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Blood Coagulation During Infancy.*

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Several years ago data were published from this laboratory[1] which showed that the plasma prothrombin level in young infants is somewhat less than one-half as high as in adults. These prothrombin measurements were made with a 2-stage titration technic.[2] In the first stage, defibrinated plasma, in suitable dilution, is treated with calcium in optimal amounts, and with thromboplastin in large excess. After allowing ample time for full conversion of the prothrombin into thrombin, the latter was determined in the second stage of the titration by the addition of fibrinogen.

Since these studies were made we have undertaken further studies on infant plasma, and have discovered that in the period between the second and sixth days of life there occurs a further, though temporary, fall in prothrombin not previously recognized. We have also made a comprehensive study of infant plasma, using the one-stage prothrombin titration technic of Quick,[3] and have found that a fall also occurs with this technic. We have also observed, contrary to expectation, that except for this brief fall, the method of Quick gives results fully as high as those obtained with normal adult plasma.

This difference in results indicates the existence of important differences between the two methods. Data already published from this laboratory[4,5] indicate the nature of this difference. It has been pointed out that the technic of Quick is one in which thromboplastin is added in large amounts to plasma. Under these conditions, thrombin is rapidly built up to the clotting level, and clotting occurs before

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1 Brinkhous, K. M., Smith, H. P., and Warner, E. D., *Am. J. Med. Sci.*, 1937, **193**, 475.

2 Warner, E. D., Brinkhous, K. M., and Smith, H. P., *Am. J. Physiol.*, 1936, **114**, 667; *J. Exp. Med.*, 1937, **66**, 801.

3 Quick, A. J., *Am. J. Physiol.*, 1936, **114**, 282.

4 Warner, E. D., Brinkhous, K. M., and Smith, H. P., *Am. J. Physiol.*, 1939, **125**, 296; PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 197.

5 Ziffren, S. E., Owen, C. A., Hoffman, G. R., and Smith, H. P., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 595.

all of the prothrombin has been converted. The rate at which thrombin can be built up to the clotting level depends not only upon the amount of prothrombin, but also upon the specific "convertibility" of the prothrombin.[4, 5] It is our belief that in plasma of newborn infants, prothrombin conversion occurs quite rapidly, compensating for a relative deficiency in amount. The technic of Quick thus measures the summation of at least two variables—amount of prothrombin and convertibility of the latter. In our opinion this does not detract from the value of the test; on the contrary, the test measures an important summation, and under restricted conditions it gives a

TABLE I.

Case No.	Age in days	Sex	2-stage Prothrombin Test (% of normal)	Quick's Test (% of normal)	New Test (% of normal)	Whole blood clotting time (min)
1	0	M	35	110	144	—
2	0	F	36	120	134	—
3	0	F	37	70	129	4.0
4	1	F	20	110	132	—
5	1	M	37	—	123	5.5
6	1	F	44	90	122	5.0
7	1	M	23	50	76	6.0
8	1	F	29	45	60	—
9	2	M	—	—	122	—
10	2	M	53	80	104	—
11	2	M	44	115	90	—
12	2	M	22	35	41	7.5
13	2	M	20	20	28	—
14	3	F	26	50	67	5.5
15	3	F	40	—	48	—
16	3	M	23	25	34	7.5
17	3	M	20	25	28	10.0
18	3	F	22	20	25	9.0
19	4	M	28	60	63	6.5
20	4	M	37	45	56	6.0
21	4	M	—	—	55	—
22	4	M	42	60	52	—
23	4	F	23	30	43	—
24	5	M	37	—	18	—
25	5	F	27	—	93	4.5
26	5	M	40	80	97	7.5
27	5	F	—	—	117	—
28	5	M	—	—	119	—
29	6	M	6	15	32	—
30	6	M	—	90	125	—
31	6	F	31	120	139	—
32	7	F	42	—	120	5.5
33	7	F	47	130	131	—
34	8	M	39	120	104	—
35	8	F	44	100	104	—
36	8	M	—	—	138	—
37	9	F	25	100	118	—
38	10	M	41	—	139	4.5

very practical measure of the tendency to bleed. It should not, however, be assumed to give a specific measure merely of the amount of prothrombin present.

Table I gives the results obtained on 38 young infants arranged in order of their ages. Blood for these analyses was obtained from the jugular vein or from the superior longitudinal sinus,† except in case 2, where the sample was drawn from the umbilical cord. The table gives prothrombin titers obtained by the 2-stage technic; also the results obtained by the method of Quick. In the latter test, we have titrated all plasmas in the undiluted state, and also at 2- and 3-fold dilution with saline. This dilution increases the sensitivity of the method, as one could predict from the dilution curves published by Quick.[6] This dilution did not appreciably alter the results, however. In addition to these two methods we have listed the results obtained with a new clotting test which we[5] have recently devised for clinical use in cases of vitamin K deficiency. This test is similar in principle to that of Quick, but in the new test thromboplastin is added directly to whole blood instead of to plasma. This is a simplification, and as we have shown the new technic has certain theoretical advantages.

