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Prothrombin Deficiency of the Newborn.

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(Introduced by P. György.)

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Many of the recent studies on blood coagulation have been concerned with the nature of prothrombin, its composition and the interrelationships of the various coagulation components that affect its conversion to thrombin.

Quick[1,2] at first claimed that prothrombin, as determined by the one stage method, appeared to be composed of at least two components which he designated as A and B. His original work was soon supported by other investigators[3–5] who reached similar conclusions. Seegers, Loomis, and Vanderbilt[6,7] have since prepared prothrombin that is electrophoretically homogeneous and have therefore rejected the multi-component hypothesis.

A number of recent studies, in particular those of Fantl,[8] Quick,[9] Ware,[10] Murphy,[11] Owren,[12,13] Munro[14] and MacMillan[15] are of special interest in that they present evidence of other new factors important in the conversion of prothrombin to thrombin. Ware *et al.*[16] have been able to prepare a globulin of high purity that both accelerates the prothrombin conversion and increases the yield of thrombin. Owren[13] has described a new and highly purified factor that also accelerates prothrombin conversion. It has not been settled as to whether or not this factor is identical with the substance described by Ware *et al.* MacMillan[15] has presented experiments showing that the blood of dicoumarolized patients is deficient in a factor that is different from that described by Owren.

In the present work an attempt was made to determine whether any of the previously described factors are concerned with the prolongation of prothrombin time observed in the newborn infant.

Subjects. Twelve full term infants, under 5 days of age, whose plasma exhibited a prolonged prothrombin time were selected for this study. Neither mother nor infant had received vitamin K and none showed clinical evidence of hemorrhagic phenomena. The method employed in the evaluation of the deficiency leading to the prolonged prothrombin time consisted of the addition to the infant's plasma of other plasma or serum known to contain or lack certain components. The plasma or serum added was chosen from groups of subjects as follows: 1) Plasma from 12 normal adults, (also used as controls in the prothrombin determination). Plasma from 6 normal adults stored for various lengths of time. Serum from 6 normal adults similarly stored (Series 1). 2) Plasma from 6 normal infants whose prothrombin times were within the normal range, and who had

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TABLE I.—Series 1.
Prothrombin Time (Seconds) of Infants Plasmas and Mixtures with Other Sera or Plasmas as Indicated.

Infant	Infants deficient plasma (A)	Adult normal plasma (B)	75% A / 25% B Calculated	75% A / 25% B Observed	Adult stored plasma (C)	Days stored	75% A / 25% C Calculated	75% A / 25% C Observed	Adult serum days stored (D)	75% A 25% D
1	39.3	20.3	32	23.0*						
2	253.0	18.5	60	28.5*						
3	64.1	20.2	30	25.6						
4	37.1	19.7	27	21.5						
5	48.6	21.3	29	25.4						
6	25.7	18.9	22	18.4						
7	36.1	17.9	25	20.2	59.6	18	39	18.2	18	18.9
8	35.9	18.8	26	21.4	96.5	21	42	22.6		
9	30.7	18.8	25	21.2	83.6	50	36	18.1	50	17.4
									21	18.2
10	41.1	18.6	26	22.3	93.0	48	46	22.1	16	19.7
11	42.3	22.3	28	19.5	176.0	160	42	24.7	120	24.3
12	30.4	19.9	24.5	20.5	200.	190	30	22.3	160	27.1

* 90% A — 10% B.

TABLE II.
Prothrombin Time (Seconds) of Infants Plasmas and Mixtures with Other Plasmas as Indicated.

	Series 2				Series 3					
Infant	Infants deficient plasma (A)	Infants normal plasma (E)	75% A Calculated	25% E Observed	Adult Dicumarolized plasma (F)	75% A Calculated	25% F Observed	Adult Liver damage plasma (G)	75% A Calculated	25% G Observed
4	37.1				30.9	36	32.4			
5	48.6				53.0	48	45.7			
7	36.1	19.5	26	24.1						
8	35.9	19.0	26	23.6						
9	30.7	19.7	24	23.8	34.8	27	25.8			
10	41.1	19.6	27	24.2						
11	42.3	18.9	27	25.5	33.5	39	25.5	27.5	36	30.5
12	30.4	18.2	24	24.2	31.5	30	29.9	31.5	30	32.5

not received vitamin K (Series 2). 3) Plasma from 5 adults who had received dicoumarol for thrombotic disease but were otherwise healthy (Series 3). 4) Plasma from two adults who were suffering from liver disease (Series 3).

Technic. The prothrombin times of the various plasmas and plasma mixtures studied were determined by use of the Quick method. Details are given in a previous paper.[17] The values given represent the averages (in seconds) of at least 3 determinations. Stored sera and plasmas were kept in a refrigerator at approximately 5°C for 18 to 190 days, as indicated in Table I.

