PROTHROMBIN TIME IN HEALTH AND DISEASE*

(ACCORDING TO QUICK'S METHOD)

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RECENT advances in basic sciences supply a satisfactory explanation of the defects in the mechanism of coagulation of blood and suggest valuable methods for the laboratory diagnosis and therapeutic control of some of the most baffling clinical conditions, namely, hæmorrhagic diseases.

Recent advances in physiology

Dam (1934; 1935) observed that chicks on synthetic diets developed hæmorrhages uncontrollable by vitamins A, C and D. He therefore attributed the bleeding tendency to lack of a specific food factor, vitamin K (coagulation vitamin). Dam and Schonheyder (1934) demonstrated that the delay in coagulation in chicks on diets deficient in vitamin K was not caused by a disturbance in the levels of fibrinogen, calcium or the cellular elements of the blood or the thrombokinase of the tissues. Dam, Schonheyder and Tage-Hansen (1936) subsequently showed the element deficient in the blood of these chicks to be prothrombin.

Investigations along a different line have thrown new and valuable light on the significance of the above researches. Numerous studies relating to the coagulation defect in jaundice had revealed that bleeding in jaundice was not due to a disturbance in fibrinogen, calcium, or formed elements, or in any other easily studied component of clotting mechanism. Only one component in blood, prothrombin, remained to be still investigated. Quick (1935; 1937; 1938) devised a method, simple but specific, to determine blood prothrombin and also succeeded in demonstrating a prothrombin deficiency in jaundiced patients with hæmorrhagic tendency. Warner, Brinkhous and Smith (1936) have developed a more accurate but somewhat complicated method. Recently, a third method has been employed and advocated by Dam and Glavind (1938; 1940).

Two interesting findings are disclosed by the above studies. The first is that the prothrombin level is constant for each species. The second is that ordinarily the level of prothrombin is much in excess of the physiologic requirements, providing for large losses. Serious hæmorrhages do not occur until 80 per cent of prothrombin is lost and the blood prothrombin sinks below 20 per cent.

With the aid of these newer methods for the determination of prothrombin, it has become possible to gain further information regarding the stages in the synthesis of prothrombin by suitable experimental procedures on animals and by investigation of clinical conditions, with frank hæmorrhages or in suspected cases of hæmorrhagic diathesis.

Hypoprothrombinæmia produced by surgical procedures in animals

(A) *Biliary fistula.*—Dogs with bile fistula develop spontaneous hæmorrhages. Greaves and Schmidt (1937) demonstrated that prothrombin could be markedly lowered by either ligation of bile duct or by making a biliary fistula. They also observed a hæmorrhagic tendency in rats with bile fistulæ. These animals had a low level of prothrombin by Quick's method. Bile is necessary for the adequate absorption of fat-soluble vitamins. Absence of bile in the intestine leads to impaired absorption of vitamin K with resulting lowering of plasma prothrombin. Both the hypoprothrombinæmia and hæmorrhages were quickly controlled by administering adequate amounts of bile salts along with the usual diets.

(B) *Experimental liver damage.*—Smith, Warner and Brinkhous (1937) reported a marked drop in blood prothrombin in dogs subjected to prolonged chloroform anæsthesia. Partial hepatectomy also reduced the prothrombin level. Total hepatectomy in dogs and rats was followed by a rapid decline of prothrombin. In rabbits, a hæmorrhagic tendency was observed after artificially produced fever (severe hepatic necrosis ?).

In view of these findings, it may be concluded that liver is essential for the formation of prothrombin and that the concentration of prothrombin is reduced in all conditions with extensive parenchymatous damage to the liver. It is also assumed that the liver is concerned in the conversion of vitamin K (derived from diet or from the action of intestinal bacteria) into prothrombin. In the jaundiced patient, the reduction of prothrombin may be due either to inadequate absorption from the bowel or faulty utilization by the damaged liver or to a combination of both defects.

