THE CONSUMPTION OF PROTHROMBIN DURING COAGULATION

THE DEFECT IN HAEMOPHILIA AND THROMBO- CYTOPENIC PURPURA

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During the process of normal blood coagulation prothrombin is converted into thrombin. As a result serum contains considerably less prothrombin than the plasma from which it is derived, the prothrombin having been consumed during the coagulation of the blood. In certain conditions this conversion is incomplete and much prothrombin remains in the serum. Often this is not obvious from the usual tests of clotting function since only a small amount of thrombin is necessary for apparently normal coagulation of blood in glass tubes. This defect is unmasked by the prothrombin consumption test which offers another method for studying the efficacy of the blood clotting process.

Bordet and Delange (1912) were the first to observe deficient prothrombin consumption in normal platelet-free plasma, and their findings were confirmed and elaborated by Quick (1947) and Quick, Shanberge, and Stefanini (1949a). A similar defect was reported in thrombocytopenic purpura (Soulie, 1948; Quick *et al.*, 1949b, and Alexander and de Vries, 1949a), while at the same time Soulier (1948) pointed out that there were a number of haemorrhagic syndromes where, although the platelets were not numerically decreased, there was defective prothrombin consumption, and he postulated a qualitative platelet defect in these conditions. Brinkhous (1939) had pointed out a prothrombin consumption defect in haemophilia, and this observation was confirmed and extended by Quick (1947), Soulier (1948), and Alexander and de Vries (1949b). The observations of Quick (1947) and Soulier (1948) showed that thrombocytopenic plasma and haemophilic plasma consumed little prothrombin during coagulation. On mixing and recalcifying these two types of plasma, a normal amount of prothrombin was used up in the clotting process. It was therefore argued that the defective consumption of prothrombin was due to a different deficiency in the two types of plasma, since each appeared to contain an excess of a factor lacking in the other. It is the purpose of this study to confirm and extend these observations.

Technique

Reagents were prepared as follows.

Brain Thromboplastin.—Fresh human brain was collected from the post-mortem room, all superficial vessels and meninges removed, and the substance ground with

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acetone in a mortar. The acetone was poured off and replaced several times and the remaining granular powder dried on a suction filter. When completely free of acetone the powder was kept in bottles at room temperature. This material can be stored for three months without deterioration.

When required for the test 0.5 g. dried brain was mixed with 5 ml. normal (0.85%) saline and incubated for 15 minutes at 37° C. It was then centrifuged at 500 revolutions per minute for one minute and the supernatant emulsion diluted with normal saline to the concentration which had been shown by trial for that particular batch to give the best results. This was usually a 1/5 dilution. A fresh suspension of brain was made daily.

Calcium Chloride Solution.—This was made up to a strength of $\frac{\text{molar.}}{40}$

Fibrinogen.—Jaques' (1943) method was followed. A 3-mol. phosphate buffer at pH 6.6 was made by dissolving 817 g. of anhydrous KH_2PO_4 in 1,000 ml. of distilled water. After warming, the volume was made up to 2,000 ml. and the solution filtered: 2 mol., 1 mol., or $\frac{\text{mol.}}{4}$ solutions were made from this stock solution.

Stored citrated human plasma was used after preliminary adsorption with an excess of barium sulphate. To 100 ml. of adsorbed plasma 100 ml. of 2 mol. phosphate buffer is added. After centrifuging, the precipitate is washed with 100 ml. of mol. phosphate buffer and then dissolved in 75 ml. of $\frac{\text{mol.}}{4}$ phosphate buffer. This precipitation with an equal volume of 2 mol. phosphate buffer, washing with mol. phosphate buffer and redissolving the precipitate in three-quarters of the volume of $\frac{\text{mol.}}{4}$ phosphate buffer, was repeated twice. The final precipitate was dissolved in 50 ml. of normal (0.85%) saline containing 0.16% sodium citrate. The solution was dialysed for 24 hours against 3% sodium citrate in normal saline, the dialysing fluid being changed after the first 12 hours. Excess solids were finally removed by centrifugation. The fibrinogen was stored at -20° C. and thawed immediately before use when its strength was adjusted with normal saline to lie between 80-120 mg. fibrinogen per 100 ml.

