Correlation of peripheral white cell and bone marrow changes with folate levels in pregnancy and their clinical significance

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SYNOPSIS Two hundred and thirty-eight 'megaloblastic' pregnancies are reviewed. Bone-marrow aspiration was performed in every case. Serum folate estimations were strongly suggestive of folic acid deficiency in only 34% of cases. If, however, the red cell folate was determined as well, there was an almost complete agreement with the changes in the erythroblasts. It is suggested that hypersegmentation of the neutrophils in association with a diminished red cell folate level is indicative of significant folic acid deficiency.

This series possibly shows a slight but statistically insignificant increase of accidental haemorrhage. No effect on birth weight or incidence of stillbirth was demonstrable.

Over a period of four and a half years (August 1960 to February 1965) 238 'megaloblastic' pregnancies occurring in 222 patients were investigated. The incidence of megaloblastic change and/or white cell 'nuclear maturation defect' (Finch, Hosain, Morgan, Marsaglia, Giblett, and Hillman, 1965) was calculated from 230 cases of a total of 10,258 (2.2%) delivered in the City General Hospital, Sheffield¹. This compares with 0.42% found by Gatenby and Lillie (1960), 2.8% by Giles and Shuttleworth (1958), and 3.8% by MacKenzie and Abbott (1960).

Blood counts, peripheral films, and bone-marrow aspirations were obtained in all cases. Serum folate assays were available in 191 and vitamin B₁₂ estimations in 219 cases.

MATERIAL AND METHODS

The patients were classed in three groups according to the bone-marrow findings. Group I comprised 70 pregnancies with frankly megaloblastic erythropoiesis; group II, 122 with intermediate megaloblasts and giant metamyelocytes; group III, 34 with more than occasional giant metamyelocytes but normoblastic erythropoiesis. Only those patients whose bone-marrow smears were available for a final re-checking (at the end of the survey) were assigned to one of the three groups; for this reason 12 cases were not classified.

Marrow aspiration was carried out if indicated by the

appearances of the neutrophils in the peripheral blood films. Such films were considered essential for the exact determination of the number of nuclear lobes in view of the morphological changes occurring in bottle samples, such as pulling apart of parts of the same lobe connected in the fresh state by a 'bridge' but not a true filament. Up to the middle of 1962 both the 'nuclear number', *i.e.*, the number of lobes in 100 neutrophils (Hynek, 1909), and the percentage of hypersegmented neutrophils (a hypersegmented neutrophil is defined as that possessing five or more nuclear lobes) (Chanarin, Rothman, and Berry, 1965), were determined but subsequently an impression gained from a careful scrutiny of peripheral films was thought to be more satisfactory. Thus, cases with fewer than 5% hypersegmented neutrophils but with obviously abnormal cells containing six or seven lobes connected with fine filaments were also investigated.

Serum folate (*L. casei*) was estimated in 191 patients (Baker, Herbert, Frank, Pasher, Hutner, Wasserman, and Sobotka, 1959; Herbert, Baker, Frank, Pasher, Sobotka, and Wasserman, 1960; Waters and Mollin, 1961). More recently the folate content of the red cells was also determined. Ascorbic acid haemolysates of whole blood (1:10 dilution of sequestrene blood in 1% aqueous ascorbic acid, Hoffbrand, Newcombe, and Mollin, 1964) were further diluted with 0.1 M phosphate buffer containing 4 mg. ascorbic acid per ml.; final pH 6.1. From this point the serum folate and whole blood folate techniques were identical. Red cell folate values were calculated from the whole blood and serum folate (Cooper and Lowenstein, 1964). Results of serum vitamin B₁₂ assays were available in 219 patients. Figlu determinations (Kohn, Mollin, and Rosenbach, 1961) were carried out initially in a small number of cases.

¹Eight patients were delivered outside the hospital and were not used in the calculation of incidence.

Received for publication 22 November 1965.

