THE LABORATORY DIAGNOSIS OF HAEMOPHILIA

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Haemophilia is the only recognized haemorrhagic diathesis which is inherited as a sex linked recessive character. It is a disease of males which is transmitted through apparently unaffected females. The classical case can be recognized with certainty since the laboratory manifestations are specific. The association of a prolonged coagulation time with a normal bleeding time and platelet count, if combined with a family history of a bleeding tendency, can hardly ever lead to any other diagnosis.

During an investigation of the nature of the defect in haemophilia it was possible to examine a large number of cases of haemorrhagic disease. It soon became apparent that the laboratory diagnosis of the haemophilic state was not as simple as had at first been assumed. Of the cases which had a familial and clinical history resembling haemophilia, many had typical laboratory findings, yet in other apparently similar cases it was difficult to obtain laboratory confirmation of the disease. Accordingly, it seemed worth while to review the pathological findings in haemophilia in an attempt to assess their significance and value in diagnosis. It is these observations which form the basis of this paper.

Material

The material for this series was derived from the records of a number of the London teaching hospitals and those of the Radcliffe Infirmary. The case records of Dr. R. G. Macfarlane and Professor L. J. Witts provided additional cases, and also a certain number of patients were referred to this hospital from other centres, either because our interest in the subject was known or alternatively because of difficulty in diagnosis. All cases which fulfilled our diagnostic criteria for haemophilia were included in this series. In all, 54 haemophilics were actually seen and examined, and records of another 18 were available for analysis.

Familial Incidence

The familial incidence of the present series is shown in Table I.

The typical family history is a valuable clue to diagnosis of haemophilia, but the occurrence of apparently sporadic cases has often been reported, and haemophilia can be diagnosed in the absence of a family history. Some of these apparently

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TABLE 1
INCIDENCE OF FAMILIAL HISTORY OF HAEMOPHILIA IN PRESENT SERIES

	Patients with Family History		Patients with No Family History
	Patients	Families	
Patients seen and examined	37	23	17
Patients from Radcliffe Infirmary records ..	7	6	11
Totals	44	29	28

sporadic cases may arise from silent transmission of the bleeding tendency through a long line of carrier females; others may arise by mutation.

It will be seen that in 28 out of 72 patients of the present series (i.e., 39%) no family history of bleeding could be elicited. Twenty-nine out of a total of 57 families had more than one affected member. Birch (1937) reported 51 out of a total of 73 families with a familial history of bleeding disease. The greater incidence of isolated cases in the present series may be due to selection, since only seven patients were normally resident in Oxfordshire. Furthermore many cases of haemorrhagic disorder without a family history were referred to us for confirmation of the haemophilic state because the absence of such a history was regarded as a strong point against the diagnosis.

Laboratory Manifestations

Bleeding Time, Platelet Count, and Tourniquet Test.—No extensive observations were made on these three tests, since almost invariably normal results were obtained. On one occasion, in an undoubted haemophilic, a positive tourniquet test was encountered. On one occasion, too, a long bleeding time was found. This served to emphasize the fact that if, in performing the test for bleeding time in haemophilia, vessels other than small capillaries are damaged, bleeding may not cease in the normal range of time of this test.

Coagulation Time.—The lengthening of the coagulation time has been rightly regarded as the classical laboratory abnormality in haemophilia. This is also reflected in the lengthening of the clotting time of recalcified blood, which seems to be peculiarly sensitive to the number of platelets present, as Gram first showed in 1920. Quick (1941) took advantage of this phenomenon in elaborating the so-called Quick's test for haemophilia. Finally, if all the platelets are removed from the plasma the blood becomes completely incoagulable (Conley, Hartmann, and Morse, 1949).

Prothrombin Consumption.—Brinkhous (1939) first showed that, when haemophilic blood clotted, the consumption of prothrombin was less than normal, and this has since been confirmed (Quick, 1947; Soulier, 1948; Alexander and de Vries, 1949; Merskey, 1950a). Prothrombin consumption can be measured on the serum from whole clotted blood, obtained by venepuncture or finger-prick, or on that obtained by recalcifying citrated plasma.

Antihæmophilic Globulin.—If a small proportion of normal blood which contains the substance usually known as antihæmophilic globulin be added to hæmophilic blood, all the defects so far mentioned are largely corrected, and this characteristic reaction is used as a test for hæmophilia. Conversely, the blood from a patient suspected of having hæmophilia can be added to known hæmophilic blood and its corrective ability compared with that of normal blood.

