FOLIC-ACID DEFICIENCY IN INFANTILE GASTRO-ENTERITIS

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Reports from different centres show considerable variation in the type of anaemia found in association with protein malnutrition.[1, 2, 14, 19-21, 23, 26, 28-30, 32] The reason for these conflicting opinions may be that the anaemia is perhaps a reflection of associated haemopoietic deficiencies prevalent in a local population group rather than a manifestation of protein depletion itself. As a preliminary to the investigation of the anaemia of kwashiorkor, infants with gastroenteritis were studied to see how their haemopoietic deficiencies compared with those in kwashiorkor. Only findings relevant to folic-acid deficiency are presented in this paper.

MATERIAL AND METHODS

Investigations were carried out on 51 non-White infants suffering from gastroenteritis between November 1962 and February 1963. All these infants were dehydrated and required intravenous therapy in the outpatient resuscitation unit. The ages ranged from 2 to 27 months. The investigations were carried out on the first 3 days of their treatment. On the first day peripheral blood smears, reticulocyte counts, bone-marrow examinations, and histidine-loading formiminoglutamic acid (Figlu) tests were performed. Serum-iron, iron-binding capacity, and serum vitamin-B_{12} levels were estimated on blood drawn on the second and third days of treatment. Haemoglobin and mean corpuscular haemoglobin concentration (MCHC) were determined on venous blood on the third day, by which time it was hoped that rehydration would have been achieved.

The bone marrows were assessed by one of us (D.McK.), who had no knowledge of the results of the other tests. The parameters for assessment of folic-acid deficiency were complete in 40 cases, and the remaining 11 cases were excluded.

When the malnutrition classification of Gomez *et al.*[8] was applied to the Boston 50th percentile, 12 of these infants fell in the well-nourished class, 11 suffered from first-degree malnutrition, 10 from second-degree malnutrition, and 7 from third-degree malnutrition. The 40 cases were subdivided into a better nourished group of infants who all showed normal serum-albumin levels (greater than 3·3 G per 100 ml.) and weighed more than 80% of the Boston 50th percentile—13 cases; a poorly nourished group who, while not clinically in the kwashiorkor category, showed abnormally low serum-albumin levels (less than 3·3 G per 100 ml.) and weighed less than 75% of the Boston 50th percentile—12 cases; and 15 infants who, by these criteria, were unqualified for inclusion in either group and were not considered further, since no conclusions could be drawn from their intermediate nutritional status. This subdivision afforded the opportunity of assessing folic-acid deficiency in 2 separate nutritional groups within the same series.

Standard methods were used in determining haemoglobin and MCHC values and in preparing reticulocyte, peripheral-blood, and bone-marrow smears. The diagnosis of megaloblastosis was based on leukocyte changes in most instances.[20, 34] In all cases so diagnosed giant metamyelocytes or giant stab cells above 18 μ were found,[6] and in 2 of them intermediate megaloblasts were also present.

Serum proteins were measured with the biuret reagent of Wolfson *et al.*,[31] standardized by Kjeldahl. Serum albumin was separated by the ether-centrifugation method[12] after precipitating the globulins with 27% sodium sulphate.[24] Mean normal albumin in a series of 31 controls was 3·89 (±0·26) G per 100 ml.

Serum-iron levels and serum iron-binding capacity were estimated by the methods of Trinder[27] and Henry *et al.*[9] respectively.

Serum vitamin-B_{12} estimations were carried out by the method of Hutner *et al.*[11] Normal serum-B_{12} concentration was taken as being between 140 and 900 $\mu\mu$g/ml.[25]

Figlu estimations were determined by conventional voltage electrophoresis on cellulose-acetate strips.[13] A loading dose of 2·5 G of histidine was chosen, by inference, from the adult

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dose of 15 G. The urine was collected for 5 hours beginning 3 hours after the loading dose and was maintained at the required pH by means of concentrated HCl. Bacterial contamination was avoided by the addition of thymol crystals and the specimen stored in a deep freeze until the performance of the test. The Figlu spot was graded from 'faint trace' to 4+. All results adjudged as + or above were counted as positive.

RESULTS

The distribution of haemoglobin values found in the 40 infants with complete parameters for the assessment of folic-acid deficiency is shown in Fig. 1, with the result of their Figlu tests above and the microscopic assessment of their bone marrows below.

