FOLINIC ACID IN MEGALOBLASTIC ANAEMIA OF PREGNANCY

BY

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Within the past ten years treatment of the megaloblastic anaemia of pregnancy has been completely revolutionized. Before Vilter *et al.* (1945), in America, and Wilkinson *et al.* (1946), in this country, published their preliminary report on the haemopoietic properties of folic acid, this pregnancy anaemia presented a vexing problem. A large number of the patients did not respond to parenteral liver, even crude preparations (Ungley, 1938; Miller and Studdert, 1942; Fullerton, 1943; Callender, 1944; Davidson *et al.*, 1948; Ginsberg *et al.*, 1950; Scott, 1954), and, on account of the gastro-intestinal upsets frequently encountered with this anaemia, few could take oral liver in sufficient quantity to produce a response (Scott, 1954). As a result many patients were labelled "refractory" and the majority had to be kept alive by repeated blood transfusions until delivery.

Folic acid has completely reversed this picture. Since supplies became available an average of 15 to 20 patients at this hospital each year have received this form of therapy, and all have responded well, provided treatment was continued throughout pregnancy and during the first few weeks of the puerperium.

In itself the anaemia is of particular interest not only to those dealing with the pregnant patient but to haematologists in general. It has been shown that the response to parenteral liver and vitamin B_{12} is very variable and seems to depend on the degree of megaloblastic change in the marrow and the extent of the maturation arrest (Scott, 1954). In contrast to this the specific effect of folic acid gives definition to the field of inquiry and allows for a more accurate determination of the place occupied by folic acid and its derivatives in the hierarchy of enzymes necessary for erythropoiesis.

Recent work (Callender and Lajtha, 1951; Swan *et al.*, 1955) indicates that folinic acid ("leucovorin"), derived from folic acid, may be the actual enzyme acting upon the marrow. When supplies of this substance became available it was decided to institute clinical trials in pernicious anaemia of pregnancy; the results were rather startling.

Materials and Methods

In all, 19 patients were given folinic acid. Seventeen were diagnosed antenatally, but four went into labour before treatment could be followed for any length of time. In the remaining two cases the anaemia was not spotted until the puerperium, and it is interesting to note that both these patients had pre-eclamptic toxaemia.

In six of the cases megaloblasts were found in the peripheral blood and diagnosis was easy, but in the remaining 13 a confirmatory sternal puncture had to be made. Smears and sections were stained by haematoxylin and eosin and May–Grünwald solution and also examined for iron by the prussian-blue reaction. During the reticulocyte response daily reticulocyte counts were made and the blood picture was examined thereafter at weekly intervals. A gastric analysis was carried out in 13 of the cases, the histamine test meal being used. Serum iron readings (Sven Dahl, 1948) were made in another four cases.

Results

All the patients had severe anaemia with haemoglobins ranging between 3.6 and 9.3 g. per 100 ml. and red cell counts between 800,000 and 3,260,000 per c.mm. The blood pictures were macrocytic in five instances, normocytic in 13, and microcytic in one. All but one of the pregnancies were complicated by some other condition. Three of the patients had an infection requiring treatment with antibiotics, and the anaemia developed subsequent to this. Cardiac disease, pre-eclamptic toxaemia, and diarrhoea and vomiting accounted for the other complications, and in addition to this there were four cases of twin pregnancy. Before starting treatment a gastric analysis was made on 13 of the cases in order to rule out, so far as was possible, an Addisonian pernicious anaemia, which, in contrast to the megaloblastic anaemia of pregnancy, shows a histamine-fast achlorhydria and responds well to vitamin B_{12} (Scott, 1954). In the present series six patients had a normal curve and six were hypochlorhydric. Only one patient had a histamine-fast achlorhydria, and when the test meal was repeated after delivery she was found to have a limited amount of free acid.

At the beginning of the trial supplies of folinic acid were scarce, and once the initial response to treatment had been observed the patients were dismissed home on a maintenance dose of folic acid. The fourth patient, however, lost her prescription for folic acid, and when she reported to the blood clinic it was found that her blood count had continued to improve and was near normal, though no further treatment had been given. This woman was 33 weeks pregnant when she first received a total of 36 mg. of folinic acid intramuscularly. At that time her haemoglobin had been 8.0 g. per 100 ml. and red cell count 2,120,000, yet seven weeks later at delivery her haemoglobin was 10.6 g. per 100 ml. and red cell count 3,280,000 (Fig. 1). In the puerperium she continued to improve and no further treatment was required apart from iron therapy.

