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

III. Effect of folic acid supplementation

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SUMMARY Seventeen infants with severe (14) or moderately severe (3) erythroblastosis were given daily oral supplements of 2.5 or 5 mg folic acid from day 16 (average) to 3.2 months (average). Their rate of weight gain, expressed as weight centiles, was followed for 1 year and was compared with that of a very similar group of 34 erythroblastotic infants without folic acid supplements. By the end of the 4th month, just after stopping additional folate intake, the median centiles for weight had risen from the 40th to the 80th centile, while in the untreated control group they rose during this period from the 35th to the 50th centile. During the second half of the year both groups declined in weight centiles, the 'treated' group ending up at the 50th centile for weight, while the control group fell to the 25th.

In the preceding papers (parts I and II, pp. 1, 7) it was shown that erythroblastotic infants have a slightly lower birthweight than well-matched controls, and that mothers with low serum folate ($<5 \mu\text{g/l}$) gave birth to 4 times as many small-for-dates infants as mothers whose serum folate was $8 \mu\text{g/l}$ or more. The growth pattern of the erythroblastotic infants over the first year showed an initial period of accelerated weight gain, but during the second half of the year the growth rate was slower than expected and about one-third ended up below their birth centile for weight at the end of the first year; furthermore, there were twice the number of infants at or below the 10th centile than would be expected (11 out of 55 infants). There was a significant correlation between rising weight centiles and serum folate levels of $8 \mu\text{g/l}$ or more; falling weight centiles were associated with serum folate values of less than $5 \mu\text{g/l}$.

Data are now presented to show the beneficial effect on growth when folic acid was given to an unselected group of erythroblastotic infants.

Patients

A group of 17 infants with erythroblastosis were randomly selected for folic acid supplementation

(2.5-5 mg/d by mouth); this was usually started after the first week (average age 16 days) and continued for about 3 months. All except 3 of the 17 infants were classified as severely affected in that they required either exchange transfusions (6 cases), or subsequent 'top-up' transfusions (4 cases), or both (4 cases). The remaining 3 infants were classified as moderately affected in that their Hb levels fell below 10 g/dl during the first 3 weeks of life though no transfusion was required.

The control group consisted of 34 infants taken from the series described in part II. 25 were severely affected and 9 moderately so. The controls were selected to match the folic acid treated group as far as possible (Table 1).

Attempts were made to collect umbilical cord blood for Hb and serum folate at delivery, and further blood samples at appropriate intervals, especially before and after folate supplementation. Exchange and subsequent 'top-up' transfusions were performed as clinically indicated. 16 of the 17 infants in the folic acid treated group were followed for one year, as were about 80% of the control group (27 of 34). When blood was taken by venepuncture for Hb determination, a sample was usually obtained for serum folate.

The weights of the infants at birth and subsequently were converted into weight centiles as described in parts I and II, due allowance being made for gestational age. The *Lactobacillus casei* assay for

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Table 1 Composition of the treated and control groups of erythroblastotic infants

	Treated	Controls
<i>Severe cases</i>	<i>n</i> =14	<i>n</i> =25
Average gestational age (w)	37	38
Average birthweight (kg)	2.95	3.0
Exchange transfusion	6	15
Supplementary transfusions	4	4
Exchange + supplementary transfusions	4	6
<i>Moderate cases</i>	<i>n</i> =3	<i>n</i> =9
Mean gestational age (w)	39	38
Mean birthweight (kg)	3.07	3.05
<i>Whole group</i>	<i>n</i> =17	<i>n</i> =34
Mean gestational age (w)	37	38
Mean birthweight (kg)	2.97	3.04
Dates of birth	February '70–February '73	August '69–March '73

serum folate was as described in part I. A total of 108 blood samples from the 17 infants receiving folic acid and 216 blood samples from the 34 controls were assayed for serum folate.