There is a wide scatter of haemoglobin levels, ranging from 8 to 13 G per 100 ml. No definite conclusions have been drawn from haemoglobin values, for in individual cases rehydration was not always satisfactory on the third day of treatment.

In the whole series there was a 55·5% incidence of megaloblastoid change in the bone marrow and a 50% incidence of positive Figlu tests. As can be seen from Fig. 1, 22 were Figlu-positive and 18 negative. In 23

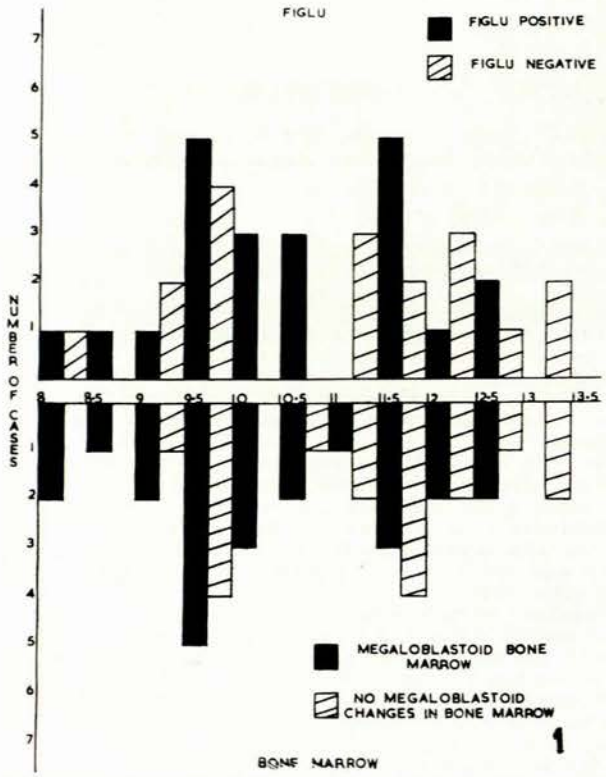

Fig. 1. Interrelationships of haemoglobin, Figlu and megaloblastoid change in bone marrow (40 cases). Figures in centre band represent venous haemoglobin (G per 100 ml.).

infants megaloblastoid changes were found in the bone marrow. Both the positive results of the cytological assessments and Figlu tests are clearly weighted toward the lower end of the haemoglobin scale, but even in those over 11 G Hb per 100 ml. there were 8 of the 19 whose bone marrows showed megaloblastoid changes and whose Figlu tests were positive.

In Fig. 2 the possible influence of age is charted against marrow and Figlu assessments. Obviously there is no difference in the marrow assessments, 50% of those above

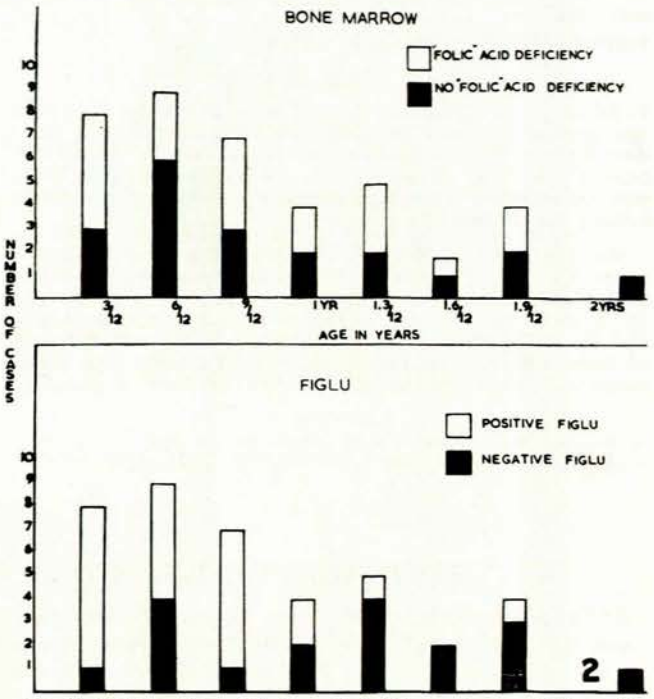

Fig. 2. Relationship of folic-acid deficiency to age (40 cases).

and below 1 year of age being adjudged 'folic-acid deficient'. With the Figlu test 75% of those under 1 year were positive as compared to 25% of those over that age. Nevertheless, the marrow and Figlu findings for the whole group when superimposed as in Fig. 3 show agreement in 70% of cases. Table I shows the relationship of the 2 criteria of folic-acid deficiency to the nutritional

