INFLUENCE OF FIBRINOGEN CONCENTRATION UPON PLASMA PROTHROMBIN TIME

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ESTIMATION of the prothrombin time of whole and diluted (12.5 per cent) plasma has been shown to have advantages over the usual one-stage procedure in which whole plasma alone is employed.[1a,b] Recently, Deutsch and Gerarde indicated that the prothrombin time of diluted plasma may be influenced by variations in the fibrinogen level.[2] Their study was on rabbit plasma. In the present communication, data are given concerning the fibrinogen concentration in human plasma in the presence of changes in diluted (12.5 per cent) plasma prothrombin time.

The following studies were made: Simultaneous estimation of prothrombin time and fibrinogen concentration of plasma in: (1) normal subjects, (2) cases of hyperprothrombinemia,[3,4] (3) cases of hypoprothrombinemia.

Methods

Estimation of the prothrombin time was made by the procedure previously described.[1a] Fibrinogen concentration was established by determination of the nitrogen content of plasma before and after the contained fibrinogen was coagulated and removed according to the method described by Peters and Van Slyke, modified for micro-kjeldahl technic.[13]

Results

In the tables given below, the prothrombin time of the diluted (12.5 per cent) plasma is given in seconds. The normal standard is 39.5 seconds, standard deviation ±2.5. (The whole plasma prothrombin time plays no part in the present discussion.) The fibrinogen values are given in milligrams per 100 ml.

Figure 1 is a scatter diagram of 76 simultaneous determinations of plasma fibrinogen and diluted plasma (12.5 per cent) prothrombin time. There is no correlation between the two.

Table 1. In 4 normal persons, simultaneous prothrombin time and fibrinogen estimations were made. The prothrombin time was normal in each case. The fibrinogen values also were normal. In 3 cases of spontaneous hyperprothrombinemia, the fibrinogen results were within the normal range.

Table 2. Normal persons and cases of liver disease were given large doses of synthetic vitamin K intravenously. Each type of response is represented: Prothrombin time unchanged, reduced, or increased. All of the fibrinogen values were within normal limits and no parallelism was observed between the shift in the prothrombin time and the fibrinogen levels.

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Discussion

The study was made in normal, hyperprothrombinemic (both spontaneous and induced) and hypoprothrombinemic bloods. Correlation between fibrinogen concentration and variations in prothrombin time is strikingly lacking. All the fibrinogen values are within the normal range while the prothrombin times extend

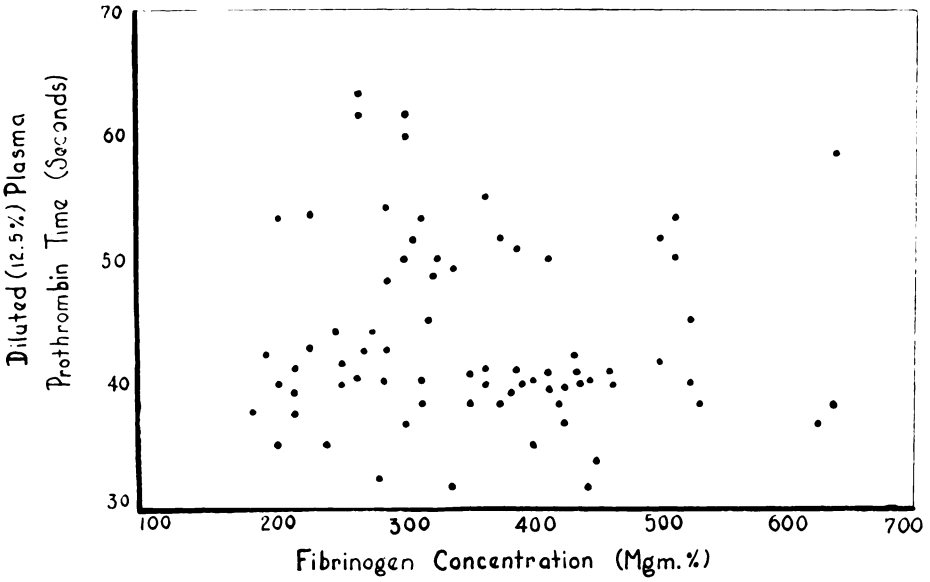

Fig. 1.—Scatter graph representing the fibrinogen concentration and diluted (12.5%) plasma prothrombin time of 76 samples of blood. The series includes cases in which the prothrombin time is normal, prolonged (hypoprothrombinemia) and reduced (hyperprothrombinemia).

Table 1.—*Simultaneous Determinations of Plasma Fibrinogen and Diluted (12.5%) Plasma Prothrombin Time in 4 Normal Subjects and in 3 Cases of Hyperprothrombinemia*[3]

Case	Prothrombin Time	Fibrinogen
	sec	*mgm. per 100 ml.*
A. Normal subject	42.4	410
B. Normal subject	40.0	310
C. Normal subject	37.8	420
D. Normal subject	39.8	205
E. Spontaneous hyperprothrombinemia	32.8	450
F. Spontaneous hyperprothrombinemia	32.8	330
G. Spontaneous hyperprothrombinemia	35.4	430

throughout the gamut of normal, increased and reduced activity. Especially instructive is the contrast between Case H and Case J. In the former, the serial prothrombin time estimations remained within the normal range despite an increase in fibrinogen content from 246 to 609 mg. per 100 ml. plasma. In Case J, a significant prolongation of prothrombin time occurred simultaneously with elevations in

fibrinogen values from 240 (at which time, the prothrombin time was normal) to 410 mg., when it was definitely at a hypoprothrombinemia level. Case I is likewise noteworthy, because in it, the fibrinogen concentration showed practically no