Collection of Plasma.—Blood was withdrawn by venepuncture into a clean dry syringe and added to 2% potassium oxalate or 3.8% sodium citrate in the proportion of one part of anticoagulant to nine parts of blood. It was then centrifuged at 1,000 revolutions per minute for 10 minutes to obtain plasma.

Collection of Serum.—From the same syringe four samples, each 1 ml., were added to four clean, dry standard test-tubes ($2\frac{1}{2}$ in. $\times$ $\frac{3}{8}$ in.) which were immediately incubated in a water bath at 37° C. The tubes were tilted every half minute until coagulation occurred, and the time between the first appearance of blood in the syringe and the coagulation was recorded as the Lee and White coagulation time. The tubes were then left undisturbed until one hour after coagulation had occurred, when the clot was gently separated from the side of the tube. The tubes were centrifuged at 1,000 revolutions per minute for three minutes and the serum separated.

Prothrombin Consumption Test Using Serum from Whole Clotted Blood

This test* is devised to measure the amount of prothrombin present in the original plasma as well as the amount remaining in the serum one hour after the blood has

*Modification of test devised by Rosemary Biggs.

clotted, the plasma and the serum being tested separately. In each case a two-stage technique is used. Any prothrombin which is present in the plasma or serum is converted into thrombin by the addition of an excess of brain thromboplastin and an optimum amount of calcium, and it is assumed that the amount of thrombin formed is proportional to the amount of prothrombin which was present originally. The amount of thrombin formed is measured by transferring measured amounts of the original incubation mixture to other tubes containing fibrinogen and the time taken for these latter tubes to clot is recorded. The more thrombin generated in the incubation mixture the more rapidly will the fibrinogen be clotted. By this means the amount of thrombin formed from the plasma and serum can be measured and the amount of prothrombin which each originally contained can be inferred.

Technique.—The standard test-tubes ($2\frac{1}{2}$ in. $\times$ $\frac{3}{8}$ in.) were used throughout and the whole test was carried out at 37° C. To 0.2 ml. plasma (or serum) 0.2 ml. 0.85% sodium chloride and 0.2 ml. brain thromboplastin is added. As rapidly as possible thereafter 0.2 ml. $\frac{M}{40}$ calcium chloride is added, starting a stop-watch at this instant. The contents of the test-tube are mixed by inversion. Samples, each 0.1 ml., are removed from this mixture at 10 seconds, 20 seconds, 60 seconds, 120 seconds, and 180 seconds after the calcium has been added by means of a pipette calibrated to "blow out." Each of these samples is added to separate tubes containing 0.4 ml. fibrinogen, and the time which elapses between the addition of the 0.1 ml. of the incubation mixture to the fibrinogen and the coagulation of this mixture is noted. The end point is taken as that at which the first strands of fibrin appear.

In performing the test it is advantageous to have the assistance of one other person. The first observer prepares the incubation mixture and transfers the 0.1 ml. specimens to the fibrinogen solution. The second observer times the clotting of the second series of tubes, starting a second stop-watch at the instant the 10-second sample is added to the fibrinogen. With a little practice it is perfectly possible for one person to do the whole test.

When the plasma is tested it will be noted that a clot forms in the incubation mixture about 14 to 18 seconds after the calcium has been added. This is because the mixture is similar to the Quick one-stage prothrombin test. This clot needs to be expressed around a wooden applicator before the later specimens can be obtained. The 20-second specimen is usually removed from the incubation mixture just before the coagulation of the mixture. Owing to the absence of fibrinogen no such difficulty is encountered in the case of normal serum, but is occasionally met with in abnormal sera, especially haemophilic.

In a typical normal case the following results were obtained (Table I).

