Folate Deficiency in Premature Infants

Zuelzer and Ogden first clearly defined the syndrome of megaloblastic anaemia in infants, which responds to folic acid, as long ago as 1946. They concluded: 'In an analysis of etiologic conditions, infections and nutritional deficiencies appear significant while age, race and possibly prematurity and maternal anemia are predisposing factors'. 5 of the 25 infants they described had been born prematurely, but it is only in the past few years that the particular susceptibility of premature babies to folate deficiency has been adequately documented.

The exact frequency with which premature babies in well-nourished communities develop the deficiency is still uncertain. Strelling *et al.* (1966), examining buffy coat preparations, found megaloblastic changes in 7 of 54 premature babies and suspected it in a further 4. The incidence of the abnormality was highest in the smallest babies since all 6 babies weighing less than 1500 g. at birth and 3 of the 18 weighing between 1500 g. and 1800 g. had a definite or 'probable' megaloblastic change. All 11 infants with megaloblastic changes were said to show a reticulocyte response with a rise in haemoglobin in response to folic acid therapy, but these responses were not recorded by the authors. Gray and Butler in 1965 described 3 premature infants who developed megaloblastic anaemia between the 3rd and 7th week of life, and they clearly showed that in each infant the anaemia responded well to folic acid therapy. Other cases of premature infants developing megaloblastic anaemia responding to folic acid therapy have been described by Zuelzer and Ogden (1946), Zuelzer and Rutzky (1953), Kho and Odang (1959), and Vanier and Tyas (1967).

The incidence of biochemical abnormalities due to folate deficiency is more fully documented than the incidence of haematological changes. As in other conditions in which folate deficiency occurs, biochemical abnormalities are found far more frequently than overt megaloblastic anaemia since megaloblastic anaemia occurs only when the deficiency is extremely severe. Thus Shojania and Gross (1964) found that 28% of all premature babies and as many as 68% of those weighing less than 1700 g. at birth developed low serum folate levels between 1 and 3 months of age. Vanier and Tyas (1967) extended these observations. In their series 65% of premature babies aged between 2 and 3 months had subnormal serum folate levels, 40% had subnormal red cell folate levels, and 24% excreted raised amounts of formiminoglutamic acid (Figlu) after a histidine load.

Clinically the deficiency in premature babies differs from that in full-term infants in two respects. First, the deficiency tends to develop in premature babies within the first few weeks or months of life. This contrasts with the peak age incidence of folate-deficient megaloblastic anaemia in full-term infants, which is in the second 6 months of life. Secondly, the deficiency may occur in premature babies but not in full-term infants without an external cause such as infection, general malnutrition, or a diet of abnormally low folate content being implicated.

Folate deficiency occurs in premature babies because demands for growth exceed intake of the vitamin and thus use up stores of folate. Serum and red cell folate levels at birth, both in premature and in full-term infants, are higher than normal adult values (Grossowicz *et al.*, 1960; Shojania and Gross, 1964; Vanier and Tyas, 1966, 1967). After birth, the serum and red cell folate levels drop rapidly in all infants, but the fall is both more rapid and more severe in infants born prematurely (Matoth *et al.*, 1964; Shojania and Gross, 1964; Vanier and Tyas, 1967). The steepest falls occur in the smallest babies. Serum folate falls more rapidly than red cell folate, the lowest levels being reached between 4 and 8 weeks, and in the series of Roberts *et al.* (1969) all but one of 13 premature infants had a serum folate less than 6·0 ng./ml. at 7 weeks of age. The data of Roberts *et al.* (1969) also suggest that the smallest prematures may be born with lower folate levels than larger premature babies, but the numbers analysed were insufficient for this to be definitely established.