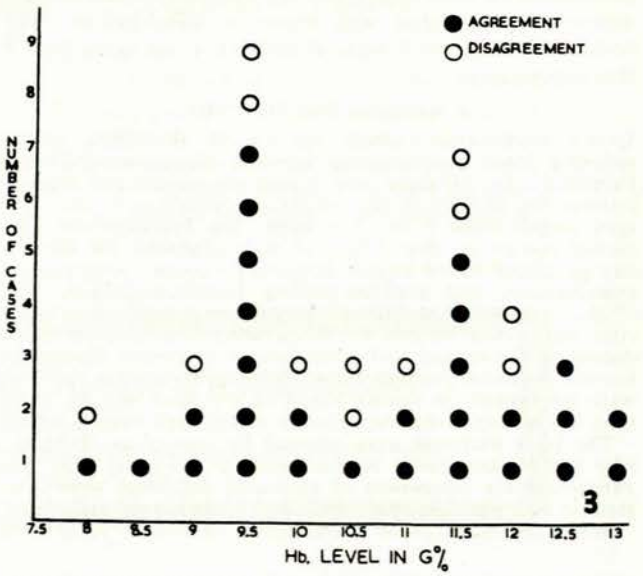

Fig. 3. Assessment of folic-acid deficiency by examination of bone marrow and histidine-loading tests.

state of 25 of the infants, i.e. the better nourished and the poorly nourished. In both nutritional groups slightly more than half showed megaloblastoid marrows. Judged by the Figlu tests the worse nourished infants showed less evidence of folic-acid deficiency.

TABLE I. FOLIC-ACID DEFICIENCY (BONE MARROW AND FIGLU) RELATED TO NUTRITIONAL STATUS

	No. of cases	*Folic-acid deficiency*	%
Wt. more than 80% of 50th percentile, and normal serum albumin			
Bone marrow	13	7	54
Figlu	13	9	69
Wt. less than 75% of 50th percentile, and low serum albumin			
Bone marrow	12	7	58
Figlu	12	3	25

Fig. 4 shows the results of bone-marrow examinations and Figlu tests when compared to percentage weight for age. Of interest is the greater number of better nourished infants in the group under 1 year of age. In this group also there is good agreement between the results of the 2 special investigations. Where there is disagreement, the Figlu test was positive while the bone marrow was normal in all cases. In the group over 1 year of age there is exact agreement of the results of bone marrow and Figlu tests in all except 5 instances, where in each case the bone-marrow examination showed megaloblastoid change while the Figlu test was negative. In these 5 cases serum-albumin levels were all below 2·78 G per 100 ml. In 15 out of the 40 cases (37·5%) folic-acid deficiency was present as judged both by bone-marrow and Figlu tests.

The peripheral blood smear was not helpful as an indication of early folic-acid deficiency, since macrocytosis was present in only 2 cases and multinucleated polymorphs were not seen.

No evidence of haemolysis was found, and serum vitamin-B_{12} levels were normal in all cases.

In the whole series, 72% of cases showed lowered serum-iron levels and in 59% of cases iron deficiency was diagnosed on examination of the bone marrow. These results will be reported elsewhere.

DISCUSSION

Zuelzer and Ogden,[34] using the diagnostic methods available in 1946, considered examination of the bone marrow 'the only reliable single criterion' of megaloblastosis in children. Our results appear to confirm this. Certain aspects of the urinary Figlu test need further examination before it can properly be compared to the bone marrow as an indication of folic-acid deficiency in all circumstances. In particular, the 12 cases in this series (30%) where there was disagreement between the results of the Figlu test and that of the bone-marrow examinations, need explanation.

In 7 cases the Figlu test was positive while the bone marrow was normal—this situation is accepted in folic-acid deficiency states,[10, 15] and will not be discussed here any further. The 5 instances where the findings were reversed necessitate critical review (Table II).