TABLE I
PROTHROMBIN CONSUMPTION TEST: TYPICAL RESULT IN A NORMAL INDIVIDUAL

Incubation time (secs.) ..	10	20	60	120	180
Plasma time (secs.) ..	30	18	16	25	46
Serum time (secs.) ..	220	165	160	205	242

The prothrombin consumption index would be recorded as:

$$\frac{\text{Most rapid plasma time}}{\text{Most rapid serum time}} \times 100$$

or in the example quoted:

$$\frac{16}{160} \times 100 = 10\%$$

The most rapid time is usually recorded in the 20- or the 60-second specimen, though occasionally one of the other specimens gives the shortest time. In most cases the time usually lengthened after the 60-second specimen, presumably from the action of antithrombin.

In normal cases the prothrombin consumption index has always been under 40%. In practically all cases of haemophilia and in blood rendered artificially thrombocytopenic by high-speed centrifugation, the prothrombin consumption index has been over 100% and in many cases even exceeding 150%. In these cases, therefore, the time taken for the incubation mixture derived from the serum to clot the fibrinogen has been less than that derived from the plasma. In other words the serum appeared to contain more prothrombin than the plasma. This phenomenon was noted by Bordet and Delange (1912) in the case of thrombocytopenic blood, and by Quick *et al.* (1949a) in both thrombocytopenia and haemophilia. It can also be inferred from the data published by Alexander and de Vries (1949b) in half of their cases. Quick *et al.* (1949a) commented that this demonstrated how inaccurate was the estimation of prothrombin in serum. It appears most unlikely that there is more prothrombin in serum than in plasma. A similar apparent increase in prothrombin can be observed if ordinary plasma is clotted with thrombin and the "serum" tested, once the thrombin added had been neutralized. The exact nature of this phenomenon remains at present unexplained.

Prothrombin Consumption Test using Serum from Recalcified Citrate or Oxalate Plasma

The test can also be done on recalcified citrate or oxalate plasma. In this modification of the test 1.0 ml. plasma in a standard test-tube is recalcified and the time taken for the tube to clot is recorded as the calcium time. In the experiments recorded in this paper the plasma was recalcified with 20 c.mm. of molar calcium chloride. Since this solution is hypertonic, care must be exercised in its use. Alternatively, a larger amount of $\frac{\text{molar}}{40}$ calcium chloride can be used. One hour after coagulation has occurred the clot is expressed around a wooden applicator, and 0.2 ml. of serum is removed and tested in a similar manner to that applied to serum from whole clotted blood.

This adaptation allows for experiments to be made with mixtures of various plasmas. It has some limitations which can modify the results. In particular, the speed and duration of centrifugation need to be very carefully standardized since these factors will alter the number and physical characters of the blood platelets. The following experiment demonstrates the effect of increasing speed and duration of centrifugation in a specimen of normal plasma (Table II).

TABLE II
EFFECT ON PROTHROMBIN CONSUMPTION OF CENTRIFUGATION OF A SPECIMEN OF PLASMA
AT SEVERAL SPEEDS

Speed and Duration of Centrifugation (R.P.M. × Minutes)	Calcium Time (Mins.)	Plasma Time (Secs.)					Serum Time (from Recalcified Citrate Plasma) in Secs.					Prothrombin Consumption Index (%)
		10	20	60	120	180	10	20	60	120	180	
1,000 × 5 ..	3½	30	27	14	15	40	360+	360+	360+	360+	360+	under 5
1,500 × 5 ..	3½						250	195	195	200	245	7
2,000 × 10 ..	5						110	70	70	140	170	20
3,000 × 10 ..	5½						140	120	53	65	72	26

As the speed and duration of centrifugation increased, the plasma became clearer and the number of platelets diminished. When each of these plasmas was recalcified less prothrombin consumption occurred corresponding with the decrease in platelets. This effect was only noted once the plasma had been recalcified, the uncalcified citrated plasma always giving the same readings whatever its content of platelets. The use of recalcified plasma introduces a number of variables which are not very easy to control. For this reason the simpler method is preferable—i.e., using serum from whole clotted blood.

