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SERUM CHOLINESTERASE TEST

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ABSTRACT OF THE DISCLOSURE

Colorimetric procedure for the determination of serum cholinesterase activity in human blood utilizing 5,5'-dithiobis-(2-nitrobenzoic acid) as a color developer reagent, quinidine sulfate as an inhibitor reagent and a halide salt of propionylthiocholine as a substrate.

Background of the invention

This invention relates to a novel diagnostic test. More particularly, it relates to an improved colorimetric method for determining serum or plasma cholinesterase activity in human blood. The cholinesterase present in the serum sample acts on propionylthiocholine releasing thiocholine which in turn reacts with 5,5'-dithiobis-(2-nitrobenzoic acid) producing a yellow color. The reaction is stopped by the addition of excess quinidine sulfate followed by the subsequent measurement of the optical density.

The determination of serum cholinesterase is of particular importance when screening individuals who may have suffered toxic effects from organic phosphate insecticides and similar chemicals which can result in depressed levels of enzyme activity. Serum cholinesterase determinations are also of importance clincally as an indicator of hepatic disease or congenital deficiency of serum cholinesterase resulting in profound sensitivity to succinylcholine.

Summary of the invention

This invention broadly comprises the steps of:

(a) Commingling a buffered aqueous solution containing about 0.01% w./v. of 5,5'-dithiobis-(2-nitrobenzoic acid) having a pH of about 7.4 at 37° C. and a sample of suspect serum, the volume ratio of said solution to said serum being approximately 200 to 1;

(b) Adding to the resulting mixture on aqueous solution containing about 0.15% w./v. of a halide salt of propionylthiocholine in an amount sufficient to provide a volume ratio of said aqueous solution to said serum of about 25 to 1; incubating and subsequently adding

(c) An amount of an aqueous solution containing about 0.5% w./v. of quinidine sulfate sufficient to provide a volume ratio of said quinidine sulfate to said serum of about 50 to 1;

And determining cholinesterase activity spectrophotometrically by measuring the optical density at a wavelength of about 410 mμ.

Experimentally, it is preferred to use the following amounts of materials when carrying out the afore described diagnostic test:

	Ml.
Serum sample	0.02
5,5'-dithiobis-(2-nitrobenzoic acid) reagent	4.0
Propionylthiocholine reagent	0.5
Quinidine sulfate reagent	1.0

Of course, it is to be understood that any equivalent volume proportions may be used in lieu of those above, however, the above amounts are preferred since the total resulting volume is appropriate for the subsequent optical density measurements.

With regard to the wavelength at which the optical density measurements are carried out, it is preferred to use a wavelength of 410mμ, however, a range from 400 to 420 is equally suitable.

Detailed description of the invention

The herein disclosed diagnostic method determines the amount of cholinesterase activity in human serum wherein said activity is measured spectrophotometrically using a spectrophotometer set at a wavelength of from 400 to 420 mμ. The resulting optical density is multiplied by 100 to express chloinesterase activity in units. The normal range for cholinesterase activity predetermined experimentally by the subject method is from about 45 to about 90 units. An abnormal or low reading, that is less than 45, indicates one of the malfunctions described earlier.

Experimentally, the diagnostic procedure is carried out in the following manner: An 0.02 ml. sample of suspect serum is mixed with 4 ml. of an 0.01% w./v. aqueous solution of 5,5'-dithiobis-(2-nitrobenzoic acid) [DTNB] having a pH of about 7.4 at 37° C. Said serum sample must necessarily be nonhemolyzed since hemolyzed serum may lead to falsely elevated cholinesterase activity due to the release of acetylcholinesterase from the erythrocytes. The aforesaid DTNB is buffered with tris(hydroymethyl) aminomethane. To this mixture is added a slight excess of an aqueous solution containing about 0.15% w./v. of propionylthiocholine iodide. Experimentally, 0.5 ml. is added. Mechanistically, the serum cholinesterase reacts with the propionylthiochloine iodide to form thiocholine which then reacts with DTNB to form the yellow anion of 5-thio-2-nitrobenzoic acid. The serum cholinesterase activity is measured preferably after allowing the aforesaid mixture to incubate for three minutes at 37° C. The reaction is then terminated by the addition of 1 ml. of an aqueous solution containing about 0.5% w./v. of quinidine sulfate (inhibitor). The resulting mixture is agitated thoroughly. If cuvettes are not used as containers for the test procedure, the solution is transferred to a typical spectrophotometer cuvette and the optical density determined within 30 minutes. Experimentally, if cuvettes other than 12 ml. are used, the optical density is converted to a 12 ml. cuvette light path by multiplying by the appropriate factor. The aforedescribed method is well suited to any laboratory adapted for routine determinations.

