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Nutritional Anemia and Megaloblastosis in Pregnancy

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ABSTRACT

Macrogranulocytic and/or erythroid megaloblastic bone marrow changes which could not be accurately predicted from the hematologic findings in the blood were present in 25% of 305 mildly to moderately anemic pregnant women attending a public antepartum clinic in Montreal. Iron deficiency was the primary cause of anemia in most instances. Serum folate activity of less than 4.1 ng./ml. and/or serum vitamin B_{12} levels of less than 100 pg./ml. were present in 90% of the 77 patients having these bone marrow changes, whereas approximately one-third of 228 patients with normoblastic marrow had these low values. Red cell folate did not correlate as well as serum folate activity with bone marrow changes. After treatment with oral folic acid in the range of 0.2 mg. to 0.8 mg., daily, for seven to 14 days, the megaloblastic and macrogranulocytic changes in patients with low serum folate activity and normal serum vitamin B_{12} values disappeared in 15 of 21 patients. Of five women having both low folate and vitamin B_{12} values, three failed to respond and two showed only partial improvement after 0.4 mg. of folic acid daily, *per os*, for 10 days. The average diet of these anemic women was suboptimal in folate and in iron.

SOMMAIRE

Des modifications macrocytaires et mégaloblastiques de la moëlle osseuse, qui n'auraient pu être prévues à partir des données des analyses hématologiques, étaient présentes chez 25% des 305 femmes enceintes, souffrant d'une anémie bénigne ou modérée, qui fréquentaient une clinique publique pour femmes enceintes de Montréal. Dans la majorité des cas, l'insuffisance de fer était la cause principale de l'anémie. On a trouvé dans le sérum une activité en folate, inférieure à 4.1 ng./ml. et des concentrations sériques en vitamine B_{12} inférieures à 100 pg./ml. (ou l'une des deux déficiences) chez 90% des 77 femmes qui présentaient des modifications de la moëlle osseuse, alors que le tiers environ des 228 femmes à moëlle normoblaste avaient ces valeurs déficientes. L'activité en folate des érythrocytes ne correspondait pas, aussi bien que l'activité en folate du sérum, aux changements de la moëlle osseuse. Après traitement *per os* à l'acide folique, aux doses de 0.2 à 0.8 mg./jour, pendant sept à 14 jours, les modifications mégaloblastiques macrocytaires observées chez des sujets ayant une faible activité en folate sérique et des concentrations sériques normales en vitamine B_{12} ont disparu chez 15 femmes sur 21. Des cinq femmes qui avaient à la fois une faible teneur en folate et en vitamine B_{12}, trois ne réagirent pas au traitement et deux furent partiellement améliorées après 0.4 mg. d'acide folique *per os*, pendant 10 jours. Le régime alimentaire moyen de ces femmes anémiques était à la limite de la déficience en folate et en fer.

MEGALOBLASTIC anemia is a common complication of pregnancy in certain tropical regions.[1-11] Until recently, the development of megaloblastic anemia was considered a rare complication of pregnancy or the puerperium in temperate areas.[12-15] With the recognition that mild to moderate anemia with transitional megaloblastic changes in the bone marrow occurs much more frequently than the severe form with classical megaloblastosis, the reported incidence has continued to rise.[16-22] These mild to moderate forms often can only be diagnosed from megaloblastic changes in the bone marrow.[16, 23, 25, 26] In fact, these morphologic changes may exist in the absence of anemia and almost invariably are indications of maternal deficiency of folate and/or vitamin B_{12}.[25, 26]

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In temperate climates, most pregnant women with this disease have responded to large (5 mg. or more) doses of folic acid,[16-21] whereas satisfactory response to vitamin B_{12} has occurred less frequently.[34] When it did occur, massive doses of vitamin B_{12} often were required, and in some instances the response even to these massive doses was incomplete.[16, 26, 32] In certain areas of the tropics, however, satisfactory response has been obtained with small doses of vitamin B_{12}.[32]

In 1955 it was suggested that deficiency of folate and, less frequently, of vitamin B_{12} was responsible for the development of megaloblastic anemia in pregnant women attending the antepartum clinic of the Royal Victoria Hospital in Montreal[16] and

that the diet of many of these patients was deficient, particularly in folate. In the last several years, a number of workers have performed microbiological and biochemical studies to test this hypothesis.

Several theories have been advanced as to the pathogenesis of megaloblastic anemia of pregnancy. There is, as yet, no general agreement as to the basic mechanism involved in this condition, owing to the wide variation in the population groups studied and the large non-specific doses of folic acid or vitamin B_{12} which have been used. Also, it has not been determined finally whether the established normal values for microbiologic and biochemical assays of these vitamins or their metabolites apply in pregnancy.[25, 34, 40]

Past observations have shown that folate and/or vitamin B_{12} deficiency may exist for some time, with low serum microbiological values only, before megaloblastic bone marrow changes and subsequent anemia develop. If megaloblastic anemia of pregnancy follows exhaustion of the body stores due to inadequate diet in the face of increased requirements, then microbiological evidence of deficiency of folate and/or B_{12} might be expected to occur more frequently than overt megaloblastic anemia. Moreover, the development of megaloblastic anemia should correlate well with low levels of folate and/or B_{12} and would represent a more severe state of deficiency than the presence of low microbiological values alone.

The present report records observations on 305 consecutively studied anemic pregnant women who attended the antepartum clinic of the Royal Victoria Hospital. Preliminary studies in some of these women have been reported previously.[23, 25, 40]

Materials and Methods

Women attending the antepartum clinic were considered to be anemic and were referred to a special clinic if their hemoglobin or packed cell volume (PCV) dropped below the following values:

	Hemoglobin	*PCV*
First 16 weeks of pregnancy.....	11.6 g.%	36%
17th to 24th week of pregnancy.....	11.0 g.%	34%
25th week to term.....	10.4 g.%	32%

At the anemia clinic, a detailed history (including dietary) was taken and a complete physical examination was carried out. Initially, and often serially thereafter, the peripheral blood and bone marrow (including iron stain) were examined by methods long used in our laboratory.[16, 24] At the first visit, and serially when indicated, the serum iron, unsaturated iron-binding capacity (UIBC), serum vitamin B_{12} concentration, serum folate activity and, more recently, erythrocyte folate activity were determined. Initial studies also included determination of blood volume and, in some cases, of serum vitamin C concentration, total serum protein and electrophoretic pattern and urinary FIGlu (formiminoglutamic acid) excretion after stressing with L-histidine.

The effect of the administration of small daily doses of folic acid or of vitamin B_{12} upon the bone marrow morphology and upon the blood folate and serum vitamin B_{12} levels was determined in some of the women having megaloblastic changes in the bone marrow.

Bone marrow aspiration was performed, usually from the sternum. The aspirates were stained with Jenner-Giemsa and smears containing marrow particles were stained for iron with potassium ferrocyanide.[35, 36] The bone marrow preparations were interpreted independently by two observers (L.L. and L.B.). Megaloblastic transition was considered to be present if any degree of megaloblastic change of nucleated red cells was present, and/or if classical late macrometamyelocytes and macrogranulocytes were found consistently[33] by both observers. The presence of increased Howell-Jolly bodies was noted in a number of cases. The amount of iron in the preparations was graded from 0 to 3+.[24]

Methods for serum iron, UIBC and plasma volume determinations and for the calculation of red cell volume and hemoglobin mass have been detailed elsewhere.[24] Oral iron administration was omitted for 48 hours before serum iron determinations. Ten drops of Lugol's solution was administered daily, for one week, before the determination of plasma volume, to minimize uptake of I^{131} by maternal and fetal thyroid.