The newborn infant's requirements for folate have been estimated to be of the order of 20–50 µg. per day, about 4–10 times adult requirements based on body weight (Matoth *et al.*, 1964; Sullivan, Luhby,

and Streiff, 1966). The folate intake of premature babies is likely to be below this figure, the intake depending on the folate content of milk, the volume of milk taken, and its method of preparation. Normally, freshly expressed breast milk contains about 50 µg. folate per litre, and most powdered milks, made up as recommended by the manufacturers, contain about this amount (Ford and Scott, 1968). Heating, and particularly prolonged boiling, destroy folate however, and this is likely to occur if the milk is heated twice, since the first heating destroys ascorbic acid which might otherwise protect folate from oxidative destruction in the subsequent heating. Ghitis (1966) found that boiling for as little as 5 seconds reduced the folate content of fresh cow's milk by about 50%. Losses of folate from artificial milk during its preparation are the probable explanation for the more rapid fall in 'whole blood' folate levels observed by Matoth et al. (1964) in infants fed artificial feeds than in breast-fed infants. The pooled expressed milk given to premature babies usually contains less than 50 µg./ litre. That examined by Roberts et al. (1969) had a folate content as little as 3 µg./litre. This extremely low figure may be partly due to losses in preparation and partly because the milk was obtained from women early in the post-partum period when the folate content of milk is at its lowest (Ramasastri, 1965).

The diagnosis of megaloblastic anaemia is made on the basis of the blood count, the finding of macrocytes and hypersegmented polymorphs in the peripheral blood film, and of megaloblastic changes in bone-marrow (or buffy coat) films. Folate deficiency is established as the cause of anaemia by assay of serum and red cell folate, and by showing that the anaemia responds to folic acid in a physiological dose, e.g. 50 µg. daily. Vitamin B12 deficiency, which is extremely rare in the first few months of life, should nevertheless be excluded as the cause of the megaloblastic anaemia by measuring the serum vitamin B12 level. Incidentally, folate assay results should not be expressed in terms of 'whole blood' folate which has been used by a number of workers in this field since the whole blood folate level, i.e. the amount of folate in 1 ml. blood, depends as much on the haematocrit value as on the folate content of individual red cells (red cell folate concentration being 30 times that of plasma).

The anaemia should be suspected in all anaemic premature babies but particularly in those with the smallest birthweight or those who have feeding difficulties or infection. It may also occur in premature babies who have had exchange transfusions since these replace neonatal blood of high folate content with blood of normal adult folate content (Strelling et al., 1966). Moreover, haemolytic disease itself may use up the infant's folate stores excessively. Causes of megaloblastic anaemia other than prematurity are rare in the neonatal period in well-nourished communities. When it does occur it may be due to folate or vitamin B12 deficiencies or occasionally to neither of these deficiencies. Severe and prolonged infection, particularly gastro-enteritis, may precipitate folate deficiency in full-term as well as in premature infants by increasing demands for folate and reducing its intake (May et al., 1952). Severe folate deficiency is rare in the first year of life in children with coeliac disease or congenital haemolytic anaemia or with epilepsy treated by anticonvulsant drugs. In poorly nourished communities, however, severe folate deficiency is common in infants of all ages, and is particularly frequent in infants with kwashiorkor or scurvy. Folate deficiency may also occur in infants fed solely on goat's milk which has an exceptionally low folate content (Becroft and Holland, 1966), and in infants with specific malabsorption of folic acid (Lanzkowsky, Erlandson, and Bezan, 1969).

Vitamin B12 deficiency megaloblastic anaemia is rare in infancy and usually presents after the first 6 months of life. It may be due to congenital pernicious anaemia (congenital deficiency of intrinsic factor) (see review by McIntyre et al., 1965), to congenital specific malabsorption of B12 with proteinuria (so called 'Imerslund's syndrome') (see review Mohamed, MacKay, and Galloway, 1966), or to severe maternal B12 deficiency (Lampkin, Shore, and Chadwick, 1966). Megaloblastic anaemia which is not due to deficiency of vitamin B12 or folate may also occur in infants. For instance it may be due to the rare disorder of pyrimidine synthesis, orotic aciduria in which there is failure of conversion of orotic acid to uridylic acid (Rogers et al., 1968). Though rare, this condition is important to recognize, since it may be successfully treated with uridine. Arakawa and his colleagues in Japan have described infants with three different inborn errors of folate metabolism. In one, megaloblastic anaemia occurred, but there were other congenital abnormalities and early death (Arakawa et al., 1967). Whether megaloblastic anaemia responding specifically to vitamin E can occur in malnourished infants as claimed by Majaj (1966) remains uncertain.

