VITAMIN K REQUIREMENTS OF THE NEW-BORN

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It has been shown in the last few years, by numerous investigators (Waddel et al., 1939; Nygaard, 1939; et al.) that there occurs an appreciable drop in the prothrombin levels of newborn infants. In an investigation on a small series of sixty infants these results have been confirmed (Toohey, 1941). The fall in prothrombin usually reaches its maximum between the second and fourth days and the level then rapidly rises to nearly normal by the seventh day. This hypoprothrombinaemia is in the majority of cases without clinical significance, but in a very small percentage, where the fall in prothrombin is extreme, the condition of haemorrhagic disease of the newborn occurs. Treatment with vitamin K rapidly restores the prothrombin level to normal with cure of the disease, except when treatment has been delayed, or the condition is extreme.

It has also been shown that prophylactic administration of vitamin K, either before delivery to the mothers or soon after birth to the infant, prevents the fall in the prothrombin level, or markedly reduces it. In a large number of infants treated prophylactically with vitamin K by numerous investigators (Hellman et al., 1940; Waddel and Lawson, 1940) no case of haemorrhagic disease occurred. It can now be assumed that vitamin K is specific for the prevention and treatment of haemorrhagic disease of the newborn. In the investigation recorded below an attempt has been made to find the required dose of a vitamin K analogue which will prevent any fall in the prothrombin level; also the time of administration giving full effect.

Estimation of prothrombin in infants

Estimation of prothrombin in infants is not the simple procedure it is in adults, where Quick's method is reliable. Repeated venepuncture in infants to obtain plasma is not a practical clinical procedure. Most investigators have used a modified form of Quick's method, with whole blood obtained by heel puncture instead of plasma, as advocated by Bray and Kelley (1940). This has been further adapted to tests carried out at the bedside. Whole blood obtained by heel puncture is mixed with an equal quantity of an active thromboplastin solution, and the time taken for a clot to form is the prothrombin clotting time. This is usually expressed as a percentage by taking a control clotting

time. The disadvantage of these tests is the indefinite end-point with blood as compared with the sharp end-point with plasma.

To avoid this Owen and Toohey (1941) obtained plasma from heel puncture by means of white-cell pipettes containing oxalate, and then carried out Quick's test. This simple method gives fairly accurate results. When numerous estimations are being carried out, however, it has the disadvantage of taking up too much time. To expedite the test and to retain the advantage of a sharp end-point, and hence more accurate clotting time, the following method was used:

Dale and Laidlaw's coagulation tubes are used. These are half-filled with an active thromboplastin solution, either brain extract or viper venom. The infant's warmed heel is pricked and the tube is completely filled with blood by capillary attraction, thus giving equal quantities of thromboplastin solution and blood. The remainder of the test is practically identical with carrying out an ordinary coagulation time by Dale and Laidlaw's method. The filled tube is immediately immersed in water at 100° F. (approximately), the ends being firmly closed by forefinger and thumb. The tube is rotated till the bead inside the tube stops, denoting that clotting has occurred. The time from the filling of the tube with blood to the bead stopping is accurately taken by a stop-watch. This is the prothrombin clotting time. The whole procedure takes less than a minute to perform and can be easily repeated two or three times on each infant. The end-point is sharp and can be accurately timed.

The advantages of the above method are the rapidity with which the test can be done at the bedside, the sharp end-point, and hence precise timing, and lastly, the standard conditions of the test. Using this method some hundreds of repeated estimations on infants have been performed and the variation in each case has been found to be usually not more than one second. Variation of more than one to two seconds in any one case occurred mainly in those infants showing a prolonged clotting time, as also occurs when Quick's original method with plasma is used.

Results of estimations on control infants

A. Using method of Owen and Toohey with plasma.

Estimations on sixty infants were made and have been reported in detail elsewhere (Toohey, 1941). A summary of these results need only be given here:

1. Sixteen infants showed prothrombin clotting times of four to ten seconds above adult controls.
2. Eighteen infants showed prothrombin clotting times of ten to twenty seconds above adult controls.
3. Five infants showed prothrombin clotting times of more than twenty seconds above adult controls.