At birth Quick's test, like the new test, gives values which equal adult values, or exceed them somewhat, and this approximate equality persists even when both plasmas are diluted 2- or 3-fold with saline. In contrast to these results, the 2-stage technic shows that the prothrombin level is somewhat less than 40% of the adult value. In making the 2-stage test, we have used thromboplastin of many sources, including lung and brain of man, ox, and rabbit; also we have used cephalin, and with all of these we have obtained substantially the same result, indicating that maximum conversion had been obtained. Furthermore, we have mixed infant plasma with an equal volume of normal adult plasma, and with the 2-stage technic have obtained values half-way between the two. Nor is there any evidence of excessive antithrombic activity in the plasma of newborn infants. In fact, the plasma is diluted 20-30-fold before the test is made, and very little antithrombic activity remains. The maximum thrombin titer developed in the first stage of the titration is well maintained for several minutes. All of the evidence at hand thus indicates that the 2-stage technic gives the full titer of prothrombin, both in infants and in adults.

During the next few days of life, both Quick's test and the new

† The fontanelle punctures were kindly performed by Drs. G. W. Wagner and E. F. VanEpps.

[6] Quick, A. J., *J. A. M. A.*, 1938, **110**, 1658.

test show a surprising fall, levels of 30-50% being quite common. In some cases the fall is apparent as early as the second day, but in others it seems to develop later. In all cases studied on the third and fourth days the decrease was evident.

A careful survey of the data also shows a fall in prothrombin by the 2-stage technic though the fall is not so great as with other tests. We have made a special study of those cases which were less than 50% by Quick's test. In this group the 2-stage titration gave an average value which was 29% of normal, whereas in all cases above this level an average of 41% was obtained. One must conclude that the prothrombin titer by the 2-stage technic does fall, but the fall by the one-stage tests tends to be greater.

In our series, whole blood clotting time was determined in a limited number of cases. In this small series there is considerable variability, but there is some evidence that the clotting time is moderately prolonged between the third and fifth days, corresponding to the fall in prothrombin. This prolongation of clotting time was also observed by Rodda.[7] It is a curious fact that whole blood clotting time does not show much prolongation until the various prothrombin tests reach very low levels. As we have pointed out already[5] this fact suggests that thromboplastin variations may compensate for prothrombin deficiency. In the various prothrombin tests, thromboplastin is added in such large amounts that these variations are effaced, and the true prothrombin deficit makes itself evident.

At the end of 5-7 days all 3 prothrombin tests returned to approximately the values prevailing at birth. Data not given in the table show that Quick's test and the new test undergo no very significant change during the next 12 months. We have also confirmed the older observation of this laboratory[1] that the prothrombin level by the 2-stage technic rises gradually to adult levels during this 12-month period. Neither in this series, nor in the older one has sex had any definite effect on the results.

The fall in prothrombin which occurs in the first week of life was not detected in the earlier studies from this laboratory, for in those studies very few readings were taken between the second and eighth days. The fall in prothrombin is particularly significant in view of the fact that it is during this period that one sees cases of hemorrhagic disease of the newborn. Among the cases previously reported from this laboratory was a case of this disease in which the 2-stage method showed a profound lowering of the plasma prothrombin level to be the cause of the bleeding. In a study of this

[7] Rodda, F. C., *Am. J. Dis. Child.*, 1920, **19**, 269.

problem one must appreciate the fact that the convertibility of prothrombin deserves attention equally with the problem of the actual prothrombin level. By combined study with one-stage and 2-stage methods it is possible to evaluate both factors.

The cause of variation in convertibility has not been determined. In rabbit plasma the prothrombin is much more readily converted than in the plasma of man,[4] despite the fact that the actual prothrombin levels in the 2 are almost identical. We have also seen human cases in which a lowering of the prothrombin level was compensated by increased convertibility. Whether the variable convertibility represents differences in the prothrombin itself, or in the amount of "antiprothrombin" or in other factors must be answered by future research.

Summary. A study of 38 normal infants confirms previous work from this laboratory that the plasma prothrombin level is low in early infancy. It is also shown that an additional fall, not previously recognized, occurs between the second and sixth days of life. Evidence is presented to indicate that the rate of thrombin formation during coagulation depends upon convertibility of prothrombin as well as upon the amount of the latter. In newborn infants rapid convertibility of prothrombin compensates for deficient quantity of prothrombin. Evidence also suggests that variations in thromboplastin serve, in some circumstances, to compensate for a deficiency in the amount of prothrombin.

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Oxidation of Citric Acid by *Coli-aërogenes* Bacteria.

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Species of *Aërobacter* and *Citrobacter* utilize citric acid as a sole source of carbon in contrast to *Escherichia.* Previous work[1] has established the nature of the anaërobic dissimilation of citrate by *Aërobacter.* The present communication reports the results of an investigation of the aërobic breakdown of citric acid by *Aërobacter indologenes* and *Citrobacter freundii.*

1 Brewer, C. R., and Werkman, C. H., 1939, accepted for publication in *Enzymologia.*