In the performance of these prothrombin consumption tests it needs to be emphasized that the test is susceptible to small alterations in technical detail. In particular, a bad venepuncture will cause an abnormally low reading. Dirty test-tubes, wet test-tubes, tubes of different size, and varying amounts of filling and tilting of the tubes during coagulation of the blood will all cause different readings.

For all these reasons the technique adopted should be rigidly followed and too much emphasis should not be laid on small differences in results.

Results

In Normals.—Observations on 37 normals are recorded in Fig. 1. These results were obtained by the use of serum from whole clotted blood. The subjects were mostly fit laboratory workers. The age and sex distribution of the controls are shown in Table III.

It will be noted that in the vast majority of cases the prothrombin consumption index is under 20% while all the

FIG. 1.—Prothrombin consumption index of 37 normal subjects.

TABLE III
SEX AND AGE DISTRIBUTION OF NORMAL CONTROLS

Age in Years :	15-20	21-25	26-30	31-35	36-40	41-45	46-50
Males ..	2	2	5	4	1	—	1
Females ..	10	8	1	1	1	—	1
Total ..	12	10	6	5	2	—	2

normals fall in the range 0-40%. These figures compare well with those obtained by Alexander *et al.* (1949) where a one-stage technique was used, and the normal range was between 0-32% with the majority falling in the range 0-16%.

In Haemophilia and Thrombocytopenia.—Nineteen cases of haemophilia have been tested, and the results on the serum from whole clotted blood were as in Table IV.

TABLE IV
PROTHROMBIN CONSUMPTION IN 19 CASES OF HAEMOPHILIA

Case No. and Name	Age (Years)	Plasma Time (Secs.)					Serum Time (Secs.)					Prothrombin Consumption Index (%)
		10	20	60	120	180	10	20	60	120	180	
1 R.E.	9	16	20	17	37	75	50	40	40	105	180	40
2 A.B.	59	60	50	20	40	93	20	15	15	25	40	133
3 V.M.	12	33	38	27	46	105	17	15	18	30	45	180
4 W.D.	6	20	20	17	28	64	26	25	12	48	60	142
5 E.G.	50	25	94	36	60	100	20	20	25	40	70	125
6 P.R.	4	45	35	45	95	125	40	35	42	80	140	100
7 T.N.	38	25	20	20	45	45	60	50	25	15	32	133
8 J.D.	42	23	23	19	27	46	17	17	14	18	30	136
9 L.B.	11	20	19	23	55	61	15	20	23	44	85	127
10 J.O.	10	40	30	25	28	42	30	25	15	30	33	167
11 A.A.	50	23	25	22	37	58	18	25	30	50	95	122
12 T.E.	10	35	18	18	26	41	52	44	30	54	—	60
13 J.L.	50	40	23	21	29	43	37	22	22	32	60	95
14 A.H.	4	25	20	18	25	48	12	12	10	21	37	180
15 P.H.	18	30	23	20	28	42	20	17	25	38	66	118
16 P.L.	10	25	17	15	30	40	15	10	12	30	40	150
17 T.H.	10	25	22	16	26	49	15	10	11	15	25	160
18 M.J.	36	27	24	18	30	41	25	18	18	29	44	100
19 S.L.	50	26	20	15	35	42	30	20	23	27	35	75

It will be seen that in almost all cases values over 100% were obtained. In a few cases a little prothrombin consumption occurred. In the case R.E. it was noted that the venepuncture was not cleanly performed, but this did not apply in the other cases.

The test has been carried out in eight cases of naturally occurring thrombocytopenia. The results, using serum from whole clotted blood, are as set out in Table V.

