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BLOOD THROMBOPLASTIN FORMATION

A STUDY OF ITS MEASUREMENT IN VARIOUS DISORDERS,

WITH PARTICULAR REFERENCE TO A RAPID SCREENING TEST.

A THESIS

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By

N. D. HICKS, M.B., B.S., ADELAIDE, 1948.

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The regulations of the University of Adelaide for the degree of Doctor of Medicine require:

- (1) A declaration that the thesis is the writer's own composition.

This declaration may be found on page 5.

- (2) An indication of where the writer considers the thesis to advance medical knowledge or practice. This subject is contained in the Summary and Conclusion on page 123.

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PART I - INTRODUCTION.

The Reasons for the Study.

The introduction by Biggs and Douglas (1953a) and Biggs, Douglas and Macfarlane (1953), of the thromboplastin generation test, provided a sensitive tool for the diagnosis of haemophilia and allied disorders of clotting function. Without this test, serious disorders can be overlooked. Subsequent surgery in these cases often produces fatal results. It is therefore imperative that thromboplastin generation be tested in every case of suspected bleeding tendency, particularly if surgery is contemplated. Other tests of clotting function such as the whole blood coagulation time and the prothrombin consumption test do not always detect disorders of thromboplastin generation. The thromboplastin generation test has been modified by Pitney (1956) and by Biggs (1957) to provide an assay of anti-haemophilic globulin, (AHG). Pitney (1956) showed that some patients with a normal thromboplastin generation test had mild bleeding tendencies due to minor deficiencies of anti-haemophilic globulin. These patients suffered from troublesome rather than severe haemorrhagic tendencies and it is felt that the thromboplastin generation test is adequate for the diagnosis of any appreciable degree of haemophilia or related disorder. Any less sensitive test is inadequate. Unfortunately, the technique is time consuming and sufficiently complicated to deter many haematologists who do not normally carry out specialised coagulation work. However, it must again be emphasised that it is misleading and dangerous to regard the coagulation mechanism of a patient as normal if this test has been omitted.

When blood is allowed to clot an extremely powerful thromboplastin

is formed. In this study it is shown that if plasma is diluted in a suitable buffer and incubated with a platelet substitute together with calcium chloride, a powerful thromboplastin is still formed even when the dilution of the plasma is as high as 1 part in 20. Thrombin is also formed in this mixture, but in relatively small amounts and it does not interfere with the measurement of thromboplastin when subsamples are taken and added to a normal plasma used as substrate. Using this observation, a test was devised to compare thromboplastin generation in different plasma samples. It was felt that if this were sufficiently sensitive it would provide a satisfactory rapid and more simple substitute for the thromboplastin generation test. It was hoped that with this procedure patients with normal coagulation could be distinguished from those with abnormalities of thromboplastin generation. The test is, of course, not specific and any abnormalities detected require further investigation to determine their exact nature. Its main purpose is to provide a rapid means of detecting haemophilia and allied disorders.

Hence-forth in this thesis the procedure is referred to as the "screening test of thromboplastin generation", or, more simply, the "screening test". Other more simple tests are required for the detection of abnormalities and defects involving the prothrombin complex, fibrinogen and platelets. The following routine is suggested for the investigation of patients with suspected bleeding disorders:

- (1) Case history, family history and physical examination.
- (2) Bleeding time.
- (3) Hess' test. (Tourniquet test)
- (4) Platelet count.
- (5) Whole blood coagulation time (tube method of Merskey, 1950b)

and examination of the clot 2 hours after coagulation to assess the degree of clot retraction and to detect any abnormal fibrinolysis.

- (6) The one-stage prothrombin test.
- (7) The screening test for disorders of thromboplastin generation.

This scheme can confidently be expected to detect any appreciable degree of capillary, platelet or coagulation abnormality of any type so far described. All tests can be carried out rapidly and are simple to perform. This provides a quick and efficient means of screening cases of possible haemorrhagic disorders.

The study detailed in this thesis was undertaken to test the sensitivity of the screening test and to compare it with the thromboplastin generation test of Biggs and Douglas and other tests of clotting function. In the investigation of some specialised aspects of coagulation, the assay of individual factors is required and the screening test has been adapted for this purpose.

A Brief Outline of the Study Undertaken.

The original work to be presented in this thesis can be summarised as follows:

1. The development of a rapid screening test for disorders of thromboplastin generation describing the results in 256 normal subjects.
2. An investigation of 62 cases of haemophilia and allied disorders, comparing the results obtained with this test with those of other tests of clotting function.
3. The study of platelet function in 13 patients.
4. The assay of anti-haemophilic globulin by a modification of the