The essential feature of this last test is that the clotting efficiency of a mixture of two hæmophilic blood samples is unlikely to be greater than either separately, and in no case can this mixture be as good as either of these hæmophilic blood samples mixed with an equivalent proportion of normal blood.

Laboratory Techniques

Coagulation Time.—Coagulation times were measured on venous blood (Lee and White, 1913) and finger-prick blood (Dale and Laidlaw, 1911–12), the latter having been shown to be the better of two methods which used capillary blood (Biggs and Macmillan, 1948).

Venous Blood.—This was collected in a paraffined syringe and 1 ml. placed in each of four dry test-tubes ($2\frac{1}{2}$ in. $\times$ $\frac{3}{8}$ in.) which were kept at 37° C. The tubes were gently tilted every half minute and the time from the first appearance of blood in the syringe to coagulation of the blood was recorded. If difficulty were encountered with venepuncture or if bubbles appeared in the syringe the result was rejected as unreliable.

Capillary Blood.—A small tube of wide capillary bore containing a freely mobile lead shot (average weight 0.006 g.) was prepared. A sharp needle was used to prick the finger and the first drop of blood which emerged was used to fill this capillary tube. The tube, held by rubber-covered forceps in a water bath at 37° C., was tilted every 10 seconds and the time between filling the capillary and that at which the lead shot ceased to move was recorded.

Recalcified Plasma.—Blood was collected into 3.8% sodium citrate, using one part of citrate to nine parts of blood. Plasma was obtained by centrifuging the blood at 1,500 revolutions per minute for five minutes. Recalcification was effected at 37° C. by the use of equal volumes of plasma, normal (0.85%) sodium chloride solution, and M/40 calcium chloride solution, and the time of the tubes to clot was recorded. These tests were performed in duplicate and the results averaged.

Quick's Test (1941) for Haemophilia.—Oxalated blood, 5 ml., was divided into two equal portions. One of these was centrifuged at 750 r.p.m. for five minutes. The second was centrifuged in an angle centrifuge at 3,000 r.p.m. for a similar time. Samples, 0.1 ml., of each of these plasmas were recalcified at 37° C. with 0.2 ml. of M/80 CaCl_2 (0.14 g. CaCl_2 , 0.42 g. sodium chloride in 100 ml. distilled water) and the time taken for the mixture to clot was recorded in each case. The test was done in quadruplicate and the results averaged. All procedures were completed within 30 minutes of taking the blood.

Prothrombin Consumption Index (P.C.I.).—This was measured by a two-stage technique (Merskey, 1950a). The test is based on the measurement of the amount of prothrombin present in citrated plasma compared with the amount present in the serum of blood in glass tubes one hour after coagulation had occurred.

Serum from Whole Clotted Venous Blood.—The serum from the four Lee and White coagulation tubes was pooled and a sample tested.

Serum from Whole Clotted Capillary Blood.—Blood was obtained by finger-prick and the finger was squeezed in order to obtain a mixture of blood and tissue fluid.

Serum from Recalcified Citrate Plasma.—Plasma was obtained from citrated blood by centrifuging the blood at 1,500 r.p.m. for five minutes. It was recalcified with an equal volume of M/40 CaCl_2 .

In all cases the tubes were incubated for one hour at 37° C. and the serum obtained by centrifuging in the case of whole blood, or by expression of the clot in the case of recalcified citrate plasma. In the latter case an allowance was made for the dilution with CaCl_2 .

In the measurement of prothrombin 0.2 ml. each of brain thromboplastin, M/40 CaCl_2 , normal saline, and serum (the serum being added last) were incubated in a water bath at 37° C., and 0.1 ml. samples removed at 20 seconds, 60 seconds, and 120 seconds thereafter and added to a second row of tubes each containing 0.4 ml. fibrinogen. The time taken for the latter tubes to clot was recorded. The plasma was treated in a similar manner and the prothrombin content of the serum was compared with that of the original plasma and expressed as a prothrombin consumption index as follows:

$$\text{P.C.I.} = \frac{\text{most rapid plasma time}}{\text{most rapid serum time}} \times 100$$

Correction of Haemophilic by Normal Blood.—The known haemophilic, the normal control, and the patient to be tested, all being of compatible ABO blood groups were venepunctured simultaneously using similar sized needles and syringes for obtaining the two latter specimens. The blood of the haemophilic and the normal control, or the haemophilic and the patient to be tested, was mixed in the proportions shown in Table II (there being approximately 40 drops per ml.). Each tube was inverted once and 1 ml. of each mixture was placed in a glass tube $2\frac{1}{2}$ in. $\times$ $\frac{3}{8}$ in. and the time taken for the contents of each tube to coagulate at 37° C. determined. One hour later the prothrombin remaining in each tube was measured.

