Response of Low Birthweight Infants to Treatment with Folic Acid

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Burland, W. L., Simpson, K., and Lord, J. (1971). *Archives of Disease in Childhood*, 46, 189. **The response of low birthweight infants to treatment with folic acid.** Folate deficiency, detected by measuring serum and red cell levels, was seen within a group of low birthweight infants who had been followed for a period of 9 months. A similar group treated with $14 \times 100 \mu\text{g}$ folic acid by intramuscular injection over a period of 28 days during the first 5 weeks of life had significantly higher folate levels. There was no evidence of megaloblastic anaemia in the untreated group and no differences from the treated group in haemoglobin concentration or other haematological index except for a significant increase in neutrophils with 5-lobed nuclei at 3 months. We conclude that folate should be given from birth to all infants of low birthweight, but its form, and the route, dosage, and duration of treatment merit further investigation.

The folate status of term and premature infants has been studied by Matoth *et al.* (1964); Shojania and Gross (1964); Matoth, Pinkas, and Soroka (1965); Strelling *et al.* (1966); Vanier and Tyas (1966, 1967), and Roberts *et al.* (1969). All noted a fall in serum and red cell folate in early infancy, the fall being most rapid and profound in low birthweight and gestationally immature infants. Demands for folate are highest in this group presumably because of rapid growth, and some of these authors and Gray and Butler (1965) described megaloblastic anaemia responding to folic acid in premature babies.

The dietary folate requirements of newborn infants have been estimated to be 20–50 μg daily, 10 times those of adults based on body weight (Matoth *et al.*, 1964; Sullivan, Luhby, and Streiff, 1966). Hoffbrand (1970) suggested that prophylactic folic acid be given, for a few months after birth, to infants weighing less than 1.5 kg at birth and to debilitated infants who have had prolonged infections or repeated replacement transfusions. This communication reports the effect of prophylaxis with folic acid on the clinical and haematological status of low birthweight infants.

Methods

A group of 10 infants of low birthweight ($<1800 \text{ g}$) were given folic acid. First a venous blood sample was collected within 5 days of birth; immediately afterwards the infants received 100 μg folic acid by intramuscular injection. This dose was repeated on alternate days for 14 injections (a total of 1.4 mg folic acid). Blood samples were taken on the 17th and 28th days of the investigation and then at 3, 6, and 9 months. The results were compared with those from a group of 20 similar but untreated infants. There was no evidence of anaemia in the mothers during their pregnancy as judged by haemoglobin concentration, but it was difficult to establish which of the mothers had received folic acid.

All infants received feeds of the same evaporated milk. No additional vitamins or iron were given during the period of treatment. Their milk intake, body weight, and longitudinal growth were recorded during the first 5 weeks. Haemoglobin concentration (Hb), packed cell volume (PCV), red blood cell count (RBC), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV), reticulocyte count, and differential neutrophil count were recorded for each blood sample. The differential neutrophil counts were made by Schilling's method and the Cooke and Ponder modification of Arneith's classification (Wintrobe, 1967), in which 100 neutrophils in a peripheral blood film were counted and classified according to the number of lobes forming the nucleus. Serum vitamin B12 was assayed according to the method of Anderson (1964), serum folate as described by Waters and Mollin (1961) and red cell folate as des-

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TABLE I

Serum and Red Cell Folate Levels in Two Groups of Low Birthweight Infants. Treated Group Received 100 µg Folic Acid i.m. on Alternate Days for 28 Days

	Serum Folate (ng/ml)				Significant Difference Between Treated and Untreated (Student's t test)	Red Cell Folate (ng/ml)				Significant Difference Between Treated and Untreated (Student's t test)
	Treated		Untreated			Treated		Untreated		
	Mean	Range	Mean	Range		Mean	Range	Mean	Range	
Pretreatment	15.8	6.3-38.8	26.5	5.0-64.0	—	389	98-733	581	310-887	—
After 17 days	26.4	8.2-80.0	4.8	1.5-9.8*	P<0.001	357	182-540	341	186-715*	—
After 28 days	19.7	4.6-29.8	4.3	1.8-10.2	P<0.001	351	198-593	215	85-352*	P<0.005
Aged 3 months	10.5	4.7-14.2*	5.0	2.5-6.8	P<0.025	273	161-418*	159	61-292	P<0.01
Aged 6 months	13.5	4.5-16.6	10.0	5.8-15.3*	—	317	190-457	333	164-448*	—
Aged 9 months	18.8	12.3-24.2	10.0	5.3-13.4	P<0.005	444	270-631	381	92-650	—

*Values at these times were significantly different from those recorded in the preceding sample (P<0.001 to <0.025).

