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'Accelerator Globulin" and "Antihemophilic Globulin" in Thrombin Formation from *Aged* Prothrombin and in Hemophilic Blood.*

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Recent studies[1-9] have shown that a newly recognized factor is important in the conversion of prothrombin to thrombin. The plasma "accelerator globulin" of Ware, Guest, and Seegers,[3,4,9] used in the present studies, contains this factor and is, in all probability, similar to Quick's "labile factor"[6] and Owren's "factor V."[1] "Antihemophilic globulin"[10] is a plasma protein fraction which restores the "thromboplastic" defect and thus accelerates the thrombin formation in hemophilic blood. The following experimental data were obtained during current *in vitro* studies of the various factors involved in the process of thrombin formation from purified prothrombin preparations.

Reagents (*cf.* [11,12]). 1. *Borate buffer* (buff.), pH 7.7, solvent and diluent throughout. 2. *Prothrombin* (PRO.) from bovine plasma, courtesy of Dr. W. H. Seegers and colleagues (Wayne Univ.) PRO. *G* partially purified (product 5 of reported procedures)[13] potency 720 "units" per mg (dry wt). PRO. *H*, highly purified (ammonium sulfate fractionation of product 8),[13] potency 990 "units" per mg (dry wt = 10 × tyrosine). 3. *Thromboplastin* (tpln.) 0.2% rabbit lung preparation (Squibb's). 4. $CaCl_2$ (Ca) M/20. 5. *Fibrinogen* (B.F.), 0.5% Armour's bovine plasma Fraction I (55% "clottable"). 6. *"Antihemophilic globulin"* (HF), Harvard[14] human Fraction I, low in fibrinogen (HF_1 37%; HF_2 18%). 7. *"Accelerator globulin"* (AcG), from bovine plasma (Seegers *et al.*), contained a small amount of prothrombin.

Methods. 0.1% solutions of PRO., AcG, and HF were divided into 2 portions, part being freshly frozen and kept at –20°C until tested and part being aged at 28°C (thermostat water bath) for 7 days. 5 cc (total vol.) of thrombic mixtures (T), in borate buffer, contained 0.1% PRO., to dilution noted in Tables I, II, 0.5 cc Ca, 0.5 cc tpln (approximately optimal), and 0.5 cc of the various agents noted. At stated incubation periods (i.t.), 0.25 cc T was added to 0.5 cc BF, at 28°C, and clotting-time (c.t.) noted.

"Antihemophilic" activity was measured by addition of 2 cc of fresh hemophilic blood to 0.1 cc of test preparations and measurement of the coagulation time at 37°C.

Results. In the data of Table I, the prothrombin (PRO *H*) after *aging* 1 week at 28°C, when diluted and activated with the stated amounts of calcium and thrombo-

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TABLE I.
Effects of "Accelerator Globulin" (AcG) and "Antihemophilic Globulin" (HF) on Activation of *Aged* and *Frozen* Prothrombin (Pro.).

T = thrombic mixtures, containing (final concentrations) Pro.*H* (1:20,000), tpln. (1:5000), Ca (M/200), and agents cited (1:10,000), with borate buffer (pH = 7.7) to 5 cc vol. Clotting times (c.t.), sec., at 28°C, for 0.25 cc T + 0.5 cc BF (0.5%), at stated incubation periods (i.t.).

T.	Pro.	Agent	Incubation time: 5m	10m	15m	20m	30m	1 hr	4 hr
			Clotting time (sec.)						
1.	Aged	—	190	129	105	72	50	30	17
2.	"	AcG (aged)	137	104	78	61	43	23	15
3.	"	" (froz.)	17	11	10	10	9½	8	8
4.	"	HF_1 (aged)	165	107	101	98	75	47	30
5.	"	" (froz.)	161	122	106	100	91	58	32
6.	Froz.	—	16	10	8½	8	8	8	8
7.	"	AcG (aged)	12	9½	8	8	8	8	8
8.	"	" (froz.)	12	8	8	8	8	8	8
9.	"	HF_1 (aged)	26	16½	13	12½	12	12	12
10.	"	" (froz.)	29	19	15	13	13	12½	12½
11.	—	AcG (froz.)	120	95	73	71	62	53	48
12.	—	" (aged)	220	220	225	220	240	300	345