Results. It will be noted that when the plasma of infants with prolonged prothrombin times was mixed with the plasma from normal adults in the proportions recorded (Table I, Series 1) a considerable shortening of the infant's prothrombin time occurred in each case, even though only a relatively small amount of adult normal plasma had been added. When the infant's plasma was mixed with adult stored plasma or serum a similar and in some cases, a more marked decrease in prothrombin time occurred. Plasma of infants with normal prothrombin times (Table II, Series 2) also decreased the prothrombin time of the infants with abnormal plasma but not as markedly as did the adult plasma. Finally when the plasma with prolonged time was mixed with plasma of dicoumarolized adults or of those with liver disease (Table II, Series 3) a variable result was obtained, but usually the decrease in prothrombin time was slight.

Discussion. Restoration of prothrombin times in the foregoing series of experiments demonstrates that more than a deficiency of prothrombin is involved, at least insofar as this is indicated by the one stage method. Considering the mixture described in the first experiment (Table I, infant No. 1), through the use of plasma dilution curves, the total theoretical prothrombin content of the mixtures may be calculated by adding the prothrombin contributed by each of the 2 component plasmas. Because of dilution, the adult plasma (assumed to be 100% before dilution) contributed 10%. The infant (containing only 20% before dilution) could contribute no more than 18%. The final mixture would be expected to contain the sum of the contributions by each plasma, or 28%, and the expected time would be 32 seconds instead of the observed 23. This discrepancy is even more obvious in the second infant studied (Table I, No. 2). This infant's plasma, having an extremely prolonged time, could contribute practically no prothrombin, and the calculated clotting time of the mixture would be that of a 10% dilution of adult plasma or 60 seconds instead of the observed 28.5 seconds. Similar reasoning applied to the other subjects is given in the column (Table I) marked "calculated". That more than a simple deficiency of prothrombin exists is further substantiated by the demonstration that the plasma of infants, normal by the one stage test, proved to be less effective than adult plasma in restoring the prothrombin times of the deficient infants.

The evidence here presented does not support the conclusions reached by Quick,[9] according to which the plasma of these infants should be deficient in the component he designates as prothrombin A. If this were so, the plasma of patients receiving dicumarol or suffering from liver disease should restore to normal the prothrombin time of the plasma of these infants. This was not evident in the seven instances studied.

Further, deficiency of Quick's[1,9] labile factor does not seem to be the basis of the deficiency seen in the newborn, because stored plasma, in which this factor is either absent or greatly diminished, proved to be as effective as fresh plasma for restoration of the prothrombin times.

Finally, and perhaps most significant was the demonstration that stored serum, from which prothrombin, thrombin and fibrinogen would have disappeared, was also effective in bringing the prothrombin times of deficient infants to normal.

It would appear from the foregoing that the prolonged prothrombin times frequently observed in the newborn, when measured by the one stage technic, cannot be adequately

[17] Randall, A., IV, and Randall, J. P., *Science*, 1948, **107**, 399.

explained as a deficiency of prothrombin itself or of any of the classical clotting factors. Rather it would seem that the newborn infant is unable to convert prothrombin to thrombin with the same speed and efficiency as the normal adult. This view would also explain the discrepancy observed in studies on such subjects in the past when their blood has been examined simultaneously by the one stage and two stage technics. Our experiments indicate that a factor is provided by normal plasma, aged plasma or serum that considerably enhances the conversion of prothrombin. Recently reported studies, as previously mentioned, have shown that one or more accelerator factors are involved in the normal blood clotting mechanism. At least one of these factors has been shown to be present in serum. It is our belief that deficiency of such a factor contributes to the clotting abnormalities of the newborn, heretofore attributed solely to a lack of prothrombin. Further investigation of this basic problem to confirm and amplify these findings is clearly required.

Two recent papers[15,18] demonstrate that a diminution of such a factor accounts for certain clotting changes observed in dicoumarolized subjects. The observation that dicoumarolized plasma exerts little effect upon the plasma of deficient infants suggests that the changes in both plasmas may be of similar nature.

Summary. 1. Evidence is presented indicating that the prolonged prothrombin times observed in the plasmas of newborn infants, studied by the one stage technic, cannot be adequately explained as a simple deficiency of prothrombin.

2. Although there may be some diminution of prothrombin, it appears that the plasma of the newborn is deficient in a factor accelerating conversion of prothrombin to thrombin. The deficiency of this factor is remedied by the addition of small amounts of normal plasma, stored plasma or serum.

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Agglutination of Sheep's Erythrocytes Sensitized with Histoplasmin.

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The need of sensitive and specific serologic tests in fungus diseases has lately arisen in cases with skin reactions to histoplasmin. Several publications[1-3] describing complement fixation tests have appeared. Recently Saslaw and Campbell[4] have reported agglutination tests using collodion particles sensitized with histoplasmin. The preparation of collodion particles, however, is very difficult. Middlebrook and Dubos[5] have reported a similar agglutination test for tuberculosis in which sheep's erythrocytes were sensitized with a specific substance extracted from tubercle bacilli.

The present report describes an agglutination test using histoplasmin-sensitized sheep's erythrocytes as antigen and sera from rabbits immunized with *Histoplasma capsulatum.*

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