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The purpose and programme of this work may be said to be threefold—

(1) The choice of a suitable method for the study of the anomalies of coagulation and hæmorrhagic diseases.

(2) The determination of prothrombin time in normal healthy adults.

(3) Observations on the variations in prothrombin time in various diseases.

Choice of the method or technique

In order to select an accurate, widely-used and well-established technique, a good deal of time was spent in wading through the extensive and rapidly increasing literature on the various laboratory procedures found useful in the study of the defects of coagulation and hæmorrhagic diathesis. Local conditions, laboratory facilities at our disposal, and the growing handicaps due to the difficulties in obtaining new apparatus and new chemicals, imposed severe restrictions on our choice. We had reluctantly to abandon all hope of starting work on the very accurate method of Warner, Brinkhous and Smith and even on the simpler method advocated by Dam and Glavind. After reviewing our present facilities and taking account of the future uncertainties, we decided to study the possibility of using Quick's method in our work. It is now well recognized that patients whose coagulation time and bleeding time is normal, as judged by the usual tests, may still develop hæmorrhages, particularly those with jaundice and biliary tract disease. It is in such cases that the new test devised by Quick reveals a potential impairment of clotting and gives a danger-signal warning against the possibility, nay probability of hæmorrhages. Quick's method is therefore a definite improvement over the older methods now in use in the hospitals and laboratories.

The principle and technique of Quick's test

The method is based on the assumptions that the rate of coagulation is a function of the concentration of thrombin and that the production of thrombin in oxalated plasma is proportional to the concentration of prothrombin, if an excess of thromboplastin is present and an optimum amount of calcium is added. The clotting time according to this method is a direct measure of prothrombin content.

Four and a half cubic centimetres of blood obtained by venepuncture is mixed immediately with 0.5 c.cm. of M/10 sodium oxalate solution and centrifuged. 0.1 c.cm. of plasma is pipetted into a clean dry test-tube and is mixed with 0.1 c.cm. of thromboplastin solution prepared according to Quick's directions. The test-tube is kept immersed in a water-bath at 37°C. for a few minutes and then 0.1 c.cm. of M/40 solution of pure anhydrous calcium chloride is quickly added. The contents of the tube are now rapidly shaken while immersed in the bath for a few more seconds. The test-tube is now removed from the bath and rocked gently, at short intervals, until the mixture is seen to coagulate. The time in seconds (accurately recorded with a stop watch) from the time of adding calcium to the moment of coagulation constitutes prothrombin time.

Whenever a new specimen of thrombokinase is prepared and whenever new solutions are made, the prothrombin time of normal controls is estimated for purposes of comparison.

Quick's method was slightly modified by Illingworth (1939). The general procedure is the same as above but the source and quantity of material used to prepare the emulsion of thrombokinase differ. Quick prepared the thrombokinase from rabbit's brain by macerating the brain with acetone, while Illingworth uses human brain tissue from the autopsy room. After removal of the meninges and vessels, the brain is thoroughly washed to remove all blood and is pulped and ground into a uniform paste. It is then spread as a thin layer over a sheet of glass and dried by an electric hot-air blower. The dried brain is scraped off and stored in a well-stoppered bottle. Before use, 2 grammes of the dried brain is emulsified in 10 c.cm. of physiological saline and incubated for 15 minutes at 37°C. After this, it is filtered through gauze to remove coarse fragments.

In our early studies, we followed Quick's original method; later, as acetone was not available locally, we followed Illingworth's modification.

Prothrombin time in normal healthy adults

In our observations on 20 adults, using Illingworth's technique, we obtained prothrombin times ranging between 15 and 25 seconds (*see* table). In an earlier series, where

TABLE I*

Prothrombin time with M/40 calcium chloride solution

Number of cases	Nature of disease	Ages: years	Prothrombin time in seconds		
			Range	Mean	S. D.
20	Normals, and non-hepatic conditions.	15 to 40	14 to 25	19.2	± 2.8
1	Jaundice	25	..	45.0	..