TABLE 2.—*Simultaneous Determinations of Plasma Fibrinogen and Diluted (12.5%) Prothrombin Time after Vitamin K*

Description of Cases	Days*	Prothrombin Time	Fibrinogen
		sec.	*mgm. per 100 ml.*
Case H. Normal subject. Prothrombin time unchanged after vitamin K. Variations in fibrinogen values marked.	1	40.8	311
	2	44.0	
	3	41.8	246
	4	39.4	609
Case I. Normal subject. Prothrombin time reduced following vitamin K. Fibrinogen values relatively constant.	1	42.4	410
	2		
	3	35.2	400
	4		
	5	31.8	450
Case J. Hepatic cirrhosis. Positive vitamin K tolerance test.[6] Prothrombin time increased following vitamin K. Fibrinogen values *increased* when prothrombin time prolonged.	1	45.8	210
	2	40.4	240
	3	41.9	360
	4	48.0	340
	5	52.0	410
Case K. Hepatic cirrhosis. Prothrombin time initially prolonged and temporarily reduced following vitamin K. Fibrinogen values not increased when prothrombin time reduced.	1	53.6	500
	2	34.8	450
	3	41.8	400
	4	52.0	510
Case L. Hepatic cirrhosis. Positive vitamin K tolerance test. Fibrinogen concentration not reduced on day prothrombin time prolonged.	1	49.0	260
	2	42.4	240
	3	41.0	205
	4	64.9	250
Case M. Hepatic cirrhosis. No correlation of increased prothrombin time with reduced fibrinogen concentration.	1	42.4	320
	2	52.4	380
	3	46.2	300
	4	54.2	370
	5	49.6	240

* Seventy-six mg. of synthetic vitamin K (2-methyl-1,4 naphthohydroquinone diphosphoric acid ester tetrasodium salt [Synkayvite]) was given intravenously on each of the first four days. Blood for prothrombin time and fibrinogen estimation was withdrawn each day before administration of the daily dose of vitamin K.

alteration at the time the prothrombin time became reduced to the hyperprothrombenemia range.

Deutsch and Gerarde,[2] working with rabbit plasma, induced in vitro a reduction of the prothrombin time of 10 per cent plasma from 33 seconds to 22 seconds by

adding 150 mg. of beef fibrinogen per 100 ml. plasma. They pointed out that there was considerable species variation in this effect. Our findings indicate that the results obtained with rabbit plasma are not applicable to man. The normal range of fibrinogen content of the human plasma is 200–600 mg. per 100 ml. If fibrinogen variations had as great an effect on human diluted plasma as is implied by the data on rabbit plasma referred to above, it is difficult to understand how the mean prothrombin time of the diluted (12.5 per cent) plasma of 39.5 seconds, obtained by studying blood from several hundred normal subjects, could have a standard deviation of only ±2.5. This fact substantiates further the belief that the usual fibrinogen range of diluted (12.5 per cent) plasma in man (30 mg. to 75 mg. per 100 ml.) does not alter significantly the accelerated clotting time.

Data offered by Owren[7] in his extensive study of the coagulation mechanism support the above conclusion. Owren demonstrated that the critical low level of fibrinogen below which clotting time increases sharply, varies with the prothrombin concentration. In 10 per cent plasma, the fibrinogen concentration must be reduced to below 20 mg. per 100 ml. before a significant effect upon accelerated clotting time is noted. It is at this dilution that the quality of the clot becomes poor and difficult to detect. The 12.5 per cent plasma yields a firm and easily discernible clot. The 8 per cent plasma often gives a rather poor clot. The range between these two dilutions, 12.5 per cent and 8 per cent, includes the critical fibrinogen concentration below which clotting time rises sharply. This is consistent with our experience of two instances in which 12.5 per cent plasma yielded poor clots and the fibrinogen concentrations were 100 mg. per cent and 120 mg. per cent. Thus, only in these very rare cases of hypofibrinogenemia (below 150 mg. per cent) does fibrinogen concentration become a factor in 12.5 per cent plasma prothrombin time determination. It appears that a poor clot, which is a rare occurrence, may be considered as a warning that the fibrinogen level is sufficiently low to cause an alteration in the accelerated clotting (prothrombin) time.

An obvious modification of the technic would be to use fibrinogen solution as diluent in place of normal isotonic saline. This has been done by Thordarson[8] and by Link and his students.[9] In man, it has been our experience that the prothrombin time of diluted (12.5 per cent) plasma may be affected in an unpredictable fashion thereby. In some instances, the prothrombin time remained unaltered, while in others it became extended. When the protein solution is used as diluent additional factors such as questionable purity and stability may be introduced. These additional variable factors do not exist if saline is used as diluent.

The data presented show considerable difference in fibrinogen concentration with no corresponding variations in prothrombin time. Reliable estimations of the diluted (12.5 per cent) plasma prothrombin time can be made at the low normal level of fibrinogen (180 mg. per 100 ml.) as well as at the high level of 650 mg. per 100 ml.

It has been found by Foster and Whipple,[10] and later emphasized by Link,[9] that fibrinogen is a very labile plasma protein and fluctuates readily in response to a variety of factors. It is important to point out that massive doses of dicumarol may depress the fibrinogen level of plasma[11] but that at therapeutic dosage levels of

dicumarol the fibrinogen concentration is maintained within the normal range. This has been demonstrated in animals[9] and in man.[12]

Summary

The effect of the normal variations of fibrinogen concentration (180 mg. per cent to 650 mg. per cent) on the diluted (12.5 per cent) plasma prothrombin time in man, as observed in this study, is not significant.

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