B. Using the method described above with blood instead of plasma.

Estimations on forty infants were made. At least two estimations during the first week of life were carried out and the estimations repeated two or three

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times on each occasion. Table 1 shows in detail the prothrombin clotting times of these infants.

TABLE 1
PROTHROMBIN CLOTTING TIMES IN 40 INFANTS—UNTREATED

NO.	1ST DAY	2ND DAY	3RD DAY	4TH DAY	5TH DAY	6TH DAY	7TH DAY	8TH DAY
1		17	19	15		14		
2		17	15			13		
3		14		15		12½		11
4	12		12½			13		
5			17					
6		14			11	14		
7		17	20			15		
8		15		18				
9		16	17		13			
10		13			13			
11		27	40	38		21½		
12	15	19		13				
13		17	16		17½		16	
14	15		16½		14½		14½	
15	15		24	16½		14		
16	17		18½		17½			
17	14		17		15½	15½		
18	14½		15½		16	13		
19	14		17½		13			
20	13		16½	19½	16½			
21	15		29	30				
22	18	18	22					
23		18½	16½	18½		13		
24		20	18	21½		13		
25		15½	16½		18½		13½	
26		16	16½		14½		11½	
27		23	17		16		12	
28				21	15½		14	
29					25	16	14	11½
30		15	15		13			
31	15	19		14½		13		
32		22	25		15		14½	
33		17½	24	17½		16		
34		16½		15				
35		13½	14					
36	25		13					
37		16	17					
38		17	17					
39		17½		23				
40	14½	14½						
Average clotting time in secs. ..	15.5	17.2	18.8	19.5	15.5	14.4	13.7	
No. of estimations ..	14	27	30	14	17	15	8	

The prothrombin clotting times obtained by the same method on adult controls were between ten and eleven seconds; eleven seconds is taken as the control time throughout this investigation. A control estimation was performed on each occasion. From table 1 it will be seen that thirty-three infants gave a prothrombin clotting time of more than four seconds above control time of eleven seconds, and of these twelve gave times longer than ten seconds above

control time. It had previously been found that a 50 per cent. prothrombin concentration raises the actual clotting time by only three to four seconds, whilst a 20 per cent. concentration usually raises the clotting time by about ten seconds. It will be seen that a rise of only a few seconds in the prothrombin clotting time denotes a considerable reduction in the prothrombin percentage. It is only when the prothrombin level is considerably reduced that prolonged clotting times of fifteen seconds or more are found. In view of the lack of correlation between the actual prothrombin clotting times, and the prothrombin percentage concentration present, it is preferable to express results of prothrombin esti-

FIG. 1.

mations in actual clotting times and not as percentages. This gives a truer clinical picture when accurate determination of prothrombin is not obtained as in all bedside tests. Fig. 1 shows the limits of variation of the clotting times for each day in these forty infants.

It will be seen from the results of repeated estimations on a hundred infants that a definite hypoprothrombinaemia exists during the first week of life.

Results of administration of vitamin K analogue to mothers and infants

Synthetic vitamin K, 2-methyl-1:4 naphthoquinone (Kapilon), was the preparation used throughout this investigation. The doses given to mothers

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and infants were decided on purely empirical grounds. From previous experience it was found that large doses of vitamin K analogue had no harmful effects on infants, so that it was decided to err on the large side. The series was divided into four groups each receiving different doses of vitamin K analogue either to the mother and infant, or infant alone.

Group I. 50 mgm. vitamin K analogue was given to twenty-nine mothers before delivery and 3 mgm. to the infants, six to ten hours after birth. The mothers received the vitamin at least two hours before delivery and usually not longer than sixteen to twenty hours. The average time of administration of the vitamin was approximately six hours before delivery for all groups.

Results of the prothrombin estimations for this group are shown in table 2.

