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Anticephalin Activity and the Prothrombin Conversion Rate of Normal and Hemophilic Plasmas.

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Important differences have been observed in the results of the quantitative determination of prothrombin by the 1-stage[1] and 2-stage[2] methods. One explanation offered for these differences has been that the 1 stage method measures, not so much the total amount of prothrombin present but the speed with which prothrombin can be converted to thrombin and its titer built up to the clotting level.[3] In the 2-stage method, an excess of thromboplastin is added to the *diluted* plasma and enough time is allowed for the complete conversion of all the prothrombin into thrombin before fibrinogen is added and the clotting time measured. Dilution of the plasma is intended principally to overcome the action of inhibitors. It has not been clear, however, just what inhibitor, if any, is responsible for slowing the prothrombin conversion rate. Moreover, demonstrations of increases in the rapidity of this rate have not been sufficiently convincing to be generally accepted.[4]

The evidence to be presented seems to indicate that the prothrombin conversion rate is influenced by the anticephalin activity of the plasma. When anticephalin is present in excess (as in hemophilic plasma)[5] the rate of conversion is slow, but since in both the 1-stage and 2-stage methods a great excess of thromboplastin is used, and (in the 2-stage method) enough time is allowed for full conversion, little or no difference will be found between the results obtained by the two methods. If anticephalin has been reduced or removed, however, the rate of conversion of the remaining prothrombin is so accelerated as to lead to a pronounced difference in the results, the 1-stage method nearly always yielding higher values.

Plasma from normal and hemophilic men collected with especial precautions[6] was used either in the intact state or after exposure to adsorbents. Anticephalin may be rapidly removed from plasma by contact with kaolin, infusorial earth, pumice stone, asbestos fibers, aluminum hydroxide, etc.[7,8] Asbestos (short fibers, A. H. Thomas Co., Phila.) was used because of the ease with which the plasma can be separated from this adsorbent after the period of contact is over. By carefully adjusting the quantities of plasma and adsorbent, the period of contact and the temperature, it is possible to remove most of the anticephalin with only a slight loss in prothrombin and fibrinogen. By "adsorbed plasma" will be meant normal or hemophilic human citrated plasma that has been in contact with asbestos fibers (10 mg asbestos for each ml of plasma) for 2 hours at 20°C in a stoppered paraffin coated tube. The prothrombin and antithrombin content of the intact normal and hemophilic plasmas were within normal limits. The asbestos adsorbed plasma does not seem to lose antithrombin, but it contains slightly less prothrombin and fibrinogen than the intact plasma.

Adsorbed hemophilic plasma becomes unusually susceptible to activation by cephalin and cannot be distinguished in this respect from adsorbed normal plasma (Table I). As will be shown elsewhere, the response of a

1 Quick, A. J., Stanley Brown, M., and Bancroft, F. W., *Am. J. Med. Sci.*, 1935, **190**, 501.

2 Smith, H. P., Warner, E. D., and Brinkhous, K. M., *J. Exp. Med.*, 1937, **66**, 801.

3 Smith, H. P., Public. No. 13, Am. Assn. for Adv. of Sci., p. 10.

4 Quick, A. J., *Hemorrhagic Diseases and the Physiology of Hemostasis*, 1942, p. 35.

5 Tocantins, L. M., PROC. SOC. EXP. BIOL. AND MED., 1943, **54**, 94.

6 Tocantins, L. M., *Am. J. Physiol.*, 1943, **139**, 265.

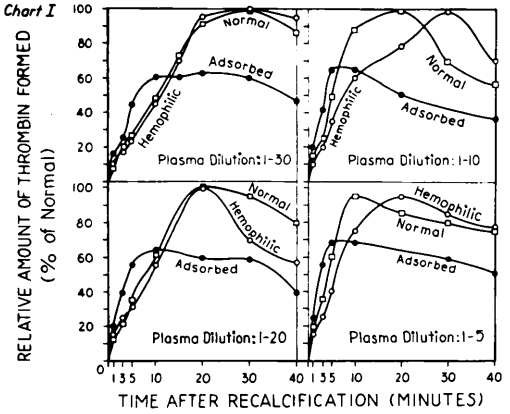
7 Tocantins, L. M., *Fed. Proc.*, 1943, **2**, 48.

8 Tocantins, L. M., *Fed. Proc.*, 1944, in press.

TABLE I.
Effect of Adsorption on Cephalin and Prothrombin Times of Normal and Hemophilic Plasmas.

	Normal		Hemophilic	
	Intact	Adsorbed	Intact	Adsorbed
Cephalin clotting time*				
0′ incubation (sec.)	84	32	177	31
5′ " "	97	30	210	31
Prothrombin time				
1-stage method† (sec)	12	8	12	9
2- " " ‡ "	16	21	16	20

* 0.3 cc plasma, 0.1 cc cephalin (0 or 5 min. incubation), 0.1 cc 0.074 M $CaCl_2$.[5]
† Quick Method.[1]
‡Smith, Warner and Brinkhous method,[2] using brain instead of lung extract and allowing 3 min. for conversion of the prothrombin.