Previously described methods[33, 38] were used for the determination of serum folate and whole blood folate activity (expressed as nanograms (ng.)/ml. of packed erythrocytes). Except on rare occasions, these determinations were performed within seven to 10 days of drawing the blood. When a greater interval was anticipated, the blood was taken in citrate, ascorbate was added and the samples were stored at –20° C.

Serum vitamin B_{12} was determined with *E. gracilis* var. *bacillaris*.[39]

Total plasma protein concentration was calculated from the refractive index increment as measured with a refractometer.* Fractionation was carried out using a modification of the horizontal strip technique. The strips were stained with amido black 10 B and were examined in a Densicord.

Results

The incidence of anemia in 2685 patients attending the antepartum clinic over a two-year period is shown in Table I.

Of the total 305 anemic women in this study, the bone marrow was completely normoblastic in 228 (75%), and some degree of megaloblastic transition was present in 77 (25%). Iron deficiency was the primary cause of the anemia in most of the

*These determinations were carried out through the courtesy and under the supervision of Dr. Eleanor E. McGarry.

TABLE I.—ANEMIA IN 2685 PREGNANCIES

	No.	% of deliveries	% of 274 anemias
Anemic patients	274	10.2	100
Megaloblastic	63	2.35	23
Normoblastic	211	7.85	77

patients of both groups, although in a small minority the anemia was due to thalassemia minor, infection, or other causes. It was rarely severe enough to give rise to symptoms.

The megaloblastic and the normoblastic groups were compared with reference to toxemia, prematurity, birth weight, congenital anomalies and neonatal morbidity. No difference was found between the two groups. There was no evidence for an increased incidence of megaloblastic anemia in the winter months. Over a two-year period, 26% of the patients were found to have megaloblastic bone marrow changes during the summer months (May 1-October 31), and 18% exhibited these changes during the winter months (November 1-April 30); there was no significant difference in the number of deliveries during these two periods. Patients who were para 3 or greater constituted 75% of the megaloblastic group and 23% of the normoblastic group. These figures suggest that repeated pregnancies play some part in the development of megaloblastic anemia.

Seventy-five anemic pregnant Greek women were included in the study and are of special interest. The Greek population of Montreal is a closely knit group, retaining many of the customs of their country of origin. Many of the women become iron deficient and develop anemia during pregnancy. Megaloblastic bone marrow changes were found in only four of the 75 anemic Greek women, an incidence of 5.3%. When the Greek women are excluded from the calculation, the incidence of megaloblastic transition in the bone marrow of the remainder was 31.7%. The median serum folate activity was 5.5 ng./ml. in the anemic Greek women, and 4.1 ng./ml. in the non-Greek anemic women. A dietitian, accompanied by an interpreter, was sent into the homes of four members of the Greek group. One of these patients was found to be destitute and in need of financial assistance. The dietitian spent some time observing the type of food purchased and the method of cooking in the homes of the other three patients. In this small sample group, it was found that the Greek patients ate liver or kidney, usually twice a week, as well as large quantities of beans and lentils, and it was felt that this diet provided an abundant source of folate. Visceral protein is seldom eaten by the Canadian women attending our clinic.

The results of the serum protein studies are depicted in Table II.

The total serum protein and the serum albumin at the 38th antepartum week were slightly lower in 20 normal, well-nourished pregnant women than

TABLE II.

Protein (g.%)	20 normal pregnant controls	45 normoblastic anemic subjects	43 megaloblastic anemic subjects
Total protein	6.52 ± 2.6	7.10 ± 1.70	7.30 ± 2.3
Albumin	3.11 ± 0.76	3.20 ± 0.62	3.20 ± 0.50
$Alpha_1$ globulin	0.48 ± 0.28	0.60 ± 0.22	0.50 ± 0.16
$Alpha_2$ globulin	0.76 ± 0.30	0.90 ± 0.33	0.90 ± 0.25
Beta globulin	1.14 ± 0.50	1.20 ± 0.47	1.30 ± 0.45
Gamma globulin	1.07 ± 0.45	1.30 ± 0.54	1.40 ± 0.57

in non-pregnant normal women. These values were not further reduced in the anemic pregnant women of either the normoblastic or the megaloblastic group.

HEMATOLOGICAL OBSERVATIONS

Severity of Anemia: The majority of patients of both groups had hemoglobin levels between 8 and 10 g. %, while over a quarter of both groups had greater than 10 g. % (Fig. 1). The anemia was more severe (6 to 8 g.) in 14% of the megaloblastic and 7% of the normoblastic group. A single patient with an acute gastrointestinal hemorrhage had a hemoglobin value of less than 6 g.

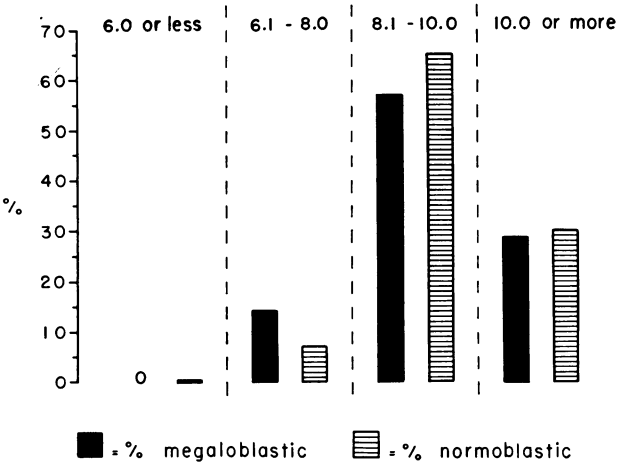

Fig. 1.—Distribution of hemoglobin values in 305 anemic pregnant women.

Anemia was observed initially before the 29th week of pregnancy in 46% of the normoblastic group and in only 15.6% of the megaloblastic group. Eight anemic patients who had normoblastic marrows when first seen developed megaloblastic changes later. They all responded to iron therapy, and when the megaloblastic changes were first observed, five had more than 10.1 g. % hemoglobin.

Evidence and Incidence of Iron Deficiency: At the time of the initial diagnosis of anemia the bone marrow iron content, the serum iron, and the mean corpuscular hemoglobin concentration (MCHC) were determined in 100 patients with normoblastic bone marrow and in 60 patients with megaloblastic

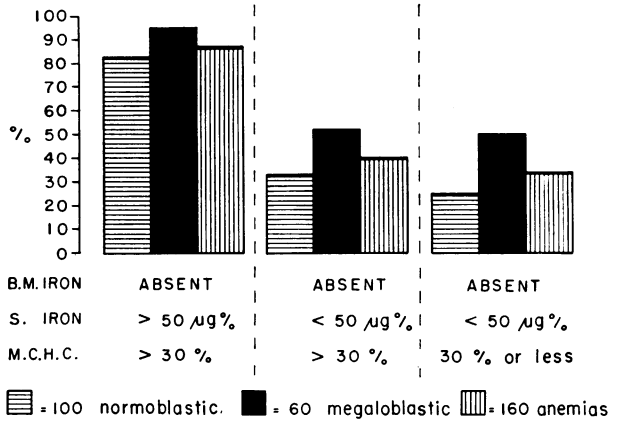

Fig. 2.—Iron deficiency in 160 anemic pregnant women.

