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Hemophilioid Factors: Acquired Deficiencies in Several Hemorrhagic States. (23030)

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It is rather well established that at least 2 types of congenital "hemophilioid" disease exist, in addition to true hemophilia. In each case, sluggish clotting of blood can be corrected in large measure by addition of either of the other types of blood. Such evidence provides the chief grounds for considering the 3 types as separate entities. It has been postulated by some that these 3 disorders are due to the presence of inhibitors in the blood stream(1). It is more widely held however, that they are due to deficiency of 3 rather ill-defined factors needed for activation of thromboplastin. The 3 factors in question have been referred to as antihemophilic factor (AHF), plasma thromboplastin component (PTC)(2) and plasma thromboplastin antecedent (PTA)(3).

Although the nature of hemophilioid factors is undetermined, study of their activity in coagulation disorders has yielded data which are useful in classification and management of these disturbances. Their levels in acquired hemorrhagic states have never been fully described. In a previous publication it was pointed out that faulty adsorption of vit K leads to lowering of serum level of PTC(4). In the present study, activity of PTA is also shown to be affected under similar conditions. The effects of parenchymatous liver-cell damage on hemophilioid factors will also be discussed.

Methods. Labile factor was measured by Wolf's(5) modifications of Quick and Stefanini's technic(6). *Stable factor* was measured by modification of the method of Owren (7). *Prothrombin* was measured by the 2-stage method of Ware and Seegers(8), modified by substitution of purified labile factor (9) and stable factor(9) in place of dilute bovine serum to stabilize the assay. The *Prothrombin time* test of Quick(10) is now known to measure combined deficiencies of stable factor, labile factor, prothrombin and more rarely of fibrinogen. A dilution technic was used, and all results were expressed as per cent of normal. Methods for quantitative assay of hemophilioid factors have been outlined in principle(4). They were based on the observation of Biggs *et al.* that platelets together with calcium, AHF, PTC and PTA can form a potent thromboplastin(11). In performing the assays we devised a standard procedure whereby a reaction mixture

containing platelets and calcium was supplied with 2 of the 3 hemophilioid factors, each in high titer. The third factor, the one to be assayed, was added as an unknown preparation. The titer of thromboplastin thus produced provided a measure of the hemophilioid factor in question. Plasma and sera with congenital deficiencies of the various hemophilioid factors are used as substrates for these assays. In our laboratory, identification of these deficiencies was made by cross matching plasma or serum with those of our earlier cases or with those from other laboratories. Plasma and serum having a deficiency of PTA were identified by cross testing with plasma obtained from Robert Rosenthal who first defined the deficiency (3).

The preparation of assay reagents, as well as details of the PTC assay, have been described(4). In assay of PTA, as now described, the serum to be assayed is the source of the factor. PTC is supplied by serum from a patient congenitally deficient in PTA but rich in PTC. Adsorbed plasma from a patient rich in AHF but congenitally deficient in PTA is used as a source of AHF. With these modifications, the PTA assay is performed as described already for assay of PTC(4). To assay for AHF, a test plasma, adsorbed with $Al(OH)_3$, is the source of AHF. Normal pooled serum serves as a source of PTC and PTA. $Al(OH)_3$-adsorbed plasma from patient congenitally deficient in AHF is also added; it serves as source of labile factor, the absence of which might influence the assay. The reagents already described(4) are mixed as follows: *Solution A*: 0.1 cc $Al(OH)_3$-adsorbed test plasma (unknown or normal control plasma); 4.4 cc 0.85% sodium chloride; 0.5 cc $Al(OH)_3$-adsorbed plasma congenitally deficient in AHF. *Solution B*: 0.2 cc normal serum diluted 1:10 with 0.85% sodium chloride; 0.2 cc human platelet suspension; 0.2 cc 0.02 M $CaCl_2$. Next, 0.2 cc of Sol. A are incubated at 37°C with 0.6 cc of Sol. B. Remainder of assay is performed as described for assay of PTC(4).