TABLE II
DILUTION SCHEME FOR TITRATION OF ANTIHAEMOPHILIC GLOBULIN CONTENT OF BLOOD

Volume of haemophilic blood (ml.)	1.0	—	1.0	1.0	1.0	1.0	1.0	2.0	4.0	8.0
Volume of patient's (or control) blood (drops)	—	40	16	8	4	2	1	1	1	1
Concentration of patient's (or control) blood in haemophilic blood (%)	0	—	29	17	9	5	2.5	1.25	0.6	0.3

Recalcified Citrate Plasma.—Blood samples from a known haemophilic, the patient to be tested, and a normal control were obtained within a short time of each other. They were citrated as before and centrifuged at 2,000 r.p.m. for five minutes to obtain plasma. Dilutions of plasma of the patient to be tested and the normal control were made separately in normal saline, suitable dilutions being 0.75%, 1.5%, 3%, 6%, 15%, and 30%. Of each of these dilutions 0.2 ml. was added separately to 0.2 ml. known haemophilic plasma, and this was recalcified with 0.2 ml. M/40 CaCl_2 and the calcium time at 37° C. determined. The final dilutions would then be one-third of the above mentioned figures. Dilutions of haemophilic plasma with 0.2 ml. saline were also recalcified at the beginning and end of the experiment. All observations were made in duplicate. One hour later the prothrombin content of the serum in each tube was measured.

Results

Coagulation Time in Normals.—The results for venous blood in 25 normal males and 25 normal females are shown in Fig. 1, where each tube has been plotted separately. There is a slight difference between males and females which is statistically significant, but for an individual coagulation time this difference can be ignored.

FIG. 1.—Histogram showing results of Lee and White coagulation times in 25 normal males and 25 normal females. The four tubes of each test have been plotted separately.

The range was found to be from two to 12 minutes for individual tubes and from three and three-quarters to 10 minutes for the average for any particular individual. An average for the four tubes in excess of 11 minutes is regarded as abnormal.

The results for capillary blood in 25 normal males and 25 normal females are shown in Fig. 2, and the mean figures and standard deviation in Table III.

TABLE III
COAGULATION TIME (DALE AND LAIDLAW METHOD) IN NORMAL CONTROLS

	Mean (Seconds)	Standard Deviation (Seconds)
Males	124.1	15.8
Females	131.8	14.8
Whole group	127.9	15.7

FIG. 2.—Histogram of results of Dale and Laidlaw coagulation times in 50 normal individuals.

Coagulation Time in Haemophilia.—In many cases of haemophilia the coagulation time by any reliable method is greatly prolonged. In 10 patients of the present series the venous blood coagulation time fell within the normal range; in 11, it was just over the upper level of normality; and it was well beyond the normal range in the remaining 30 patients (Fig. 3).

Reliability of Dale and Laidlaw Method.—Quick, Honorato, and Stefanini (1948) make the statement:

“It must be emphasized that the coagulation time of capillary blood, irrespective of the method used, is worthless and unreliable for clinical purposes.”

Even admitting the superior reliability of the coagulation time of venous blood, this criticism of capillary blood methods seems unduly harsh. To investigate the point further the Dale and Laidlaw coagulation time of each case was compared with the Lee and White time to see how often a prolonged coagulation time would be observed by either method.

Consecutive Tests on Same Patients.—Two haemophiliacs with prolonged Lee and White coagulation time were tested approximately every two days over a two to three week period. The results are shown in Fig. 4. It will be seen that the Lee and White time was prolonged on all occasions, whereas on four occasions the Dale and

Dale and Laidlaw (1911–12), using a slightly larger average shot, found an average clotting time of 101.5 seconds with a mean error of 4.3 seconds. Abrahams and Glynn (1949) tested 16 normals and found a range from 120 seconds to 210 seconds with an average of 165 seconds, but the temperature at which these observations were made was not specified. It is obvious, therefore, that minor alterations in technical detail, and especially in the bore of the capillary tube and the size of the lead shot, may affect the results, and individual laboratories should evaluate their own range of normal. In this laboratory figures in excess of 180 seconds are regarded as being outside the normal range.