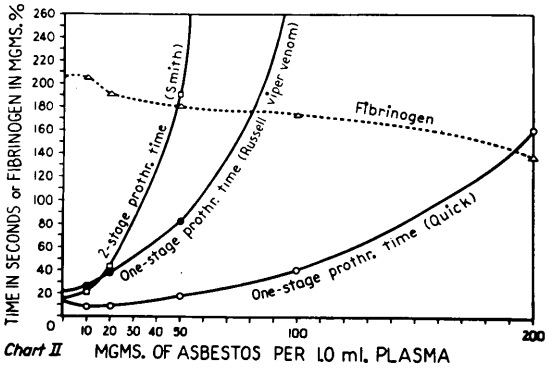


CHART I.
The rate of thrombin formation in 3 types of plasma, using cephalin. The shortest clotting time obtained with each dilution on normal plasma was considered to represent 100% thrombin formation for that specimen of plasma. The times equivalent to further dilutions (75%, 50%, etc.) of that plasma were determined and a curve plotted out. The percent of thrombin formed by that and the other plasmas at any period after recalcification was then calculated from their respective clotting times. Clotting mixtures as in Table II.

CHART II.
Effect on prothrombin time (3 methods) and fibrinogen of exposure of 1 ml of normal plasma to various amounts of asbestos for 60 min at 20°C. Prothrombin-free fibrinogen may be prepared in this manner, as the starting step.

given plasma to activation by cephalin may serve as an indication of its anticephalin (or antithromboplastin) activity provided its prothrombin is not under 50% of normal and there is no excess of antithrombin.

The adsorbed plasmas display shorter than normal prothrombin times by the 1-stage method but a longer time when tested by the 2-stage method (Table I). That this disagreement is brought about by an increase in the conversion rate of the adsorbed plasma prothrombin is shown in Chart 1 and Table II, In low dilutions, conversion of prothrombin by cephalin is slowest in the plasma with the most anticephalin activity (hemophilic) and fastest in that with the least (adsorbed). Diluting the plasma 1-20 or more weakens the inhibitor, thereby effacing most of the difference in anticephalin activity between hemophilic and normal plasma and tending to equalize their prothrombin conversion rates. Higher dilutions (1-80, 1-100), however, are necessary to reduce the anticephalin activity of the normal plasma to the level of that of the adsorbed plasma, before equalization of the prothrombin conversion rate of these two plasmas is brought about. When a potent thromboplastin solution instead of

TABLE II.
The Prothrombin Conversion Rate of Undiluted and Diluted Intact and Adsorbed Normal Plasma, Using Thromboplastin.

Type of plasma	Time (sec.) after recalcification before addition of fibrinogen.* 0	4	10	15	20	30	60	120	180	300	Dilut. of Plasma†
	Clotting time in seconds										
Intact	18	11	8	4	2						0
Adsorb.	13	7	4	2							0
Intact	34	26	21	19	13	11	9	9	9	9	1:5
Adsorb.	20	15	12	11	11	11	11	12	12	13	1:5
Intact	55		43		32	29	18	16	16	17	1:20
Adsorb.	47		38		32	29	22	22	22	25	1:20

* Contents of each tube: 0.3 cc incubation mixture ($CaCl_2$, 0.85% NaCl, thromboplastin, imidazole buffer),[2] 0.1 cc plasma, incubation for 0, 4, 10, etc., sec., then 0.1 cc fibrinogen.
† Defibrinated by addition of thrombin.

cephalin is employed (Table II), similar differences in the prothrombin conversion rate between normal and adsorbed plasmas may be observed, though lower dilutions of plasma must be used. The undiluted adsorbed plasma builds up a clotting titer of thrombin faster than the intact plasma and thus gives the impression that it has more prothrombin. When both plasmas are diluted 1-5 or 1-20, however, and enough time is allowed for the full conversion of all the prothrombin, the intact plasma gives a higher yield of thrombin, though, even at the high dilution, the adsorbed plasma still shows a slightly faster prothrombin conversion rate.

Exposure of 1 ml of plasma to quantities of the adsorbent greater than 10 mg intensifies the disagreement between the two methods (Chart II). When Russell viper venom, however, is used instead of brain extract, in the 1-stage method, the results are more in agreement with those obtained with the 2-stage method. Variations in prothrombin conversion rate apparently do not influence the action of Russell viper venom, probably because this venom acts directly on prothrombin, and is invulnerable to anticephalin.[7] The variations in conversion rate that have been pointed out for the prothrombin of various species[9] may be due to differences in anticephalin activity, in addition to chemical differences in the prothrombin itself. A study of the various animal prothrombins after exposure to adsorbents to remove anticephalin, may throw some light on this point.

Summary. Contact with certain adsorbents reduces the anticephalin activity of normal and hemophilic plasmas and accelerates the conversion rate of the prothrombin of such plasmas. Increases in the rate of conversion of prothrombin appear to be due to diminution of anticephalin activity and may be one cause of the disagreement in the results of quantitative determination of prothrombin by the 1-stage and 2-stage methods.

9 Warner, E. D., Brinkhous, K. M., and Smith, H. P., PROC. SOC. EXP. BIOL. AND MED., 1940, **40**, 197.