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HUMAN PROTHROMBIN: QUANTITATIVE STUDIES ON THE PLASMA LABILE FACTOR AND THE RESTORATIVE EFFECTS OF NORMAL, HYPOFI-BRINOGENEMIC, AND HEMOPHILIC PLASMA ON THE PROTHROMBIN OF STORED PLASMA

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Research Article

HUMAN PROTHROMBIN: QUANTITATIVE STUDIES ON THE PLASMA LABILE FACTOR AND THE RESTORATIVE EFFECTS OF NORMAL, HYPOFIBRINOGENEMIC, AND HEMOPHILIC PLASMA ON THE PROTHROMBIN OF STORED PLASMA [1]

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In 1943 Quick (1) reported on the multiple nature of plasma prothrombin. On the basis of his data, which have been repeatedly confirmed (2–4), he concluded that prothrombin consists of two essential factors combined with calcium. The two components, "prothrombin A" and "prothrombin B," had different properties. The former was labile in oxalated or citrated plasma stored at refrigerator temperatures, not adsorbable by aluminum hydroxide, fairly heat stable, and not reduced in dicumarolized blood. Its deterioration was said to account for the high prothrombin time of stored plasma. "Prothrombin B," on the other hand, remained intact in refrigerated plasma, was removed by aluminum hydroxide, was labile at 60° C. for 15 minutes, and was decreased by the administration of dicumarol.

More recently other entities were described which affect the conversion of prothrombin to thrombin by thromboplastin plus calcium (5–7). These are apparently distinct from both "prothrombin B" and the factor which deteriorates in stored plasma. The term "prothrombin A" is now reserved by Quick (5) for one of these substances, and the term "labile factor" for the moiety which is found wanting in stored plasma. Normal prothrombin activity, as determined by the one-stage method, requires the presence of adequate amounts of all these substances.

Loomis and Seegers (4) found that the prothrombin activity of aged plasma could be restored remarkably by the addition of purified fibrinogen. Accordingly, they concluded that deterioration of fibrinogen accounts for lengthening of the prothrombin time in stored plasma and that "reactive fibrinogen" is necessary for prothrombin activity by the one-stage technique. This conclusion is not in accord with those of Munro and Munro (8) and Honorato and Quick (9). The last authors (9) demonstrated that purified human fibrinogen was incapable of reactivating the prothrombin of stored human plasma. Furthermore, it has also been shown that plasma defibrinated by thrombin could reactivate the prothrombin (9, 10).

The availability of afibrinogenemic plasma from a subject with spontaneous fibrinopenia [3] permitted decisive resolution of this conflict. It is the purpose of this report to present data supporting the evidence for the existence of a labile factor which is distinct from fibrinogen and whose deterioration as plasma ages accounts for the increasing prothrombin time. Also included are additional observations on the prothrombin activity of stored plasma and on the restorative effects of hemophilic plasma.

METHODS

Prothrombin activity was determined by the Quick method (11, page 312) with the following modification (12): Commercial (Difco) rabbit brain was used as the source of thromboplastin. Into each of many prothrombin-time tubes were pipetted 0.1-cc. aliquots of a uniform batch of freshly prepared thromboplastin, which were then stored at − 10° C. until prothrombin determinations were made. Although thrombloplastin thus kept frozen in ready-to-use units maintains full potency for eight months, new thromboplastin preparations were made every two weeks. The control prothrombin value, determined on normal plasma with each fresh batch, served as the control for the two-week period.

In some experiments the prothrombin time was determined on whole plasma unaltered or mixed with afibrinogenemic or hemophilic plasma. In others, the plasma or plasma mixtures were diluted with normal plasma ren-

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dered essentially free of prothrombin by prior adsorption with barium sulfate according to the technique of Rosenfield and Tuft (13). The prothrombin time of the diluted mixture was then determined. By comparing the values thus obtained with those of normal plasma diluted serially in the same manner actual prothrombin activities in per cent of normal were calculated.

Fibrinogen[4] was measured by the method outlined by Quick (11, page 323). To 1.0 cc. of oxalated or citrated plasma were added 30 cc. of H_2O, 2.5 cc. of 0.02 M $CaCl_2$, and two drops of a saline solution containing 50 units of thrombin (Topical thrombin, Parke-Davis) per cc. After incubation at 37° C. for 15 minutes, the clot was wound on a glass rod, washed with physiological saline, hydrolyzed with sodium hydroxide and its tyrosine content determined. Since it is almost impossible to remove completely the fibrin clotted in stored plasma by winding on a glass rod, a technique was devised whereby the fibrin was filtered through glass wool, washed and then hydrolyzed in the usual manner. The procedure together with observations on changes in plasma fibrinogen during storage will be reported in detail in a subsequent communication (14).

