Influence of folic acid on birthweight and growth of the erythroblastotic infant

II. Growth during the first year

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SUMMARY The growth of 73 erythroblastotic infants (18 mild, 19 moderate, and 36 severe) was followed. In all three groups the average centiles for weight rose during the first half of the year. There was a fall in centiles during the second half of the year in 35 of 55 infants, 17 of them dropping below their birth centiles. Throughout the first year of life there was a strong correlation between serum folate of 8 $\mu\text{g/l}$ or more and rising weight centiles, and 5 $\mu\text{g/l}$ or less and falling centiles. None of the infants had macrocytic anaemia and their haemoglobin level rose even when their weight gain fell below the expected rate.

It is suggested that in some erythroblastotic infants a shortage of folic acid may be a limiting factor for a normal growth rate.

In the preceding paper (p. 1) it was shown that the average birthweight of infants with erythroblastosis was less than that of nonerythroblastotic infants matched for sex and gestational age. The reduction in weight was related to the severity of the disease, and mothers with a low serum folate gave birth to 4 times as many infants at or below the 10th centile as would be expected from a normal distribution of birthweights. If a moderate deficiency continued or occurred after birth, this would lead to failure to maintain a normal rate of weight gain, without necessarily showing the classical signs of folate deficiency, i.e. macrocytic anaemia. Though much has been written about folate deficiency in low-birthweight infants, there is not much information about infants with erythroblastosis and their growth rate during the first year. In this part of the investigation low serum folate and low rate of weight gain are shown to exist during the first year, especially from the 6th to 12th months.

The term folic acid is used throughout to denote all forms of folate or tetrahydrofolate that are registered by *Lactobacillus casei*. The growth rate of the infants is expressed throughout in terms of centiles for weight; this parameter was chosen in order to allow easy comparison between infants of different sexes and gestational ages.

Material

Haemoglobin level, folic acid status, and growth rate in the erythroblastotic infant. A total of 73 infants with erythroblastosis was studied. One or more exchange transfusions were performed on 29 of the 73 infants. 34 of these were also included in the birthweight analysis (part I). For the present series an attempt was made to collect the following samples: venous blood from the mother for serum folate on the day of delivery (results reported in part I), and cord blood for Hb and serum folate. The assay method is described in part I. Gestational age was calculated from the last menstrual period, and birthweights were recorded and converted into centiles according to the charts of Thomson *et al.* (1968), due allowance being made for sex, gestational age, and parity.

After discharge from hospital the babies were seen in a follow-up clinic, the frequency and number of visits being determined by the clinical status of the infant and, at a later date, by the willingness of the mother to bring the child. After each visit the infant's weight was converted into a centile value, using the data of Tanner *et al.* (1966). The calculated centiles took into account the degree of prematurity, e.g. if an infant was born at 36 weeks' gestation then 4 weeks were deducted from the chronological age. Blood samples were obtained (usually by venepuncture) for Hb and reticulocyte count as often as was

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clinically indicated. When sufficient blood was available serum folate was also determined. Supplementary 'top-up' transfusions at 1-5 weeks were needed in 14 infants.

In order to obtain information about the growth pattern of infants with erythroblastosis, an attempt was made to follow at least some of them for the first year of life; this prolonged follow-up could not be achieved in all cases and blood samples were only obtained relatively infrequently after the first 4-8 weeks of life.

The 73 erythroblastotic infants in this part were categorized according to the severity of the disease into three groups. (1) *Mildly affected infants*. All had a positive Coombs's test, but required neither an exchange nor subsequent 'top-up' transfusions. Hb values never fell below 10 g/dl at any time, and no specific treatment was required. (2) *Moderately affected infants*. As in group 1, except that Hb values fell below 10 g/dl during the first 2 months of post natal life, though no 'top-up' transfusions were required. (3) *Severely affected infants*. Required one or more exchange and/or subsequent 'top-up' transfusions. Only 15 of the 73 infants were premature (37 weeks or less). The remaining 58 (80%) were considered mature, i.e. 38 weeks or more.