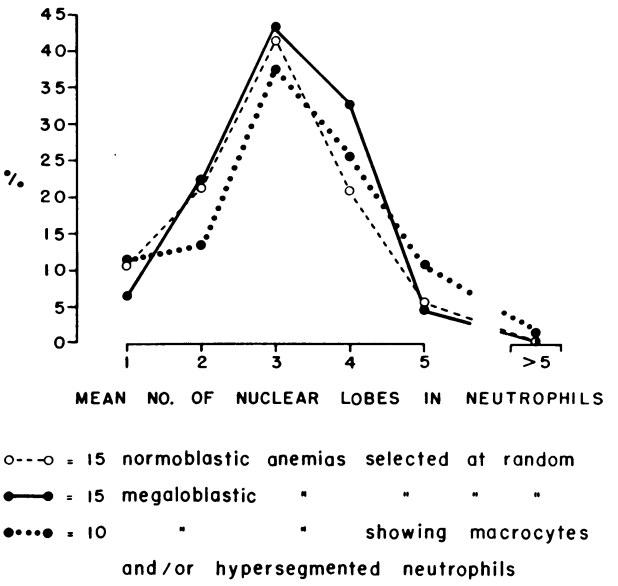

Fig. 3.—Mean Arneth counts in anemia in pregnancy.

marrow. These results are depicted in Fig. 2. Serum iron, UIBC and MCHC were determined in the remaining patients, but their marrow was not stained for iron. The peripheral blood smears of all patients were also examined for hypochromia, but this was found to be a late sign of iron deficiency and not as reliable an indicator as the other parameters.

Stainable iron was absent from the bone marrow in 83% of the 100 patients in the normoblastic group and in 95% of the 60 patients in the megaloblastic group, or in 87.5% of the total 160 patients. The bone marrow contained no iron and the serum iron was less than 50 µg. % in 34% of the normoblastic group and 52% of the megaloblastic group, or in 40% of the total 160 patients. Bone marrow iron was absent, the serum iron was less than 50 µg. % and the MCHC was 30% or less in 25% of the normoblastic and 50% of the megaloblastic group, or 34% of the total 160 patients.

In 41 patients there was no stainable iron in the bone marrow and the MCHC was 30% or less, although the serum iron was greater than 50 µg. % but only ranged between 51 and 70 µg. %. Satisfactory response to iron therapy provided further evidence of iron deficiency in 28 of these patients, and thalassemia minor was present in another eight of these patients, but in view of the borderline serum and absent iron stores, iron deficiency may have contributed to their decreased MCHC. The remaining five of these 41 patients had plasma hypervolemia with normal erythrocyte and hemoglobin mass for late pregnancy. In another 14 patients, all in the normoblastic group, the MCHC was 30% or less, although stainable iron was present in the bone marrow and the serum iron was not decreased. Ten of these patients had thalassemia minor, and one had pyelitis.

Morphological Evidence of Megaloblastic Transition in the Peripheral Blood and Bone Marrow

The MCV of the normoblastic group ranged from 72 to 100 cu.µ., with a mean of 90.3, while that of the megaloblastic group ranged from 82 to 100 cu.µ., with a mean of 91.4. Arneth counts were performed in 15 patients with normoblastic erythropoiesis and in 15 patients whose bone marrow showed megaloblastic changes (Fig. 3). These patients were selected at random from their respective groups. There were no significant differences in the Arneth counts of these two groups. Counts were performed in another 10 patients having morphological changes in the erythrocytes in their blood smears, suggesting the presence of megaloblastosis. This group had a slightly greater percentage of five-lobed neutrophils than the former two groups.

On examination of blood smears obtained at the same time as the initial bone marrow aspiration, macrocytes and/or hypersegmented neutrophils were found in 37% of the 77 patients with megaloblastic bone marrow changes. Oval macrocytes were present in 18% and hypersegmented neutrophils in 22%. When macrocytes were observed in the peripheral blood, their greatest diameters measured 8-9 µ. Thus, the presence of megaloblastic changes in the bone marrow could not be predicted from the morphological changes in the peripheral blood in the majority of cases.

The marrow aspirates of 70% of the 77 patients in the megaloblastic group contained macrogranulocytes, but no erythroblasts undergoing megaloblastic transition; these changes were present in both granulocytic and erythroid cells in the remaining 30% of patients. Increased numbers of Howell-Jolly bodies were noted in the erythroblasts of some patients, but this finding was not as constant as the presence of macrogranulocytes and was not noted in their absence. The degree of megaloblastic changes in the bone marrow could not be related to the degree of anemia, which in most cases varied directly with the severity of iron deficiency.

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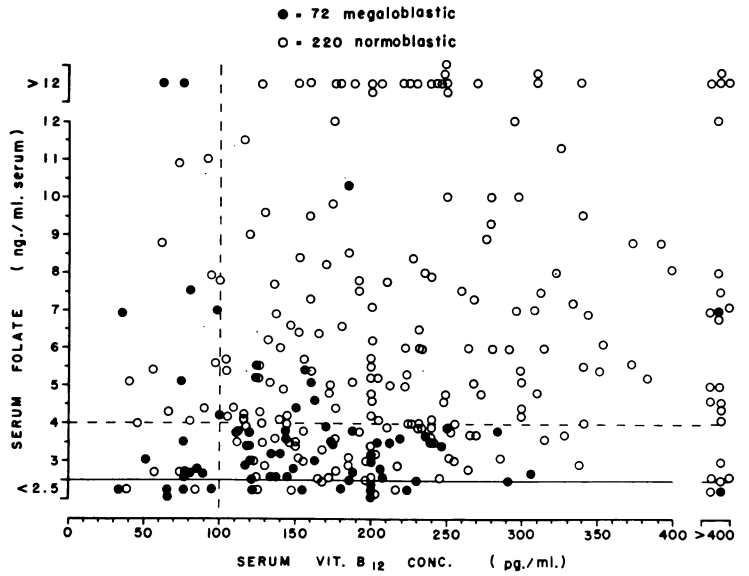

Fig. 4.—Serum folate and serum vitamin B_{12} determinations on 292 anemic pregnant women.

Relation of Blood Folate and Serum Vitamin B_{12} Levels to Bone Marrow Findings

The serum folate activity was determined in all 305 patients. In six of the megaloblastic group, and in eight of the normoblastic group, serum vitamin B_{12} determinations were not obtained. From the scattergram (Fig. 4) of the initial serum folate and vitamin B_{12} values in the two groups, obtained at the same time as the initial bone marrow aspirate, it is apparent that the presence of low serum folate activity and/or low serum vitamin B_{12} correlates reasonably well with the presence of megaloblastic changes in the bone marrow, but correlates poorly in those patients having only normoblastic erythropoiesis. Thus, there is considerable overlap of microbiological values in the normoblastic and megaloblastic groups. It may be seen from Fig. 5 that the serum folate activity was 4 ng./ml. or less, and/or the serum vitamin B_{12} was < 100 picograms (pg.)/ml. in approximately 90% of patients with megaloblastic bone marrow changes. These levels were exceeded in only 10% (eight patients) of this group (discussed below). In the normoblastic group, 37% of the patients had similar low values.