Oxalated (1 cc. of 0.1 M sodium oxalate to 9 cc. of blood) or citrated (1 cc. of 2.5 per cent sodium citrate to 9 cc. of blood) normal blood was stored at 4–5° C. until its prothrombin time became prolonged. Sterile precautions were used and specimens were cultured to exclude bacterial contamination. Aliquots were then mixed with varying amounts of fresh normal, fresh or freshly frozen fibrinopenic, fresh hemophilic plasmas, or with fresh barium sulfated[5] plasma, and prothrombin time determined.

RESULTS

Effect of $BaSO_4$ adsorption on plasma clotting constituents: The prothrombin time of $BaSO_4$ plasma exceeds 180 seconds, indicating a prothrombin activity of less than 1 per cent of normal. The question arises whether $BaSO_4$ removes important clotting constituents other than prothrombin. Although plasma so treated loses 100 mgm. of protein per 100 cc. (13) its fibrinogen concentration and antihemophilic potency were found unchanged. Also (see below) it is fully capable of rectifying the delayed prothrombin time of stored plasma. It is noteworthy that $BaSO_4$ removes far less prothrombin from citrated than from oxalated plasma.

Prothrombin activity of aged plasma and restorative ability of $BaSO_4$ plasma: The activity of normal oxalated plasma stored at 4–5° C. decreases to about 3 per cent of normal within three weeks (Table I). Citrated plasma, on the other hand, deteriorates more slowly. These observations agree with those of Quick (15). At body temperature the loss is much more rapid although here, too, citrated plasma is more stable. Full restoration of the prothrombin activity to stored refrigerated plasma is induced not only by the admixture of fresh normal citrated or oxalated plasma (as found by others [1, 8]) but also by $BaSO_4$ plasma (Tables I, II). Substantial restoration to incubated plasma can similarly be obtained (Table I). Indeed, mixtures of fresh $BaSO_4$ plasma with oxalated plasma aged for more than 14 days at 4–5° C. exhibit much more prothrombin activity than the original plasma before, or within the first week

TABLE I

Effect of temperature and anticoagulant on prothrombin activity in stored plasma

1. Pools of oxalated and citrated plasma, subdivided and stored at 4–5° C.*

Storage	Oxalated				Citrated			
	Undiluted plasma		Diluted 1:10 with fresh $BaSO_4$ plasma		Undiluted plasma		Diluted 1:10 with fresh $BaSO_4$ plasma	
	Prothrombin		Prothrombin		Prothrombin		Prothrombin	
days	*time sec.*	*per cent*	*time sec.*	*per cent†*	*time sec.*	*per cent*	*time sec.*	*per cent†*
0	14.8	42	21.4	118	12.1	100+	18.3	180
3	15.7	32	23.4	100	12.2	100+	21.5	115
7	21.9	11	24.0	96	11.5	100+	22.0	110
10	43.6	4	19.1	160	14.8	41	21.4	115
17	60.0	2.8	17.6	215	15.9	30	17.8	210
32	50.0	3.2	19.1	160	21.7	11.3	17.2	220
35	58.0	3.0	18.8	172	—	—	—	—
45	60.0	2.8	20.2	130	—	—	—	—
49	—	—	—	—	61	2.8	18.2	194
54	—	—	—	—	38	4.2	21.2	118

2. Oxalated and citrated plasma (from same donor) kept at 37° C.

hours								
0	14.0	50	28.0	75	11.9	100+	27.3	80
1	14.2	45	29.4	68	13.2	72	28.2	74
3	16.2	25	29.7	67	13.1	80	30.1	65
6.5	17.1	23	31.9	58	13.4	65	32.2	58
24	29.7	6.8	33.2	55	18.8	17	33.6	50

* Culture of a representative sample of both oxalated and citrated plasma, performed on the seventh day of storage, showed no growth. The pH of both on this day was 8.52 and 8.06 respectively.

† Corrected for the 1:10 dilution with the barium sulfated plasma.