The following examples are given to more fully illustrate the present invention. It is to be understood that these examples are for illustrative purposes only and that the invention is not meant to be limited to the specific details of the example.

Preparation of reagents

(A) Buffered aqueous solution containing 0.1% w./v. 5,5'-dithiobis-(2-nitrobenzoic acid) [DTNB].—To a one liter flask is added 100 mg. of DTNB and 6.64 g. of NaCl. To this solution is added 650 ml. of an HCl-tris mixture (prepared by combining 400 ml. of 0.1 N HCl and 250 ml. of 0.2 M tris(hydroxymethyl) amino-methane (tris) solution. Without stirring, sufficient distilled water is then added to give a 1 liter solution. The mixture is then stirred to permit dissolution of all reagents, and adjusted to a final pH of 7.4. If below 7.4, 1 N NaOH is used whereas if the mixture is above 7.4, 1 N HCl is added.

(B) Propionylthiocholine iodide.—To approximately 600 ml. of distilled water in 1 liter volumetric flask is added 7.5 g. of propionylthiocholine iodide with stirring to allow dissolution, followed by the addition of sufficient water to give a 1 liter solution. This solution is dispersed into vials, each vial receiving 1 ml. of solution. Lyophilization of these vials are out at a temperature of 70°–80° F. When ready to use, the vial is reconstituted with 5.0

ml. of distilled water resulting in a 0.15% w./v. solution (1.5 mg./ml.).

(C) Quinidine sulfate.—To approximately 600 ml. of distilled water in a 1 liter volumetric flask is added 5.0 g. of quinidine sulfate with stirring to allow dissolution, followed by the addition of sufficient water to provide a 1 liter solution.

Example I

A sample of non-hemolyzed suspect serum (0.02 ml.) is combined with 4.0 ml. of reagent A as prepared by the procedure outlined above. If foaming occurs, the foam is allowed to dissipate before proceeding further. Reagent B (0.5 ml.) is then added and the reaction mixture agitated and allowed to incubate for 3 minutes at 37° C. Reagent C (1.0 ml.) is then added, the resulting mixture mixed well and the solution transferred to a 12 mm. spectrophotometer cuvette. The wavelength of the spectrophotometer is set at 410 mμ, adjusted to zero optical density with a blank, and the optical density of the sample determined.

The optical density experimentally is 0.502 which is multiplied by 100 to express cholinesterase activity in "units." Hence, the sample contains 50 cholinesterase units.

Example II

The procedure of Example I is repeated wherein propionylthiocholine bromide is used in preparing Reagent B instead of propionylthiocholine iodide. Equivalent results are obtained.

Example III

The procedure of Example II is repeated wherein the following amounts of sample and reagents are used:

(I)

	Ml.
Sample	0.04
Reagent A	8
Reagent B	1
Reagent C	2

(II)

Sample	0.08
Reagent A	16
Reagent B	2
Reagent C	4

Equivalent results are obtained.

What is claimed is:

1. An improved method for determining serum cholinesterase activity in human blood which comprises the steps of:

(a) commingling a buffered aqueous solution containing about 0.01% w./v. of 5,5'-dithiobis-(2-nitrobenzoic acid) having a pH of about 7.4 at 37° C. and a sample of suspect serum, the volume ratio of said solution to said serum being approximately 200 to 1;

(b) adding to the resulting mixture an aqueous solution containing about 0.15% w./v. of a halide salt of propionylthiocholine in an amount sufficient to provide a volume ratio of said aqueous solution to said serum of about 25 to 1; incubating and subsequently adding

(c) an amount of an aqueous solution containing about 0.5% w./v. of quinidine sulfate sufficient to provide a volume ratio of said quinidine sulfate to said serum of about 50 to 1;

and determining cholinesterase activity spectrophotometrically by measuring the optical density at a wavelength of 400 to 420 mμ.

2. The method of claim 1 wherein said buffering agent is tris(hydroxymethyl)aminomethane.

3. The method of claim 1 wherein said halide salt of propionylthiocholine is propionylthiocholine iodide.

4. The method of claim 1 wherein said incubating period is 3 minutes at 37° C.

5. The method of claim 1 wherein the amounts of said serum sample, said 5,5'-dithiobis-(2-nitrobenzoic acid) solution and said quinidine sulfate solution are 0.02 ml., 4 ml. and 1 ml., respectively.

References Cited

UNITED STATES PATENTS

3,378,463 4/1968 Guilbault et al. ..... 195—103.5

OTHER REFERENCES

Wilkinson, An Introduction to Diagnostic Enzymology, Edward Arnold Publishers, London, pp. 197–199 (1962).

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