DIAGNOSTICS BASED ON TETRAZOLIUM COMPOUNDS

CROSS-REFERENCE TO PRIOR APPLICATION

This is a continuation-in-part of U.S. application Ser. No. 09/161,876, filed on Sep. 28, 1998, now U.S. Pat. No. 5,902,731.

BACKGROUND OF INVENTION

1. Field of the Invention

This invention relates to diagnostic compositions that permit the measurement of analyte concentrations in hemoglobin-containing biological fluids. The compositions are based on tetrazolium dye precursors and involve suppressing the hemoglobin-induced reduction of them.

2. Description of the Related Art

Adipose tissue is one of the most abundant forms of energy storage in the body. It releases stored fatty acids into the circulatory system to be metabolized primarily by the liver. In the process, fat is consumed and energy is released and made available to the body. Normally, little fat is consumed, the fatty acids are completely metabolized to carbon dioxide and water, and the conversion does not upset the delicate pH balance of the body. However, if insufficient amounts of carbohydrates are present in the body, due, for example, to dieting, then fat consumption and fatty acid production can increase to potentially harmful levels. In addition to dieters, insulin-dependent patients are vulnerable, because of their impaired carbohydrate metabolism. When excessive fatty acid is used to supply a body's energy demand, then large quantities of acetoacetate, acetone, and beta-hydroxybutyrate are produced. These intermediates are referred to as ketone bodies, and the condition is known as ketoacidosis.

The ketone bodies can normally be recycled into other forms by the body, provided it is not overwhelmed. Therefore, a healthy individual accumulates a negligible amount of these analytes. When a large quantity of fats is being metabolized in a relatively short period or when most of the energy is derived from fats, massive amounts of ketone bodies are produced. Excessive production of these fat metabolites can cause certain neurologic disorders, if the problem is not corrected promptly.

Ketone bodies are present in blood and, if a threshold is exceeded, are excreted via the urine. They are easily detected by a modern clinical analyzer. On average, the percentages of beta-hydro xybutyrate, acetoacetate, and acetone are 78%, 20% and 2%, respectively. Because of its relatively low concentration and high volatility, acetone is seldom measured. Instead, acetoacetate is quantitatively determined by a nitroprusside reaction and the beta-hydroxybutyrate is quantified with an enzymatic method. Acetoacetate test strips have been available for decades. They are based on a nitroprusside ion coupling reaction with aldehydes and ketones. An alkaline urine sample or a serum specimen is allowed to react with the nitroprusside for some minutes, and a purple color is developed. The intensity of the color indicates the acetoacetate concentration. However, acetone interferes with the test, resulting in higher readings. Further, as the patient recovers from a ketoacidosis episode, the acetoacetate level in urine and in blood increases, thus making the diagnosis difficult.

The beta-hydroxybutyrate test is more useful for monitoring ketone body concentrations. It is based on the oxidation of beta-hydroxybutyrate with the corresponding dehydrogenase in the presence of nicotinamide adenine dinucleotide (NAD) cofactor. (Strictly speaking, only D-beta-hydroxybutyrate is naturally present and oxidized, but we omit the "D" for brevity throughout this specification and the appended claims.) Upon the oxidation, NADH is produced, and its concentration is measured directly with a UV spectrophotometer. Hence, the corresponding signal change in the spectrum is proportional to the analyte's concentration. Unfortunately, the excitation of NADH occurs in the UV region; thus, this mode of detection is suitable only for laboratory instruments. Another method for monitoring beta-hydroxybutyrate is by oxidizing the NADH with a tetrazolium compound.

Tetrazolium compounds are generally very sensitive to strong bases and to light. Thus, special care must be exercised to ensure the integrity of these compounds. Nevertheless, tetrazoliums have played an important role in studies of tissue metabolism. For example, this class of compounds has been used in probing anaerobic oxidation and reduction reactions in cells. Further, they are commonly used in clinical diagnostics. The compounds are typically light-colored or colorless compounds that undergo a reduction reaction, in the presence of a reducing agent, to yield a highly colored formazan. Reducing agents such as ascorbates, sulfhydryls, or variants of NADH, NADPH, and $PQQH_2$ (reduced PQQ—pyrrolo-quinoline quinone) are capable of forming the dye.

In clinical diagnostics, these dyes have been found to be invaluable for monitoring the formation of NAD(P)H from their parent compounds, NAD(P)+, in anaerobic reactions. (See, for example, U.S. Pat. No. 5,360,595, issued on Nov. 1, 1994 to D. Bell et al.) The redox reaction is rapid and is not sensitive to oxygen. The resulting dye color is very intense and has low solubility in water.

In principle, tetrazolium dye precursors can be used to measure ketone bodies and glucose in whole blood. However, the tetrazolium can be reduced non-enzymatically by hemoglobin (Fe(II)) to form a colored formazan, if the hemoglobin is not contained within the red cells of the blood. Thus, free hemoglobin causes serious interference with the measurements. In fact, due to hemolysis and the resultant abundance of free hemoglobin relative to the analyte of interest, in a typical ketone body measurement, the interfering signal from hemoglobin could exceed the intended signal. Glucose measurements, particularly in the normal concentration or above, are not affected as adversely. When the reaction is carried out in high hematocrit samples or at a higher temperature, where the hemoglobin oxidation reaction is faster, interference with glucose measurements is significant, as well. Since the hemolysis of red blood cells, which causes free hemoglobin to be present, cannot easily be avoided, red blood cells must be removed from samples prior to testing, if tetrazolium is to be used for the analysis.

Red blood cells can be removed from samples by filtering with membranes and filters, by trapping with chemical reagents, or by a combination of both methods. Filtration methods for separating red cells from whole blood are costly and require rather large sample volumes. An example of a blood ketone (betahydroxybutyrate) test that uses filtration to eliminate red cells from a whole blood sample is the KetoSite® test available from GDS Diagnostics, Elkhart, Ind. (See Tietz Textbook of Clinical Chemistry, 2nd Ed., ed. by C. Burtis et al., W. B. Saunders Co., Philadelphia, Pa., 1994, p. 974.) The "Test Card" used in that test has two filter layers, which makes the card rather costly and necessitates a large (25 μL) blood sample. Further, the blood must not be hemolyzed.