Results

At birth the treated (17) and control (34) groups of infants (Table 1) were comparable in terms of the severity of disease, centiles for birthweight, dates of birth, serum folate, and cord blood Hb (Tables 2–4). Over the first 2 weeks of life both groups showed a decline in weight centile from an average of about 40 and 35 at birth to 30 (Table 2). This of course is accounted for by the physiological drop in weight during the first few days of life. Both treated and control groups also showed a comparable fall in serum folate levels from an average of 16 $\mu\text{g/l}$ at birth to about 9 $\mu\text{g/l}$ by the 2nd to 3rd week (Table 3). Folic acid supplementation was started after the

Table 2 Weight centiles for the two groups

Infants	Treated		Untreated	
	<i>n</i>	Mean $\pm$ SEM, range, median	<i>n</i>	Mean $\pm$ SEM, range, median
At birth	17	40 $\pm$ 5.6 0–95 40	34	35 $\pm$ 4.2 0–90 35
At approx day 16 (before treatment)	17	30 $\pm$ 5.4 0–95 30	34	30 $\pm$ 4.4 0–90 25
At 4th m (after treatment)	17	70 $\pm$ 6.7 30–100 80	32	50 $\pm$ 5.0 0–100 50
At 6th–8th m	12	60 $\pm$ 8.1 15–100 60	27	45 $\pm$ 6.5 5–95 50
At 10th–12th m	16	50 $\pm$ 5.9 20–95 50	27	35 $\pm$ 5.6 0–95 25

first week (average age 16 days), most infants were given 5 mg/d, but some received only 2.5 mg/d. Treatment was continued for just over 3 months.

Table 3 Serum folate ($\mu\text{g/l}$) of the two groups

Infants	Treated				Untreated			
	<i>n</i>	Mean, range, median	Distribution		<i>n</i>	Mean, range, median	Distribution	
At birth	15	16.5 4.6–32.0 14.3	<5 5–8 >8	2 — 13	30	16.0 0.1–50.0 11.0	<5 5–8 >8	7 2 21
At approx day 16 (before treatment)	17	8.3 2.5–19.5 7.4	<5 5–8 >8	4 4 9	29	9.1 0.32.5 6.3	<5 5–8 >8	14 3 12
At 4th m (after treatment)	14	35.7 9.7–90.0 28.0	<5 5–8 >8	— — 14	21	7.0 0.6–38.8 4.6	<5 5–8 >8	11 5 5
At 6th–8th m	7	7.8 5.3–10.5 7.3	<5 5–8 >8	— 4 3	18	6.7 1.2–19.8 5.2	<5 5–8 >8	7 4 7
At 10th–12th m	8	5.6 0.8–10.4 5.4	<5 5–8 >8	3 3 2	17	7.1 0.4–28.0 5.2	<5 5–8 >8	5 7 5

Growth rate during the first 4 months of life (Table 2, Figs. 1, 2). Both the treated and control groups showed a rise in average centiles for weight during this period (similar to the pattern described in part II). There was, however, a striking difference in that the treated group had risen from the 40th centile at birth to the 70th (median 80) at the end of folic acid therapy. The control group, on the other hand, only increased their average centile from 35 at birth to 50 (median 50) at 4 months; the difference was significant ($0.025 > P > 0.0125$). The average weight gain of the treated infants was 1.0 kg per month during this period, while the control group gained only 0.72 kg per month.

Growth rate from the 4th to 12th months (Table 2, Figs. 1, 2). The average weight centile of the treated group declined after stopping treatment from 70 to 60 by the 6th to 8th month, and ended up at the 50th centile by the end of the year, i.e. only slightly better than that predicted by their centile at birth. The average weight centile of the control group dropped steadily from 50 at the 4th month to below 40 (median 25) by the end of the year. Though the treated group had a consistently higher centile than

Fig. 1 Average centiles for weight of the 17 erythroblastotic infants receiving folic acid supplements for 3.2 months (average) and a control group of similarly severe disease but receiving no folate supplement. Vertical bars = SEM. The difference at 4 months is significant ($P < 0.05$). The number of infants whose birthweights were available at birth, at start of treatment (about day 16), at approximately the 4th month (after stopping folate supplements), at 6–8 months, and at 10–12 months are indicated.

