensity indicative of glucose content when the predetermined test conditions are employed. The practice of this invention is illustrated further by the following example of preferred practice of my invention.

The protein coagulant which preferably is employed in the practice of this invention is a suspension of zinc hydroxide. According to the specific exemplified practice, this suspension is prepared by adding a 20% solution of sodium hydroxide to a 10% solution of zinc sulfate until the pH is at 6.5. The suspension is permitted to stand for approximately twelve hours whereupon the supernatant liquid is decanted and discarded. The resulting suspension contains substantially 4% solids and is substantially neutral. The reagent chromogenetically responsive to the presence of glucose which preferably is employed is a solution containing glucose oxidase, peroxidase and o-tolidine in phosphate citrate buffer. While such test reagents are well known, the preparation of the preferred reagent may be exemplified as follows:

To 100 ml. of a solution which is prepared by combining 0.1 M Na_2HPO_4 with 0.05 M citric acid to give a solution with a pH of 5.6 are added 1.0 gm. commercial glucose oxidase, 0.04 gm. commercial peroxidase and 0.02 gm. o-tolidine hydrochloride. The solution is filtered and is used as such.

The following is illustrative of the test procedure employed in the practice of this invention. A drop of blood

expressed from a finger prick is deposited into a small depression in a porcelain test-plate or other similar small container. Two drops of the above-described zinc hydroxide suspension are added to the drop of blood from a standard dropping pipette and the resulting mixture is stirred with a fine rod until the resulting coagulum is of substantially uniform consistency throughout. One drop of this coagulum then is deposited from a standard dropping pipette onto a strip of filter paper which may be approximately 30 x 6 mm. and approximately 0.15 mm. in thickness. The coagulated protein remains localized at the point of application, whereas the aqueous solution of the non-coagulated blood constituents including the glucose spreads laterally so as to wet the paper strip. When the paper strip has become completely wet with the diffusing solution, a drop of the glucose testing reagent is added to the center of the wet area of the paper strip using a constricted dropper which delivers approximately 0.025 ml. The application of the reagent is taken as zero time and after one minute has elapsed the color of the paper is compared with a previously prepared color chart by matching the color that has developed in comparison with a series of color standards produced utilizing standard solutions of glucose covering the clinical range from 0.025–0.40% glucose, the standard colors of the color chart having been prepared by the foregoing procedure except for the source of the glucose solution.

As a further example of the practice of this invention, the reagents used are the same as those described in the preceding example and the same procedure may be employed for obtaining the small sample of blood wherein the protein has been coagulated. According to this example, the glucose test reagent is previously applied to the test paper strip and the strip is dried so as to permit storing until such time as the strip is to be used in actually making a test. The test procedure that is employed is the same as that described in connection with the preceding example except that zero time is taken as the instant of the deposit of the coagulated blood on the test strip. As above described, the color thus developed may be compared with a series of standard colors appearing on a color chart previously prepared by the application of standard glucose solutions of the character aforesaid to the treated test paper strips according to the test technique that has been described.

In the practice of this invention the protein coagulant which preferably is employed is a substantially neutral zinc oxide suspension. Such a suspension has been found to effectively coagulate the protein and to permit the ready separation of a substantially colorless filtrate from the coagulated protein. Moreover, the zinc oxide suspension is desirable because its substantial neutrality is favorable to desired control of the enzymatic chromogenetic response to the presence of glucose. The rate of response is affected somewhat by variation in pH and it is desirable, therefore, that the pH of the protein coagulant that is used be the same both in testing and in setting up the standard color chart that is used. Moreover, the enzymatic reagent that is used is especially effective at a substantially neutral pH.

While a zinc hydroxide suspension preferably is employed in the practice of this invention, other protein coagulants may be employed such as a 3% solution of perchloric acid or a 10% solution of trichloracetic acid. Using these reagents, two drops may be added to one drop of blood as in the examples hereinabove described. Another protein coagulant which may be employed is tungstic acid and in this case the technique employed is that of adding to one drop of blood one drop of a 10% solution of sodium tungstate followed by one drop of two-thirds normal sulphuric acid. When a reagent such as perchloric acid, trichloracetic acid or tungstic acid as employed, the pH of the reagent is substantially below 7. Nevertheless, an enzymatic chromogenetic reaction will take place. It is important, however, in setting up the testing procedure that the particular reagent that is selected shall always be at essentially the same pH.

While a particular enzymatic reagent chromogenetically responsive to the presence of glucose has been exemplified hereinabove, the formulation and preparation of such reagents may be varied somewhat according to known procedures. However, for comparison with a standard color chart it is necessary that essentially the same reagent be used in making a test as that which was used in making up the color chart.

I claim:

1. In a method of determining the glucose content of blood utilizing a chromogenetic enzymatic reagent chromogentically responsive to the presence of glucose, the improvement which comprises mixing a predetermined quantity of blood with a predetermined quantity of protein coagulant of predetermined composition effective to coagulate the protein content of said quantity of blood as a coagulum of coagulated proteinous material dispersed in residual fluid, depositing a predetermined quantity of said coagulum on a piece of absorbent paper, said coagulated proteinous material remaining located at the site of its application and said fluid diffusing laterally by capillary action in said paper away from said coagulated proteinous material in a test area wet with said fluid and freed of said coagulated proteinous material, and contacting said fluid absorbed by said filter paper in said area with said chromogenetic enzymatic reagent.

2. A method according to claim 1 wherein said protein coagulant is a substantially neutral aqueous zinc hydroxide suspension.

3. A method according to claim 2 wherein said aqueous zinc hydroxide suspension contains approximately 4% solids.

4. A method according to claim 1 wherein said piece of paper is of predetermined size wettable throughout the lateral extent thereof by the coagulum deposited thereon.

5. A method according to claim 4 wherein said absorbent paper is substantially white filter paper.

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