Results. Activities of hemophilioid factors were compared with activities of other clotting factors in a variety of acquired hemorrhagic states:

Hepatocellular disease: Blood specimens were collected from 20 patients having a variety of hepatocellular diseases, and from 30 normal controls. Various coagulation factors were measured, with special emphasis on PTA, PTC and AHF. In addition, hepatic status was evaluated on clinical grounds and by performing routine tests of hepatic function. Patients with liver disease were divided into 2 groups, acute and chronic (Table I). The former included infectious hepatitis and homologous serum hepatitis; the latter included portal cirrhosis, Wilson's disease, Gaucher's disease and carcinoma metastatic to the liver. In acute disorders, levels of PTA activity ranged from 21% of normal in patient with severe homologous serum hepatitis, to 43% of normal in patient recovering from infectious hepatitis. In chronic disorders, levels of PTA ranged from 16% in patient who died shortly thereafter with acute yellow atrophy, to 95% of normal in patient with mild portal cirrhosis. PTC values in these patients had a similar range. In both acute and chronic groups, data for serum proteins, bilirubin and cephalin flocculation also varied considerably but these data corresponded roughly with data for the clotting factors. None of these tests differentiated clearly between acute and chronic disorders. Blood levels of PTA and PTC seemed often to provide a more sensitive index of dysfunction than did levels of prothrombin, stable factor or labile factor. This too can be concluded from recent data published on PTC by Cowling (12).

Vit K deficiency: The influence of vit K on prothrombin has been known for many years and its influence on stable factor and PTC has been investigated previously(13,4). Influence of the vitamin on PTA has never been reported. Several patients with inability to adsorb vit K were therefore studied. Diagnoses included non-tropical sprue, obstructive jaundice and fibrocystic disease. Representative data, obtained on patient with obstructive jaundice are shown in Table II.

TABLE I. Levels of Activity of Plasma Thromboplastin Component (PTC), Plasma Thromboplastin Antecedent (PTA) and Other Clotting Factors in Both Acute and Chronic Hepatic Disorders.

	Proth. time											
	1 stage	2 stage	Lab. fac.	Stab. fac.	PTC	PTA	AHF	A/G ratio, g %	Bilirubin total, mg %	Alk. phos., B.U.	Ceph. floc.	Clinical status
	(In % of normal)											
Normal												
Mean avg	100	100	100	100	100	100	100	4.6/2.0	<.8	1-4	0	
Stand. dev. of mean					± 6.2	± 7.4	± 5.9					
Acute hepatocellular disease												
Infectious hepatitis	77	89	85	56	54	43	100	3.8/3.0	4.0	6.7	3	Recovering
Idem	70	100	81	60	68	40	95	4.0/3.3	3.0	7.3	4	"
Serum hepatitis	35	64	80	29	22	21	100	3.0/2.9	26.0	6.3	3	Severe
Idem	75	100	75	67	60	33	95	3.7/2.5	6.3	7.1	3	Recovering
Serum hepatitis and	80	91	90	93	25	37	7	3.3/3.6	16.5	7.4	3	"
hemophilia recovered from hepatitis	100	100	100	100	100	90	5					Recovered
Chronic hepatocellular disease												
Portal cirrhosis	98	87	100	72	87	95	100	3.1/3.9	.7	8.3	4	Mild
Idem	37	43	36	26	28	35	89	2.5/2.9	6.2	3.9	4	Severe
"	37	40	40	34	40	42	97	2.2/3.4	1.4	4.6	4	"
"	74	53	72	60	50	49	100	3.3/3.5	.5	2.7	0	Advanced
"	54	35	28	27	18	21	100	3.2/4.3	4.0		4	"
"	66	59	97	40	66	31	100	4.2/2.6	.8	2.8	4	Compensated
"	69	89	70	60	71	57	95	3.2/1.5	.4	5.8	0	"
"	55	53	43	53	75	32	100	3.6/3.1	1.2	2.7	4	"
"	79	84	67	57	42	46	100	4.9/1.0	.5		2	"
"	45	65	45	22	75	49	100	2.5/2.7	2.0	1.6	4	Severe
" & acute atrophy	9	38	18	5	6	16	80				3	Died in 8 hr
Wilson's disease	87	85	63	39	45	38	100	4.4/1.7		5.4	0	Advanced
Gaucher's disease	64	44	50	33	66	21	100	4.1/2.1	1.5	5.7	0	"
Carcinoma, metastatic to liver	71	100	108	34	53	30	86	4.8/2.8	.9	20.3		Died in 2 wk

TABLE II. Effect of Vit. K Replacement on Levels of PTA, PTC and Other Clotting Factors in Several Patients Unable to Adsorb Fats.