TABLE I

Group	HAEMATOLOGICAL FINDINGS AND SERUM VITAMIN LEVELS					
	Haemoglobin (g./100 ml.)		P.C.V. (%)		Percentage of Cases with	
	Range	Mean	Range	Mean	Serum Folate < 1.5 ng./ml.	Serum Vitamin B ₁₂ < 80 pg./ml.
I	1.9-12.3	8.5	6.5-35	29.7	33.9 (20/59) ¹	3.2 (2/63)
II	4.0-12.3	9.5	22-38	30.7	9.1 (9/99)	5.5 (6/110)
III	5.9-12.6	9.8	23-39	33	9.1 (3/33)	0 (0/46)

¹20 of 59 cases

RESULTS

HAEMATOLOGICAL FINDINGS Haemoglobin, packed cell volume, and vitamin assay results are recorded in Table I.

The serum folate values showed an overlap with those found in the last trimester in patients without bone-marrow changes, but in megaloblastic pregnancies values under 1.5 ng./ml. were rather more frequent than in normoblastic ones. In these latter levels less than 1.5 ng./ml. were infrequent.

The red cell folate was diminished (<150 ng. per ml.) in nearly all patients with megaloblastic marrow so examined (22), but in less than half of patients with white cell changes only (Fig. 1).

FIG. 1. Red cell folate levels in 35 pregnant women (nine group I, 13 group II, and 13 group III) with megaloblastic change in the bone marrow, 26 'normal' pregnant women in the last trimester and 30 non-pregnant normal women.

ACCIDENTAL HAEMORRHAGE Complete hospital records of this complication were available only for the period 1962-64. Accordingly Table II gives the incidence of this complication which occurred in 6,310 non-megaloblastic pregnant women delivered during this period and in 230 'megaloblastic' pregnancies over four and a half years. The figures shown in Table II compare with an incidence of 8% in megaloblastic pregnancies given by Hibbard (1964).

TABLE II
ACCIDENTAL HAEMORRHAGE

	No. of Cases	Accidental Haemorrhage
Non-megaloblastic pregnancies	6,310	248 (3.9%)
Megaloblastic pregnancies (groups I and II)	186	11 (5.8%)
Megaloblastic pregnancies (group III)	44	2 (4.5%)

A t test at 95% level does not show any significant difference between the frequency of accidental haemorrhage in the various groups. Comparing the normal group and combined groups I and II, which show the largest difference, results in a t value of approximately 1.2, which is not significant.

STILLBIRTHS Table III is self-explanatory. Statistical analysis shows no significant difference between frequencies of stillbirths in these two groups.

TABLE III
INCIDENCE OF STILLBIRTHS

	Live Births	Stillbirths
Megaloblastic pregnancy	243	5 (2.0%)
Non-megaloblastic pregnancy	9,956	271 (2.6%)

BIRTH WEIGHT The weights of 214 infants born in non-multiple 'megaloblastic' pregnancies are shown in Table IV. They are compared with 250 infants born to randomly selected normal mothers. Analysis of this contingency table shows no significant difference between the two groups.

TABLE IV
BIRTH WEIGHTS IN 214 MEGALOBlastic
AND 250 NORMAL PREGNANCIES

No. of Infants	<5lb. 8oz. 6lb. 7oz.	5lb. 8oz.- 6lb. 7oz.	6lb. 8oz.- 8lb. 7oz.	8lb. 7oz. or More	Total
Megaloblastic pregnancy	19	28	141	26	214
Normal pregnancy	21	38	157	34	250

RECURRENCE OF THE FOLIC ACID DEFICIENCY STATE
There was a tendency for megaloblastic change to recur in subsequent pregnancies. So far 30 women out of the 222 included in the review are known to have had recurrences, 28 once and two twice.

In 10 of these patients we were able to examine the folate levels in the non-pregnant state. None of them showed any morphological evidence of folic acid deficiency; two had serum folate levels <3 ng. and six red cell folate levels <150 ng./ml. (Table V).