TABLE II*

Prothrombin time with M/10 calcium chloride solution

Number of cases	Nature of disease	Ages: years	Prothrombin time in seconds		
			Range	Mean	S. D.
20	Normals or non-hepatic diseases.	15 to 55	41 to 65	53.1	± 7.6

*Summarized by Editor.

thrombokinase from rabbit's brain was used, we noticed that the prothrombin times were two to three times longer than in Quick's experience. The prothrombin times of 24 adults with no recognizable disease of the liver or of blood were between 40 and 60 seconds (table II). This undue prolongation of clotting time, we later found to be due to the fact that the calcium solution used in this early series was made by dissolving 1.11 grammes in 100 c.cm. of distilled water as mentioned by Quick (1938). This figure is obviously a wrong one*, as Quick and other workers, using the same method, mention repeatedly that a M/40 solution of calcium is to be used. Such a solution is prepared by dissolving 0.2775 gramme in 100 c.cm. of distilled water. Tests repeated with M/40 solution gave a prothrombin time of 15 to 25 seconds.

Prothrombin in various clinical conditions

With the kind co-operation of the staff of the King George Hospital, a number of patients from the out-patient as well as in-patient departments were examined for prothrombin time. Patients with skin diseases, patients with even surgical conditions (like carbuncle neck, iliac abscess, vesical calculus) or patients with medical complaints (like lumbago, lymphadenitis, peripheral neuritis, chronic arthritis and round worms) did not show any definite and significant alterations from the normal. The only cases which gave prolonged prothrombin time were those suffering either from severe jaundice or cirrhosis of the liver. One patient with jaundice has been diagnosed as cancer of the stomach with large multiple deposits in the liver. These cases came into the hospital in the early days of our observations when the normal adults gave a prothrombin time ranging between 40 to 60 seconds. Two patients with obstructive jaundice and two with cirrhosis of the liver gave prothrombin times ranging between 2½ minutes and 3 minutes (*see* table III). As far as we have been able to ascertain, none of these four patients had so far manifested any tendency to bleeding.

Determination of prothrombin time with the improved dilution technique, on similar cases and on other types of cases, is being continued. Further, work is also in progress to prepare a chart like that of Quick indicating the relation of clotting time to the concentration of prothrombin, with ultimate object of providing clinicians with a ready and convenient scale to estimate the risk of hæmorrhage in any patient.

* In a private communication, Quick has pointed out that this was a printing error; 100 c.cm. should have read 400 c.cm.

[*Note.*—Napier and Das Gupta (*Indian Med. Gaz.*, **76**, 229) copied this wrong figure into their paper, but these writers have actually been using a weaker solution of calcium chloride, and they now confirm the present writers' observation that more accurate results are obtained with M/40 solution.—EDITOR, *I. M. G.*]

TABLE III

Prothrombin time in clinical conditions, with M/10 calcium chloride solution

Number	Age	Disease	Prothrombin time in seconds
		HEALTHY MEDICAL STUDENT SHOWED A PROTHROMBIN TIME OF 57 SECONDS	
1	25	Jaundice	165
2	40	Cirrhosis of the liver	169
3	50	Do.	150
4	35	Jaundice—cancer liver	140
5	40	Enteric fever 19th day (bloody motions on the 17th day).	85
6	30	Peripheral neuritis	52
7	50	Ing. hernia	50
8	30	Iliac abscess	75
9	22	Vesical calculus	67
10	57	Carbuncle neck	76

Control.

The last four were pre-operative cases.

Summary

1. Prothrombin time of a number of healthy adults was determined according to Quick's method. In the earlier series when M/10 calcium chloride solution was used the time ranged between 40 and 60 seconds. In the second series where M/40 calcium chloride was used the time ranged between 15 and 20 seconds.

2. Prothrombin time was markedly prolonged in cases of obstructive jaundice and cirrhosis of the liver. It was more than 2½ minutes when the normals gave a prothrombin time of 40 to 60 seconds, and 45 seconds when the controls gave a prothrombin time of 15 to 25 seconds.