[4] The term "fibrinogen" in this paper refers to that plasma protein component which is clotted by the addition of thrombin.

[5] Hereafter referred to as "$BaSO_4$ plasma."

TABLE II

Restorative effect of normal or prothrombin-free plasma on lost prothrombin activity of stored plasma (4–5° C.)

Plasma mixture	Prothrombin		
	Time	Concentration (*per cent of normal**)	
		Expected	Found
	sec.		
Fresh cit. plasma	12.0	100	100
Stored (45 days) oxal. plasma	116.0	—	1.3
One part cit. plasma plus 1 part stored oxal. plasma	12.7	51	80–100
Fresh oxal. plasma	13.8	100	100
$BaSO_4$ oxal. plasma	more than 15 min.	0	0
One part fresh oxal. plus 9 parts $BaSO_4$ plasma	25.9	10	9
One part fresh oxal. plus 19 parts $BaSO_4$ plasma	40.2	5	5
Stored (7 days) oxal. plasma	28.7	—	7
One part stored plus 9 parts $BaSO_4$ fresh plasma	21.6	0.7	11.5
One part stored plus 1 part fresh plus 18 parts $BaSO_4$ fresh plasma	23.3	5.3	10
One part fresh plus 9 parts stored plasma	21.0	16.3	12
One part fresh plus 2 parts stored plasma	15.9	38	30
Fresh oxal. plasma	14.5	100	100
$BaSO_4$ oxal. plasma	more than 15 min.	0	0
One part fresh plus 9 parts $BaSO_4$ plasma	25.9	10	9
Stored (10 days) oxal. plasma	41.5	—	4.3
One part stored plus 9 parts $BaSO_4$ plasma	23.9	0.4	10
One part fresh plus 9 parts stored plasma	21.8	13.9	11
Two parts fresh plus 8 parts stored plasma	17.6	23.4	21
Three parts fresh plus 7 parts stored plasma	16.4	33	25
Four parts fresh plus 6 parts stored plasma	15.0	42.6	40–80
Five parts fresh plus 5 parts stored plasma	14.2	52	100

* Calculated on the basis of fresh normal plasma having 100 per cent prothrombin activity.

of storage despite the fact that the prothrombin time of the undiluted plasma has become very prolonged.

Prothrombin activity of congenital afibrinogenemic plasma and its restorative properties on prothrombin activity of stored plasma: As is evident in Table III, afibrinogenemic plasma from a patient with congenital afibrinogenemia can fully reactivate the prothrombin of stored plasma. This is all the more significant since a mixture of equal parts of both contains half the fibrinogen concentration of the stored plasma alone. Fibrinogen, therefore, is not the factor whose deterioration accounts for the retarded prothrombin time of stored plasma.

The afibrinogenemic plasma failed to clot following the addition of thromboplastin plus calcium. According to the orthodox one-stage method of determining prothrombin, this would be interpreted as indicating zero prothrombin. When, however, measured by the $BaSO_4$ plasma dilution technique its prothrombin activity was normal.

Relation between prothrombin activity and plasma fibrinogen concentration: The prothrombin time of mixtures of fresh normal plasma with congenital afibrinogenemic plasma or plasma defibrinogenated by the addition of thrombin does not change significantly until the fibrinogen drops to approximately 100 mgm. per cent (Table IV). Below this the prothrombin time increases until at 17–25 mgm. per cent the apparent prothrombin activity is about 11 per cent of normal. Furthermore, at this point the clot becomes translucent and coagulation is not abrupt. In three weeks old plasma (4–5° C.), on the other hand, prothrombin activity is about 3 per cent of normal but coagulation is sharp, the clot is opaque and fibrinogen concentration is well above 100 mgm. per cent (14). These observations also support the interpretation that deterioration of fibrinogen does not explain the elevated prothrombin time of stored plasma.