TABLE 2
PROTHROMBIN CLOTTING TIMES IN 29 INFANTS—TREATED
Mother given 50 mgm. vitamin K before delivery
Infant given 3 mgm. vitamin K 6 to 10 hours after birth

NO.	1ST DAY	2ND DAY	3RD DAY	4TH DAY	5TH DAY	6TH DAY	7TH DAY	8TH DAY
1		17½		13½		13		12½
2		15		13		12		
3		13		14		13		13½
4	15		14		14		13½	
5		16			15		14	12½
6		16		14		13½		13
7		12½			13½		13	
8		12½			17	17		13½
9	15	13½		13½		13		
10	16		14		15		15	
11		13		14		14		
12		15		15				12
13		15		15				12½
14		13		16				12
15		14		13½		12½		11
16		15		15		13		
17		14		12½		12		
18	14		12½		12½			
19	13		12½		12½			
20		13		12				11
21	11			12		11		11½
22				13		12		11½
23			16		13		11½	
24			14		13½		12	
25		12		11½		11		
26	17		15½	15				12
27	12½		13½	12				
28	12½		13½				11	
29	11		11					
Average times ..	13.7	14.1	13.6	13.5	14	12	12.8	12.1
No. of estimations ..	10	17	10	18	9	13	7	13

Eight infants gave prothrombin clotting times of more than four seconds above adult controls of eleven seconds. The longest clotting time for this group was, however, only 6½ seconds above controls. The average time for each day is shown at the end of the table and it will be seen that the longest average time above control time is only three seconds. Fig. 2 shows the variation from shortest to longest clotting times for each day. It will be

readily seen that the limits of variation are considerably reduced when compared to fig. 1, representing the untreated infants.

FIG. 2.

Group 2. 20 mgm. vitamin K analogue was given to twenty-four mothers before delivery and 10 mgm. to each infant four to eight hours after birth. Table 3 shows the results of the prothrombin estimations.

TABLE 3
PROTHROMBIN CLOTTING TIMES IN 24 INFANTS—TREATED
Mother given 20 mgm. vitamin K before delivery
Infant given 10 mgm. vitamin K 4 to 8 hours after birth

NO.	1ST DAY	2ND DAY	3RD DAY	4TH DAY	5TH DAY	6TH DAY	7TH DAY	8TH DAY
1	13		13		12			
2		14		13		12		
3	13		13½		13			
4	15		14	13½			12½	
5		14		12		11½		
6		13		12		12½		
7	15	13			12½			
8		13	13½				11	
9		12		12½				11
10	13½	13½	13½				11½	
11	12½	11½				11½		
12	12½	12				11		
13			12	11		11		
14		13	13		12		11	
15		13		11		12		11½
16		13		11		12		11½
17		13		14	12		13	12
18		14	14		12½		13	13
19		12½		11		11		
20		12½		11		11		
21	13		12		11		11	
22	11½		13½	11	11			
23	12		11		11			
24	13		12½	11		12	11	
Average times ..	13.1	12.9	12.9	11.8	11.8	11.5	11.7	11.8
No. of estimations ..	11	16	12	13	9	11	8	5

No infant gave a prothrombin clotting time of more than four seconds above control time of eleven seconds, the longest time for this group being four seconds above controls. The longest average time for any one day was only two seconds above control time. Fig. 3 shows the limits of variation of clotting

FIG. 3

times for each day. It is at once seen that these are markedly reduced as compared to fig. 1 for untreated group.

Group 3. 20 mgm. of vitamin K analogue was given to seventeen mothers before delivery and 5 mgm. to each infant four to six hours after birth. Table 4 shows the prothrombin clotting times for the group.