Laidlaw method gave results which were within normal limits. Immediate repetition of three of these Dale and Laidlaw estimations gave figures which were definitely abnormal. The fourth was unfortunately not repeated.

Fifty-two Parallel Observations on 46 Haemophiliacs.—Six patients were re-examined on a second visit to the hospital. It will be noted that the Dale and Laidlaw method failed to disclose a greatly prolonged coagulation time in four instances when the Lee and White time was over 15 minutes. Three of these four were immediately repeated, and the readings were 420, 210, and 165 seconds. Thus the Dale and Laidlaw method might be misleading in two of these 25 cases. When the Lee and White coagulation time was 15 minutes or less the capillary blood method seemed to be at least as reliable as the method using venous blood. Occasionally, definitely abnormal readings were obtained with the Dale and Laidlaw method, when the Lee and White coagulation time was normal. In these cases the capillary blood method appeared to give more information than the use of venous blood (Table IV and Fig. 5).

FIG. 3.—Histogram of results of Lee and White coagulation times in 51 haemophilic patients. The four tubes of each test have been plotted separately.

FIG. 4.—Comparison of two methods of measuring coagulation time. Two haemophiliacs with prolonged coagulation time tested every second day. Note that four readings by the Dale and Laidlaw method fall in or near the normal range, but repetition of three of them (circles) all fall well outside the normal range. The broken lines indicate the normal range of each test.

Recalcified Plasma.—Observations made on the clotting time of recalcified citrate plasma showed that, in general, this tended to be parallel to the Lee and White time. It was greatly increased when the Lee and White time was prolonged and even moderately lengthened in some of the cases where the Lee and White time was near normal. The figures obtained varied considerably, and, since the results were affected by the age of the plasma and the number of platelets present, it did not appear to be as reliable a method of evaluating a case of haemophilia as the use of whole blood without anticoagulant.

Quick's Test for Haemophilia.—The results of this test in 18 cases of haemophilia and 20 normal controls are shown in Fig. 6. The case records of the Radcliffe Infirmary were searched and another 10 examples of Quick's test in haemophilia discovered. These are also shown in the same diagram. In this diagram the percentage increase of the calcium time caused by high speed centrifugation has been plotted against the original (slow speed) calcium time. Some

TABLE IV
COMPARISON OF TWO METHODS OF MEASURING COAGULATION TIME

Lee and White Coagulation Time	Dale and Laidlaw Coagulation Time		
	Over 240 Seconds	180-240 Seconds	Under 180 Seconds
Over 15 minutes	21	3	1
10-15 minutes	8	6	4
Under 10 minutes	1	3	5

haemophilic blood samples were undoubtedly more affected by centrifuging than the normal controls, increases of 160%, 180%, and 191% being recorded in the three extreme cases. But in other cases very little increase was found. In the

FIG. 5.—Comparison of two methods of measuring coagulation time (52 parallel determinations on 46 haemophiles). Note the close correlation recorded by the two methods, the Dale and Laidlaw method failing to indicate a prolonged coagulation time in only four instances. Three of these readings were immediately repeated (crosses). The broken lines indicate the normal range of each test.

FIG. 6.—Results of Quick's test for haemophilia.

normals the increases were by no means negligible, and in two cases a normal was increased by over 100%, while in five cases the percentage increases exceeded 60%.

The percentage increase in the calcium time of nine cases of haemophilia was beyond the normal range. In 19 cases the increase was no greater than the normal range. The test was no more accurate in cases with a long as compared with those with a short calcium time. It was therefore only of slight value in the diagnosis of haemophilia.

Prothrombin Consumption Index (P.C.I.)—Under the conditions specified the P.C.I., using serum from whole clotted venous blood in normal controls, is usually less than 20% and always under 40% (Merskey, 1950a), while figures for serum from clotted capillary blood or recalcified plasma are usually less than this and often under 5%.