Normal levels in adults: serum folate 5-23 ng/ml.

Red cell folate 160-640 ng/ml (from the same laboratory).

scribed by Hoffbrand, Newcombe, and Mollin (1966). Feeds were prepared by diluting 1 part of evaporated milk with 2½ parts of water. Assays for total folate and free folate activity were carried out on milk prepared for feeding, on 4 other makes of milk reconstituted, human milk (10th day), and pasteurized cow's milk. Assays were carried out according to the method described by Butterworth, Santini, and Frommeyer (1963), with ascorbic acid added to the initial buffer used.

Results

Clinical. The group of infants treated with folic acid consisted of 5 males and 5 females with a mean birthweight of 1530 g (range 920 to 1790 g). The mean gestational age at birth was 33 weeks (range 30 to 36). The untreated group comprised 11 males and 9 females, mean birthweight 1560 g (range 920 to 1870 g), mean gestational age 33 weeks (range 26 to 40). The milk intake, gain in body weight, and longitudinal growth were each not significantly different for the two groups.

Serum and red cell folate levels. Serum and red cell folate levels in the two groups of infants are shown in Table I and the Fig.

After 17 days there was a very significant fall in the serum folate level in untreated infants (P<0.001). There was no further significant fall at 28 days and 3 months, but there was a significant rise at 6 months (P<0.001). In the treated group the levels of folate in the serum at 17 and 28 days were not different from those before treatment began. At 3 months, there had been a significant fall (P<0.02) but the mean level was within the range of normal values in adults and the lowest level recorded in the group was 4.7 ng/ml.

There was a very significant fall in red cell folate in untreated infants after 17 days (P<0.001) and a further significant fall by 28 days (P<0.005). The fall of folate in the red cell was more prolonged than that in the serum, but a significant rise occurred at 6 months paralleling the rise in the serum level. In the treated group there was no significant difference in the red cell folate at 17 days and 28 days nor between these levels and those at the start of the investigation. There was however a significant fall at 3 months compared with previous levels (P<0.02) followed by a rise parallel with that seen in the serum folate.

Comparing the treated and untreated groups there was a very significant difference in serum folate level at 17 and 28 days (both P<0.001). The differences between the two groups at 3 and 9 months were significant (P<0.025; P<0.05). Differences in red cell folate were only significant at 28 days (P<0.005) and 3 months (P<0.01). There were no differences in red cell folate between the two groups at 17 days, due to the higher levels in the initial samples from untreated infants.

Serum vitamin B12 levels. There was a pronounced fall in the serum vitamin B12 levels in both groups during the first 4 weeks (Table II) but the levels rose again at 3 months. There were no significant differences between the groups at any time during the study. Low borderline levels of serum vitamin B12 were seen in individual infants in the treated group. One infant had 90 pg/ml before treatment, 2 had 92 pg/ml at 17 days, 3 had levels of 80, 110, and 115 pg/ml at 28 days, and 1 had 110 pg/ml at 3 months. None of these showed evidence of megaloblastic anaemia though

FIG.—Serum and red cell folate level in the two groups of low birthweight infants.

TABLE II

Serum B12 Levels in Two Groups of Low Birthweight Infants (Treated Group Received 100 µg Folic Acid i.m. on Alternate Days for 28 Days)

	Serum B12 (pg/ml)			
	Treated		Untreated	
Pretreatment	270	90-560	357	140-640
At 17 dy	195	92-345	220	135-330
At 28 dy	151	80-230	199	120-235
At 3 mth	260	110-415	359	170-630
At 6 mth	401	235-550	497	330-825
At 9 mth	361	230-600	585	290-1120

Normal levels in adults (from the same laboratory) 120-925 pg/ml. 80-120 pg/ml is considered 'borderline'.

low levels were found consecutively in 3 infants. All these infants had serum and red cell folates within the normal range.