TABLE V
PROTHROMBIN CONSUMPTION INDEX IN 8 CASES OF NATURALLY OCCURRING THROMBOCYTOPENIA

Case No. and Name	Disease	Platelet Count (per c.mm.)	Bleeding Time (Min.)	Purpura	Hess Test	Plasma Time (Secs.)					Serum Time (Secs.)					Prothrombin Consumption Index (%)
						10	20	60	120	180	10	20	60	120	180	
1 G.F.	Aplastic anaemia ..	4,000	20+	+	+	50	40	15	20	37	20	17	15	27	40	100
2 F.G.	Subacute myeloid leukaemia	Under 30,000	—	—	—	40	20	17	24	60	35	30	23	50	75	74
3 B.S.	Idiopathic thrombocytopenic purpura ..	0	25+	++	++	25	20	18	25	40	35	33	35	75	180	55
"	14 days later ..	28,000	4	—	±	20	15	24	58	105	75	75	75	180	180	20
4 M.J.	Thrombocytopenia (due to gold)	6,000	15+	++	++	25	20	12	21	45	25	20	15	23	60	80
5 D.H.	Idiopathic thrombocytopenic purpura	11,000	15+	++	++	17	15	20	34	—	22	15	24	32	—	100
6 A.J.	Pernicious anaemia in relapse	80,000	4	—	—	30	22	16	31	57	290	240	220	300+	—	7
7 D.P.	Thrombocytopenia purpura ? toxic	7,000	20	++	±	25	15	13	20	—	15	10	10	20	—	130
8 S.H.	Idiopathic thrombocytopenic purpura	12,000	30+	+	+	15	18	23	40	—	22	17	23	41	—	88

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It will be seen that the prothrombin consumption defect is present in thrombocytopenia of varying aetiology. Provided the platelet count has been sufficiently reduced, the defect is present. Values over 100% are much rarer, possibly because some platelet fragments remain or because the platelets have not been entirely eliminated from the circulation.

If citrated plasma is centrifuged at high speed (15,000 r.p.m.) for 20 minutes, the supernatant plasma contains very few platelets. If this plasma is recalcified no prothrombin consumption occurs. In many cases values over 100% are obtained. In fact, if such a value is not obtained, the plasma should be recentrifuged, when almost invariably the result will exceed 100% (Table VI).

TABLE VI
PROTHROMBIN CONSUMPTION INDEX IN NORMAL PLASMA RENDERED THROMBOCYTOPENIC BY HIGH-SPEED CENTRIFUGATION

Source of Normal Plasma	Plasma Time (Secs.)					Serum Time (Secs.)					Prothrombin Consumption Index (%)
	10	20	60	120	180	10	20	60	120	180	
Donor A	50	30	17	16	35	15	15	20	30	55	107
" B	22	14	22	37	60	14	13	17	28	—	108
" C	22	14	22	37	60	14	13	17	28	—	108
" D	26	16	16	19	38	14	11	12	19	50	145
" E	30	16	14	19	35	15	15	21	34	—	93
" F	17	14	15	19	24	18	14	16	18	38	100

It will be seen that in four out of six cases values over 100% were obtained, so that it appears likely that the mere removal of platelets has stopped prothrombin consumption. While the calcium time of these plasmas is usually prolonged to about eight to ten minutes they clot firmly so that an adequate amount of fibrinogen remains. In one case where the fibrinogen was estimated before and after high-speed centrifugation, it had fallen from 360 mg. per 100 ml. to 246 mg. per 100 ml. after 30 minutes at 17,000 r.p.m. The one-stage (Quick) prothrombin time, and the two-stage plasma time as measured in this test remained unaltered, so that it was unlikely that any material alteration in prothrombin or factor V had occurred in the plasma. It seemed fair therefore to ascribe the differences in behaviour of this plasma to the removal of the platelets.

TABLE VII
EFFECT OF MIXTURES OF NATURALLY OCCURRING THROMBOCYTOPENIC PLASMA AND HAEMOPHILIC PLASMA ON PROTHROMBIN CONSUMPTION

Volume of Haemophilic Plasma (ml.)	Volume of Thrombocytopenic Plasma (ml.)	Prothrombin Consumption Index (%)
1.0	—	180
—	1.0	80
0.9	0.1	8
0.1	0.9	35
0.5	0.5	9

TABLE VIII
EFFECT OF MIXTURES OF ARTIFICIALLY PREPARED THROMBOCYTOPENIC AND HAEMOPHILIC PLASMA ON PROTHROMBIN CONSUMPTION

Volume of Haemophilic Plasma (ml.)	Volume of Thrombocytopenic Plasma (ml.)	Prothrombin Consumption Index (%)
1.0	—	108
—	1.0	108
0.9	0.1	30
0.1	0.9	50
0.5	0.5	21

In Mixtures of Thrombocytopenic* and Haemophilic Plasmas.—Fresh citrated haemophilic plasma was mixed with fresh citrated plasma from a case of subacute myeloid leukaemia with a platelet count of under 5,000 per c.mm. The mixture was recalcified and tested as previously described (Table VII).