TABLE V
COAGULATION TIME AND PROTHROMBIN CONSUMPTION INDEX IN CASES OF THIS SERIES

No.	Age (Years)	Coagulation Time		Prothrombin Consumption Index (%)			Other
		Lee and White Method (Mins.)	Dale and Laidlaw Method (Secs.)	Whole Clotted Blood		Recalcified Citrate Plasma	
				Venous	Capillary		
1	16	150	1,140	108	100	135	Repeat Dale and Laidlaw reading 420
2	11	84	—	126	—	—	
3	13	55	735	200	110	200	
4	60	54	480	145	133	177	
5	50	53	630	150	—	—	
6	4	52	630	110	—	100	
7	59	51	510	133	—	125	
8	50	49	480	122	—	—	
9	50	48	—	71	—	—	
10	10	47	340	166	—	—	
11	4	42	580	166	187	150	
12	5	42	1,040+	150	188	157	
13	40	37½	150	158	85	137	
14	2	37½	708	212	190	190	Repeat Dale and Laidlaw reading 165
15	7	37½	605	183	122	147	
16	12	37	600	107	100	94	
17	44	37	450	210	100	100	
18	9	37	570	188	75	—	
19	42	36	540	136	125	108	
20	10	35	190	160	80	—	
21	7½	34	450	170	77	121	
22	39	34	420	188	155	—	
23	40	33	—	125	—	—	
24	4	33	480	180	—	—	
25	18	17½	370	100	12	79	
26	4	16	—	112	—	—	Repeat Dale and Laidlaw reading 210
27	10	16½	—	60	—	—	
28	13	16	195	100	<8	34	
29	7	15	540	190	100	—	
30	36	15	270	100	—	58	
31	36	14½	155	94	<5	115	
32	20	14½	300	146	14	25	
33	37	14	220	140	<5	156	
34	57	12½	332	44	<5	<5	
35	9	11½	330	48	6	80	
36	24	11½	260	83	<5	42	
37	29	11½	230	74	<5	31	
38	30	11½	190	64	<5	<5	
39	40	11½	185	64	<5	<5	
40	19	11½	215	58	6	6	
41	37	11	165	35	<5	<5	
42	67	10½	293	100	<5	<5	
43	24	10	205	60	12	25	
44	9	9½	190	36	<5	45	
45	19	9½	200	47	<5	75	
46	19	8½	220	27	5	20	
47	38	8½	165	115	<5	<5	
48	17	8	152	41	12	12	
49	35	7½	155	65	10	71	
50	17	7	153	82	<5	7	
51	3½	6½	255	113	16	100	
52	2½	—	720+	—	At least 100	Venous blood unobtainable Serum prothrombin compared with normal plasma	
53	20/12	—	680	—	At least 100		
54	9	—	460	—	At least 100		

FAMILIES: 3, 54; 4, 9, 27; 13, 17; 34, 38, 39, 41, 42; 35, 36, 37, 51; 40, 43, 45, 46.

Haemophilia.—The P.C.I. was found to vary in a manner roughly proportional to the coagulation time, and the readings for the whole series of cases are recorded in Table V and Fig. 7, where the cases have been arranged in relation to their Lee and White coagulation time.

It will be seen from Table V and Fig. 7 that the longer the coagulation time the more likely are all three methods of measuring the P.C.I. to be abnormal. As the coagulation time shortens, the capillary blood is the first to give normal readings.

FIG. 7.—The prothrombin consumption index related to the Lee and White coagulation time in 51 haemophilic patients. Note that the P.C.I. falls as the coagulation time shortens. The use of serum from whole clotted venous blood is especially valuable when the coagulation time is in or near the normal range.

As it shortens further the recalcified citrate plasma readings become normal whereas the serum from whole clotted venous blood remains abnormal in the vast majority of instances including even cases with normal coagulation time. The degree of abnormality in the latter is less than in those with a long clotting time and approaches very close to normal in quite a number. In three instances it falls into the normal range.

Correction of Haemophilic Blood by Normal Blood.—The effect of the addition of increasing proportions of blood of one normal female and one normal male to that of a haemophilic with prolonged coagulation time is shown in Table VI. It will be seen that in both cases the coagulation time shortens progressively and the prothrombin remaining in the serum rapidly diminishes. There seems to be no significant difference between the ability of the blood of the normal male and the normal female to correct haemophilic blood.

TABLE VI

EFFECT ON COAGULATION TIME AND PROTHROMBIN CONSUMPTION INDEX OF HAEMOPHILIC BLOOD BY ADDITION OF NORMAL BLOOD

Percentage Blood Added	Lee and White Coagulation Time (Mins.)		Prothrombin Consumption Index (%)	
	Normal Male	Normal Female	Normal Male	Normal Female
Nil	34		188	
0.6	16½	13½	81	100
1.25	13½	11½	81	71
2.5	11	11½	41	85
5.0	11½	9½	48	38
9.0	9	7½	40	46
17.0	7½	7	38	31
35.0	7	6½	—	22
Control	6½	7½	<5	<5

FIG. 8.—Shortening of the calcium time of known haemophilic plasma produced by plasma from six normal controls. The percentage of plasma added is plotted on a logarithmic scale. The calcium time of the haemophilic plasma alone was 1,230 seconds.