The most obvious possibility is that the histidine load was not absorbed because of vomiting or intestinal hurry. As all the infants who were known to have vomited were excluded, this factor can be ignored. The subjects were all

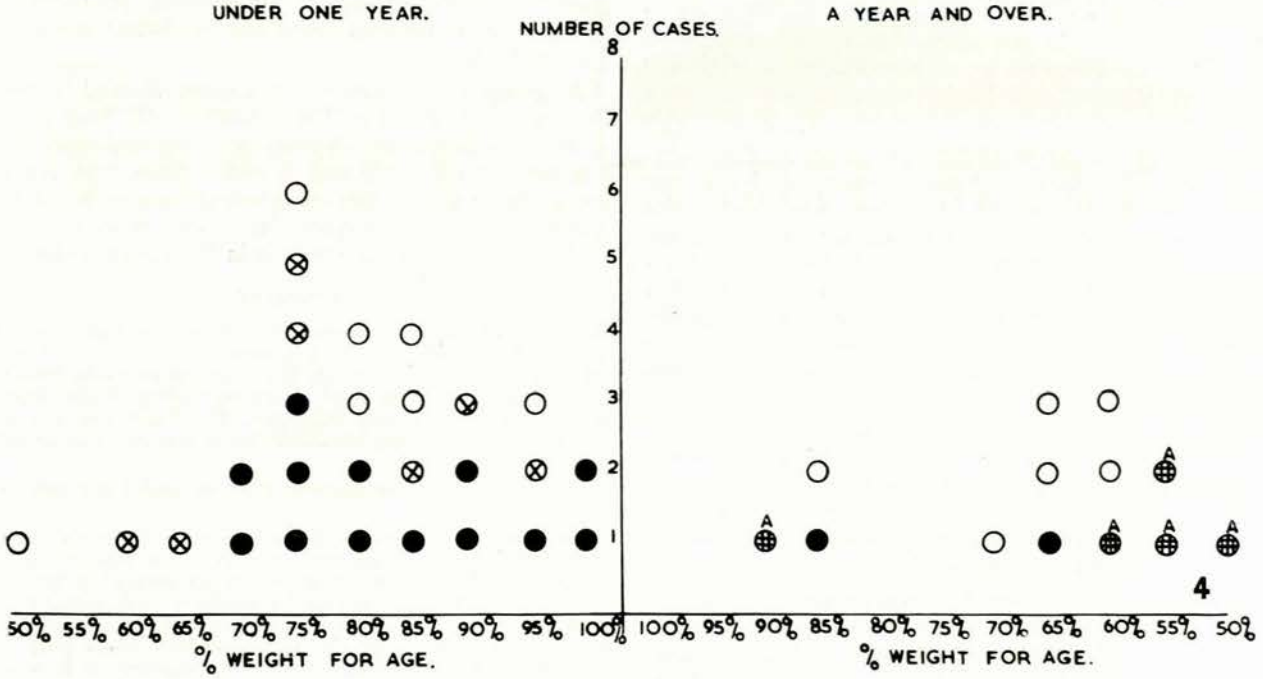

Fig. 4. Relationship of bone marrow and Figlu to % weight for age. Black circle=positive urinary Figlu test and megaloblastoid bone-marrow change. White circle=negatve Figlu test and normal bone-marrow cytology. Cross in circle=positive Figlu test and normal bone-marrow cytology. Double cross in circle=negative Figlu test and megaloblastoid bone-marrow change. A=infants with serum-albumin levels less than 2·78 G per 100 ml.

TABLE II. CASES WITH MEGALOBLASTOID CHANGE IN BONE MARROW AND NEGATIVE URINARY FIGLU TESTS

Name	*Stool wt.*	*Electrophoretic pattern*			B_{12} $\mu\mu g/ml.$	*Fe* $\mu g/100$ ml.	*Total iron binding* $\mu g/100$ ml.	*Bone marrow*	*Age (months)*	*Weight*	*Ser. alb.* G/100 ml.
		Histidine	*Glycine*	*Figlu*							
S.W.	—	+	Trace	—	688	16	173	Fe deficiency	13	18 lb. 7 oz.	2·03
C.B.	—	2+	+	—	1240	100·5	232	No Fe deficiency	12	12 lb. 1 oz.	2·77
J.G.	—	2+	2+	—	1420	73·2	Insufficient	No Fe deficiency	14	13 lb.	1·97
D.H.	54 G/20 hrs.	4+	4+	—	500	47·1	163	No Fe deficiency	21	14 lb. 1½ oz.	2·28
C.F.	—	3+	+	—	1168	67·5	101	No Fe deficiency	17	11 lb. 15 oz.	2·15

under treatment for gastroenteritis and intestinal hurry of some degree must have been present, but it cannot be assessed because the stool weight was not known except in one infant, and in that one the stool weight was normal. Nor is there any informaion available on the state of intestinal absorption in these infants. The fact that so many positive Figlu tests were found in other similar cases of gastroenteritis suggests that the histidine load is not usually difficult to absorb even in the presence of enteritis. Similarly, a change in blood volume is not a likely explanation of the negative Figlu tests in these 5 cases; there was no difference between the serum vitamin-B_{12} levels of these infants and the others. It seemed possible that these 5 marrow assessments were at fault, but re-examination of the smears did not corroborate this.