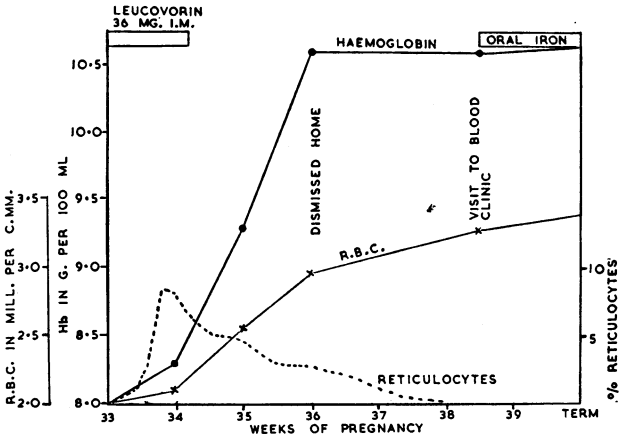

FIG. 1.—Case 4. A para-3 aged 20. Effect of treatment on haemoglobin, red blood cells, and reticulocytes.

This remarkable response to such a small amount of folinic acid encouraged us to withhold treatment in all subsequent cases, once they had obtained their maximum reticulocyte response. In all, 13 cases were followed. Eight were antenatal patients, their pregnancies ranging from 28 to 39 weeks, and the remaining five were in the puerperium. By varying the amount of folinic acid administered daily in each case and watching the reticulocyte response, it was found that the optimum dosage lay somewhere in the region of 6 mg., and in the last 10 cases the following regime was adopted. On the first day of treatment 12 mg. of folinic acid was administered intramuscularly. Thereafter daily injections of 6 mg. were given until the reticulocytes reached a peak, usually between 8 and 19%. This commonly occurred around the eighth day, when just over 50 mg. had

been injected. These reticulocyte levels may seem low in comparison with those obtained in Addisonian pernicious anaemia, but it has been shown already (Scott, 1954) that this is the usual finding in pernicious anaemia of pregnancy. Commonly it occurs around the eighth day and falls gradually thereafter, normal levels not being reached until the fourth week.

In the present series no further treatment was given once the reticulocyte peak had been reached, yet all 13 patients continued to improve. At term the antenatal patients had an average haemoglobin of 10.4 g. per 100 ml. and a red cell count of 3,350,000 (see Table), and two

Results Before Therapy and at Term in Antenatal Anaemias

Case No.	Initial Hb (g./100 ml.)	Initial R.B.C. (mill./ c.mm.)	Weeks Pregnant	Folinic Acid Total Dose (mg.)	Hb at Term (g./100 ml.)	R.B.C. at Term (mill./ c.mm.)
4	8·0	2·12	33	36	10·6	3·28
6	5·7	1·57	32	45	11·9	3·56
7	7·0	2·50	28	48	11·6	3·77
11	7·5	2·56	39	48	7·1	2·47*
12	7·9	2·90	36	54	9·0	3·25
14	7·5	2·24	34	48	12·3	4·40
15	7·7	1·75	37	54	10·5	3·10
16	5·4	1·29	30	54 48†	10·3	2·96
17	9·1	2·21	30	50	12·1	3·95
18	6·6	1·8	35	50	10·6	3·45
19	8·8	3·26	29	50	12·2	3·95

* Lower levels due to blood volume increase commonly found at this stage of therapy.
† Second course given; showed no secondary reticulocytosis.

months after delivery the average for the whole series was 12.7 g. per 100 ml. and 4,430,000. The latter figures do not include the results for two antenatal patients who discharged themselves irregularly from hospital after delivery. Seven of the patients reported six months after delivery. All were well and had normal blood counts.

At this stage in the investigation an oral form of folinic acid (calcium leucovorin) became available, and the next three patients (Cases 17, 18, and 19) received this form of therapy, the dosage being left unchanged. On the first day two tablets (10 mg.) were given half an hour before breakfast, and on subsequent days this was reduced to 5 mg. All three were antenatal cases, their pregnancies ranging from 29 to 35 weeks. Each received a total of approximately 50 mg., and without exception all three showed responses equal to those obtained with intramuscular therapy. As before, improvement continued though therapy was discontinued. The results are shown in the Table.