The next experiment (Table VIII) was similar to the last, except that normal citrate plasma freed from platelets was used as a source of thrombocytopenic plasma.

Similar results were obtained with both experiments. Neither the haemophilic nor the thrombocytopenic plasmas were capable of consuming prothrombin. Yet mixtures of these two plasmas had this capacity. On the whole it seemed as if the platelet-free plasma was able to correct the haemophilic plasma more easily than the haemophilic plasma was able to correct the platelet-free plasma. For this reason it was decided to measure these defects quantitatively.

Quantitative Experiments.—In the first experiment decreasing amounts of normal plasma were added to haemophilic plasma and the mixtures recalcified as before (Table IX).

TABLE IX
EFFECT OF THE ADDITION OF DECREASING AMOUNTS OF NORMAL TO HAEMOPHILIC PLASMA

Volume of Haemophilic Plasma (ml.)	Volume of Normal Plasma (ml.)	Final Concentration of Normal in Haemophilic Plasma (%)	Prothrombin Consumption Index (%)
1.0	—	—	133
0.9	0.1	10	10
0.9	0.01†	1	17
0.9	0.001†	0.1	66
0.9	0.0001†	0.01	92

† Made up to 0.1 ml. with normal saline.

It will be seen that 10% of normal plasma is quite adequate, and 1% of normal plasma is also capable of exerting an almost maximal effect. Even 0.1% of normal plasma has some effect. Thus normal plasma is capable of correcting haemophilic plasma as far as the prothrombin consumption defect is concerned when added in very small amount. This effect is quite independent of the platelets added and can

* In order to simplify reading only the prothrombin consumption index has been recorded.

TABLE X
EFFECT OF ADDITION OF INCREASING AMOUNTS OF PLATELET-CONTAINING PLASMA TO
PLATELET-FREE PLASMAS

Volume of Platelet-free Plasma (ml.)	Volume of Platelet-containing Plasma (ml.)	Prothrombin Consumption Index (%)
1.0	—	100
—	1.0	8
0.95	0.05	40
0.90	0.10	28
0.85	0.15	19
0.80	0.2	17.5
0.75	0.25	15

be obtained with platelet-free plasma. The amount of plasma required was found to be considerably less in this patient than that reported by Alexander and de Vries (1949b). Unlike at least two of the cases reported by these authors this patient had not been receiving plasma transfusions.

In the next experiment platelet-containing plasma was added to platelet-free plasma prepared as previously described. The blood from which the platelet-containing plasma was derived had been centrifuged at 2,000 r.p.m. for 5 minutes (Table X).

This experiment was performed on a number of occasions since one tube was often "out of step." Nevertheless the result was always the same; as the volume of platelet-containing plasma increased, so did the prothrombin consumption. This result, however, depended to some extent on the number of platelets in the platelet-containing plasma, and in general the faster the plasma was spun the less its effect. This was only to be expected since centrifugation at faster speeds removed the platelets more efficiently. If haemophilic plasma was used as a source of platelets, these

TABLE XI
EFFECTS ON PROTHROMBIN CONSUMPTION OF MIXTURE OF PLATELET-FREE AND PLATELET-CONTAINING
NORMAL AND HAEMOPHILIC PLASMAS

