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Significance of Variations of Prothrombin Activity of Dilute Plasma.

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The introduction of the hemorrhagic principle, Dicoumarol, (3,3′ methylenebis (4-hydroxycoumarin)) has supplied a new impetus to the study of the prothrombin mechanism of plasma. Several investigators[1-4] have suggested that dilute plasma may be a more sensitive indicator of variations in prothrombin activity and consequently may be more efficaciously used to follow Dicoumarol therapy. During the course of this investigation on prothrombin activity of serial dilutions of human plasma, data have been obtained which suggest a physiological basis for clinical conditions manifesting an intravascular disturbance of the coagulation mechanism. Quick's one stage method applied to dilute plasma showed a difference in coagulation activity of "normal" plasma and plasma obtained from individuals having intravascular disturbances.

Prothrombin activity curves for "normal" plasma and plasma obtained from individuals in various clinical conditions were constructed by serial dilution with physiological saline. Quick's[5] method was followed meticulously in every detail. The clotting time for oxalated undiluted plasma (12-15 seconds) was repeatedly corroborated with regard to the narrow limits of variation that occur and found to be in complete agreement with the above mentioned investigator. However, with 12.5% and 25% plasma the clotting time deviated markedly from the values reported by Quick, indicating less prothrombin activity for these dilutions. Reproducible activity curves have been obtained from 60 "normal" individuals by the present method. Fig. 1 shows the limits of variation for "normal" human plasma for all dilutions compared with Quick's published data. It is to be noted that the greatest difference was encountered when 12.5% plasma was used. The factors responsible for the difference found are being subjected to a critical study.

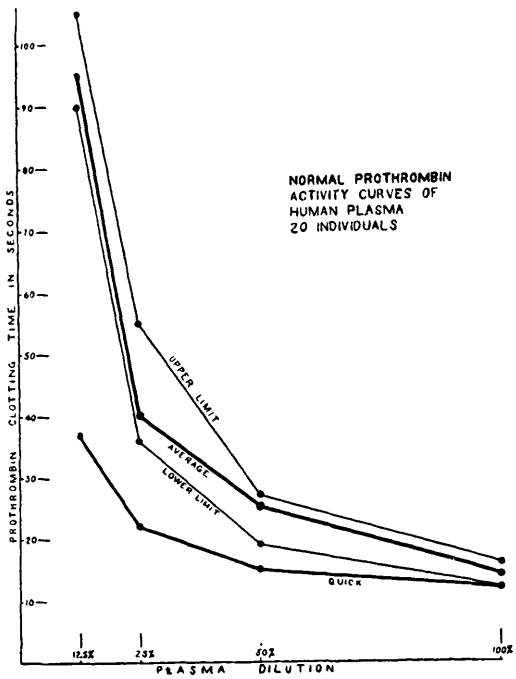

Fig. 1.

Comparison of Quick activity curve with average "normal" curve obtained in the present study. Abscissæ represent plasma dilutions with physiological saline and ordinates prothrombin clotting time in seconds. The curve showing the greatest activity was obtained from Quick's data, and deviates markedly from the values obtained in the present investigation. The limits of variation are indicated by thin lines.

1 Stahman, M. A., Huebner, C. F., and Link, K. P., *J. Biol. Chem.*, 1941, **138**, 513.

2 Campbell, H. A., Smith, W. K., Roberts, W. L., and Link, K. P., *J. Biol. Chem.*, 1941, **138**, 1.

3 Wright, I. S., and Prandoni, A., *J. Am. Med. Assn.*, 1942, **120**, 1015.

4 Shapiro, S., and Sherwin, B., *N. Y. State J. Med.*, 1943, **43**, 45.

5 Quick, A. J., *The Hemorrhagic Diseases and the Physiology of Hemostasis*, Charles C. Thomas, Springfield, Illinois, 1942.

TABLE I.

	Prothrombin clotting time, sec			
	Undil. plasma	12.5% plasma	No. individ.	
Normal	12-14	90±10	60	
Phlebitic	12-14	60±10	30	
Maternal	12-14	50±10	50	*
Fetal	15-18	110±20	50	*
Preoperative	12-14	90±10	5	†
3-5 days postoperative	12-14	60±10	5	†
10-14 " "	12-14	90±10	5	†

* Maternal and fetal blood taken simultaneously at time of delivery.

† Same individuals.

Increased plasma prothrombin activity expressed in terms of decreased clotting time was observed in certain clinical conditions when 12.5% plasma was used while undiluted plasma gave the established normal value. The data presented in Table I shows that an acceleration of the clotting power of dilute plasma (12.5%) was found in phlebitis, post-operative surgical trauma and during childbirth.