Results

Haemoglobin values for all three groups (Table 1). As expected the Hb levels at birth were lowest in the severe group (mean 12.5 g/dl) while anaemia was almost absent in the mild group. In all three groups the Hb fell during the first 2 months. This

Table 1 Haemoglobin values (g/dl) for the 3 groups of erythroblastotic infants

Time	Mild	Moderate	Severe
At birth (n=72)			
Mean	17.2	15.5	12.5
Range	14.5-20	12.6-18	5.8-22
Median	17.8	15.7	12.5
n	19	17	36
3rd-6th m (n=55)			
Mean	12.4	11.9	11.9
Range	10.3-14	10.8-12.6	8.4-17.1
Median	12.2	12.2	11.8
n	14	15	26
7th-12th m (n=39)			
Mean	12.4	12.2	12.5
Range	11-13.5	10.1-13.3	11.2-15.2
Median	12.7	12.0	12.6
n	9	12	18

was by definition more pronounced in the severe and moderate than in the mild group. From the third month on, the means converged to 12 g/dl and

none of the infants was anaemic during the second half of the first year, when disease no longer affected erythropoiesis.

Centiles for weight among the three groups. The growth pattern of the three groups is shown in Fig. 1a (mean centiles for weight) and 1b (median values of centiles for weight). If the growth pattern of the erythroblastotic infants had been near normal, the curves would have been approximately straight horizontal lines for both mean and median values of weight centiles throughout the first year. Differences between Fig. 1a and b illustrate the skewed distribution in all three groups. During the first half of the year the rate of weight increase was greater than that given in the standard growth charts, while during the second half of the year, there was a definite decline in weight centile for all three groups (see Discussion p. 11).

Mildly affected (Table 2). In the mild group the mean weight centile rose from 50 at birth to 75 during the first half of the year. This rise was due to 11 of the 15 infants gaining weight at a faster rate than expected, while three maintained their weight centiles and only one declined (from 40 to 20). During the second half of the year 14 of the original 19 infants were seen, and 10 of these showed only small changes in weight centiles: the mean dropping from 75 to 65, but 4 infants fell below their weight centiles at birth.

Moderately affected (Table 3). The mean centile for weight rose from 40 at birth to 55 during the first half of the year. This was due to 9 of the 15 infants seen between the 3rd and 6th month improving their weight centiles, while in 6 of the infants the centile rating dropped from an average of 20 to less than 3. During the remainder of the year the mean centile for weight fell from 55 to 35. Only 4 of the 14 infants seen then improved or maintained their centiles for weight, and 10 showed a decline which in 5 was sufficiently severe to cause their average weight to fall to the 10th centile.

Severely affected (Table 4). During the first half of the year 29 infants were followed. Their mean centile for weight rose from 30 at birth to 50. This was due to a clear-cut rise in 19 infants, while 6 maintained and 4 dropped below their weight centiles at birth. During the second half of the year 27 infants were seen. Their mean weight centile dropped from 50 to 40 (median 25). This was due to 14 of the infants showing a fall in their weight centiles from 50 at mid-year to 15 by the end of the year, and 8 of these dropped below their birth centiles. Only 5

Fig. 1 Changes in weight centiles of the mild, moderate, and severe groups. The number of infants is shown for each group. (a) Average centiles with standard errors of the mean. The values for the mild group are significantly higher than those of the severe group at all ages ($P < 0.01$). (b) Median values for centiles for the 3 groups at birth, 3–6 months, and 7–12 months. This illustrates the pattern of rising and falling centiles more clearly.

Table 2 Mild group: serum folate and centiles

	Serum folate ($\mu\text{g/l}$)				Centiles	
	n	Mean, range, median	Distribution		n	Mean, range, median
At birth	16	14.8 (3–29) 10.5	<5 5–8 >8	1 1 14	19	50 (5–100) 55
3rd–6th m	14	8.8 (1.2–22) 7.8	<5 5–8 >8	3 4 7	15	75 (50–100) 75
7th–12th m	9	5.0 (0.1–14) 4.9	<5 5–8 >8	5 2 2	14	65 (45–90) 70