Recalcified Citrate Plasma.—The ability of six normal controls (four males and two females) to correct the calcium time of haemophilic blood is shown in Fig. 8. In addition the effect of each of these on the prothrombin consumption index of haemophilic plasma is shown in Table VII. Once again no significant difference is apparent in all six normals.

TABLE VII
EFFECT ON THE P.C.I. OF HAEMOPHILIC PLASMA OF ADDITION OF PLASMA FROM SIX NORMALS

Percentage Plasma Added	Prothrombin Consumption Index (%)					
	Male (1)	Male (2)	Male (3)	Male (4)	Female (1)	Female (2)
Nil	177					
0.25	12	15	41	18	14	53
0.5	<5	12	32	11	47	19
1.0	<5	<5	<5	16	<5	<5
100.0	11	<5	<5	9	5	25

Haemophilia with Normal Coagulation Time.—It was found that cases of bleeding diathesis resembling classical haemophilia in practically all respects but having

FIG. 9.—Shortening of the calcium time of known haemophilic blood produced by plasma of one normal control and two haemophiliacs with normal coagulation time. The percentage of plasma added is plotted on a logarithmic scale. The calcium time of the haemophilic plasma alone was 765 seconds.

a normal or near normal coagulation time were able to correct haemophilic blood to some extent but not nearly to the extent possessed by normal blood. A typical example incorporating two such cases (patients 44 and 47 in Table V) and one normal control is shown in Fig. 9.

Discussion

Coagulation Time of Whole Blood.—The results of this series confirm the observation that the coagulation time in haemophilia is often greatly prolonged. There appears to be a gradation of clotting times from those which are greatly prolonged to those which are only slightly, and some which are not at all, prolonged. Provided that only technically perfect venepunctures were considered and no blood transfusion had been given immediately before the test, it was found that in general the coagulation time in any one patient remained of the same order over long periods of time. In one patient, for instance, a Lee and White time of 150 minutes was found a year later to be 52 minutes: both these figures would be regarded as being greatly prolonged and therefore of the same order. Often the readings for the coagulation time did not vary by more than 20% over a year or more. With one exception, no patient having a coagulation time of 15 minutes or less on one occasion recorded a time in excess of 20 minutes on any other occasion. In some of these cases observations were available over a number of years. Even in the one exception the discrepancy was small. Much of course depends on the venepuncture, and unless this is efficiently performed misleading results may be obtained.

The capillary blood coagulation time as measured by the Dale and Laidlaw technique also proved reasonably satisfactory. In three children when only capillary blood was available, as adequate venous blood could not be obtained, it was the only available method. The Lee and White method is preferable and should always be done where possible; if veins are poor, or if repeated tests need to be done to evaluate the effect of therapy or for any other reason, valuable information can be obtained by the use of the Dale and Laidlaw method. It is important that the needle used be sharp and that the first drop of blood issuing be used, as the coagulation time shortens with the second drop (Gibbs, 1924; Christie, 1927). Furthermore the test tends to give false short rather than false long times, so that in all cases where a normal result is obtained it ought to be repeated on at least one occasion.

Quick's test for haemophilia was only occasionally helpful. Both normal and haemophilic blood are sensitive to the removal of platelets, and occasionally haemophilic blood may have even a greater degree of sensitivity. If the calcium time is initially prolonged, high speed centrifuging may increase the calcium time by many minutes, but the proportionate increase might still be no greater than that produced in normal blood. The important feature would then only be the initially prolonged calcium time. Finally, the test is non-specific, and other causes of a prolonged coagulation time, such as circulating anticoagulants, may give a "positive" result (Chargaff and West, 1946; Dreskin and Rosenthal, 1950). The test can only play a small part in the diagnosis of haemophilia.

Prothrombin Consumption Index.—The prothrombin consumption defect of haemophilia is amply demonstrated in this series of cases. It will be seen that by the use of serum from whole clotted venous blood abnormal prothrombin consumption

was found in all but three patients. In haemophilia with prolonged coagulation time the P.C.I. is almost invariably over 100%, but as the coagulation time shortens so the P.C.I. diminishes and its value may fall under 100%. In cases with a Lee and White coagulation time of 15 minutes or less where the diagnosis of haemophilia might be in doubt, the P.C.I. was of definite help in the diagnosis in all but three cases. It is unfortunately not in itself diagnostic because prothrombin consumption defects occur also in thrombocytopenic states (Soulier, 1948; Quick, Shanberge, and Stefanini, 1949; Merskey, 1950a; and others). Even a "functional platelet deficiency" has been described with normal number of platelets and abnormal prothrombin consumption (Soulier, 1948), and I have encountered similar cases. Nevertheless it still remains an important laboratory observation.