Prothrombin and prothrombin restorative activity of hypofibrinogenemic blood from a patient

TABLE III

The effect of the addition of plasma from patient with congenital afibrinogenemia on the prothrombin activity of stored plasma

Plasma mixture (Parts)						Prothrombin	
Afibrinogenemic* (Citrated)	Saline	Stored†	Fresh normal	Barium sulfated normal	Barium sulfated stored‡	Time	Concent.
						sec.	*per cent of normal§*
—	—	—	1	9	—	29.1	70
—	—	1	—	—	—	68	2.2
—	—	1	—	9	—	23	103
1	—	—	—	—	—	∝	
—	—	1	1	—	—	12.6	100±
1	—	1	—	—	—	12.5	100±
—	—	9	1	—	—	19.2	158
1	—	9	—	—	—	18.0	200
—	—	—	2	—	8	29.2	35
1	—	—	1	—	8	27.7	33.5
—	1	—	1	—	8	53.5	30
1	—	—	—	9	—	26.5	83

* This plasma was citrated. All the others were oxalated. 1 cc. of this plasma to which was added 30 cc. of physiological saline, 2.5 cc. of 0.02 M $CaCl_2$ solution ,and two drops of thrombin solution (400 units per cc.) showed no clot within four hours.

† Stored 19 days at 4–5° C. Cultured and found sterile.

‡ The plasma which was stored for 19 days was then treated with barium sulfate to remove prothrombin activity.

§ Corrected for dilution.

with liver disease: In a patient with fibrinopenia [6] and liver disease prothrombin activity as determined by the usual procedure was about 10 per cent of normal. When, however, prothrombin was measured on a mixture of the subject's plasma with prothrombin-free $BaSO_4$ plasma, higher but still subnormal prothrombin values were obtained. Clearly the $BaSO_4$ plasma provided a clotting agent, either fibrinogen, or other non-prothrombin substances, which was inadequate in the fibrinopenic plasma and which is necessary for full prothrombin activity. That normal prothrombin activity was not found in the mixture was to be expected in view of the extensive liver disease which this patient had (carcinoma of pancreas with diffuse involvement of the liver).

As with the congenital afibrinogenemic plasma, this fibrinopenic plasma lowered the elevated prothrombin time of stored plasma although not to the same degree as did normal plasma.

The effect of defibrination of plasma by thrombin on its ability to reactivate the prothrombin of stored plasma: We were able to confirm the ob-

[6] Plasma fibrinogen concentration was 53 mgm. per 100 cc.

TABLE IV

Relationship between plasma fibrinogen concentration and prothrombin activity

Experiment 1. Afibrinogenemic plasma from subject with congenital fibrinopenia

Plasma mixture (parts)		Fibrinogen concent.	Prothrombin		Properties of clot observed		
Afibrinogenemic	Normal		Time	Concent.	Volume	Opacity	Sharpness of endpoint
		*mgm. per cent**	*sec.*	*per cent*			
0	1.0	250	13.0	100	normal	opaque	sharp
1.0	3.0	188	12.5	100	normal	opaque	sharp
1.0	1.0	125	12.8	100	normal	opaque	sharp
2.3	1.0	76	14.7	40	small	translucent	sharp
4.0	1.0	50	16.5	25	small	very translucent	sharp
9.0	1.0	25	22.2	11	very small	very translucent	not sharp
Experiment 2. Normal plasma defibrinated by addition of thrombin (5 units to 1 cc. of plasma)							
0	1.0	171	13.7	100	normal	—	sharp
3	7.0	120	13.2	100	normal	—	sharp
5	5.0	86	13.3	100	normal	—	sharp
6	4.0	68	14.2	46	small	—	sharp
7	3.0	51	14.4	43	small	—	sharp
8	2.0	34	16.5	25	small	—	sharp
9	1.0	17	22.8	10	small	—	sharp
19	1.0	8.5	45.0	4	very small	—	not sharp

* Assuming 250 mgm. per cent fibrinogen in the normal plasma. (This value was obtained on the same subject one month later.) The fibrinopenic plasma was shown to be completely devoid of fibrinogen but normal in prothrombin content.

servations of others (10, 16) that plasma defibrinated by thrombin can partially reactivate the prothrombin of stored plasma. That it cannot do so fully is perhaps due to some inactivation of labile factor by the prolonged incubation (one hour) required for complete defibrination and inactivation of whatever excesses of thrombin may have been present. Another possibility is partial removal of the labile factor by the fibrin clot.

The effect of heat defibrinogenation of plasma on its ability to restore prothrombin activity to stored plasma: Human plasma heated at 55½–56° C. for two minutes retains some prothrombin (27–40 per cent) but has lost its ability to reactivate the prothrombin of stored plasma. Although such treatment removes fibrinogen, the loss in prothrombin restorative ability was not referable to defibrination since adequate amounts of fibrinogen were provided in the mixture of heat-treated with stored plasma.