Table 3 Moderate group: serum folate and centiles

	Serum folate ($\mu\text{g/l}$)				Centiles	
	n	Mean, range, median	Distribution		n	Mean, range, median
At birth	16	17.6 (0.1–43) 17.8	<5 5–8 >8	4 2 10	18	40 (3–90) 35
3rd–6th m	15	5.9 (1.4–13.0) 4.2	<5 5–8 >8	9 0 6	15	55 (<3–100) 70
7th–12th m	11	7.7 (0.6–19.8) 6.0	<5 5–8 >8	3 4 4	14	35 (<3–85) 25

Table 4 Severe group: serum folate and centiles

	Serum folate (μg/l)				Centiles	
	<i>n</i>	<i>Mean, range, median</i>	<i>Distribution</i>		<i>n</i>	<i>Mean, range, median</i>
At birth	31	16 (0·1–50) 18	<5 5–8 >8	7 4 20	36	30 (0–90) 25
3rd–6th m	25	5·5 (0·7–15·4) 4·0	<5 5–8 >8	15 3 7	29	50 (<3–100) 50
7th–12th m	19	6·2 (0·4–17) 5·2	<5 5–8 >8	8 5 6	27	40 (<3–97) 25

Table 5 Patterns of changes in weight centiles during the second half of the first year in the 3 groups

Weight centiles	Mild (14)	Moderate (14)	Severe (27)	Total
Improved or maintained	4	4	13	21
Declined	10(4*)	10(5*)	14(8*)	34(17*)
Infants falling below their weight centiles at birth (%)	29	36	30	31

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*No. of infants dropping below the weight centiles at birth.

infants showed a rise, and 8 maintained the centile rating they had reached at mid-year.

Table 5 shows the pattern of weight centiles of the severely affected group during the second half of the first year and, for comparison, that of the mildly and moderately affected groups. Thus it appears that the severity of the disease does not significantly change the relatively low increase in weight of the infants during the second half of the first year, but other factors may underlie the reason (or reasons) why about 60% of all infants (34 of 55) grew less well during this period than could be expected from standard growth charts; 17 of 55 dropped below their weight centile at birth and 11 ended up below the 10th centile.

Serum folate values in the three groups. By the nature of the primary disease a certain number of infants could be expected to have used up substantial proportions of their folate stores and might, therefore, become more dependent on a continual supply than a normal infant. None of the infants, however, developed a macrocytic anaemia.

Mildly affected (Table 2). The mean serum folate showed the physiological decline from a high level at birth for 14 of the 16 infants to 8.8 $\mu\text{g/l}$ during the 3rd to 6th month. By then, however, 3 infants had values below 5 $\mu\text{g/l}$. During the second half of the year the mean dropped to 5 $\mu\text{g/l}$ with 5 of the 9 infants below this value.

Moderately affected (Table 3). The mean serum folate of 15 infants seen during the 3rd to 6th month fell from the normal mean levels at birth to 5.9 $\mu\text{g/l}$ (median 4.2) with 9 infants below 5 $\mu\text{g/l}$. During the second half of the year the mean serum folate for 11 infants was 7.7 $\mu\text{g/l}$ (median 6.6) with 3 infants below 5 $\mu\text{g/l}$ (3.0, 1.4, and 0.6 μg) and 4 infants with values between 5 and 8 μg which may be considered low borderline values.

Severely affected (Table 4). The mean serum folate level of the 25 infants seen between the 3rd and 6th month fell from a normal value at birth to 5.5 $\mu\text{g/l}$ (median 4.0) with three-fifths (15) below 5 $\mu\text{g/l}$. Only one-fifth had been deficient at birth i.e. below this value. During the second half of the year the mean serum folate was 6.2 μg (median 5.2) for 19 infants. Of these 8 were below 5 $\mu\text{g/l}$ and 5 had low borderline values (between 5 and 8 μg).

To summarize the changes in serum folate in the course of the first year: the means in all three groups showed a similar decline, though at birth there were more infants with low serum folate in the severely affected group. By the end of the first year only 12 of the 39 infants had serum folate levels of over 8 $\mu\text{g/l}$, 11 had low borderline values (between 5 and 8 μg) and 16 infants, i.e. 40%, were definitely below a level satisfactory for 7 to 12 month-old infants.