Serum Iron Investigations

During the reticulocyte response daily estimations of serum iron were made on four of the patients, and the results are shown in Fig. 2. It can be seen that the earliest

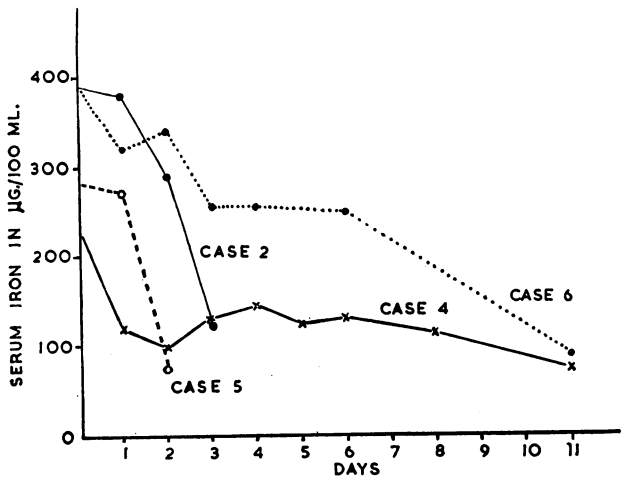

FIG. 2.—Serum iron curves in four cases.

fall in serum iron occurred in the patient with the lowest reading (Case 4). Case 5 went into labour on the second day of therapy, and this was probably responsible for the sudden drop shown by her the next morning (Scott, 1954). The remaining two patients had very much higher readings and their serum iron values fell less steeply. Case 6 had the largest amount of stainable iron in her marrow, and, though she showed a slight initial drop in her serum iron, it remained well above normal levels during the first week of therapy.

Hawkins (1955) stated that the 48-hour serum iron test was a reliable method of assessing the effect of haematinics in the macrocytic anaemias. This is possibly true in the majority of cases, but it would appear that in pregnancy at least the exception can arise. The rate of fall would also appear to be proportional to the initial serum iron reading, and this in turn seems to bear a direct relationship to the amount of stainable iron in the marrow.

Discussion

It is now generally agreed that the megaloblastic anaemia of pregnancy represents only a temporary upset in erythropoiesis and that specific antianaemic therapy can usually be discontinued in the puerperium once the blood count has reached normal levels. Until recently this has been the procedure adopted with all such anaemias in this hospital, but the results with folinic acid now show that prolonged therapy is no longer necessary.

From the review of the findings two salient features become evident. Firstly, it was found that folinic acid had to be given in sufficiently large amounts initially to produce a satisfactory response. Trial and error showed that the required dosage lay somewhere in the region of 6 mg. daily intramuscularly and that a total of 50 mg. had usually to be given. For example, Case 3 received only 12 mg., and 16 days later she showed a secondary reticulocyte rise of 5% when maintenance therapy with folic acid was begun. On the other hand, no such secondary rise in reticulocytes was observed with folic acid in those patients given adequate amounts of folinic acid initially.

The second point is that once a satisfactory reticulocyte response was obtained folinic acid could be discontinued. Independent of the degree of anaemia, 13 patients in the present series continued to improve, and repeat sternal punctures, carried out in three instances, showed that erythropoiesis was again normoblastic.

This being so, it seems unlikely that the megaloblastic anaemia of pregnancy can be due to an absolute deficiency of folic acid arising out of the pregnancy. One would expect that, if such were the case, all eight antenatal patients given short-term therapy would have required maintenance treatment until delivery at least. A likelier suggestion is that folinic acid acts more as a catalyst at some stage in erythropoiesis, and, owing to its absence or to the "resistance" which Badenoch *et al.* (1955) say may develop in pregnancy, megaloblastic change takes place in the marrow. It is conceivable that the reversal of such a process might require a large amount of catalyst initially, and this would also explain why all 16 cases in the present series needed fairly large doses of folinic acid to begin with and why the total amount given seemed to be independent of the degree of anaemia.

It is more difficult, however, to ascertain whether the initial defect on haemopoiesis is caused by a temporary deficiency of folinic acid or whether an actual "resistance" does develop in pregnancy. Thompson (1950) and Lajtha (1950) believe that there may be an inhibitor present in Addisonian pernicious anaemia, but Feinmann *et al.* (1952) and Swan *et al.* (1955) were unable to find any evidence to support this thesis. Until proof of its existence in the pregnancy anaemia is forthcoming, the simpler explanation of a temporary deficiency seems the more logical and could be supported by the fact that the anaemia occurs more readily in multiple pregnancy. In the present series four

patients were delivered of twins. Other factors, too, would seem to aggravate or even precipitate the onset of the anaemia: diarrhoea and vomiting, debility from repeated pregnancies, infection, pre-eclamptic toxaemia, and cardiac disease were found in that order in the present series. Only one patient appeared to have had a normal pregnancy, and she had been treated for an iron-deficiency anaemia at the seventh month. With hospitalization and treatment it was possible to deal with these secondary complications and in this way remove any factors which might possibly enhance a deficiency.