Fig. 2 Median values of weight centiles of the control group and the 'treated' group (receiving folate supplements) are shown. Note: the median values give a clearer picture of the centile distribution in the two groups, e.g. at 4 months one-half of the treated infants had reached weight centiles of 80 or more.

the controls during this period, the differences were not significant.

Although 3 of 17 infants in the treated group had been at or below the 10th centile for weight at birth, after stopping folate supplements (i.e. by the 4th month) none stayed at such a low centile throughout the remainder of the year. In the control group 6 of 34 infants were at or below the 10th centile at birth and by the end of the year 6 out of 27 were still at this low value.

Serum folate (Table 3). The treated and control groups had similar values at birth and shortly thereafter. At the end of the treatment (at about 4 months) the average serum folate of the treated group had risen to 35.7 $\mu\text{g/l}$ and none had a level $< 8 \mu\text{g/l}$. The control group at this time had an average of 7 $\mu\text{g/l}$ (median 4.6) and over half the infants had $< 5 \mu\text{g/l}$ (Table 3). During the 6th to 8th month, after stopping treatment when only 7 of the infants had blood taken for serum folate, the average value had dropped to normal levels, though none had 5 μg or less, while one-third of the control group had $< 5 \mu\text{g/l}$. By the end of the year both groups had similar levels of serum folate, with 3 out of 8 previously treated infants having $< 5 \mu\text{g/l}$. Similarly, 5 out of 17 control infants of whom serum folate values were available had $< 5 \mu\text{g/l}$ towards the end of the

year. As could be expected, the infants who had received folate supplements 6 to 8 months previously no longer showed any changes in serum folate levels by the end of the year, when compared with the control groups.

Haemoglobin levels (Table 4). The average Hb of the treated and the control groups was not significantly different at birth or during the first and second half of the year. No effect on the level of Hb was demonstrable in the folic acid treated group.

Discussion

In parts I and II of this study (pp. 1, 7) it was shown that erythroblastotic infants have a lower average birthweight than carefully matched controls. During the first 6 months after birth the erythroblastotic infants gained weight at a slightly greater rate than would be predicted from standard growth charts, but during the second half of the year about one-third of the infants failed to maintain their expected growth rate as shown by a declining centile for weight. Furthermore, it was suggested that a maternal shortage of folic acid might play a role in the lower average birthweight and that a shortage of folate in the infant later on might lead to a reduction in growth rate. There was a significant correlation between declining centiles for weight and low levels of serum folate ($<5 \mu\text{g/l}$). Maintenance of weight centiles was usually associated with satisfactory serum folate levels ($\geq 8 \mu\text{g/l}$).

This pilot study was undertaken in order to ascertain whether folic acid supplementation given for a limited period of time would have a beneficial effect on the rate of weight gain (expressed as centiles for weight) in a small group of erythroblastotic infants who, though anaemic, showed no signs of a

shortage of folic acid other than a frequently low serum folate and a deficit in weight centile rating, particularly during the second half of the year.

In the present study 34 erythroblastotic infants served as controls, 25 were severely and 9 moderately severely affected. The folic acid supplemented group of 17 consisted of 14 with severe and only 3 with moderately severe disease. Neither group included mildly affected infants. Thus, if there is any bias in the composition of the treated and the control group it would be in favour of the control group, because moderately affected infants show a slightly faster growth rate during the first half of the year than severely affected ones (see part II).