Haemoglobin and serum folate levels of all three groups (Table 6). No correlation could be found

Table 6 Lack of correlation between haemoglobin and serum folate levels in the 3 groups of erythroblastotic infants

Group	Serum folate <5 µg/l		Serum folate ≥5 µg/l	
	n	Hb (g/dl)	n	Hb (g/dl)
<i>Data from 3rd–6th m</i>				
Mild	8	11.9	3	11.9
Moderate	3	11.4	10	12.5
Severe	8	11.9	5	12.0
Total no. and mean	19	11.8	18	12.3
<i>Data from 7th–12th m</i>				
Mild	4	12.5	4	13.1
Moderate	3	11.9	8	12.3
Severe	8	12.8	8	12.9
Total no. and mean	15	12.5	20	12.7

between Hb concentration and serum folate levels. During the 3rd to 6th months the mean Hb of infants with serum folate <5 µg/ml was 11.8 g/dl (19 infants), while those with folate ≥5 µg/l had Hb 12.3 g/dl (18 infants). Similarly between the 7th and 12th months the 15 infants with serum folate < or >5 µg/l had comparable Hb values (see Table 6). This apparent lack of correlation between Hb and serum folate indicates that none of the infants suffered from such a degree of prolonged folate deficiency that would lead to a megaloblastic anaemia.

Rising and falling weight centiles and serum folate levels. Is there a correlation between the infant's serum folate and a rising or falling centile during the first year of life? An answer to this question was sought in three different ways.

(a) There were 18 infants who showed a clear pattern of an initial rise or maintenance of weight centile, followed by a substantial fall. 2 further infants showed an almost continuous decline in weight centile throughout the whole year. From these 20 infants 60 determinations of serum folate were made during the periods of rising or maintained centiles: the average serum folate was 9.5 µg/l ±1.23 SE. In contrast, the average serum folate of 60 determinations obtained while their centiles were falling was 4.7 µg/l ±0.66 SE. The difference between these two means was highly significant ($t=3.38$, $P<0.001$).

(b) There were 24 infants who showed a steady rise in centile over an average period of 9 months and 135 determinations of serum folate were made on them; the mean was 8.8 µg/l which is close to the value for the 20 infants (of test *a*) whose average had been 9.5 µg/l before their centile began to drop. Thus an average of 8.8 (or 9.5) µg FA/l serum permits an infant up to one year old either to maintain or improve its growth rate, while

4.5 µg/l could readily be associated with a drop in centile.

(c) In order to obtain further information about the relation between satisfactory folate levels and maintained or increasing centiles for weight on the one hand, and low serum folate and declining centiles on the other hand, data from all 3 groups were compiled for a χ^2 analysis. For this purpose the following conventions were adopted. (1) Cord blood serum folate levels were excluded (since this largely represents activity of the trophoblast). (2) At subsequent follow-up visits the centile for weight for individual infants was assessed and compared with the immediately preceding value. These observations were classified as rising (or maintained) if the centile values were higher by more than 5 centiles (or the same). If, on the other hand, their growth rate had declined by more than 5 centiles, they were classified as falling. (3) At each visit when a sample for serum folate was taken, the result was classified as <5 µg, 5–8 µg, or >8 µg/l.

The data for this analysis (Table 7) gave a highly significant correlation ($\chi^2=13.5$, $0.005>P>0.001$).

Table 7 Weight centiles and serum folate (all groups)

Centile	Serum folate (µg/l)			Total
	>8	5–8	<5	
Rising or maintained	108	59	113	280
Falling	12	11	42	65
Total	120	70	155	345

Thus 90% of the 120 determinations over 8 µg/l were associated with a rising or maintained growth rate, and two-thirds of all infants with a falling centile had serum folates below 5 µg/l.

It should nevertheless be pointed out that there was a substantial number of determinations below 5 µg/l (113 of 280) that were associated with a rising centile. As most of the falling weight centiles tended to occur during the second half of the year, i.e. during the period when serum folate levels might be slightly lower than normal, the question arose whether there was any difference in the serum folate values of infants whose weight centiles fell either early or late in the year. No difference was detected, whether values for the first 4 months were compared with those for the last 8 months (mean serum folate 3.2 and 4.0 µg/l) or for the two halves of the year (4.3 and 4.4 µg/l).