Individuals during childbirth reveal the most striking results. The undiluted maternal plasma and plasma obtained from the umbilical cord at the time of delivery have nearly identical prothrombin clotting time values; 12.5% maternal plasma under the same conditions coagulate with a markedly accelerated rate (50-60 seconds) while cord (fetal) blood plasma has a normal clotting time (110 ± 20 seconds). On the basis of the preceding data, it is suggested that a protective physiological mechanism may be set up by the maternal organism producing plasma prothrombin activators leading to a decreased clotting time during this critical period. This protective mechanism may function as part of a hormonal system antecedent to hemorrhage at the time of delivery. Hormones as factors affecting the coagulation system may be an explanation for the phenomenon observed. The fetal circulation, on the other hand, is not interrupted, consequently excess prothrombin activators are not essential and a normal prothrombin activity curve results. Fig. 2 shows the limits of variation for maternal and fetal plasma from 50 deliveries. A marked difference in activity curves between the two plasmas can be observed and furthermore, the limits of variation do not overlap except for undiluted plasma.

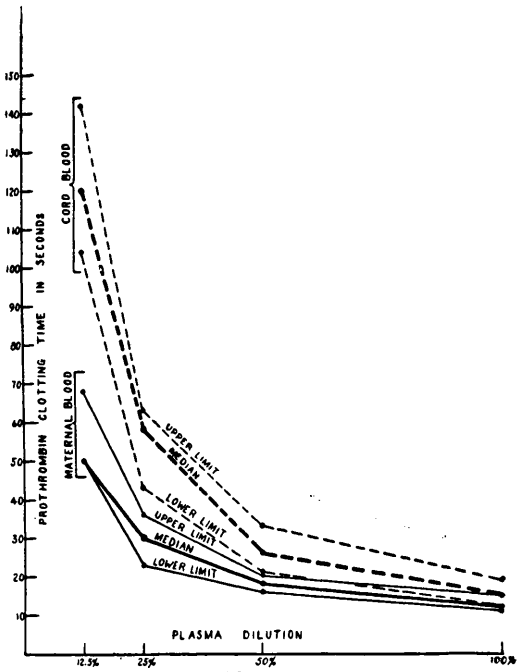

FIG. 2.

These curves show the limits of variation for maternal and fetal plasma from 50 deliveries. A marked difference in activity curves between the two plasmas can be observed, and that the limits of variation do not overlap except for undiluted plasma. Maternal plasma shows an increased activity while fetal plasma exhibits essentially normal activity. Abscissæ represent plasma dilutions with physiological saline and ordinates prothrombin clotting time in seconds.

During post-operative surgical trauma a decreased clotting time for dilute plasma (12.5%) was found for all individuals studied. After 10 days the accelerated coagulation was restored to the normal condition. (Table I). During this period, prothrombin activators may be produced in excess as part of a protective mechanism of the organism in response to hemorrhage.[6] Failure of the accelerated mechanism to return to normal may undoubtedly be one of the contributory causes leading to clinical post-operative thrombophlebitis. The phenomenon following post-operative surgical trauma is similar

[6] Cannon, W. B., *The Wisdom of the Body*, W. W. Norton, New York, 1939.

to that observed in childbirth.

Individuals with clinical symptoms of phlebitis manifest a decreased clotting time for dilute plasma (12.5%) and a normal value for undiluted plasma (Table I). In this clinical condition the prothrombin activators may possess a potency sufficient to produce intravascular clots and embolus formation. Silberberg[7] postulates biochemical factors that may be responsible for accelerated coagulation. It is of special interest in this connection that patients receiving the hemorrhagic principle, Dicoumarol, exhibit intravascular coagulation difficulties when the effects of the drug cease and the prothrombin clotting time of 12.5% plasma again shows a decreased coagulation time compared with a normal individual.

Dicoumarol* has been used to disrupt the prothrombin mechanism intravascularly. Individuals with chronic clinical phlebitis do not respond as readily as normal individuals to this hemorrhagic agent and return in a short time to their previous condition. Dicoumarol therapy can be followed most satisfactorily when dilute plasma (12.5%) is used. Variations in clotting time can be detected with this dilution that are not manifest when undiluted plasma is used. No toxic symptoms were observed in any of the individuals receiving this hemorrhagic principle.

Since the foregoing observations demonstrate increased prothrombin activity of the plasma in some pathological states, an attempt was made to induce this condition in "normal" individuals by intravenous injections of repeated large doses of a synthetic vitamin K derivative (Hykinone).† No increase in coagulation activity was found in any of the dilutions of normal plasma. These findings are in keeping with all previous observations that vitamin K derivatives do not accelerate the prothrombin coagulation mechanism above normal limits but rather activates an organ, *i.e.*, the liver, to produce this thrombin precursor until an optimum amount is available for normal processes.

Quick's simple one stage procedure for evaluating the prothrombin clotting time of oxalated plasma has been successfully adapted to provide an index to the prothrombin activity occurring in the various above mentioned clinical conditions. By this means, employing dilute plasma (12.5%), it is possible to measure accelerated coagulability of plasma. The demonstration of prothrombin "activators"[7] as a protective mechanism or as a pathological state is suggested to account for the difference in clotting activity encountered. The nature of these "activators" is being subjected to a critical biochemical and physiological study.

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7 Silberberg, M., *Physiol. Rev.*, 1938, **18**, 197.

* The Dicoumarol (3,3′-methylenebis (4-hydroxycoumarin)) used in this study was supplied by the Abbott Laboratories, Chicago, Ill.

† The vitamin K derivative (Hykinone) was supplied through the generosity of the Abbott Laboratories, Chicago, Ill.