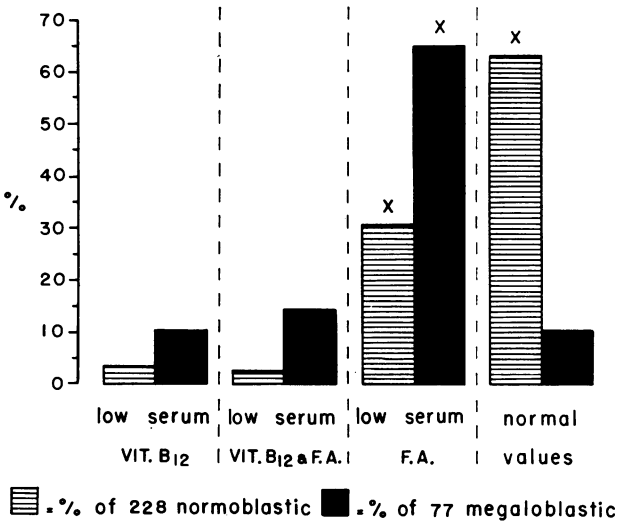

Fig. 5.—Serum folate activity and serum vitamin B_{12} in 305 anemic pregnant women.

The serum folate was decreased and vitamin B_{12} concentration was normal in 65% of the megaloblastic group and in 31% of the normoblastic group. All patients of the megaloblastic group upon whom serum vitamin B_{12} determinations were not performed were found to have a low serum folate activity. Fig. 5 also shows that approximately 25% of all patients having megaloblastic bone marrow changes had serum vitamin B_{12} concentrations of less than 100 pg./ml., that over half of these also had low serum folate activity, and that low serum vitamin B_{12} only was observed in 10%. Only three patients, all in the megaloblastic group, had serum vitamin B_{12} concentrations of less than 60 pg./ml. An additional 12 patients, or 16.7% of the megaloblastic group, had serum vitamin B_{12} determinations of 100 to 130 pg./ml. at the time of the initial observation. Although these latter values would be considered abnormally low in the non-pregnant subject, such significance has not yet been proved in the pregnant subject.

In our laboratory, non-pregnant patients with proved folate deficiency have had whole blood folate activities of less than 175 ng./ml. of packed red cells,[25, 38] and those without folate deficiency have exhibited values above this level.

The determination of whole blood folate activity was performed simultaneously with the serum folate and vitamin B_{12} determinations at the time of diagnostic bone marrow examination in the last 148 patients investigated. The serum folate is plotted against the whole blood folate for each of these cases in Fig. 6. Whole blood folate levels showed a definite trend, with 50% of the megaloblastic group having levels of less than 175 ng./ml. as compared with 25% in the normoblastic group. The whole blood folate, however, did not correlate as well with the bone marrow findings as did the serum folate. Only one of the megaloblastic patients with low serum vitamin B_{12} levels had elevated serum folate activity, and the red cell folate activity of this patient also was elevated.

Serial observations of bone marrow, serum folate activity and vitamin B_{12} concentrations were made in 29 megaloblastic patients who did not receive folic acid or vitamin B_{12}. Initially, eight of these patients had megaloblastic bone marrow changes with normal serum values (Fig. 7). The serum folate activity decreased to 4 ng./ml. in one and to 2.5 ng./ml. or less in five of these patients. The

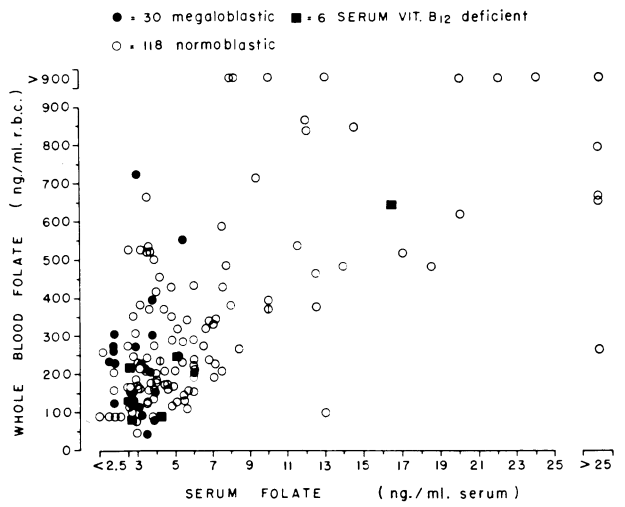

Fig. 6.—Whole blood and serum folate determinations on 148 anemic pregnant women.

serum vitamin B_{12} concentration decreased to 70 pg./ml. in the seventh patient. If the results of the initial and the serial observations are combined, only one of the 77 patients with megaloblastic bone marrow maintained serum values within the normal range, and in that case the value was 4.2 ng./ml. Low serum values preceded megaloblastic changes in the bone marrow in seven patients. Low serum values and megaloblastic changes were both present in 14 patients at the time of the initial observation. The low serum values persisted in these 14 patients and in some the marrow showed progressive increase of the megaloblastic changes.

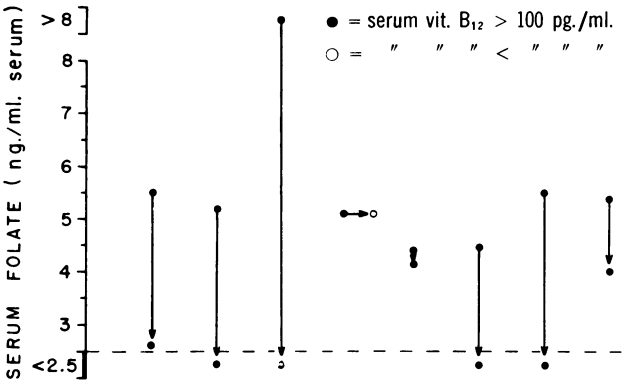

Fig. 7.—Serial folate studies in eight anemic pregnant women with megaloblastic bone marrow changes.

RESPONSE TO SPECIFIC ANTI-ANEMIC THERAPY

Iron.—In initial reports[23, 40] it was found that these pregnant women with mild to moderate iron deficiency anemia and megaloblastic bone marrow changes responded satisfactorily to oral or intramuscular iron therapy. In the present larger series, this experience was confirmed. After iron therapy, the macrogranulocytic and/or megaloblastic erythroid transition either remained unchanged or became somewhat more marked, but showed no different trend from the bone marrow changes observed in the 29 serially studied patients who received no antianemic therapy.