Discussion

Role of folic acid as a general growth factor. In clinical medicine folic acid is considered to be a

specific antianaemia factor, and it is generally accepted that a deficiency of folic acid leads to a megaloblastic anaemia, associated with characteristic changes in erythropoiesis and leucopoiesis and similar 'macrocytic' changes in some epithelia, as in the alimentary tract.

In biochemistry it is fully established that folic acid in its functional forms as tetrahydrofolate is an essential coenzyme in the *de novo* synthesis of the purine ring for RNA and DNA and in the synthesis of thymidylate for DNA. Therefore, every growing tissue requires folic acid. It has long been known that every dividing cell requires folic acid (Jacobson, 1952, 1954a, b, c).

There is an important difference between the adult and the growing child with regard to folic acid requirements. Both need folic acid for the maintenance of a normal complement of red and white blood cells, regeneration of certain epithelia, and for all those processes involving synthesis of RNA in nongrowing cells and for transmethylation reactions. The infant, on the other hand, requires folate not only for all these processes, but also for cell multiplication in virtually every organ and for the extensive myelination that takes place in the central nervous system.

Herbert (1962) has shown that if an adult is placed on an experimental diet containing no more than 5 µg folic acid per day, it takes about a week for the serum folate to drop significantly. Red cell folate does not decrease to any great extent before the end of the second month, and it is only in the 5th month of severe folate deprivation that a megaloblastic anaemia begins to occur. Evidence for folic acid deficiency on the biochemical level (e.g. FIGLU-excretion), however, becomes evident by the 3rd month.

The serum folate level is a direct measure of folic acid available to the tissues at the time of sampling. Red cell folate determinations, on the other hand, have a different significance; they give 'historical' information about the folate status 2-3 months previously, when the erythroblasts took up folic acid in the bone marrow. Most of this red cell folate is held firmly against a concentration gradient throughout the life span of the erythrocyte and is not readily available to the tissues. It is interesting that red cells can retain up to over 40 times higher concentrations of folate than serum. In adults the average ratio of folate in red cells to serum is 32:1 (Hoffbrand *et al.*, 1966). It is not clear what function folate fulfils inside the mature red cell. As to storage, the amount of folate in red blood cells is insignificant, as 1 ml packed red cells contains only a fraction of 1 µg when compared with a requirement of 50 to several hundred µg/d for a neonate or one-year-

old infant. The loss of folate due to exchange transfusion is negligible: the replacement of, for example, 120 ml plasma and, at most, a similar amount of packed red cells by adult blood, amounts to a loss of a fraction of a single day's folate requirement of the erythroblastotic infant, which would be at least 50 000 ng (50 µg).

Thus, if the growth of the whole infant is considered, rather than focusing solely on haematological parameters, it is evident that red cell folate values do not reflect the amount of folate available to all the other tissues in the body.

Haemoglobin and serum folate during the first year.

By definition the mildly affected infants had an almost normal Hb, whereas about one-half of the moderate group and over 80% of the severe group were anaemic at birth (Hb < 15 g/dl). During the first 2 months after birth the Hb levels in the moderately and severely affected infants fell in a manner characteristic of the disease, and though some infants had low values of serum folate, there was no evidence that this later anaemia was caused by a folate deficiency. Not only were there no macrocytes, but frequently infants were seen with a severe anaemia (Hb 8 g/dl or less), yet they had satisfactory serum folate levels (10 infants). By the end of the first year the average Hb was approximately the same in the infants of all three groups. None had values below 10 g and only 2 out of 39 had values below 11 g/dl. Thus there was no evidence that folic acid was the limiting factor in erythropoiesis.

Growth rate during the first year (Fig. 1a, b). There was an increase in the mean centile values for weight during the first 3 to 6 months in all three groups. About two-thirds rose significantly in their weight centiles, but about one-quarter (14 out of 59) dropped in weight gain during the first 6 months, so that at mid-year they were below their centile rating at birth.

An almost complete reversal of the growth pattern was noticed, starting from the 5th to 6th month: 34 of 55 infants declined in their weight centiles to varying degrees (Table 5). Thus of 34 infants with declining centiles, 17 dropped below their rating at birth. The data for weight centiles of the three groups over the whole year are summarized in Fig. 1a and b (for mean and median values).