Whether prophylactic folic acid should be given routinely to all premature babies is undecided. In the vast majority of premature infants the deficiency is not sufficiently severe to cause anaemia, and

biochemical tests for folate deficiency become normal spontaneously (though rather slowly) when the infant takes a mixed diet. For instance, Shojania and Gross (1964) found a rise in the serum folate level in their infants after two months when they began to take solid feeds while Vanier and Tyas (1967) found serum and red cell folate levels rose spontaneously in their infants between 6 and 8 months when they began mixed feeds. Two groups of workers have studied the effects of prophylactic folic acid therapy. Windmiller, Whitaker, and Sartain (1963) reported that the routine addition of folic acid to the diet of premature infants failed to influence haemoglobin levels. W. L. Burland, J. Lord, and K. Simpson (personal communication) divided neonates weighing less than 1800 g. into two groups, and gave one group 100 μg. folic acid intramuscularly on alternate days for the first 4 weeks of life, the other group acting as control. They also found that haemoglobin levels were similar at all ages in the two groups. The untreated prematures, however, had a significantly lower mean serum folate level and an excess of hypersegmented polymorphs at 3 months. At 9 months, these differences were no longer apparent. There is little evidence from these studies that folic acid therapy should be given to all premature babies. Nevertheless, the incidence and severity of folate deficiency in certain babies seem sufficiently high for them to be treated with folic acid prophylactically. It can be recommended for all babies weighing less than 1500 g. at birth, and for larger premature babies, and possibly all neonates, who suffer prolonged infections or other debilitating diseases impairing milk intake in the first few weeks of life, or for those who have exchange transfusions. It can also be recommended for infants who are given diets known to lack folate (such as goat's milk or the artificial diet that was given to babies with phenylketonuria (Royston and Parry, 1962)).

The best route and optimum amount of folic acid to be given have not been determined. A number of alternative procedures are available—addition of folic acid directly to milk, administration of folic acid to lactating mothers to raise the folate content of milk, or repeated oral or intramuscular administration of folic acid to the baby. The ability of newborn premature infants to absorb folic acid is unknown, and studies of this would help to decide which route and the amount of folic acid to be given.

Some workers have found that neonatal red cell folate levels are proportional to maternal red cell folate levels (Roberts *et al.*, 1969). It may be that the widespread use of prophylactic folic acid therapy in pregnancy may reduce the incidence of folate deficiency in their premature offspring. In severely malnourished communities routine folic acid therapy in pregnancy may even reduce the incidence of prematurity itself (Baumslag, Edelstein, and Metz, 1970).

Whichever method of administration of prophylactic folic acid is ultimately chosen, it is important that this therapy should not be continued beyond the first few months of life, since this might mask anaemia and lead to nervous damage in the albeit rare premature infant who develops vitamin B12 deficiency.

A. V. HOFFBRAND

Department of Haematology,
Royal Postgraduate Medical School,
London W.12.

REFERENCES

Arakawa, T., Narisawa, K., Tanno, K., Ohara, K., Higashi, O., Yoshiyuki, H., Honda, Y., Tamura, T., Wada, Y., Mizuno, T., Hayashi, T., Hirooka, Y., Ohno, T., and Ikeda, M. (1967). Megaloblastic anemia and mental retardation associated with hyperfolic-acidemia; probably due to N^5-methyl tetrahydrofolate transferase deficiency. *Tohoku Journal of Experimental Medicine*, **93**, 1.

Baumslag, N., Edelstein, T., and Metz, J. (1970). Reduction of incidence of prematurity by folic acid and supplementation in pregnancy. *British Medical Journal*, **1**, 16.

Becroft, D. M. O., and Holland, J. T. (1966). Goat's milk and megaloblastic anaemia of infancy. *New Zealand Medical Journal* **65**, 303.

Ford, J. E., and Scott, K. J. (1968). The folic acid activity of some milk foods for babies. *Journal of Dairy Research*, **35**, 85.