In 2 of these 5 infants iron deficiency, as judged by serum-iron levels, was present—in one of them confirmed by bone-marrow examination. Some authors have stated that giant metamyelocytes may be present in the bone marrow in iron-deficiency anaemia.[5, 16] Dawson and Bury[6] investigated the significance of giant metamyelocytes in anaemia and were of the opinion that their presence in iron deficiency indicated an associated B_{12} or folic-acid deficiency. MacIver and Back[20] reported that the giant metamyelocyte was pathognomonic of a B_{12} or folic-acid deficiency, and our experience to date confirms their view.

The possibility that the histidine load was inadequate must be considered, since the dose given was standard for all the tests despite variations in age and weight in the group. As can be seen from Fig. 4, 4 of these 5 infants with negative Figlu tests, though all over the age of 1 year, weighed considerably less than other, better-nourished, infants whose Figlu tests were positive. It is therefore considered unlikely that the loading dose was inadequate.

Histidine katabolism need not necessarily proceed along pathways leading to the formation of Figlu. In under-nutrition it is possible that the histidine load may be utilized in the formation of new body protein (Chanarin *et al.*[3]). The 5 infants under discussion all had a serum albumin of less than 2·78 G per 100 ml.

Chanarin[4] reports cases of megaloblastosis with low serum folates, high urinary urocanic-acid levels, and negative urinary-Figlu tests. He suggests that in long-continued folic-acid deficiency, tissue levels of Figlu may be so elevated that urocanase production is inhibited, and 'false negative' Figlu tests produced. It is stated that enzyme function may be depressed in kwashiorkor,[7] and perhaps this may happen in lesser degrees of malnutrition also. This could explain the finding of 'false negative' urinary-Figlu tests even in the presence of folic-acid deficiency. Whether depression of enzyme systems is governed by protein deprivation or liver dysfunction in such cases is not yet clear but there is some evidence in favour of the latter hypothesis.[22]

Luhby *et al.*[17] found the Figlu test a sensitive index of folic-acid deficiency in kwashiorkor. With the method we used, this has not consistently been our experience in poorly nourished infants with gastroenteritis, in whom we have found 'false negative Figlu tests' in association with low serum-albumin levels (Fig. 4).

It is difficult to draw any definite conclusions from this trial about the influence of gastroenteritis in producing a folic-acid deficiency state. It is possible that gastroenteritis may increase the demand for folic acid and hinder its absorption, thus increasing the incidence and severity of folic-acid deficiency.[18, 33] However, this aspect and the part played by malabsorption in determining folic-acid deficiency in gastroenteritis could not be studied in this out-patients trial.

A group of asymptomatic infants showed a 64·7% incidence of positive Figlu tests.* If bone-marrow evidence of folic-acid deficiency or low serum-folate levels can subsequently be found in such infants, then previous dietary deficiency of folic acid would seem to be the most important single aetiological factor contributing to folic-acid deficiency in gastroenteritis in the group studied.

SUMMARY

An investigation of haemopoietic deficiencies was undertaken in 51 infants suffering from gastroenteritis. Data obtainable allowed 40 of these to be studied and compared by means of bone-marrow cytology and histidine-loading Figlu tests as parameters of folic-acid deficiency. The Figlu test was positive in 50%, and megaloblastoid bone marrow was found in 55%, of these 40 infants.

In 70% of cases the marrow cytology and Figlu tests were in agreement.

The possible reasons for disagreement in the other 30% are considered and the general conclusion is arrived at that experience of the Figlu test is not at present sufficient to justify a confident opinion on its merits in very young infants as the sole criterion of folic-acid deficiency in all circumstances. In 37·5% of infants in the series positive Figlu tests were confirmed on examination of the bone marrow. It is possible that folic-acid deficiency existed in a further 5 cases (12·5%)

*See article 'Assessment of histidine-loading test in infancy' by R. Friedman *et al.* on page 67 of this issue.

discussed in the text. This result will be of some importance in assessing any future study of folic-acid deficiency in kwashiorkor cases in Cape Town.

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