Validity of growth charts used in this investigation.

As the majority of the infants in all three groups grew faster during the first 6 months and slower during the second 6 months than the growth charts indicated, the question arose whether the data of Tanner *et al.* (1966) were valid for infants born in

East Anglia between 1968 and 1973. Therefore all data from 40 weeks' gestational age onwards, and duly corrected for prematurity, were transferred to the growth charts for boys and girls as determined by Stuart *et al.* (data copied from Nelson, 1964). These data are based on the growth of term infants in the USA and have been in use at the Children's Hospital in Boston for over 30 years. No greater divergences than 5 centiles for weight were observed in any of the data of all 73 erythroblastotic infants, i.e. the differences were insignificant. Thus our charts, based on the prenatal data of Thomson *et al.* (1968) and the postnatal data of Tanner *et al.* (1966), were considered to be representative of expected weight gains.

Another possible explanation for at least part of the initial rise in growth rate may be that the data used for the weight centile charts (Tanner *et al.*, 1966) are no longer strictly applicable, particularly at around 3 months of age. Infant feeding in the UK has altered considerably, and during the period of study there was a vogue for the introduction of solid foods during the first 2-3 months. In order to determine the rate of weight gain of normal, term infants in the Cambridge area, 30 such infants* were followed and their weight centiles calculated as previously described. At birth the mean centile was 50 (median 50), by the 6th month it had risen to 65 and towards the end of the first year it had fallen to 55 (median 55) for 28 of the 30 infants. Thus the normal nonerythroblastotic infants showed a slight initial rise in weight centile, followed by a subsequent fall during the second half of the year, so that they ended up at almost the same value as at birth. None of the infants were at or below the 10th centile at any time. These findings contrast with those observed in the severe and moderate groups of erythroblastotic infants as illustrated by their median values for weight centiles (Fig. 1b) which fell to 25 by the end of the first year (Tables 3 and 4). The group of mildly affected infants corresponded fairly closely to unaffected infants.

Rate of weight gain and serum folate. There is no doubt that a multiplicity of factors determines an infant's rate of weight gain, and an attempt to single out one item is bound to meet with easy objections. The initial rise in weight centile could easily be explained on the basis that the 'disease' had impaired the infant's growth during the perinatal period (the average lower birthweight fits this assumption) and the high rate of erythropoiesis had consumed much folic acid. A typical example illustrates this: Fig. 2 shows the relevant data of an infant born at 40

Fig. 2 Data from one infant to illustrate the drop in serum folate and weight centile (after a slight rise) in the course of one year. Note: Hb improves from the 5th to 12th week and is maintained, while serum folate stays low and centiles for weight drop below 3. X indicates maternal serum folate at delivery.

weeks' gestation with moderately severe disease. The Hb level improved during the 3rd and 4th month and the level of Hb was maintained at 12 g/dl for the remainder of the year, despite serum folate values of 3 μg/l and less; her centiles for weight, however, dropped steadily. Enough folate was available for erythropoiesis, but insufficient for the maintenance of her weight centile.

In order to get an idea whether or not folic acid could have been a limiting factor in the growth of the infants, two critical appraisals are available. Firstly, to supplement a group of infants with folic acid for a number of months to see whether or not this would improve their growth rate. The favourable results of this procedure are reported in part III (p. 16).

A second critical appraisal of a connection between centile for weight and serum folate was a statistical analysis. Three such correlations of statistical significance were established. (1) There was a highly significant difference in serum folate values of a group of infants (20) while their centiles for weight were rising (or being maintained), and while their centiles were falling: 9.5 μg and 4.7 μg (test *a*, p. 11). (2) The mean serum folate levels of all those infants (24) whose centile values were steadily rising throughout the whole observation period was 8.8 μg (test *b*, p. 11), almost the same as the value obtained for the rising phase of the infants in (1).

*Data from 20 of these were kindly supplied by Dr. Joy Dauncey.