plastin, showed very slow activation (T_1) which was incomplete even after 4 hours incubation. *Aged* AcG exerted only a negligible improvement (T_2) as compared with *frozen* AcG (T_3) which was able, in less than an hour, to restore the full thrombic potency as shown by the 8″ c.t. identical with that reached in T_6, T_7, T_8. The prothrombin kept in the *frozen* state, when activated in the usual manner with Ca and tpln. (T_6) completed the thrombin formation in less than 20 min., and showed only slight acceleration by *aged* AcG (T_7) and still minor improvement (10 min. i.t.) by *frozen* AcG (T_8). HF_1 did not show any accelerator effects with either prothrombin, but, on the contrary, evinced some definite interference with thrombin formation, though somewhat less with *aged* HF (T_4, T_9) than with *frozen* HF (T_5, T_{10}). Small amounts of fine flocculant fibrin which appeared during the incubation of these mixtures did not interfere with the tests and were not considered significant.

Controls. The fibrinogen (BF) contained a mere trace of prothrombin which caused negligible fibrin shreds to appear in about 25 minutes when tested with the same amounts of Ca and tpln. (in buff.) as used in the other tests. HF controls, similarly activated, barely increased these traces of fibrin, now appearing in about 20 minutes. The AcG preparation, as expected, did show a little content of prothrombin estimated at less than 10%, in the *frozen* solution (T_{11}). The *aged* AcG showed no prothrombin activity but did contain a trace of active thrombin which deteriorated somewhat as the incubation proceeded (T_{12}). It is not conceivable that these very minor sources of prothrombin and thrombin could be at all significant in explaining the "accelerator" effect noted in T_3.

Similar experiments with a less purified prothrombin (PRO. *G*) gave exactly the same results. In Table II, A the data are presented as "percentages" of thrombin yield, derived from the clotting-times (Table II, B) obtained by a series of dilutions of T_2 at maximal activity (4 hrs). T_1: the *aged* PRO., diluted and activated in the usual manner with optimal Ca and tpln., is only about one-third activated in 4 hrs but is estimated as 80% complete in 3 days. T_2: the addition of 1 cc *fresh* (frozen) 1:1000 AcG gave over 90% of maximal thrombin formation in 10 min. and 100% (complete activation) in less than 4 hours. T_3: HF_2, another "antihemophilic globulin" preparation, one-tenth vol. (0.5 cc) of *fresh* 1:1000 soln., showed the same kind of retarding action previously noted with HF_1 (Table I), the best value for thrombin yield (i.t. 2-3 days) not exceeding 20%.

Although PRO. *G* was elsewhere[11] shown

TABLE II.

A. Thrombin Yield, "percentage" activation, after varying periods of incubation (i.t.), in mixtures (T) containing PRO.*G.* (1:12,500) aged 7 days at 28°C, tpln. (1:5000), Ca (M/200), and added agents (mg per cc) noted. 0.25 cc T + 0.5 cc BF. 28°C. pH = 7.7.

T.	Agent	5 min	10 min	1 hr	4 hr	1d	2d	3d (i.t.)	Clot lysis (37°)
1.	buff.	tr.	tr.	17	34	50	75	80	None (5 days)
2.	AcG (0.2)	44	93	95	100	100	96	80	" "
3.	HF_2 (0.1)	tr.	tr.	3	6	15	20	20	" "

B. "Percentage" Dilutions of T_2 (4 hr incub. time): clotting-times (sec.).

Dil.	100%	80%	60%	40%	20%	10%	5%
Coag. time (sec)	10	15	18	21½	37	46½	89

TABLE III.

"Antihemophilic" Activity.

2 cc freshly drawn hemophilic blood added to 0.1 cc 0.1% solutions of agents cited. Coagulation times (C.T.), min., at 37°C.

Agent:	Saline (control)	AcG (froz.)	AcG (aged)	HF (froz.)	HF (aged)
Coag. time (min.)	142	98	98	27	60

to contain a trace of protease contaminant, the clot-lysis tests recorded in Table II, A show no appreciable enzyme activity in the dilution used.

In other (not cited) experiments with PRO. *G* solution freshly prepared and activated (a) with and (b) without fresh (frozen) AcG, a maximal thrombic potency represented by c.t. of 8 sec. was reached in (a) 20 min. or (b) 30 min., respectively. This confirms the evidence in Table I (T_5, T_7) showing that these *fresh* or frozen prothrombin preparations contain an abundance of the AcG factor. In earlier experiments,[11] testing stronger (0.2-0.4%) solutions of this same prothrombin, identical activation data (with optimal Ca and tpln.) were obtained with fresh solutions as compared with the same solutions after 3-4 weeks storage at ice-box temperature (5°C).