Folic Acid and Vitamin B_{12}—Table III summarizes the response of the bone marrow to small oral daily doses of folic acid administered to patients in the megaloblastic group who had low serum folate and/or vitamin B_{12} values. Morphological changes in the bone marrow before treatment were compared with those present 48 hours after the last dose of folic acid. Eleven of 16 patients receiving 0.4 mg. daily, by mouth, for periods of 10-14 days, showed complete disappearance of the megaloblastic bone marrow changes. The marrows of two patients remained unchanged after receiving this dosage for four and six days respectively. Two of the patients who failed to respond to the 0.4-mg. dose daily for 14 days subsequently responded to 0.8 mg. daily *per os* for 14 and 42 days respectively, while a third patient, who failed to respond to the 0.4-mg. daily dose, also failed to respond to 0.8 mg. of folic acid given daily *per os* for 10 days. Two additional patients receiving 0.2 mg. daily *per os*, one for seven and the other for 10 days, showed complete reversion of the bone marrow to normal.

TABLE III.—ANTEPARTUM MEGALOBLASTIC ANEMIAS WITH FOLATE DEFICIENCY. RESPONSE TO SMALL ORAL DOSES OF FOLIC ACID

No. of cases	*Daily dose (μg.)*	*No. of days*	*Bone marrow response*		
			Yes		*No*
			complete	*partial*	
16.....	400	4-14	11		5
2.....	200	7, 10	2		
3*.....	800	10, 14, 42	2		1
5†.....	400	10		2	3

* = Failure to respond to previous dose of 400 μg.
† = Serum vitamin B_{12} < 100 pg./ml.

Five patients in whom both serum folate and vitamin B_{12} levels were low received a daily oral dose of 400 μg. of folic acid for 10 days. The marrows of three failed to improve, and two showed only partial improvement. Fig. 8 shows the serum folate levels before and after daily oral administration of 0.4 mg. of folic acid to some of the patients included in Table III; the serum folate determinations were performed 24 to 48 hours after the last dose of folic acid. The serum folate level responded variably to these small doses of folic acid, and increased at least as much, if not more, in those patients whose bone marrow showed no reversion to normal. Many patients responded satisfactorily to the administration of 5 mg. or more of folic acid.

Several additional patients seem worthy of mention because of their unsatisfactory response to either large or small doses of folic acid and/or vitamin B_{12} (Table IV). One patient who had no lowering of serum folate activity or of serum vitamin B_{12} concentration failed to respond to a daily oral dose of 400 μg. of folic acid for 10 days, then

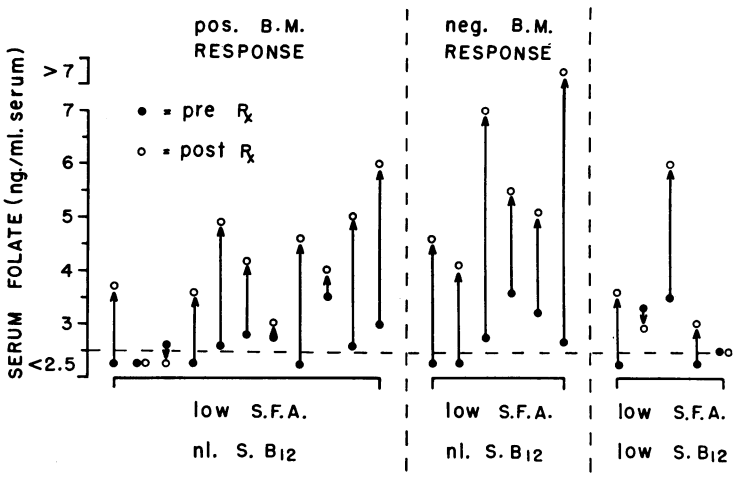

Fig. 8.—Serum folate response to 400 μg. pteroylglutamic acid (folic acid) *per os* daily A.P. in megaloblastic anemia in pregnancy.

to 100 μg. of vitamin B_{12} intramuscularly, but did show complete disappearance of megaloblastic changes after oral administration of 15 mg. of folic acid daily for 10 days. Two patients had serum folate activity of less than 4 ng./ml. with normal serum vitamin B_{12} levels. Partial bone marrow response was obtained in one after injection of 16.2 mg. of folic acid, and in the other after injection of a single dose of 5 mg. of folic acid. Two patients had microbiological evidence of combined deficiencies of folate and of vitamin B_{12}. One showed

TABLE IV.—ANTEPARTUM MEGALOBLASTIC ANEMIAS—UNSATISFACTORY THERAPEUTIC RESPONSE IN EIGHT CASES

Case No.	Microbiol. deficiency	Therapy	No. of days	Bone marrow response: Yes complete	Yes partial	No
1	—	F.A. p.o. 400 μg.	10			√
	—	B12 I.M. 100 μg.	1			√
	—	F.A. p.o. 15 mg.	10	√		
2	Folate	F.A. I.M. 16.2 mg.	1		√	
3	Folate	F.A. I.M. 5.0 mg.	1		√	
4	Folate B12	F.A. I.M. 16.6 mg.	1			√
5	Folate B12	B12 I.M. 21 μg.	1			√
6	B12	F.A. p.o. 400 mg.	14			√
7	B12	B12 I.M. 30 μg.	1		√	
8	B12	B12 10 μg.	10		√	

no response to the intramuscular injection of 16.6 mg. of folic acid and the other failed to respond to the intramuscular injection of 21 μg. of vitamin B_{12}. Two other patients with pure vitamin B_{12} deficiency microbiologically showed partial responses of the bone marrow; in one, to the administration of 30 μg. of vitamin B_{12} intramuscularly, and in the other, to 10 μg. of vitamin B_{12} daily, by mouth, for 10 days; a third such patient failed to respond to oral folic acid, 0.4 mg. daily for 14 days.

DISCUSSION

The relatively low incidence of megaloblastic changes reported in Table I is due to the fact that a marrow examination was carried out only on patients who were anemic, in most cases because of iron deficiency. We, as well as others, have found that megaloblastic changes in the bone marrow occur frequently in pregnant iron-deficient women[2, 16, 18, 25, 42] and may be present in the absence of anemia.[2, 16, 20, 37, 43, 47] In this study, 25% of bone marrows examined and in another investigation of non-anemic pregnant women from the same population group[25] 28% of bone marrows examined were found to have megaloblastic changes. Thus, the true incidence of bone marrow megaloblastic changes in our clinic patients is closer to 25% than to 2.35%. The relatively high intake of food folate in the Greek women probably accounts for the much lower incidence of megaloblastic marrow changes and somewhat higher blood folate values than were found in the anemic women of other ethnic origins. Recently, Dawson[41] and Chanarin *et al.*[42, 43] examined a proportion of anemic pregnant women in England and found an incidence of approximately 10%, while Hansen,[44] in Sweden, found that a high proportion of pregnant women had morphological changes in the marrow. A number of patients with depleted iron stores were found to have serum iron levels in the range of 50-70 μg. %. These women showed a rise in hemoglobin level in response to iron administration, if sufficient time elapsed prior to parturition. Iron stores were depleted in 87.5% of patients, and additional evidences of iron deficiency were present in 40%. In some cases, hypochromia was due to thalassemia minor without iron deficiency. The low serum iron level was found to be a better indicator of depleted iron stores than was a decrease in the MCHC.

In 70% of the patients classified as having megaloblastic bone marrow changes, erythropoiesis was entirely normoblastic and the abnormalities were found in granulocytes only. Late giant metamyelocytes and band forms were present in significant numbers in these patients. The remaining 30% had erythroid megaloblastic changes as well. That macrogranulocytosis alone may present as the earliest stage in the development of megaloblastic bone marrow has been demonstrated in the past by ourselves[16, 23, 37] and others.[5, 26-28, 41] Only 37% of the megaloblastic patients showed blood changes suggesting this type of marrow. We found no significant shift to the right of neutrophils, a change suggested by Herbert[45, 46] to be of diagnostic value.