TABLE 4
PROTHROMBIN CLOTTING TIMES IN 17 INFANTS—TREATED
Mother given 20 mgm. vitamin K before delivery
Infant given 5 mgm. vitamin K 4 to 6 hours after birth

NO.	1ST DAY	2ND DAY	3RD DAY	4TH DAY	5TH DAY	6TH DAY	7TH DAY	8TH DAY
1	11½		11		11½		10	
2		14	12	11½		12		12
3	13		11		11½		11	
4	12		13	12½		11½		
5	12½		11½			11		
6		13	12			11		11
7	12	12			11½		11½	
8	12½	11		12		11		
9	14		12		12½		11	
10	12½	12		13½		12½		
11			11	11		12		
12	13	13	13		11½		13	
13	13	11		14		14		
14	12½	12½		13		13		
15	11	13	14		13			
16	12	14		14		14		
17	11½			14	14		14	
Average times	12.3	12.5	12	12.7	12.2	12.2	11.7	11.5
Estimations	14	10	10	9	7	10	6	2

The longest prothrombin clotting time was three seconds above control of eleven seconds. The longest average time for any one day was less than two seconds above control. Fig. 4 shows the limits of variation of prothrombin

FIG. 4.

clotting times. It will be seen that the results in this group are practically identical with those of group 2.

Group 4. 10 mgm. of vitamin K analogue was given to twenty-eight infants five to eight hours after birth in a single dose. No vitamin was given to the mothers. Table 5 shows the results of the prothrombin estimations.

While twelve infants showed a rise greater than four seconds above control, the longest prothrombin clotting time was only six seconds above control time. The longest average time for any one day was less than four seconds above control time. Fig. 5 shows the limits of variation of clotting times for

FIG. 5.

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this group. It will be seen that while the prothrombin times for this group are considerably lower than the untreated infants, they are definitely longer than those of groups 2 and 3.

TABLE 5
PROTHROMBIN CLOTTING TIMES IN 28 INFANTS—TREATED

Infants given 10 mgm. vitamin K 5 to 8 hours after birth

NO.	1ST DAY	2ND DAY	3RD DAY	4TH DAY	5TH DAY	6TH DAY	7TH DAY	8TH DAY
1		12		16		14		
2		11½		15		13		
3		15		13		14		
4	13		13		11			
5	12		13½		11½			
6		14	13		12		14	
7		16½	16	13½	12			14
8		12½	15		15		15	13
9		14½	15		14			13½
10	13		17	16		15		14
11	15	16½	15	15			14	15
12		13½		15½		13½		
13		16	16	15½		15½		15
14		13	15½		16		13½	
15		14½	14½			12		
16			15	14½		14½		12½
17		15	15		15		12	
18		15	15½		15		13	
19		15		15		14½		14½
20	15½		15½		13		13½	
21		15½	14½		15		14	14½
22	14	13		12½		12		
23		13		14½		12½		12½
24		14		13		13½		
25		13		13½		12½		
26		16	15		15½			
27		16	14½	15				11
28		12	12½					
Average times ..	13.7	14.2	14.7	14.5	13.7	13.5	13.6	13.4
No. of estimations ..	6	23	19	15	12	13	8	10

Discussion

Prothrombin estimations were carried out on forty infants on at least two occasions during the first week of life. Whole blood obtained by heel puncture drawn into Dale and Laidlaw coagulation tubes, previously half-filled with an active thromboplastin solution, was used for these estimations. Thirty-three infants gave prothrombin clotting times of more than four seconds above adult controls, and of these, twelve infants gave clotting times longer than ten seconds above controls. These times, according to investigations carried out previously, represent approximately a prothrombin concentration of 50 per cent. and 20 per cent. respectively. Two of these infants showed a marked prothrombin deficiency giving clotting times of thirty and forty seconds. None of the infants showed any sign of haemorrhagic tendencies, except that in most infants slight

bruising occurred at the site of the heel puncture; in contrast to this, no infant who had vitamin K analogue showed the slightest sign of haemorrhage around the heel puncture.

In addition to these forty infants, prothrombin estimations on sixty infants, using plasma instead of blood, have previously been reported. A summary of these results shows that thirty-six infants gave prothrombin clotting times of more than four seconds above controls, and of these twenty-three showed clotting times of more than ten seconds above controls. It is therefore evident that a definite hypoprothrombinaemia exists during the first week of life.