The result of folic acid supplementation for about 3 months showed that the 17 infants improved their weight centiles during this period to a significant degree, such that the group rose from an average of 40 at birth to 70 with a median value of 80, i.e. half of them were at or above the 80th centile, and none were at or below the 10th centile for weight (only 1 of the 17 had been over the 75th centile at birth). The control group, on the other hand, only rose from an average of 35 at birth to 50 over an equivalent observation period; this was significantly lower than the treated group. After folate supplementation had stopped 7 of the 17 treated infants maintained the rating they had reached, but 10 declined and 5 of these, seen towards the end of the first year, had fallen below their birth centile for weight. The control group, of which 27 were seen at the end of the first year, had a similar proportion of infants who dropped below their weight centiles at birth (9 of 27). Thus by the end of the year only a slight difference in the distribution of weight centiles could be observed (Figs. 1, 2), the effect of folic acid supplementation 6–8 months earlier had almost worn off.

Table 4 Hb values (g/dl) of the two groups

Infants	Treated		Untreated	
	n	Mean, range, median	n	Mean, range, median
At birth	17	13.5 4.5–18.5 13.0	34	13.1 5.8–22.0 13.2
At approx day 16 (before treatment)	17	10.6 7.4–14.0 10.6	33	10.4 6.3–14.7 10.8
At 4th m (after treatment)	15	11.9 9.6–13.1 12.0	26	12.2 9.6–15.5 11.6
At 6th–8th m	9	12.1 9.5–13.7 12.1	18	12.6 10.2–15.2 12.4
At 10th–12th m	8	12.0 11.2–13.8 11.7	14	12.6 11.2–14.4 12.4

In this study no effect of folate supplementation on Hb levels could be shown (see Table 4). Strelling *et al.* (1966) and Strelling (1973) found low whole-blood folate in some erythroblastotic infants: in 2 (out of a total of 9) who were very severely anaemic, a haematological response was obtained with daily intramuscular folic acid 0.12–0.48 mg. Their rate of weight gain is not referred to. Matoth *et al.* (1964) reported the results of folic acid supplements on infants with low whole-blood folate due to malnutrition, diarrhoea, and infections, but without macrocytic anaemia, and found no striking haematological response. They stated, 'the effect of folic acid on weight gain was dramatic in some cases'; no further data are given.

It appears that the rate of weight gain of infants short of folic acid and without macrocytic anaemia has not been studied to any extent. Kendall *et al.* (1974) gave 50 µg folic acid/day by mouth from the second day to the 6th month to a group of infants weighing less than 2.5 kg at birth. By the end of the 6th month their weight was the same as that of the untreated control group. Nevertheless the authors say that 'folate supplements might, however, be necessary in premature infants suffering from a haemolytic anaemia'. Our series of erythroblastotic infants contained only 4 infants in the control group and 2 in the treated group with a birthweight less than 2.5 kg. The treated group were given 2500–5000 µg/d and there was a definite effect on the weight centiles at the end of supplementation. The control group showed only a slight rise in weight centiles which was marginally better than the growth spurt observed by Fitzhardinge and Steven (1972a, b) for nonerythroblastotic, small-for-dates babies during the first 6 months.

Dallman (1974) gave intramuscular folic acid, 100 µg every other day, to preterm infants for 2 weeks. Their serum folate levels rose, but the effect on the rate of weight gain is not reported. The very high levels of serum folate in all our samples obtained from treated infants indicates that absorption of folate from the intestinal mucosa was not a problem. This is in full agreement with the findings of Samuel *et al.* (1973).

The question arises as to whether there are any possible long-term effects of a low average centile for weight in a group of infants who are not anaemic, but have a low serum folate. At first sight it appears to be reasonable to assume that erythroblastotic infants have managed to develop fairly normally during the last 20 years without a folate supplement. Walker *et al.* (1974) have published a most careful follow-up study of survivors of Rh-haemolytic disease. Among these children they found the following abnormalities at 7–14 years of age: mild sensory-

neural hearing loss in 57 of 260 children (those with kernicterus being excluded), and mild or moderate handicap in language attainment in 43 of 255 children. The authors say, 'it is possible that factors unrelated to events in the neonatal period are involved and may account for the fact that 100 children in this study had impaired reading attainment . . .'. Fitzhardinge and Steven (1972a, b) found among 8-year-old children who had been small-for-dates infants a prevalence of defective language attainment and poor school performance (the latter in 50% of the boys and 36% of the girls). The role of a shortage of folic acid during the first year of life—a period so important for the biosynthesis of myelin—is not referred to.