Can hemophilic plasma restore prothrombin activity to stored plasma? The admixture of hemophilic plasma to stored plasma resulted in complete reactivation of its prothrombin activity. The coagulation defect of hemophilic blood cannot therefore be attributed to deficiency in labile factor, which is in accord with the normal prothrombin activity in this disease.

Amount of labile factor necessary to reactivate the prothrombin of stored plasma: The reactivation of stored plasma prothrombin by progressively increasing admixtures of fresh plasma is shown in Table V. As the per cent of fresh plasma is increased, the prothrombin time decreases, but does not become normal until the mixture contains at least 50 per cent fresh plasma. It is noteworthy that up to 40 per cent the increment in prothrombin activity induced by the fresh plasma is practically equivalent to the absolute amount of prothrombin activity added with the fresh plasma. Above 40 per cent, relatively normal prothrombin times are approached which unfortunately cannot be accurately translated into prothrombin concentrations since they are in the least accurate range of the curve which relates prothrombin time to prothrombin concentrations.

Additional experiments (Table VI) were done in which increasing amounts of fresh plasma were added to stored plasma which had been incubated for 24 hours prior to the experiment. In this way, the labile factor of the stored plasma was completely destroyed whereas its "prothrombin B" was only partially inactivated. Similar mixtures were made using $BaSO_4$ plasma which was also incubated.

TABLE V

Relation between prothrombin activity and ratio of fresh to stored plasma in mixture of both

Plasma* mixture		Prothrombin	
Normal	Stored (7 days, 4–5° C.)	Time	Concentration
per cent	*per cent*	*sec.*	*per cent of normal*
100	0	13.8	100±
0	100	28.7	7.3
10	90	21.0	12.0
29	71	17.0	23.0
33	67	15.9	40±
50	50	14.7	—
	(10 days, 4–5° C.)		
100	0	14.5	100±
0	100	41.5	4.3
10	90	21.8	11.2
20	80	17.6	21.3
30	70	16.4	25.0
40	60	15.0	40±
50	50	14.2	—

* Oxalated plasma throughout.

TABLE VI

Relation between concentrations of labile factor and plasma prothrombin activity

Plasma mixture (Parts)					Prothrombin	
Stored* incubated	Fresh† normal	Incubated barium sulfated	Mixt. cont. theoretical amounts of labile factor	"Proth. B"‡	Time	Activity
			per cent of normal	*per cent of normal*	*sec.*	*per cent of normal*
0	1	—	100	96	13.0	100
0.2	0.8	—	80	106	13.1	100
0.3	0.7	—	70	103	13.1	100
0.4	0.6	—	60	101	13.7	60–100
0.5	0.5	—	50	99	15.0	39
0.6	0.4	—	40	92	16.5	25
0.7	0.3	—	30	96	20.6	12.3
0.8	0.2	—	20	91	26.3	8.4
0.9	0.1	—	10	99	58.3	2.9
1.0	0	—	0	95	>3 min.	<1
	Normal barium sulfated	Stored incub. barium sulfated				
0.5	0.5	—	50	49	18.2	19
0.5	0.4	0.1	40	49	20.4	12.8
0.5	0.3	0.2	30	49	24.6	9.4
0.5	0.2	0.3	20	49	28.3	7.5
0.5	0.1	0.4	10	49	51.0	3.2

* Stored 39 days at 4–5° C. Incubated for 24 hours at 37° C.

† The normal plasma contained 336 mgm. fibrinogen per 100 cc.

‡ Determined by adding 0.1 cc. of the mixture to 0.9 cc. of fresh normal barium sulfated plasma according to the technique of Rosenfield and Tuft (13).

A sharp drop in prothrombin activity ensues when the amount of labile factor is decreased below 60 per cent of normal. Of interest, also, is the fact that a given deficiency of labile factor has a greater effect on prothrombin activity when the "prothrombin B" concentration is 50 per cent of normal than when it is normal.

It should be mentioned that the stored incubated plasma (Table VI) had no demonstrable fibrinogen. Nevertheless, the data are still valid, since amounts of fibrinogen requisite for substantial prothrombin activity are certainly provided by those mixtures containing at least 30 per cent of normal, or normal $BaSO_4$ plasma (see Table IV).