Iron deficiency has occurred concurrently with megaloblastic anemia of pregnancy in many areas.[2, 16, 18, 25, 42] In some instances, the megaloblastosis may be intermediate in degree but may

become more marked after administration of iron,[18,22,48] and some cases of severe dimorphic anemia do not respond initially to iron therapy, probably owing to concomitant severe folate and occasionally vitamin B_{12} deficiency. Similarly, we have observed other pregnant and non-pregnant patients with severe dimorphic anemia in whom iron therapy was associated with conversion of intermediate to classical megaloblastosis, together with failure of improvement of the anemia. In the present patients the megaloblastic changes persisted unchanged or increased slightly after iron therapy and in no instance did classical megaloblastosis develop, probably because severe folate or vitamin B_{12} deficiency was not present. Eight cases, initially normoblastic, developed megaloblastic bone marrow changes while responding to iron therapy. The magnitude of these changes did not differ from that observed in the serial bone marrow examinations of those patients who received no iron.

Iron administration has been noted to produce partial or complete response of mild to moderate dimorphic anemia and even of some cases of severe anemia.[2, 19, 25, 40, 42] The anemia in our patients was of mild to moderate severity and responded satisfactorily to adequate iron therapy.

Chanarin, Rothman and Berry[42] state that iron deficiency *per se* contributes to the production of "folic acid deficiency in pregnancy". This statement is based on their claim that the *L. casei* levels of pregnant women taking iron supplements did not fall, whereas those of pregnant women not taking such supplements did fall in late pregnancy. Our findings and those of others[2, 18, 19, 25, 49, 50] do not support this statement but indicate that nutritional deficiencies of both iron and folate commonly occur together in pregnant women. Iron administration in our cases did not alleviate folate deficiency as judged by serial bone marrow and *L. casei* blood folate findings. Inspection of Chanarin's data shows that at 35 weeks the women receiving iron supplements had a lower mean serum *L. casei* folate value and at 39 weeks a higher mean level than those of the women receiving no iron. The median values, however, did not differ in the two groups at these stages of pregnancy, and it is well known that the mean serum *L. casei* values can be misleading owing to the skew distribution of these values. Furthermore, there was no significant difference in the percentage of patients with megaloblastic marrow changes when groups receiving lactose, those receiving iron and those receiving iron plus 20 μg. of folic acid daily were compared. Their conclusions, based on the numbers of circulating hypersegmented neutrophils, do not seem justified.

Serum *L. casei* folate activity is now accepted as a reasonably accurate indicator of folate deficiency in the non-pregnant subject and may be decreased for some time before the bone marrow becomes megaloblastic.[33, 45, 50, 51, 63, 64] This may occur in nutritional folate deficiency,[52, 53] malabsorption, or disease states in which there is increased folate requirement. The low *L. casei* values found in a large percentage of normal pregnant women could be due to depletion of folate stores. The normal range of values in pregnancy may differ, on the other hand, from the non-pregnant state.[23, 25, 40, 54-56, 65-69] Ball and Giles,[57] applying an observation of Waters and Mollin,[56] concluded that measurement of serum "labile" *L. casei* folate activity was more accurate in detecting megaloblastic anemia in pregnancy than measurement of total serum *L. casei* activity. Their interpretation has been questioned and requires confirmation.[43]

Serum vitamin B_{12} levels of < 100 pg./ml., using assay with *E. gracilis*, are generally accepted as indicative of vitamin B_{12} deficiency, except for falsely low values found in patients receiving sulfonamides and certain antibiotics.

Of the 77 patients in our megaloblastic group, 69 or 90% had initial *L. casei* serum folate activity of 4 ng./ml. or less and/or serum vitamin B_{12} levels of <100 pg./ml. Serum *L. casei* values decreased subsequently in seven of the remaining eight patients, although they were found to have megaloblastic changes when the initial microbiological values were normal. In other cases, the presence of low serum values preceded or coincided with the presence of megaloblastic changes. Thus, considering the megaloblastic group alone, there was excellent correlation of the low serum microbiological values and the morphological changes in the marrow.

In the normoblastic group, the incidence of low serum values was 37%. Thus, there was a considerable overlap of low folate and B_{12} levels between the two groups.

Although it has been suggested that *L. casei* whole blood or erythrocyte folate activity is a more accurate indicator of folate deficiency than *L. casei* serum folate activity,[44, 54, 58] this could not be confirmed in the 148 patients in whom these two assays were performed simultaneously. Many of the megaloblastic patients had red cell folate values of greater than 175 ng./ml. It is noteworthy that, in contrast to Addisonian pernicious anemia in relapse,[38] none of the megaloblastic patients with low serum B_{12} values had high serum and low red cell folate *L. casei* activity.

In a previous report, Lowenstein, Pick and Philpott[16] reported a deficient diet in 75% of patients with megaloblastic anemia and emphasized that this is not a homogeneous syndrome, but represents a heterogeneous group of anemias with a common manifestation: megaloblastosis in the marrow. The results of a therapeutic trial in the present series with small as well as large doses of folic acid and of vitamin B_{12} would seem to confirm this hypothesis. Although over two-thirds of patients with low serum folate values responded fully to short trials of small oral doses of folic acid, some failed to respond to these doses, some responded to moderate increase in dose, and although most responded to

large doses, a few failed to respond to massive doses of folic acid. The presence of low serum B_{12} values in addition to low *L. casei* values seemed to impair the response to daily oral doses of 0.4 mg. of folic acid in five patients. The data seem to militate against malabsorption as a cause for failure to respond, in that in the group receiving small doses there was a rise in the serum *L. casei* folate values in patients who showed no marrow improvement and, furthermore, there was a failure to respond to large parenteral doses in a few of the others. Failure of megaloblastic anemia to respond ante partum to large doses of folic acid has led some to suggest that impaired utilization of folate is of major etiological importance.[18, 21, 25, 26, 59, 60]

In the present cases, although impaired ability to utilize folate seemed important in a minority and may have been of lesser importance in others, most cases seemed to result from maternal nutritional deficiency, especially of folate which was not available in sufficient amount to meet the increased requirements of mother and fetus. Support for this view is afforded by the correlation of low microbiological values for folate with megaloblastic changes in the bone marrow and the satisfactory response of the bone marrow of most patients receiving small doses of folic acid. If our interpretation of the data gathered in this paper is correct, i.e. that a reduction of serum and/or erythrocyte *L. casei* folate values in the blood of pregnant women is indicative of maternal folate deficiency, it seems likely that such low values often precede the development of megaloblastic changes in the marrow and thus produce a considerable overlap between the normoblastic and megaloblastic cases. If further studies confirm this view, the determination of blood folate values may evolve as a convenient method for determining the incidence of folate deficiency in pregnant populations. In this regard, it should be noted that fetal folate requirements increase greatly during the last trimester, before which time megaloblastic anemia is rarely seen, and that folate stores may be depleted in a little over a month after normal individuals are placed on a folate-deficient diet. In view of the adequacy of vitamin B_{12} stores to meet the body needs for three to six years in a non-pregnant normal individual, it is difficult to explain the response of some patients to vitamin B_{12} and serum B_{12} levels of less than 100 pg./ml. observed in 25% of the megaloblastic patients in whom there was no evidence of protein malnutrition.