TABLE V
FOLATE LEVELS IN 10 NON-PREGNANT
WOMEN WITH A HISTORY OF RECURRENCE

Patient	P.C.V. (%)	Serum Folate (ng./ml.)	Red Cell Folate (ng./ml.)	Time since Folic Acid Therapy (yr. mth.)
J.E.	45	2.2	113	3
P.E.	40	2.2	122	1 5
E.C.	43	3.8	135	4
M.D.	39	3.8	107	1 5
A.H.	38	3.7	133	1 7
B.D.	42	3.3	185	4
A.B.	42	4.4	200	2
H.L.	41	7.8	490	4
A.T.	41	3.5	216	2
M.B.	41	4.3	98	1 11

DISCUSSION

DIAGNOSIS Like Chanarin and his colleagues (1965) we, too, found an approximately 75% correlation between the presence of at least 5% hypersegmented neutrophils and megaloblastic erythropoiesis. In 18 out of 24 megaloblastic (groups I and II) cases in which an actual segment count was performed, there were 5% or more hypersegmented neutrophils. On the other hand, there were cases with this degree of hypersegmentation in which the marrow changes were limited to the white series (group III); this situation is probably met with in early or mild folate deficiency or in the spontaneous recovery phase, post partum, where a reversal to normoblastic erythropoiesis might already have taken place. In no case did we find megaloblastic erythropoiesis in the absence of the described haematological findings. All cases of anaemia not responding to iron were

carefully checked by inspecting the peripheral blood films. In the few cases with low haemoglobin levels where a bone-marrow puncture was performed despite the negative blood picture, erythropoiesis was found to be normoblastic.

Thirty normal women all had above 150 ng./ml. of folate in the red cells; eight of nine in group I, 12 of 13 in group II, six of 13 in group III, and five of 26 'normal' pregnant women had under 150 ng./ml. (Fig. 1). Thus the red cell folate level would appear to be a very good indicator of significant folic acid deficiency. Pregnant women with hypersegmentation but with normal red cell folate levels have probably a minimal but clinically insignificant deficiency.

Iron deficiency or, in some cases, urinary tract infection were presumably the only causes of anaemia in patients with normoblastic erythropoiesis (group III). Iron deficiency might also have been the cause of hypersegmentation (Chanarin *et al.*, 1965) in these cases.

Six of 10 non-pregnant women known to have had megaloblastic erythropoiesis in at least two pregnancies had red cell folate levels <150 ng./ml. and, two of these, serum folate levels <3.0 ng./ml. (Table V). This observation would favour Hansen's view (1964) that an enzyme necessary for the transformation of folic acid into N⁵-methyl-tetrahydrofolic acid might be deficient in some cases. Whilst this hypothesis is certainly worth exploring, we feel that in most cases folic acid deficiency is mainly subject to supply and demand.

Figlu excretion tests in our hands (Kohn *et al.*, 1961) as in others (Chanarin, Rothman, and Watson-Williams, 1963; Hansen, 1964; Chisholm and Sharp, 1964) gave inconclusive results, although recently Scott and Sommerville (1965) have reported a good agreement of a modified test with the morphology of white cells.

OBSTETRIC ASPECTS The patients were usually treated on diagnosis but, in some cases, only after the next antenatal visit. In 32.7% they were diagnosed before the 36th week, in 41.6% between the 36th week and term, and in 23.5% at term or after delivery.

We found no obvious connexion between the incidence of underweight babies and folic acid deficiency in the mother, which is in keeping with the observation that folate levels are higher in cord blood than in the mother's circulation (Grossowicz, Aronovitch, Rachmilewitz, Izak, Sadovsky, and Bercovici, 1960). Stillbirths were no more numerous in megaloblastic than non-megaloblastic pregnancies.

The number of megaloblastic anaemia cases investigated does not permit a definite conclusion

as to whether accidental haemorrhage is more frequent than in non-megaloblastic pregnancy.

We are indebted to Professor C. S. Russell and Mr. R. L. Lunt, consultant obstetricians, City General Hospital, for permission to study patients under their care and for their helpful suggestions and advice, and to Dr. G. F. Yeo, University of Sheffield, for statistical help. The cooperation of Mr. J. M. Bevington, Mr. S. Hewitt, and the rest of the Department of Haematology technical staff, is gratefully acknowledged.

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