DISCUSSION

That afibrinogenemic plasma from a patient with spontaneous fibrinopenia is fully capable of reactivating the prothrombin of stored plasma is conclusive evidence that the restorative factor provided by fresh plasma is not fibrinogen. This is further substantiated by the restorative properties of normal plasma rendered fibrinogen-free by thrombin. Although restoration under the latter conditions was only partial, any reactivation by fibrinogen-free plasma excludes fibrinogen as being the restoring agent. The discordant results obtained with fibrinogen by Loomis and Seegers (4) are probably referable to contamination of their fibrinogen with labile factor.

Thus far the existence of labile factor is predicated upon observations that the prothrombin time increases as plasma ages although its prothrombin concentration does not change, and that this can be rectified by the admixture of whole fresh plasma, or plasma devoid of fibrinogen and prothrombin. This does not necessarily imply that it exists as an entity, albeit independent of these coagulation substances. It is conceivable that the above properties are referable to a particular configuration in the molecule of a plasma constituent distinct from fibrinogen and prothrombin, and essential for prothrombin activity as determined by the orthodox one-stage technique.

The prolonged prothrombin time of fibrinopenic plasma, of stored plasma, and of the plasma from recently described patients whose clotting defects were not referable to true hypoprothrombinemia (5, 17) demand reevaluation of the method for measuring prothrombin by the one-stage procedure. Its inadequacies derive from the fact that in all these instances prolonged prothrombin times are observed despite normal prothrombin (B) concentrations. It is also pertinent to point out that the use of physiological saline as a plasma diluent in order to bring the prothrombin time into a range more suitable for accurate conversion into prothrombin content may aggravate these inadequacies by diluting, simultaneously with the prothrombin, other coagulation factors whose concentrations may already be critical. Clearly, these weaknesses of the one-stage technique can best be obviated by diluting the test plasma with plasma which has been rendered prothrombin-free in some manner and which still retains all other clotting factors. As far as is known, $BaSO_4$ plasma fulfills these criteria, although we are cognizant of the statement by Owren (6) that $BaSO_4$ removes some clotting "factor V" from plasma. (The exact amount of $BaSO_4$ required to do this is not indicated.)

Little is known regarding the exact quantitative relationships between labile factor concentration and apparent prothrombin activity. Munro and Munro (18) claim that there is practically no change in prothrombin time over a wide proportion of labile factor and "prothrombin B" and that a deficiency of one component can be partially compensated for by an excess of the other (3). This is not entirely in accord with our finding.

Thus far no case is recorded in which a deficiency of labile factor has been observed. It appears that reducing it below 50 per cent of normal can result in substantial prolongation of the prothrombin time. The experiments on our patient with extensive liver disease suggest that this subject was somewhat deficient not only in prothrombin and fibrinogen, but also in labile factor since his plasma could not completely reactivate stored plasma prothrombin. That this cannot be referable to low fibrinogen concentration is clear from the quantitative relationships between fibrinogen concentration and prothrombin time (Table IV). It is possible that with a more practical technique for labile factor determination such as that of Stefanini and Quick (16) more patients will be found with coagulation defects related to insufficiency of this entity.

Our observations on the heat sensitivity of labile factor are not in accord with those of Quick

(1), who claimed that it is fairly heat stable. In our experience, the labile factor is quite rapidly inactivated at body temperature, and completely destroyed at 55½–56° C. within two minutes, which agrees with the finding of Honorato (10). Under these conditions "prothrombin B" is more stable.

The reason why $BaSO_4$ does not remove all the prothrombin from citrated (in contrast to oxalated) plasma is obscure. In this connection it also is noteworthy that aluminum hydroxide adsorbs prothrombin less effectively from heparinized than from oxalated plasma (1).

The enhanced prothrombin activity of plasma stored for more than two weeks (when determined with $BaSO_4$ plasma as diluent) is striking and cannot at present be explained. Honorato (10) similarly found that a mixture of stored plasma with fresh plasma treated with $Al(OH)_3$ shows more prothrombin activity than the fresh plasma alone. He attributes this to qualitative changes in fibrinogen consequent to storage which renders it more easily coagulable. Experiments which will be presented in a subsequent communication (14) indicate that this cannot be the explanation since the fibrinogen of stored plasma becomes progressively less coagulable while at the same time the prothrombin activity, when measured by the $BaSO_4$ plasma dilution technique, increases. It is unlikely that more prothrombin can develop as plasma ages. Furthermore, no such increase in prothrombin is found by actual isolation or when the two-stage technique for prothrombin determination is used (19). It is possible that a naturally occurring clotting inhibitor (20) disappears as plasma is aged.