The adverse effect of severe maternal folate deficiency on the motor and mental development of infants and children has been reported by Gross *et al.* (1974). They found 8 of 14 such children affected, and Arakawa *et al.* (1969 and 1970) had come to similar conclusions. The effect of low serum folate levels on the central nervous system in adults has been reviewed recently by Melamed *et al.* (1975). Further work is required to show whether a shortage of folic acid during the first year could not only cause a lower rate of weight gain, but also lead to impaired higher functions in the central nervous system at a later age.

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References

- Arakawa, T., Mizuno, T., Honda, Y., Tamura, T., Sakai, K., Tatsumi, S., Chiba, F., and Coursin, D. B. (1969). Brain function of infants fed on milk from mothers with low serum folate levels. *Tohoku Journal of Experimental Medicine*, **97**, 391–398.
- Arakawa, T., Mizuno, T., Honda, Y., Tamura, T., Watanabe, A., Komatsushiro, M., Takagi, T., Iinuma, K., and Yamauchi, N. (1970). Longitudinal study on maturation pattern of EEG brain waves of infants fed on milk from mothers with low serum folate levels. *Tohoku Journal of Experimental Medicine*, **102**, 81–90.
- Dallman, P. R. (1974). Iron, vitamin E and folate in the preterm infant. *Journal of Pediatrics*, **85**, 742–752.
- Fitzhardinge, P. M., and Steven, E. M. (1972a). The small-for-dates infant. I. Later growth patterns. *Pediatrics*, **49**, 671–681.
- Fitzhardinge, P. M., and Steven, E. M. (1972b). The small-for-dates infant. II. Neurological and intellectual sequelae. *Pediatrics*, **50**, 50–57.
- Gross, R. L., Newberne, P. M., and Reid, J. V. O. (1974). Adverse effects on infant development associated with

- maternal folic acid deficiency. *Nutrition Reports International*, **10**, 241–248.
- Kendall, A. C., Jones, E. E., Wilson, C. I. D., Shinton, N. K., and Elwood, P. C. (1974). Folic acid in low birthweight infants. *Archives of Disease in Childhood*, **49**, 736–738.
- Matoth, Y., Zamir, R., Bar-Shani, S., and Grossowicz, N. (1964). Studies on folic acid in infancy. II. Folic and folinic acid blood levels in infants with diarrhea, malnutrition and infection. *Pediatrics*, **33**, 694–699.
- Melamed, E., Reches, A., and Hershko, C. (1975). Reversible central nervous system dysfunction in folate deficiency. *Journal of Neurological Sciences*, **25**, 93–98.
- Samuel, P. D., Burland, W. L., and Simpson, K. (1973). Response to oral administration of pteroylmonoglutamic acid or pteroylpolyglutamate in newborn infants of low birthweight. *British Journal of Nutrition*, **30**, 165–169.
- Strelling, M. K. (1973). Should low birthweight infants be given supplements of folic acid? *Archives of Disease in Childhood*, **48**, 827.
- Strelling, M. K., Blackledge, G. D., Goodall, H. B., and Walker, C. H. M. (1966). Megaloblastic anaemia and whole-blood folate levels in premature infants. *Lancet*, **1**, 898–900.
- Walker, W., Ellis, M. I., Ellis, E., Curry, A., Savage, R. D., and Sawyer, R. (1974). A follow-up study of survivors of Rh-haemolytic disease. *Developmental Medicine and Child Neurology*, **16**, 592–611.

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