Table III shows the antihemophilic activity of AcG and HF_1. Even in the high (<0.005% *final*) dilution, frozen HF_1 showed marked acceleration of clotting. This activity was greatly diminished by aging. AcG both frozen and aged showed only minimal effects.

Discussion. The data clearly show that the major change in "aged" prothrombin is an impaired ability to form thrombin with the classical activators, namely, calcium and thromboplastin. Fresh (or frozen) "accelerator globulin" greatly speeds up the conversion and restores the thrombin yield (*cf.* [7]), in fact completely in T_3 (Table I), but "aging" of the AcG causes it to lose this property. Fresh (or frozen) prothrombin evidently contains an adequacy of this factor, as a contaminant [6] but it deteriorates on aging at or above room temperature. This deterioration is emphasized by dilution of the prothrombin preparation. There is little doubt that the validity of estimations of the thrombin-yielding potency of prothrombin depends upon adequate control of the "accelerator" factor.[8] Our findings in regard to highly purified (Seegers) prothrombin are comparable to the data of other workers investigating the thrombin-yield changes in stored and otherwise treated plasma. Interpretation in terms of presence or instability of AcG possibly explains most of the divergence of viewpoints noted in the literature and accounts for the prothrombin preserving and activity restoring powers credited to plasma[15] or certain fractions,[1,5,15] including some fibrinogens.[7,8,16]

Questions of possible deterioration of prothrombin proper or of thrombin in highly dilute solutions[11] are raised by the later data of Table II, but the analysis of these prob-

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lems is beyond the scope of the present inquiry. The same is true of the explanation for the evident interference in thrombin formation by our weak HF solutions and the only conclusion, for the present, is that "antihemophilic globulin" lacks the accelerator effect.

We have shown elsewhere[12] that HF contains a protease precursor which, when activated by streptokinase, evinces proteolytic (*e.g.*, fibrinogenolytic, etc.) and "thromboplastic" (aiding thrombin formation) properties, similar to other plasma protease preparations. The negative clot-lysis tests included in Table II, A, show that, for reasons of dilution and lack of activation such protease plays no part in the present results.

AcG has been studied in several types of experiment with completely negative results as regards any indication that it may be related to the plasma protease[17] system, whether as active enzyme or precursor, or as activator or inhibitor of added precursor, streptokinase, or active protease. It is an important point, therefore, that the aid which the "accelerator globulin" affords to thrombin formation has no demonstrable relation to the plasma protease system (*cf.* [1]).

It is also of interest to note that AcG has no significant "antihemophilic" activity, which, of course, is to be expected from the normal prothrombin clotting times obtained in hemophilia, indicating that hemophilic blood has adequate amounts both of prothrombin and the accelerator factor.

Conclusions. 1. There is a new factor, or group of factors, (Quick's "labile factor," Owren's "factor V," Ware, Guest and Seegers' "accelerator globulin"), to be recognized in the first phase of the blood-clotting system. At present it may be considered an "accessory" activator distinct from calcium, thromboplastin, and thromboplastic enzyme. 2. This factor is found abundantly in fresh plasma and prothrombin (purified by the usual methods, *e. g.* [13]), but deteriorates on "aging," independently of the prothrombin. High dilution may be necessary to demonstrate this. The addition of fresh AcG will restore the original potency of the prothrombin, but aged AcG loses this property. 3. AcG is not "antihemophilic" and so-called "antihemophilic globulin" is not able to restore the activity of aged prothrombin. 4. "Owren's disease,"[18] a specific hemorrhagic disorder associated with a plasma deficiency of this new factor, is, in all probability, a true clinical entity, which suitable tests can differentiate from "idiopathic hypoprothrombinemia," hemophilia, and other bleeding diseases.

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Prothrombin Conversion Factor of Dicumarol Plasma.*

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Dicumarol *in vivo* is generally believed to cause a deficiency of plasma prothrombin. The therapeutic value of serum[1,2] in the treatment of cattle with "hemorrhagic sweet clover disease," a dicumarol-induced diathesis is therefore puzzling.

An investigation was conducted on dogs to which dicumarol was administered orally or

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