The two other methods of measuring the P.C.I. are less sensitive and offer very little advantage over the method using whole clotted venous blood. There is perhaps a case to be made out for the use of capillary blood in that, if an abnormal result is obtained, the diagnosis of haemophilia is extremely probable (Soulier, 1948). An abnormal result was obtained with this test only in one case where the Lee and White time was under 34 minutes; here the Dale and Laidlaw time was nine minutes. In other words, the P.C.I. of capillary blood is only likely to be abnormal if the case is one of haemophilia with prolonged coagulation time, and in this case the Dale and Laidlaw coagulation time should be abnormal. If venous blood is unavailable then it may be a useful confirmatory test in the diagnosis of haemophilia. It is also of interest in that it demonstrates that the tissue fluid of certain haemophilics seems to contain something which is absent in other haemophilics and this may account for some of the clinical differences observed.

Correction of Haemophilic by Normal Blood.—Patek and Stetson (1936) showed that there was a substance present in normal plasma and markedly deficient or unavailable in haemophilic plasma which in relatively small proportions effectively shortened the clotting time of haemophilic blood. This observation has been used as a diagnostic test for haemophilia by many authors. Furthermore it has recently been shown that a small proportion of normal blood can correct the prothrombin consumption defect of haemophilic blood (Quick, 1947; Soulier, 1948; Alexander and de Vries, 1949; and Merskey, 1950a).

In the present paper the test has been placed on a rough quantitative basis in an effort to "assay" the degree by which the test case and the normal control could correct these defects. Normal plasma can be added to the plasma of the patient and the effect on the calcium time of the latter recorded. Alternatively the blood or plasma of the patient can be added to that of a known haemophilic and the effect of this compared with that produced by a normal control.

Addition of Normal to Patient's Plasma.—If the calcium time of the patient is long, the shortening produced by the addition of small proportions of normal plasma may be very suggestive of a diagnosis of haemophilia. If the calcium time of the patient is only slightly prolonged the test has less scope since one soon reaches normal limits. In order to be completely certain in cases with a normal coagulation time it may be necessary to obtain known haemophilic blood and to perform the test in the reverse manner by testing the effect of the patient's blood on the known haemophilic blood.

Addition of Patient's to Known Haemophilic Blood.—It is easier to use citrate plasma rather than whole blood for this test, and the shortening of the calcium time seems to be at least as informative as the improvement in the P.C.I. Apparently no difference exists between donors of either sex and most people seem to be suitable as controls. This confirms the observations of Alexander and Landwehr (1948).

This test has been used as the final criterion in deciding whether or not to classify a doubtful case of haemophilia as such or whether to discard it from the group of true haemophilia. A number of suspect cases were thus excluded, and it was interesting to observe that the clinical picture of these was significantly different. The blood of patients with a circulating anticoagulant may fail to shorten the coagulation time of haemophilic blood but will not react to additions of normal blood and will, in fact, lengthen its clotting time when added in a small proportion. The difficulty of the test is to obtain haemophilic blood. It may perhaps be possible in the future to use haemophilic dog's plasma since the coagulation time of this is shortened by normal dog's plasma or by the use of a fraction prepared from human plasma (Graham, Buckwalter, Hartley, and Brinkhous, 1949), but no canine haemophilic plasma was available for trial.

It was of great interest to note the amount of normal blood required to correct the defects of haemophilic blood. Whereas under 1% of normal blood had a marked influence on these defects, 10% or even more was required for full correction of the coagulation time and prothrombin consumption index. When citrated plasma was used the prothrombin consumption index was restored to normal on the addition of under 1% of normal plasma, but the calcium time practically never reverted to normal even if over 10% of normal plasma was used. There appears to be a great difference between an improvement in these defects and full correction of them. Furthermore, very much more whole (uncitrated) blood seems to be required to bring the prothrombin consumption index back to normal than when citrated plasma is used. Very small proportions of whole blood do not seem to correct the coagulation defects of haemophilia.