Another aggravating factor which may also increase the demand for folinic acid once the upset in erythropoiesis has been established is that of more rapid blood destruction. We have been unable to demonstrate the existence of an actual haemolysin in the megaloblastic anaemia of pregnancy, but in all the buffy coats examined there is evidence of erythrophagocytosis, and Pappenheimer bodies, similar to those seen in haemolytic anaemias, are present in some of the nucleated red cells. Ungley and Thompson (1950) reported a more rapid elimination of transfused cells in one of their cases and severe haemolysis in another. With folic acid therapy they found that the haemolysis ceased and the rate of elimination of transfused cells fell to normal. In our cases the erythrophagocytosis disappeared. These findings suggest a possible increase in the rate of red cell destruction, though obviously it is not present to the same degree in all cases. Since treatment appears to reduce this process, one would expect that replacement with new cells would also fall to normal and, in this respect at least, the need for extra folic or folinic acid would be less.

Thus with treatment for both the anaemia and its complications it is possible that the demand for folinic acid may be somewhat reduced, and, with normoblastic erythropoiesis established, the pregnant woman may once more be able to keep herself in balance and maintain satisfactory blood levels. From what has been said previously, however, it is unlikely that the initial effect of folinic acid is purely one of replacement therapy. The evidence rather points to some catalytic action, and it may be that folinic acid has multiple duties, not only associated with the bone marrow, but also with those processes concerned with the production and utilization of the whole folic acid system of enzymes.

Summary

Nineteen patients with megaloblastic anaemia of pregnancy and the puerperium have been treated with folinic acid (" leucovorin ") and the results have been reviewed.

Sixteen received treatment over a limited period till the reticulocyte response reached its peak.

Eleven of these were antenatal anaemias, and though their treatment was discontinued all continued to improve. At term their average haemoglobin was 10.4 g. per 100 ml. and red cell count 3,350,000 per c.mm.

In order to produce a satisfactory response with this short-term therapy, it was found that the folinic acid had to be given in sufficiently large amounts initially (approximately 50 mg.) and that the amount necessary appeared to be independent of the degree of anaemia and the route of administration.

From these findings it seems unlikely that the megaloblastic anaemia of pregnancy is due to an absolute deficiency of folinic acid. Instead the latter would appear to act more as a catalyst at some stage in the haemopoietic process, and evidence is brought forward to show how the demand for folinic acid itself may be further reduced once normal erythropoiesis is established.

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RECTAL HYDROCORTISONE

BY

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Local administration of hydrocortisone in the treatment of ulcerative colitis was mentioned by Truelove and Witts (1955) and by Park (1955). For this purpose the steroid may be given as an ester—acetate or hemisuccinate—or as the free alcohol. The esters may well be inactive until hydrolysed to free steroid, and it is not known whether the large bowel contains suitable enzymes. Enemata of hydrocortisone and its esters could be dispensed as solutions or suspensions, but the free steroid is only sparingly soluble in water. Truelove (1956) used a solution containing 50 mg. of hydrocortisone per 100 ml. of 4.5% alcohol in saline; apparently this concentration of alcohol did not cause any local discomfort, but we preferred to avoid any solution that might aggravate the inflammation of the colonic mucosa. As 1% solutions of hydrocortisone have been used extensively in dermatological practice and the solvent does not seem to irritate acutely inflamed skin, we have used diluted 1% hydrocortisone skin lotion for these studies. A further aspect of the problem about which there is very little information is the extent to which hydrocortisone is absorbed from the bowel. Liddle (1956) has reported 26% absorption of hydrocortisone acetate administered per rectum as suppositories or in an ointment.

We have studied the absorption of various preparations of hydrocortisone administered locally in five patients with ulcerative colitis. We are not in a position to offer any opinion about the value of this form of treatment; this report is concerned mainly with its endocrine implications.

Methods.—The patients were men with mild or moderately severe ulcerative colitis, thought to be suitable for steroid therapy. Daily urine collections were made from 6 a.m. to 6 a.m. Excretion of hydrocortisone and its metabolites was estimated by the total 17-