Haematological findings. Hb (g/100 ml) and RBC are shown in Table III. There was no

difference in these, between the groups, nor were any differences seen in PCV, MCV, MCHC, and reticulocyte count. The results of differential neutrophil counts in the treated group and in 10 of the untreated group showed that there was a tendency throughout, except at 6 months, for untreated infants to have more neutrophils with 3 or 4 lobes. This was reflected in the figures for mean lobe counts shown in Table IV. There were no significant differences in the mean lobe counts between the groups however. Neutrophils with 5 lobes were only seen in significantly greater numbers in the untreated group at 3 months when folate levels were at their lowest. 8.3% of cells in untreated infants had 5 lobes at 3 months as opposed to <1% in treated infants ($P < 0.05$). Two infants in the untreated group having neutrophils with 5 lobes showed mean lobe counts greater than the upper limit of the mean counts in treated infants. Neutrophils with 5 lobes were found in only one infant in the treated group in the presence of a low serum B12 (110 pg/ml at 3 months).

TABLE III

Haemoglobin Concentration and Red Cell Counts in Two Groups of Low Birthweight Infants

		Hb Concentration (g/100 ml)				Red Cell Count ($\times 10^6/\text{mm}^3$)			
		Treated		Untreated		Treated		Untreated	
		Mean	Range	Mean	Range	Mean	Range	Mean	Range
Pretreatment		16.7	14.1-18.7	16.3	12.3-20.7	4.3	3.6-5.0	4.5	3.5-4.8
At 17 days		12.3	9.9-15.7	11.6	8.9-13.5	3.4	2.8-4.2	3.3	2.6-4.1
At 28 days		9.8	8.2-13.3	9.3	7.3-13.3	3.0	2.5-3.2	2.9	2.1-3.8
At 3 months		11.0	9.1-14.7	10.4	8.6-11.5	3.4	3.1-3.7	3.5	2.7-4.2
At 6 months		11.4	9.7-13.2	10.9	8.3-12.4	4.6	4.2-5.3	4.3	3.4-5.0
At 9 months		10.5	8.9-11.5	11.6	9.9-13.2	4.4	4.1-4.9	4.6	4.0-5.6

TABLE IV

Neutrophil Lobe Count in Two Groups of Low Birthweight Infants (figures for untreated infants represent 10 infants investigated and figures for serum and red cell folate are shown because they do not compare with those shown in Table I which represents results in 20 infants)

		Treated			Untreated			Mean Serum Folate (ng/ml)	Mean Red Cell Folate (ng/ml)
		Mean Lobe Count	% Cells with 4 Lobes	% Cells with 5 Lobes	Mean Lobe Count	% Cells with 4 Lobes	% Cells with 5 Lobes		
Pretreatment		1.8	2.4	<1	1.8	1.5	<1	24.7	560
At 17 days		1.9	2.2	<1	2.3	10.0	1.14	5.2	365
At 28 days		1.9	2.3	<1	2.1	12.5	<1	4.4	233
At 3 months		2.0	3.25	<1*	2.5	8.0	8.33*	5.4	171
At 6 months		2.2	4.75	<1	2.1	2.0	<1	7.4	328
At 9 months		2.1	3.8	<1	2.3	8.0	<1	12.2	396

*Significant ($P < 0.05$).

Discussion

This investigation has confirmed the pattern previously described in low birthweight infants, namely a rapid fall of serum folate and a slower fall of red cell folate. Low levels of serum folate were found sooner in life in this study than in that by Roberts *et al.* (1969), but the lowest recorded at 3 months here (2.5 ng/ml) was not as low as recorded by those authors (0.6 ng/ml). Neither birthweight nor gestational age could be correlated with initial serum or red cell folate in either of the groups studied here.