Grades of Haemophilia.—From the evidence presented so far it seemed justifiable to grade the defect in the various haemophilics of the series. They appeared to fall roughly into three grades (Fig. 10).

Grade A : Lee and White Coagulation Time of Over 15 Minutes.—In this grade practically all the tests for haemophilia were positive. By a combination of a prolonged Dale and Laidlaw coagulation time and abnormal prothrombin consumption of capillary blood a reasonably certain diagnosis of haemophilia could be reached on finger-prick blood alone.

Grade B : Lee and White Coagulation Time of Between 10 and 15 Minutes.—In these patients laboratory tests showed fewer positive results. In particular, capillary blood usually consumed prothrombin during coagulation, while the figures for the prothrombin consumption index of venous blood tended to be lower than those recorded in Grade A.

Grade C : Lee and White Coagulation Time Under 10 Minutes.—In these patients laboratory tests showed still fewer positive results. In no case could abnormal prothrombin consumption on capillary blood be demonstrated. In 25% of these cases too even prothrombin consumption of venous blood became normal.

FIG. 10.—The “grades” of haemophilia based on Lee and White coagulation times, and the percentage of abnormal results found by other tests in patients of this series. *Grade A*, Lee and White time > 15 minutes. *Grade B*, Lee and White time 10–15 minutes. *Grade C*, Lee and White time < 10 minutes.

Fig. 10 demonstrates the percentage of positive results with the Dale and Laidlaw coagulation time and the three methods of measuring prothrombin consumption in the three grades of haemophilia. The degree of defect remained constant in any one individual and in any particular family (Table V) even over a number of generations.

While these three grades of the condition are described, the overall impression gained from this series was that all grades of the disease exist and one grade slides imperceptibly into the next.

It is even possible that the step from normal subjects to the milder grades of haemophilia may not be very large, but no evidence is at present available on this point. Finally it will be of great interest to observe the safety or otherwise of surgical procedures in the varying grades of this condition.

Correlation of Laboratory Findings and Clinical Picture.—The clinical picture was almost invariably severe when the Lee and White coagulation time was over 15 minutes (Grade A). It did not seem to matter by how much this figure was exceeded. But under 15 minutes (Grade B) the clinical picture seemed, in some cases, to be a little milder, and once the Lee and White time fell into the normal range (Grade C) a mild clinical syndrome was the rule rather than the exception. Of the 11 families whose Lee and White coagulation time was less than 15 minutes, seven had a mild clinical syndrome, one was intermediate, and three were very severe. In the seven families with the milder syndrome haemorrhages tended to be much less frequent and less severe, and complications, especially haemarthroses, slight or absent. Many of the patients were able to lead normal lives and were only slightly incapacitated. Yet even in these patients operations were poorly tolerated; they bled excessively after dental extractions, and practically all of them had had at least one severe haemorrhagic episode seeming to endanger life. Of the three families with a normal coagulation time and a severe clinical syndrome, two patients had had almost every known complication of haemophilia and were severely incapacitated, and two members of another family had died of apparently spontaneous gastro-intestinal haemorrhage. Nine of these families are described in detail in other papers (Merskey, 1950b, c).

Summary and Conclusions

An analysis has been made of the value of the diagnostic procedures used in haemophilia. The series consisted of 72 patients. In 39% of these it was impossible to trace a family history of haemorrhagic disease.

While the lengthening of the coagulation time remained a very valuable diagnostic sign, a normal coagulation time by no means excluded the condition.

The Lee and White method of measuring the coagulation time was adequate provided technically imperfect procedures were rejected. The Dale and Laidlaw technique of measuring coagulation time was a little less reliable, but by no means valueless, and much information was obtained from its use. In three children only capillary blood was available and the test was then invaluable.

Quick's test for haemophilia was found to be of small diagnostic value only.

The measurement of the prothrombin consumption defect was a useful confirmatory test of haemophilia. The greatest information was obtained if this was measured on the serum from whole clotted venous blood. Serum from capillary

blood was only of value in cases with prolonged coagulation time. Serum from recalcified citrate plasma was intermediate in value between these two.

In doubtful cases, titration of the amount of antihaemophilic globulin in the plasma was of great value in establishing a diagnosis.

By the use of all these tests three grades of haemophilia were distinguished. The grades were not very clearly defined and intermediate cases existed. This gradation was reflected in both the clinical and the laboratory manifestations of the disease.

The degree of the defect in any one case seemed to remain constant over long periods of time.

The degree of the defect was constant in any family even over a number of generations.

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