3. The prothrombin time was normal in subjects with certain complaints (warts, gonorrhœa, lumbago, enlarged lymph glands and ophthalmic conditions).

4. Prothrombin time was slightly prolonged in a case of carbuncle, in iliac abscess, enteric fever, periostitis, secondary syphilis with rash, etc.

5. Recent improvements in the technique of the test, using serial dilutions, may bring out more definitely minor degrees of prothrombin deficiency in cases of malnutrition involving subclinical multiple vitamin deficiency, and in medical and surgical conditions, specially common in the tropics.

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Medical journals received in India during the last three months (and after the completion of this paper) contain many articles dealing with further work on the subject. Three important lines of development deserve to be specially noticed:—

1. Allen, Julian and Dragstedt (1940) advocate a modified one-stage technique using serial dilutions in the determination of prothrombin. The authors refer to the advantage of serial dilution in cases in which prothrombin response to vitamin K is minimal or sluggish. They conclude that the more dilute plasmas more readily reflect minor changes in the clotting time.

2. Souter and Kark (1940) prepared a stable thromboplastin by utilizing the method of 'lyophilization', thus avoiding the rather laborious and time-consuming processes involved according to Quick. The thromboplastic reagent for the test could be readily and easily obtained for immediate use by the addition of distilled water alone.

Fullerton (1940) dispenses with the elaborate method of preparing the thromboplastic substance from brain or other tissues, and used Stypven solution (0.1 gm. of venom being dissolved in 1 c.cm. of distilled water). In working with serial dilutions, he noticed that the end point could be more easily determined when Stypven is used than with emulsions of tissue extracts. Hobson and Witts (1940) however point out, in a preliminary communication, that snake venom must be supplemented with lecithin, and promise a full account of their experiments in the near future. They employed Rusven (Boots).

3. Fitzgerald and Webster (1940) record their observations with a bed-side test. They prepare thromboplastin according to Quick's method. 0.1 c.cm. of thromboplastin solution is placed in a small test-tube, the blood to be tested is added to make up 1 c.cm. Clotting time is noted and compared with the clotting time of normal blood. The authors recommend this test for ward work.

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THE STERILITY AND POTENCY OF INJECTABLE SUBSTANCES

(*iii*) CHOLERA VACCINES

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Pasricha *et al.* (1938) recorded the results of the examination of fourteen cholera vaccines. The vaccines were tested for sterility, freedom from abnormal toxicity, antigenic response in rabbit and man, and protective value in guinea-pigs. It was found that whereas the six cholera vaccines prepared by recognized laboratories gave satisfactory antigenic response and protected guinea-pigs against two M.L.D. of *Vibrio cholerœ*, only four of the eight commercial preparations produced cholera agglutinins and four vaccines gave uniformly negative results. It was further shown that the comparatively simple test of direct agglutination gave valuable information as to the antigenic structure of the organism used in the preparation of the vaccine, and that the results obtained by direct agglutination test corresponded closely to the results of the more costly and time-consuming agglutinogenic test in animals.

A further series of 200 samples of cholera vaccines have been tested with the following results :—

Number of vaccines that did not pass the sterility test—68 or 34 per cent.

Number of vaccines that were not satisfactory by the direct agglutination test—102 or 51 per cent.

Number of vaccines that passed both the sterility and potency tests—74 or 37 per cent.

The opacity of each vaccine was compared with Brown's opacity tubes and controlled with a vaccine made in the laboratory and stored for different periods so that the test vaccine was compared with a control vaccine of approximately the same age. The opacity of the different samples varied considerably; some were very thick suspensions (corresponding to about 16,000 millions per c.cm. or even more, to others which were so light that the opacity could not be estimated). All samples were claimed to contain 8,000 million organisms per c.cm. The final readings were made by comparing with Brown's opacity tubes 1 to 4, and, if the vaccine was more opaque than tube 4, the vaccine was diluted so that the diluted suspension came

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