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A Modification of the Hicks and Pitney Test for the Distinction between Haemophilia A and Christmas Disease

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The thromboplastin generation test devised by Biggs and Douglas (1) is the most reliable test for the differentiation of haemophilia A from Christmas disease. On the other hand, the screening test described by Hicks and Pitney (3) provides a rapid and sensitive means of detecting the abnormalities of thromboplastin generation, but being non-specific it cannot distinguish between haemophilia A and Christmas disease. Hence, when the screening test gives abnormal results, one has to proceed further to the performance of the orthodox thromboplastin generation test.

In the present paper a modification of the Hicks and Pitney test is described which enables the distinction to be made between haemophilia A and Christmas disease.

Material and Methods

Principle: When with the usual Hicks and Pitney incubation mixture the substrate clotting time is prolonged, the patient has either haemophilia A or Christmas Disease. If a second incubation mixture containing in addition diluted normal serum gives a normal clotting time, the plasma in the incubation mixture is deficient in Christmas factor; if not the plasma is deficient in factor VIII.

Reagents: a) Platelet suspension prepared as described by Biggs and Macfarlane (2), b) citrated plasma prepared as described by Hicks and Pitney, diluted 1 : 10 with imidazole buffer pH 7.3, c) imidazole buffer prepared as described by Biggs and Macfarlane (2), d) normal serum prepared as described by Biggs and Macfarlane and diluted 1 : 10 with imidazole buffer, e) substrate plasma prepared as described by Hicks and Pitney and f) 0.025 M-$CaCl_2$.

Procedure: Two incubation mixtures are prepared. The first consists of equal volumes of platelet suspension, plasma to be tested, diluted 1 : 10, imidazole buffer and solution of $CaCl_2$; in the second imidazole buffer is substituted by equal volume of normal serum, diluted 1 : 10. The clotting times of the substrate at the 4th and 5th minute are recorded. In details the technique of Hicks and Pitney is followed. A parallel incubation mixture containing normal plasma, diluted 1 : 10, is used as control.

Results and Discussion

Samples of plasma from 10 haemophiliacs and 3 patients with Christmas disease were used. The results are given in table I. When the incubation mixture contained plasma of patients with Christmas disease, the addition of diluted serum corrected completely the substrate clotting time; when the incubation mixture contained haemophilic plasma, complete correction did not occur.

Table I

Results of the modified Hicks and Pitney test.

	Incubation mixture: Plasma diluted 1:10	Buffer	Normal serum diluted 1:10	Clotting time (sec.) 4 and 5 min after the addition of the incubation mixture: 4 min	5 min
1	normal	+	—	9	9
2	Haemophilia	+	—	45	45
3	Haemophilia	—	+	23	23
4	Christmas disease	+	—	55	55
5	Christmas disease	—	+	9	9

Whilst the original Hicks and Pitney test is non-specific, the present modification renders the distinction between haemophilia A and Christmas disease possible. The present test compared to the orthodox thromboplastin test has the following advantages: it is rapid and requires few manipulations; small volumes of blood are needed since only plasma, and not plasma as well as serum, is necessary. The results are available in 15 minutes, whereas in the orthodox thromboplastin generation test the results are available only the following day since the serum has to be uncubated for 24 hours. The present test is especially suitable for children from whom often only small volumes of boold can be obtained. As for the sensitivity of the test, the observations of Hicks and Pitney have shown that the screening test is at least as sensitive as the orthodox test.

As it can be noticed in the table, serum shortens the substrate clotting time also with haemophilic plasma in the incubation mixture; but the difference of correction between haemophilic plasma and Christmas disease plasma is quite considerable, and no difficulty of differentiation arises. For the interpretation of this phenomenon investigations have been carried out, and the results will be reported elsewhere.

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Summary

A modified Hicks and Pitney test is described which enables the distinction between haemophilia A and Christmas disease. The test is sensitive, reliable, rapidly performed, requires small amounts of blood and few manipulations.

Résumé

Description d'une modification du test de Hicks et Pitney permettant la distinction entre l'hémophilie A et la maladie de Christmas.

Zusammenfassung

Es wird eine Modifikation des Tests von Hicks und Pitney beschrieben, welche die Unterscheidung von Hämophilie A und B ermöglicht.

References

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