CONCLUSIONS

1. The prothrombin activity of stored plasma as determined by the one-stage technique can be fully reactivated by the admixture of fresh normal plasma, congenitally afibrinogenemic plasma, $BaSO_4$ plasma (prothrombin-free) or hemophilic plasma. The restorative factor (labile factor) is not fibrinogen.

2. The prothrombin time of plasma can be greatly influenced by alterations in the concentrations of (*a*) prothrombin, (*b*) labile factor and (*c*) fibrinogen.

3. Prothrombin-free $BaSO_4$ plasma has a normal amount of labile factor, fibrinogen, and antihemophilic activity. Its use as a diluent in measuring prothrombin by the one-stage technique is advantageous since in this way these non-prothrombin variables are adequately controlled.

4. Plasma stored for more than two weeks shows enhanced prothrombin activity when measured by the $BaSO_4$ plasma dilution technique. The explanation of this phenomenon is obscure.

BIBLIOGRAPHY

1. Quick, A. J., On constitution of prothrombin. Am. J. Physiol., 1943, **140**, 212.
2. Oneal, W. J., and Lam, C. R., Experiments on components A and B (Quick) of prothrombin. Am. J. Med. Sc., 1945, **210**, 181.
3. Munro, F. L., Hart, E. R., Munro, M. P., and Walkling, A. A., Changes in components A and B of prothrombin in dog following hepatectomy. Am. J. Physiol., 1945, **145**, 206.
4. Loomis, E. C., and Seegers, W. H., Is prothrombin a unitary principle or a complex? Am. J. Physiol., 1947, **148**, 563.
5. Quick, A. J., Components of the prothrombin complex. Am. J. Phys., 1947, **151**, 63.
6. Owren, P. A., The coagulation of blood. Investigation on a new clotting factor, thesis. Acta med. Scandinav., Supp. **194**, 1947.
7. Ware, A. G., Guest, M. M., and Seegers, W. H., A factor in plasma which accelerates the activation of prothrombin. J. Biol. Chem., 1947, **169**, 231.
8. Munro, M. P., and Munro, F. L., The reversible inactivation of prothrombin: a factor responsible for its partial reactivation. Am. J. Phys., 1947, **150**, 409.
9. Honorato, R., and Quick, A. J., The relation of fibrinogen to the coagulation factor which diminished in stored plasma. Am. J. Physiol., 1947, **150**, 405.
10. Honorato, R., The plasmatic cofactor of thromboplastin: its adsorption, with prothrombin and fibrinogen, by alumina and tricalcium phosphate gels. Am. J. Physiol., 1947, **150**, 381.
11. Quick, A. J., The Hemorrhagic Diseases and the Physiology of Hemostasis. Charles C. Thomas, Springfield, 1942, p. 312.
12. Blumgart, H. L., Freedberg, A. S., Zoll, P. M., Lewis, H. D., and Wessler, S., The effect of dicumarol on the heart in acute experimental coronary occlusion. Am. Heart J., 1948, **36**, 13.
13. Rosenfield, R. E., and Tuft, H. S., Estimation of prothrombin level from prothrombin time. Am. J. Clin. Path., 1947, **17**, 405.
14. Alexander, B., de Vries, A., and Braun, E., The determination of fibrinogen, with observations on the fibrinogen of stored plasma. In preparation.

15. Quick, A. J., Influence of decalcification on the determination of prothrombin. Federation Proc., 1946, **5**, 150.
16. Stefanini, M., and Quick, A. J., The concentration of the labile factor of prothrombin in the blood of various species. Federation Proc., 1948, **7**, 191.
17. Owren, P. A., Parahemophilia, hemorrhagic diathesis due to absence of a previously unknown clotting factor. Lancet, 1947, **1**, 446.
18. Munro, F. L., and Munro, M. P., The interaction of prothrombin A and B. Am. J. Physiol., 1947, **149**, 95.
19. Ware, A. G., Guest, M. M., and Seegers, W. H., Stability of prothrombin. Am. J. Physiol., 1947, **150**, 58.
20. Overman, R. S., and Wright, I. S., A new blood clotting inhibitor. J. Biol. Chem., 1948, **174**, 759.