Synthetic vitamin K, 2-methyl-1:4 naphthoquinone, was given in varying doses to mothers and infants in ninety-eight cases. These were divided into four groups. In group 1, twenty-nine mothers received 50 mgm. vitamin K analogue at least two hours before delivery and the infants 3 mgm. some hours after birth. In group 2, twenty-four mothers received 20 mgm. vitamin K analogue and the infants 10 mgm. In group 3, seventeen mothers received a similar dose of 20 mgm., but the infants received 5 mgm. In group 4, twenty-eight infants received 10 mgm. of vitamin K analogue, the mothers none. In all four groups the administration of the vitamin had a very appreciable effect. The longest prothrombin clotting time in all groups of ninety-eight cases was $6\frac{1}{2}$ seconds above controls; this compares with thirty-five infants out of one hundred showing clotting times of more than ten seconds above controls in the untreated series. The best results were obtained by giving a dose of 20 mgm. of the vitamin to the mothers before delivery and 5 or 10 mgm. to the infant some hours after birth. A single dose of 10 mgm. to the infant alone while not producing quite so good a result, was totally effective in preventing any material drop in the prothrombin level during the first week. The administration of the above doses of 2-methyl-1:4 naphthoquinone to the mothers and infants practically restores the prothrombin concentration to normal adult levels. A smaller dose than 20 mgm. to the mother was not tried in the small series, but it is probable that a reduction to 10 mgm. may be just as effective. The vitamin in all cases was given orally, in tablets to the mothers, and in liquid solution to the infants. No harmful effects were observed even in the largest doses used. No diarrhoea occurred in any of the infants.

There does not seem any need to give vitamin K analogue to mothers during the last days of pregnancy as a prophylactic measure. The vitamin is effective if given during labour and at least two hours before delivery and combined with a small dose to the infant after birth. If the mother has not been given the vitamin, an adequate dose to the infant some hours after birth is sufficient to prevent any material fall in the prothrombin level.

Considerable interest has lately been aroused in the value of vitamin K therapy in lowering the incidence of intracranial haemorrhage in the newborn. Vitamin K is here supposed to act by preventing oozing from damaged capillaries in infants exposed to intracranial trauma, in forceps delivery and difficult labour. There is no doubt that in infants with a severe hypoprothrombinaemia and hence prolonged clotting times, the occurrence of trauma during labour would be likely to result in some degree of intracranial haemorrhage. In these cases

prophylactic treatment with vitamin K would be of benefit. The occurrence of hypoprothrombinaemia sufficiently severe to show haemorrhagic tendencies (such as bleeding from cord stump) is, however, rare. Hence, to hope that the neonatal mortality from intracranial haemorrhage will be materially reduced by prophylactic treatment with vitamin K is optimistic. Some authors have published reports on some hundreds of cases with and without treatment with vitamin K, and showing that the neonatal mortality is considerably lower in the treated group. Before the value of vitamin K in relation to neonatal mortality can be truly assessed, many thousands of cases will have to be carefully analysed, as so many factors enter into neonatal deaths. Huber and Shrader (1941) report two fatal cases of intracranial haemorrhage out of six neonatal deaths in a series, all of whom had vitamin K analogue. As these authors rightly point out, actual clotting is only one of the factors in the control of haemorrhage. The permeability and fragility of the capillaries must also play an important role.

Summary

1. A simple bedside method for the estimation of prothrombin in infants is described.

2. Prothrombin estimations on one hundred infants show that a definite hypoprothrombinaemia occurs during the first week of life. Seven infants showed a fairly severe prothrombin deficiency but in no case was there any haemorrhagic manifestation.

3. 2-methyl-1:4 naphthoquinone in doses of 20 mgm. to the mother before delivery and 5 mgm. to the infant raise the prothrombin level to almost adult control levels. A single dose of 10 mgm. to the infant is entirely effective in preventing any appreciable fall in the prothrombin concentration.

4. It is sufficient to administer vitamin K analogue during labour to be effective, if it is given at least two hours before delivery.

The vitamin K analogue 2-methyl-1:4 naphthoquinone (Kapilon) used in this investigation was kindly supplied by Glaxo Laboratories, Ltd.

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