Ghitis, J. (1966). The labile folate of milk. *American Journal of Clinical Nutrition*, **18**, 452.

Gray, O. P., and Butler, E. Blanche (1965). Megaloblastic anaemia in premature infants. *Archives of Disease in Childhood*, **40**, 53.

Grossowicz, N., Aronovitch, J., Rachmilewitz, M., Izak, G., Sadovsky, A., and Bercovici, B. (1960). Folic and folinic acid in maternal and foetal blood. *British Journal of Haematology* **6**, 296.

Kho, L. K., and Odang, O. (1959). Megaloblastic anemia of infancy and childhood in Djakarta. *American Journal of Diseases of Children*, **97**, 209.

Lampkin, B. C., Shore, N. A., and Chadwick, D. (1966). Megaloblastic anemia of infancy secondary to maternal pernicious anemia. *New England Journal of Medicine*, **274**, 1168.

Lanzkowsky, P., Erlandson, M. E., and Bezan, A. I. (1969). Isolated defect of folic acid absorption associated with mental retardation and cerebral calcification. *Blood*, **34**, 452.

McIntyre, O. R., Sullivan, L. W., Jeffries, G. H., and Silver, R. H. (1965). Pernicious anemia in childhood. *New England Journal of Medicine*, **272**, 981.

Majaj, A. S. (1966). Vitamin E-responsive macrocytic anemia in protein-calorie malnutrition. *American Journal of Clinical Nutrition*, **18**, 362.

Matoth, V., Pinkas, A., Zamir, R., Mooallem, F., and Grossowicz, N. (1964). Studies on folic acid in infancy. I. Blood levels of folic and folinic acid in healthy infants. *Pediatrics*, **33**, 507.

May, C. D., Stewart, C. T., Hamilton, A., and Salmon, R. J. (1952). Infection as cause of folic acid deficiency and megaloblastic anemia. *American Journal of Diseases of Children*, **84**, 718.

Mohamed, S. D., McKay, E., and Galloway, W. H. (1966). Juvenile familial megaloblastic anaemia due to selective malabsorption of vitamin B12. *Quarterly Journal of Medicine*, **35**, 433.

Ramasastri, B. V. (1965). Folate activity in human milk. *British Journal of Nutrition*, **19**, 581.

Roberts, P. M., Arrowsmith, D. E., Rau, S. M., and Monk-Jones, M. E. (1969). Folate state of premature infants. *Archives of Disease in Childhood*, **44**, 637.

Rogers, L. E., Warford, L. R., Patterson, R. B., and Porter, F. S. (1968). Hereditary orotic aciduria. I. A new case with family studies. *Pediatrics*, **42**, 415.

Royston, N. J. W., and Parry, T. E. (1962). Megaloblastic anaemia complicating dietary treatment of phenylketonuria in infancy. *Archives of Disease in Childhood*, **37**, 430.

Shojania, A. M., and Gross, S. (1964). Folic acid deficiency and prematurity. *Journal of Pediatrics*, **64**, 323.

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.

Sullivan, L. W., Luhby, A. L., and Streiff, R. R. (1966). Studies of the daily requirement for folic acid in infants and the etiology of folate deficiency in goat's milk megaloblastic anemia. (Abstr.). *American Journal of Clinical Nutrition*, **18**, 311.

Vanier, T. M., and Tyas, J. F. (1966). Folic acid status in normal infants during the first year of life. *Archives of Disease in Childhood*, **41**, 658.

——, and —— (1967). Folic acid status in premature infants. *Archives of Disease in Childhood*, **42**, 57.

Windmiller, J., Whitaker, J., and Sartain, P. (1963). The influence of exogenous folic acid on the early anaemia of prematurity. Society for Pediatric Research, May 1, 1963, Atlantic City, N.J. (Abstr.).

Zuelzer, W. W., and Ogden, F. N. (1946). Megaloblastic anemia in infancy. *American Journal of Diseases of Children*, **71**, 211.

——, and Rutzky, J. (1953). Megaloblastic anemia of infancy. *Advances in Pediatrics*, **6**, 243.