It is impossible from these observations to estimate the daily folate requirements in pregnancy. All of these patients were from a low-income group. In a separate nutritional study of patients from the same clinic population the daily average food iron and folate intakes were found to be suboptimal. In this respect, the better folate intake and the lower incidence of megaloblastic changes in the marrow of the Greek women are of interest. Most, but not all, patients in the megaloblastic group receiving 0.2-0.8 mg. folic acid daily for seven to 14 days exhibited satisfactory response. In the non-pregnant healthy subject, approximately 0.05 mg. of folate, as folic acid, seems to be the daily requirement.[52, 61] Less is known about food folate requirements. Hansen and Weinfeld[58] have described a non-pregnant patient with megaloblastic anemia due to folate deficiency who failed to respond to 0.1 mg. of folic acid daily, but who did respond to 0.2 mg. daily. The failure of some of our pregnant patients to respond to 0.4 mg. daily,[25] of Tasker's[3] pregnant Malayan women to respond optimally to 0.33 mg. daily, and of a patient of Pritchard's[62] to respond to 0.4 mg. daily suggests that, in the last trimester, the pregnant woman utilizes several-fold more folate than the non-pregnant subject.

In view of the demonstration that some antepartum women with megaloblastic bone marrow changes have varying degrees of impaired ability to utilize folate, the daily requirement of folate in pregnancy would seem best determined by investigating these requirements in sizeable groups of normal pregnant women.

SUMMARY AND CONCLUSIONS

The incidence of megaloblastic changes in the bone marrow of anemic antepartum women attending a public clinic in Montreal was 25%. The primary cause of the anemia in most of these patients was iron deficiency. The presence of megaloblastic changes in the bone marrow could not be accurately predicted from hematological findings in the blood.

Serum folate values of 4 ng./ml. or less and/or serum vitamin B_{12} levels of less than 100 pg./ml. correlated well with the development of megaloblastic bone marrow changes; low serum folate values were found in 80% and low serum B_{12} values in 25% of these patients. However, over one-third of patients with normoblastic bone marrow had similar low serum folate values. Red cell folate values did not correlate as well as serum folate values.

Serum folate values of 4 ng./ml. or less, as performed in our laboratory, probably are indicative of folate deficiency in pregnancy, and the development of these low values may precede the development of megaloblastic bone marrow changes.

When treated for short periods with small doses of folic acid, the megaloblastic bone marrow changes of most pregnant women, but not all, responded satisfactorily.

Mild to moderate folate deficiency develops commonly in this pregnant population.

The megaloblastic bone marrow changes in a minority of these women showed varying degrees of refractoriness to folic acid, suggesting impaired utilization of the administered vitamin.