(3) Comparing all serum folates (total 345) that were determined during a rising (or steady) growth rate with those during a declining phase (test c, p. 11), there were strong correlations between serum folate (a) $>8 \mu\text{g}$ and a rising rate of weight gain, and (b) $<5 \mu\text{g/l}$ and a falling one (Table 7).

Since all growing tissues are dependent on a supply of folate, the question arises as to what constitutes an adequate serum level. 44 of the 73 infants had at one time or another serum folate levels of $<3 \mu\text{g/l}$. After the 2nd month, i.e. when the immediate effects of the haemolytic process should have been overcome, 25 of the 44 infants had values of $3 \mu\text{g/l}$ or less on at least one occasion. Almost all infants were on a normal diet which obviously did not maintain the serum folate above $3 \mu\text{g}$. Thus many infants had such low levels of serum folate that their supply and stores were likely to be insufficient for optimal growth. Vanier and Tyas (1966) give the following values for serum folate for normal term infants: $12 \mu\text{g/l}$ (range 5–30) at 3–4 months, $7.7 \mu\text{g}$ (3.5–16) at 6–8 months, and $9.3 \mu\text{g}$ (3–35) at one year. Similar figures are given by Navarro (1973): 3–6 months $8.4 \mu\text{g}$ (5.2–14.3) and $8.7 \mu\text{g}$ (5.6–14.5) from 6th–18th month. For preterm infants—and some of our erythroblastotic infants were preterm—Roberts *et al.* (1969) give serum values of 2.6, 2.3, and $3.1 \mu\text{g/l}$ for the 4th, 5th, and 6th month respectively; these preterm infants happened to be kept by chance on a low folate diet (see Roberts *et al.*, 1972). Shojania and Gross (1964) found a mean value of $9 \mu\text{g/l}$ for preterm infants between the 3rd and 7th month.

The average serum folate level of our 55 erythroblastotic infants seen during the second half of the first year was only $6.2 \mu\text{g/l}$, i.e. slightly below the level reported for normal infants of this age group. There is no doubt that some infants can thrive with serum folates below this level, as about 40% of all periods of rising centile were associated with serum folates $<5 \mu\text{g}$ (see Table 7). In contrast, over 80% of all periods of falling centiles were associated with serum folates $<8 \mu\text{g}$. Thus to assure optimal conditions for growth, $8 \mu\text{g}$ appears to be a safe level to aim at in a group of infants who go through a period of additional demands for folic acid early in life.

Folate deficiency in preterm infants has been well reviewed by Hoffbrand (1970). He pointed out that a folate deficiency anaemia of prematurity would develop mainly in the first 6 months, while in term infants such an anaemia would occur during the second 6 months. He also referred to the high demand for folate in Rh and other haemolytic anaemias.

Dawson (1972) gave a group of 12 infants with a birthweight of less than 2 kg a daily supplement of

1 mg folic acid. After 3 months the Hb values were the same as in the untreated group of 11 infants of similarly low birthweight. The serum and red cell folate levels were significantly higher in the treated than in the control group after 3 months, but their growth rate is not reported. He mentions 6 erythroblastotic infants, not included in his investigation, all of whom had low serum folate and these might have benefited from folate supplement.

Thus the conclusion from the literature is that most low birthweight infants would not benefit haematologically from folate supplements, but the effect of low serum folate on the infants' growth rate seems to have been overshadowed by haematological considerations. In fact, the immature red and white cells are almost the last ones to be adversely affected by a shortage of this essential vitamin; the demand for it, in fast growing infants, is not the prerogative of immature red and white cells.

This study underlines the importance of folic acid for the growth of erythroblastotic infants. The lower average birthweight (part I) is correlated with a low maternal serum folate at delivery. The postnatal rate of weight gain, as far as folic acid is concerned, may be influenced initially by folate stores at birth, rate of postnatal haemolysis, and dietary intake; later on, haemolysis ceases and stores may be low. Then a supply of folic acid can become the limiting factor in the maintenance of a normal rate of weight gain. This was suggested by a strong correlation of rising growth rate for weight and high serum folate, contrasted with falling growth rate and low serum folate. There is considerable evidence that the supply of folic acid, especially during the second half of the first year, may be a limiting factor in the growth of some erythroblastotic infants.

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