	Proth. time 1 stage	Proth. time 2 stage	Labile factor	Stable factor	PTA	PTC	AHF
	In % of normal						
Obstructive jaundice							
Before treatment	22	66	91	15	40	23	96
26 hr after Hykinone (10 mg)	86	100	89	38	100	75	94
Fibrocystic disease							
Before treatment	11	34	82	9	7	11	100
24 hr after Hykinone (15 mg)	82	79	82	86	73	78	100
Sprue, non-tropical							
Before treatment	5		90	7	9	7	95
28 hr after Vit. K_1 (75 mg)	81		92	79	68	86	95

A deficiency of vit K in this patient was accompanied by depression in levels of prothrombin, stable factor, PTC and PTA. Ability of vit K to correct these deficiencies was tested by intramuscular administration of 10 mg of menadione sodium bisulfite (Hykinone). Twenty-six hours later, all aforementioned clotting factors had either increased by 100% or had returned to normal levels. PTA activity increased from original level of 40% before treatment, to 100% of normal after treatment. Other patients with inability to adsorb vit K showed a similar response when the vitamin was replaced (Table II).

Effect of anti-vit K. Deficiencies of prothrombin(14), stable factor(15) and PTC (4) have been reported. A fall in PTA has not previously been reported. A number of patients were studied while receiving 3-3'-methylenebis (4-hydroxy coumarin) (Dicumarol). Two representative patients, suffering with thrombophlebitis, were treated. Assay values of coagulation factors are shown in Table III. Depressions of prothrombin, stable factor, PTC and PTA accompanied treatment with this compound. Upon recovery from thrombophlebitis, the ability of vit K to correct these deficiencies was tested by intramuscular administration of vit K and Hykinone. Within 24 hours, factors previously depressed had risen almost to normal levels. In one case, PTA activity increased from original level of 22% before treatment to 89% of normal after treatment.

Discussion. Disturbances in blood coagulation associated with hepatic disease are not simple ones. Significant deficiencies of a number of circulating coagulation proteins exist. These deficiencies may be related both to destruction of hepatic cells and to hypovitaminosis K of biliary obstruction. Blood levels of prothrombin, stable factor, labile factor, PTC, fibrinogen and PTA are reduced in disease processes that are predominantly hepatocellular. Levels of prothrombin, stable factor, PTC and PTA are also dependent upon an adequate supply of vit. K. In any

TABLE III. Effect of Vit. K_1 and Hykinone on Levels of Clotting Factors Altered by Administration of Dicumarol.

	Proth. time 1 stage	Proth. time 2 stage	Labile factor	Stable factor	PTA	PTC	AHF
	In % of normal						
Patient A							
Before Dicumarol	100	100	97	100	98	100	95
After 6 days of Dicumarol	26	68	97	17	22	13	95
20 hr after Hykinone (10 mg)	70	89	94	60	89	85	95
Patient B							
Before Dicumarol	100	100	100	100	96	100	100
After 9 days of Dicumarol	11	21	100	13	24	29	100
22 hr after vit. K_1 (40 mg)	48	42	100	51	58	61	100

hepatic disorder, the relative importance of hepatocellular damage and vit. K deficiency varies. Summation of the two frequently attains clinical importance. If the hemorrhagic crisis is predominantly due to hepatic cell destruction, vit K will be ineffective, and deficient factors must often be replaced by transfusion of blood or plasma. Fortunately, stored blood preparations commonly available retain high levels of PTA, PTC, prothrombin, stable factor and fibrinogen. However, their titers of labile factor are often low. Therefore, when significant deficiencies of labile factor exist, hemostasis may be restored only by transfusion of fresh blood or plasma. Thus it is that analysis of specific deficiencies may be important.

Dependence of several coagulation factors upon hepatocellular function is not surprising, since a wide variety of blood proteins are similarly dependent. However, several of these factors have other properties which suggest that the relationship between them may be a close one. Prothrombin, stable factor, PTC and PTA have physical properties in common (*e.g.* precipitation, adsorption, stability). In addition, these 4 clotting factors must share at least one common metabolic pathway since all 4 are dependent upon vit K. Other blood proteins have not been shown to be dependent upon this vitamin.

Summary. Deficiencies of hemophilioid factors are not necessarily genetic in origin. Serum activities of plasma thromboplastin antecedent (PTA) and of plasma thromboplastin component (PTC) were depressed in 20 patients with a variety of hepatocellular disorders. PTA and PTC activities were also reduced in several patients who developed a deficiency of vit K subsequent to inability to adsorb fats. PTA and PTC were depressed in a number of patients following administration of 4-hydroxy coumarin compound. In these latter 2 groups, PTA and PTC activities rapidly increased toward normal following administration of vit K analogue. Significant deficiency of antihemophilic factor (AHF) was not found in any of the above conditions.

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