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REFERENCES

1. WOODRUFF, A. W.: *Brit. Med. J.*, **1**: 1297, 1955.
2. TASKER, P. W. G.: *Trans. Roy. Soc. Trop. Med. Hyg.*, **53**: 291. 1959.
3. *Idem*: *Ibid.*, **54**: 578, 1960.
4. RYAN, B.: *Med. J. Aust.*, **2**: 89, 1961.
5. GOOSEN, C. J.: *J. Obstet. Gynaec. Brit. Comm.*, **68**: 994, 1961.
6. TASKER, P. W. G., RICHARDSON, A. M. AND LLEWELLYN-JONES, D.: *J. Obstet. Gynaec. Brit. Emp.*, **63**: 409, 1956.
7. FULLERTON, W. T. AND TURNER, A. G.: *Lancet*, **1**: 75, 1962.
8. MENON, M. K. K. AND RAJAN, L.: *Obstet. Gynec. Survey*, **17**: 812, 1962.
9. KARTHIGAINI, S., GUANASUNDARAM, D. AND BAKER, S. J.: *J. Obstet. Gynaec. Brit. Comm.*, **71**: 115, 1964.
10. HOO, S. T.: Megaloblastic anaemia in pregnancy, *In*: Transactions of the Second Asiatic Congress of Obstetrics and Gynaecology, Calcutta, January 23-25, 1962, **1**: 23, 1962.
11. BARUA, R. N. AND FOLL, C. V.: *J. Trop. Med. Hyg.*, **63**: 225, 1960.
12. DAVIDSON, L. S. P., DAVIS, L. J. AND INNES, J.: *Quart. J. Med.*, **11**: 19, 1942.
13. CALLENDER, S. T.: *J. Path. Bact.*, **58**: 586, 1946.
14. THOMPSON, R. B. AND UNGLEY, C. C.: *Quart. J. Med.*, **20**: 187, 1951.
15. CLARK, J. R.: *Edinb. Med. J.*, **59**: 274, 1952.
16. LOWENSTEIN, L., PICK, C. AND PHILPOTT, N. W.: *Amer. J. Obstet. Gynec.*, **70**: 1309, 1955.
17. FORSHAW, J. W. B.: *Postgrad. Med. J.*, **34**: 222, 1958.
18. GILES, C. AND SHUTTLEWORTH, E. M.: *Lancet*, **2**: 134, 1958.
19. GILES, C. AND BURTON, H.: *Brit. Med. J.*, **2**: 636, 1960.
20. MACKENZIE, A. AND ABBOTT, J.: *Ibid.*, **2**: 1114, 1960.
21. GATENBY, P. B. B. AND LILLIE, E. W.: *Ibid.*, **2**: 1111, 1960.
22. LAYRISSE, M. *et al.*: *Blood*, **15**: 724, 1960.
23. LOWENSTEIN, L. *et al.*: Nutritional deficiency and anaemia in pregnancy, *In*: Proceedings of the Eighth Congress of the European Society of Haematology, Vienna, 1961, S. Karger, Basel, 1962, p. 337.
24. DE LEEUW, N. K. M., LOWENSTEIN, L. AND HSIEH, Y. S.: Iron deficiency and hydremia in normal pregnancy, *Medicine (Balt.)*, To be published.
25. LOWENSTEIN, L. *et al.*: The relation of erythrocyte and serum *L. casei* folate activity to folate deficiency in certain megaloblastic anaemias, *In*: Proceedings of the Ninth Congress of the European Society of Haematology, Lisbon, 1963, S. Karger, Basel, 1963, p. 364.
26. FORSHAW, J. W. B. *et al.*: *J. Obstet. Gynaec. Brit. Emp.*, **64**: 255, 1957.
27. ADAMS, E. B.: *Brit. Med. J.*, **2**: 398, 1956.
28. ADAMS, E. B. AND FORBES, P. F. V.: *South Afr. J. Lab. Clin. Med.*, **3**: 291, 1957.
29. MOORE, H. C., LILLIE, E. W. AND GATENBY, P. B. B.: *Irish J. Med. Sci.*, **6**: 106, 1955.
30. BERRY, C. G.: *Brit. Med. J.*, **2**: 819, 1955.
31. METZ, J., BRANDT, V. AND STEVENS, K.: *Ibid.*, **1**: 24, 1962.
32. TASKER, P. W. G., MOLLIN, D. L. AND BERRIMAN, H.: *Brit. J. Haemat.*, **4**: 167, 1958.
33. COOPER, B. A. AND LOWENSTEIN, L.: *Canad. Med. Ass. J.*, **85**: 987, 1961.
34. LOWENSTEIN, L. *et al.*: *Amer. J. Clin. Nutr.*, **8**: 265, 1960.
35. RATH, C. E. AND FINCH, C. A.: *J. Lab. Clin. Med.*, **33**: 81, 1948.
36. STEVENS, A. R., JR., COLEMAN, D. H. AND FINCH, C. A.: *Ann. Intern. Med.*, **38**: 199, 1953.
37. LOWENSTEIN, L. AND BRAMLAGE, C. A.: *Blood*, **12**: 261, 1957.
38. COOPER, B. A. AND LOWENSTEIN, L.: *Ibid.*, **24**: 502, 1964.
39. MOLLIN, D. L. AND ROSS, G. I. M.: *J. Clin. Path.*, **5**: 129, 1952.
40. LOWENSTEIN, L. *et al.*: *Postgrad. Med.*, **31**: 72, 1962.
41. DAWSON, D. W.: *J. Obstet. Gynaec. Brit. Comm.*, **69**: 38, 1962.
42. CHANARIN, I., ROTHMAN, D. AND BERRY, V.: *Brit. Med. J.*, **1**: 480, 1965.
43. CHANARIN, I. *et al.*: *Brit. J. Haemat.*, **11**: 557, 1965.
44. HANSEN, H. A.: On the diagnosis of folic acid deficiency: a methodological and clinical study with special reference to the incidence and etiology of megaloblastic anaemias in pregnancy and puerperium, Amqvist & Wiksells Boktryckeri, Stockholm, 1964.
45. HERBERT, V.: *Med. Clin. N. Amer.*, **46**: 1365, 1962.
46. *Idem*: *New Eng. J. Med.*, **268**: 201, 368, 1963.
47. GILES, C.: *Lancet*, **2**: 1063, 1960.
48. IZAK, G. *et al.*: *A.M.A. Arch. Intern. Med.*, **99**: 346, 1957.
49. FRANCIS, H. H. AND SCOTT, J. S.: *Lancet*, **2**: 1033, 1959.
50. COOPER, B. A. AND LOWENSTEIN, L.: Vitamin B_{12}-folate interrelationship in megaloblastic anaemia, *Brit. J. Haemat.*, To be published.
51. BAKER, H. *et al.*: *Clin. Chem.*, **5**: 275, 1959.
52. HERBERT, V.: *Trans. Ass. Amer. Physicians*, **75**: 307, 1962.
53. *Idem*: *Arch. Intern. Med. (Chicago)*, **110**: 649, 1962.
54. IZAK, G. *et al.*: *Amer. J. Clin. Nutr.*, **9**: 473, 1961.
55. BAKER, H. *et al.*: *Nature (London)*, **191**: 78, 1961.
56. WATERS, A. H. AND MOLLIN, D. L.: The folic acid activity of serum in normal subjects and patients with megaloblastic anaemia, *In*: Proceedings of the Eighth Congress of the European Society of Haematology, Vienna, 1961. S. Karger, Basel, 1962. p. 332.
57. BALL, E. W. AND GILES, C.: *J. Clin. Path.*, **17**: 165, 1964.
58. HANSEN, H. A. AND WEINFELD, A.: *Acta Med. Scand.*, **172**: 427, 1962.
59. BADENOCH, J. *et al.*: *Brit. Med. J.* **1**: 1245, 1955.
60. GIRDWOOD, R. H.: *Quart. J. Med.*, **25**: 87, 1956.
61. HERBERT, V.: *Med. Clin. N. Amer.*, **46**: 1365, 1962.
62. PRITCHARD, J. A.: *Amer. J. Obstet. Gynec.*, **83**: 1004, 1962.
63. HERBERT, V.: *J. Clin. Invest.*, **40**: 81, 1961.
64. GROSSOWICZ, N. *et al.*: *Proc. Soc. Exp. Biol. Med.*, **109**: 770, 1962.
65. SOLOMONS, E. *et al.*: *J. Obstet. Gynaec. Brit. Comm.*, **69**: 724, 1962.
66. HANSEN, H. A. AND KLEWESAHL-PALM, H. V.: *Scand. J. Clin. Lab. Invest.*, **15**: (Suppl. 69): 78. 1963.
67. BAKER, H. *et al.*: *Brit. Med. J.*, **1**: 978, 1958.
68. BALL, E. W. AND GILES, C.: *Lancet*, **1**: 951, 1962 (abstract).
69. HERBERT, V. *et al.*: *Blood*, **15**: 228, 1960.

PAGES OUT OF THE PAST: FROM THE JOURNAL OF FIFTY YEARS AGO

CONFOUND THOSE GRITS

In 1874 I was summoned to Sir John A. Macdonald, in the Commons, where he was suddenly seized with acute pain in the region of the left kidney, which proved to be due to the passage of a renal calculus to the bladder. The pain was so intense that he was removed to a room in the left block of Parliament Buildings, a hypodermic administered and perfect rest enjoined. After some hours the difficulty was removed, and a normal state of the system was restored. The following day Sir John asked me what this kidney stone really was, when I stated, "A small gritty calculus." At once Sir John replied, "Confound those Grits, I knew they would be the death of me some day."

In 1873 I was called to Stadacona Hall, and on arrival found Sir John had a slight cold. I was invited into his studio, where he sat in a large armchair, warming his feet by the fire and reading a book in which I noted a yellow marker. This, Sir John drew out, and asked me to read. It was a cable received the night previous from Grenfell, London, England, stating that arrangements had been completed for construction of the Canadian Pacific Railway. "Well," said Sir John, "after such a cable, I thought the best thing I could do this Sunday morning was to read my Bible, and thank God for what He had done for Canada."

In July 1860, I was summoned to an accident in Cumberland, Ontario, by a messenger on horseback. A farmer crossing his fence, carrying a large scythe on his shoulder, had accidentally punctured an artery, and blood was flowing freely. At 2 a.m., raining cats and dogs, I mounted my horse, and off we set a distance of twenty-five miles. On arrival I found the thigh fortunately tightly tied by a pocket handkerchief, and the wound in the popliteal region tightly packed with softened tobacco. This I at once removed, washed the wound, and found the popliteal artery had been seriously punctured by the scythe in crossing the fence. I at once secured the best light possible—a strip of woollen cloth saturated in sweet oil in a small cup, in fact the old Roman lamp. To save life, and without delay, I found it necessary to ligate the femoral artery, and slackened the compress when bleeding from the wound was still active. The compress was at once replaced. I examined the femoral artery carefully, and was obliged to ligate it higher up owing to an abnormal subdivision of the artery. The compress was then removed, and there was no return of bleeding from the popliteal wound. The parts were carefully dressed, and the case placed under the charge of a local surgeon. In a few weeks an excellent recovery followed. In those days country roads were not inviting and household effects were not encouraging, and yet it is surprising how life is saved, "roughing it in the bush".—J. Grant, *Canad. Med. Ass. J.*, 6: 302, 1916.