In our study it has been shown that a total of 1.4 mg folic acid injected intramuscularly over a period of 4 weeks was sufficient to increase significantly serum and red cell folate compared with untreated infants and to maintain them at normal levels for 9 months. At 3 months, 5 of the 20 untreated infants had a subnormal red cell folate, though no infant showed evidence of megaloblastic anaemia or an unduly low haemoglobin concentration. A greater number of neutrophils with 5 lobes were seen in the untreated group at 3 months. Hypersegmentation of neutrophils (a shift to the right) is characteristically seen in vitamin B12 or folate deficiency and the findings might therefore suggest subclinical folate deficiency.

The results of folate assays of milks are shown in Table V. It appears by calculation and from the results that the infants in our study did not receive sufficient folate from their diet. For example, an infant weighing 1400 g at birth received at the age of 1 week 200 ml milk daily containing 13.6 µg total folate (10.6 µg free folate) and at 5 weeks 600 ml daily containing 40.8 µg (31.8 µg). Even if absorption and utilization were complete this amount is unlikely to be adequate. Free folate is immediately available to the infant but other folates are in the

form of polyglutamates and require the action of intestinal mucosal enzymes before being available for absorption. Since the activity of intestinal pteroylpolyglutamate hydrolase in the premature infant is unknown the deficit may be larger. The folate activities of the milks examined were similar to those found by Ford and Scott (1968), and no other milk would have provided significantly more folate to the infant than that used to feed the infants in our investigation.

Hussain and Wadsworth (1968) found a reduced concentration of liver folate in infants of <37 weeks gestation who weighed <2.5 kg and died in the perinatal period. It appears therefore that low birthweight infants become deficient in folate very soon after birth because of an inadequate dietary intake in the presence of insufficient reserves.

Folate derivatives are important in the biosynthesis of DNA precursors, and deficiency may produce arrest or disruption of DNA synthesis (Menzies *et al.*, 1966). Protein synthesis may be limited through the failure of amino acid biosynthesis, which may also restrict formation of essential enzymes and interfere with cell growth (Blakely, 1969). Fetal development in the rat is known to be adversely affected by folate deficiency (Nelson *et al.*, 1955, 1956), and the human fetus is very sensitive to folate antagonists such as aminopterin (Thiersch, 1952). These data suggest that infants especially those who are of low birthweight or gestationally immature, should be ensured an adequate folate intake. There was no evidence of enhanced weight gain or longitudinal growth in the group of infants treated with folic acid when these were measured accurately during 5 weeks after birth. If the untreated group were so depleted of folate that growth was affected, it was not significantly apparent by 5 weeks. An effect might have been expected at 3 months when the results suggested that the nadir for folate status had been reached in our infants. Unfortunately it was not possible to measure growth accurately at that time, but no obvious differences in the groups were apparent.

Although overt deficiency did not occur in the untreated group in the study, our findings suggest that low birthweight infants require prophylactic folate in the early months to prevent depletion and subclinical folate deficiency. If other stresses such as infection were to be present in the untreated infant severe deficiency and megaloblastic anaemia might ensue. Hoffbrand (1970) suggested that infants weighing less than 1500 g at birth should be treated. However Strelling *et al.* (1966) found megaloblastic

TABLE V

Folate Activity in Evaporated Milk Feeds Given to Two Groups of Infants (results of assays for 4 other proprietary milk foods, prepared according to manufacturer's instructions, human milk, and pasteurized cow's milk are also reported)

Feed	Total Folate (µg/l.)	Free Folate (µg/l.)
Evaporated milk	68.0	53.0
Milk A	35.2	19.4
Milk B	73.6	64.0
Milk C	55.2	51.6
Milk D	51.6	51.2
Human milk	64.0	32.0
Pasteurized cow's milk	60.0	58.0

changes in buffy coat preparations in 3 of 18 infants weighing 1500–1800 g at birth. We included infants weighing up to 1870 g in our study, and it might be argued that those weighing up to 2000 g should receive additional folate. The method of administration of folic acid chosen for this investigation was not entirely convenient and it remains to be seen whether oral administration of folic acid for a similar short period would be as effective. Further investigation is required to establish the best form and mode of administration of folate to young infants.

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