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3,183,159 STABILIZED PARTIAL THROMBOPLASTIN REAGENT

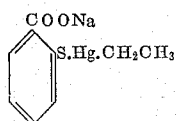
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The present invention relates to a method of stabilizing partial thromboplastin reagent and to the stabilized partial thromboplastin reagent so obtained.

The partial thromboplastin time test was originally developed by Langdell, Wagner and Brinkhous as a method of following the therapy of hemophilic patients. These investigators found this test to be extremely sensitive to changes in Factor VIII brought about by whole blood or plasma transfusions. Extensive studies of the partial thromboplastin time test by Rodman, Barrow and Graham have shown that this test is sensitive to plasma deficiencies of Factor II (Prothrombin), Factor V (AcG, Labile Factor), Factor VIII, Factor IX (PTC), Factor X (Stuart Prower Factor), Factor XI (PTA), and Factor XII (Hageman Factor). In patients deficient in any of the above factors prolonged partial thromboplastin time values are obtained. However, the partial thromboplastin test has not been generally employed as widely as might be warranted for the reason that the partial thromboplastin reagent must be freshly prepared and standardized just prior to use or else prepared in a dry form necessitating reconstitution.

It is an object of the present invention, therefore, to provide a partial thromboplastin reagent that will remain stable in the liquid form upon storage over an extended period of time so that accurate results will be obtained irrespective of the time that the reagent is used.

It has now been discovered that a partial thromboplastin test reagent may be stabilized by the addition to the reagent of small quantities of sodium ethylmercurithiolosalicylate having the formula:



When present at a concentration of 1 part in 10,000 (0.01%), the sodium ethylmercurithiolosalicylate acts as an effective stabilizing agent without effecting the activity of the partial thromboplastin reagent or altering the partial thromboplastin time. Larger amounts of the sodium ethylmercurithiolosalicylate are no more effective in stabilizing the partial thromboplastin reagent and it is recommended that greater concentrations of this sodium salt (more than 0.01%) be avoided as the mercury ion in higher concentrations could interfere with the partial thromboplastin test. The addition of appreciably smaller quantities of sodium ethylmercurithiolosalicylate (less than 0.001%) should also be avoided as too little a quantity of sodium ethylmercurithiolosalicylate may not provide the desired stability under all conditions of storage.

In order that those skilled in the art may better understand how the present invention may be carried out, the following examples are given by way of illustration and not by way of limitation.

EXAMPLE I

Preparation of partial thromboplastin reagent

The stable partial thromboplastin reagent of the present invention is an ether extract of acetone dried brain tissue. Bovine brain is used as the starting material. The meninges are removed, and the brain is washed under tap water. About 100 grams of wet brain are covered with acetone and allowed to stand for 1 hour. The tissue is gently mashed under acetone in a mortar with a pestle. The supernatant is decanted and discarded and the process repeated until the tissue becomes flaky and the acetone remains clear. The tissue is then ground into small particles and spread on a flat pan to dry. The yield of dry powder so obtained is 18 grams. The 18 grams of dry brain powder (in an Erlenmeyer flask) is covered with ether, and stoppered. The dry material is suspended by shaking and allowed to stand overnight at room temperature. The following morning, the supernatant is filtered until clear, and the clear filtrate evaporated to dryness with a stream of air. The dry residue is washed twice with 40 ml. boiling acetone, then air-dried. This residue (cephalin) is a pale yellow waxy material and weighs 3.0 grams. This material is suspended in 3 ml. of physiological saline, and the volume brought to 100 ml. by the slow addition of physiological saline with careful mixing. The 3 percent suspension so obtained is opaque and white in color.

One gram of sodium ethylmercurithiolosalicylate is dissolved in 10,000 grams of physiological saline, and 1,900 ml. of the solution so obtained is added with stirring to the 3% suspension of cephalin (100 ml.) described above. The diluted suspension is sealed in vials and may be stored indefinitely at 5° C. without loss of activity. This product may be used as a reagent in running the partial thromboplastin test.

EXAMPLE II

The partial thromboplastin test

Human blood samples are collected using as an anticoagulant a 0.1 M aqueous solution of sodium oxalate. The anticoagulant is added to the blood in the proportion of one part by volume anticoagulant to nine parts by volume of blood. The blood is then centrifuged and the plasma is separated.

The human blood plasma, 0.1 ml., collected as described above, is placed in a 10 x 75 mm. test tube. To this is added 0.1 ml. of the partial thromboplastin test reagent prepared as described in Example I above, and the test tube is placed in a water bath heated to 37° C. for 30 seconds. Calcium chloride solution is then added to the test tube (0.1 ml. of 0.02 CaCl₂) and a stop watch is started. The tube is tapped gently 2 or 3 times to mix the materials and placed in the water bath until the stop watch reads 60 seconds. The test tube is then removed from the water bath and gently tilted until a clot forms. The stop watch is stopped at this point and the elapsed time is recorded as the partial thromboplastin time. The test is performed both on a control and patient's plasma. The normal range is 60 to 90 seconds.

EXAMPLE III

Two lots of partial thromboplastin reagent prepared as described in Example I above, but at different times, are tested immediately after their preparation with normal human plasma. The partial thromboplastin time in each instance averages 72–75 seconds.

The quantities of partial thromboplastin reagent from each batch are sealed in clear glass vials and stored for 14 months at 5° C. The following table shows the partial thromboplastin time (PPT) in seconds as determined with normal human plasma after 14 months storage. It

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will be noted that the partial thromboplastin reagent has not lost activity during storage.

Vial	PTT, Lot 1		PTT, Lot 2	
1-----	71.7	70.4	72.7	70.5
2-----	70.5	71.0	71.9	74.0
3-----	73.7	74.3	72.0	75.4
4-----	70.6	70.9	73.3	75.4
5-----	70.0	69.9	71.2	69.4
6-----	70.8	73.8	75.4	81.2
7-----	67.9	70.1	73.4	74.2
8-----	81.0	74.1	74.0	72.9
9-----	72.0	74.8	76.1	80.0
10-----	77.7	79.2	74.0	80.3

The invention described and illustrated hereinbefore and secured by these Letters Patent is defined in the following patent claims.

What is claimed is:

- 1. A reagent for the partial thromboplastin time test comprising ether soluble cephalin derived from brain tissue stabilized by the presence of from about 0.001% to about 0.01% sodium ethylmercurithiolsalicylate.
- 2. A reagent for the partial thromboplastin time test comprising an ether extract of brain tissue that has been

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previously extracted with acetone, stabilized by the presence of from about 0.001% to about 0.01% sodium ethylmercurithiolsalicylate.

3. A reagent according to claim 2 wherein the brain tissue is bovine brain tissue.

4. A reagent for the partial thromboplastin time test comprising a suspension of ether soluble cephalin derived from brain tissue in physiological saline, stabilized by the presence of from about 0.001% to about 0.01% sodium ethylmercurithiolsalicylate.

5. A reagent for the partial thromboplastin time test comprising a physiological saline suspension of an ether extract of brain tissue that has previously been extracted with acetone, stabilized by the presence of from about 0.001% to about 0.01% sodium ethylmercurithiolsalicylate.

6. A reagent according to claim 5 wherein the brain tissue is bovine brain tissue.

References Cited in the file of this patent

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