	Volume of Citrated Plasma Recalcified (ml.)				Prothrombin Consumption Index (%)
	Platelet-free		Platelet-containing		
	Normal Plasma	Haemophilic Plasma	Normal Plasma	Haemophilic Plasma	
A	1.0	—	—	—	127
B	—	1.0	—	—	160
C	—	—	1.0	—	15
D	—	—	—	1.0	127
E	0.1	—	—	0.9	20
F	—	—	0.1	0.9	17
G	0.5	0.5	—	—	120
H	—	0.9	0.1	—	75
I	—	0.8	0.2	—	60
J	—	0.7	0.3	—	22

platelets were as good as, if not better than, normal platelets. It will be seen that the volumes which require to be added to platelet-free plasma to correct the prothrombin consumption defect are very much larger than those required to correct the defect in haemophilic plasma. This would then be an additional argument in favour of the identity of the two factors which are the cause of the prothrombin consumption defect in these two types of plasma.

As a final check of the correctness of the hypothesis that at least two factors are required for the consumption of prothrombin, the experiments were repeated using both platelet-containing and platelet-free normal and haemophilic plasma (Table XI).

Rows A to D show that the only plasma capable of consuming prothrombin is the platelet-containing normal plasma. Rows E and F show that either platelet-containing or platelet-free normal plasma is capable of correcting a platelet-containing haemophilic plasma, while row G shows that even a much larger quantity of platelet-free normal plasma is incapable of correcting a platelet-free haemophilic plasma.

Rows H to J demonstrate once again that the platelets require to be added in sufficient amount to haemophilic plasma before adequate prothrombin consumption occurs.

It would thus appear that both platelets and the addition of a small amount of normal plasma are required in haemophilic plasma before adequate prothrombin consumption occurs, normal or haemophilic platelets being equally effective.

Discussion

The experiments recorded in this paper substantially confirm the work of the authors quoted earlier. Whatever else may be needed for adequate prothrombin consumption, a sufficient number of platelets is necessary as well as a small amount of a substance present in normal plasma. In both thrombocytopenia and haemophilia defective prothrombin consumption occurs. Thrombocytopenic normal plasma contains a vast excess of the factor which is capable of correcting haemophilic plasma, and this could be the substance which is present in normal plasma and known as "anti-haemophilic globulin." Only a very small amount of normal plasma, of the order of 1%, is required to be added to haemophilic plasma to render this indistinguishable from normal plasma in respect of its prothrombin consumption. On the other hand, platelet-containing haemophilic plasma can correct the defect in thrombocytopenic plasma provided enough platelets are added. In this respect haemophilic plasma behaves at least as well as normal plasma, which would be another argument in favour of the inherent normality of haemophilic platelets. The amount of platelet-containing normal plasma which needs to be added largely depends on the platelet content of the plasma, and thus varies from patient to patient and with the speed and duration with which the plasma has been centrifuged. This does not exclude the possibility that some platelets may be more efficient than others. Much more platelet-containing plasma is required to restore the prothrombin consumption of platelet-free plasma than is required to correct haemophilic plasma. These facts would tend to show that the defects in the two instances are different.

Summary

Observations have been presented on the prothrombin consumption defect present in thrombocytopenic and haemophilic plasma.

Whereas in normal subjects less than 40% of the prothrombin present in the plasma remains in the serum one hour after coagulation of the blood has occurred, in these two conditions anything over 70% may be present. In many of these cases the apparent amount of prothrombin in the serum is greater than in the plasma.

After removal of platelets from normal plasma by high-speed centrifugation, prothrombin consumption ceases.

By mixing the plasmas of haemophilia and thrombocytopenia it can be shown that each of these is capable of correcting the defect in the other. Furthermore, the quantities which need to be added in the two instances are not the same, and these two facts appear to be in favour of the separate identity of these two substances.

The addition to haemophilic plasma of quantities of normal plasma comparable to those which correct the defect in the coagulation time, of the order of 1%, prove equally adequate with regard to prothrombin consumption. It is likely that the substance acting is the same in both instances and is probably "antihaemophilic globulin."

In platelet-free plasma, platelets need to be added in very much larger quantity before the defect is corrected. Depending on the speed and duration of centrifugation, between 10% and 30% or more may be needed. In this respect haemophilic platelets are as good as normal platelets.

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