Section of Pathology

President—Professor J. W. S. BLACKLOCK, M.D.

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Folic Acid Deficiency and the Megaloblastic Anæmias

By I. CHANARIN,[1] M.D., D.C.P., D. L. MOLLIN,[2] M.B., and B. B. ANDERSON, B.Sc.
London

MEGALOBLASTIC anæmia is the consequence of deficiency or interference with utilization of vitamin B_{12} or folic acid. The ætiology of those megaloblastic anæmias which respond completely to treatment with vitamin B_{12} is fairly simple: it is due to deficiency of vitamin B_{12} and this is almost invariably due to a failure of its absorption; rarely to an inadequate dietary intake. The megaloblastic anæmias which we shall discuss do not respond adequately to vitamin B_{12}, and folic acid is required in their treatment.

The conditions with which these megaloblastic anæmias have been associated are shown in Table I. Of these, only two occur with any degree of frequency in temperate climates,

TABLE I.—MEGALOBLASTIC ANÆMIA WHICH REQUIRES TREATMENT WITH FOLIC ACID OCCURS IN

1. Idiopathic steatorrhœa	5. Cirrhosis
2. Pregnancy	6. Tropical sprue
3. Anticonvulsant treatment of epilepsy	7. Nutritional megaloblastic anæmia
4. Leukæmia and related disorders	8. Kwashiorkor

namely the megaloblastic anæmia of pregnancy and the megaloblastic anæmia associated with idiopathic steatorrhœa. Megaloblastic anæmia requiring folic acid is far more common in tropical and semi-tropical countries, and again is often associated with pregnancy and with sprue.

The study of vitamin B_{12} in this connexion has been simplified by the introduction of two techniques. The first is a method for the assay of the vitamin B_{12} content of serum as a result of which B_{12} deficiency could be assessed (Ross, 1950; Rosenthal and Sarett, 1952; Mollin and Ross, 1952); the second is the introduction of methods for the study of the absorption of vitamin B_{12} (Heinle *et al.*, 1952).

The purpose of this paper is to describe methods serving a similar function in relation to folic acid. The first is concerned with the assessment of folic acid deficiency. The second is concerned with the measurement of folic acid absorption. I shall then review these techniques in relation to the various groups of megaloblastic anæmia.

ASSESSMENT OF FOLIC ACID DEFICIENCY

Folic acid deficiency is assumed to be present if a patient with a megaloblastic anæmia has a normal serum B_{12} level or if treatment with vitamin B_{12} fails to produce an adequate response. A subsequent response to folic acid is usually considered to indicate folic acid deficiency. This ignores the possibility that such a megaloblastic anæmia may be due, not to folic acid deficiency, but to interference with utilization of this vitamin, and the best-known example of this group is the megaloblastic anæmia due to the use of anticonvulsant drugs.

The plasma clearance of folic acid.—We have attempted to assess folic acid deficiency by the rate of clearance from the plasma of an intravenous dose of folic acid (Chanarin, Mollin and Anderson, 1958). The dose we used was 15 μg./kg. body weight. A person weighing 70 kilograms, therefore, received 1 mg. of folic acid. This was given intravenously and a blood sample collected three minutes, fifteen minutes and thirty minutes after the injection. The folic acid content of the serum of these samples was estimated by microbiological assay using a mutant of *Streptococcus fæcalis* as the test organism (Jukes, 1955; Chanarin, Anderson and Mollin, 1958).

The clearance in normal subjects.—The results of such a clearance test in 50 control subjects are shown in Fig. 1.

The serum folic acid concentration three minutes after the injection ranged from 75 μmg.–186 μmg./ml. (mean 127 ± 14 μmg.). It fell to 21 μmg.–80 μmg./ml. at fifteen minutes (mean 40 ± 2 μmg.) and to 4 μmg.–49 μmg./ml. at thirty minutes (mean 20 ± 9 μmg.).

[1]Joseph Senior White Research Fellow, Royal College of Physicians.
[2]In receipt of a grant from the Medical Research Council.

There are two phases in the normal clearance of injected folic acid, for, if at three minutes all the injected folic acid was still in the plasma the calculated serum level would be over 300 μmg./ml. The mean level was, in fact, 127 μmg./ml. 60% of the injected dose has, therefore, been cleared from the plasma in the first three minutes after the injection. The subsequent rate of clearance was slower. The first rapid phase represents immediate uptake of folic acid by tissues, little of this dose being excreted by the kidneys (Spray *et al.*, 1951; Chanarin, 1957). The tissues which are normally "unsaturated" for folic acid become "saturated" in this phase so that the rest of the folic acid is then removed more slowly.

If this degree of "unsaturation" of the tissues for folic acid is reduced—and this can be done by giving the subject some folic acid twenty-four hours before the clearance test—then the three-minute serum level is considerably higher, and the subsequent rate of clearance slower.

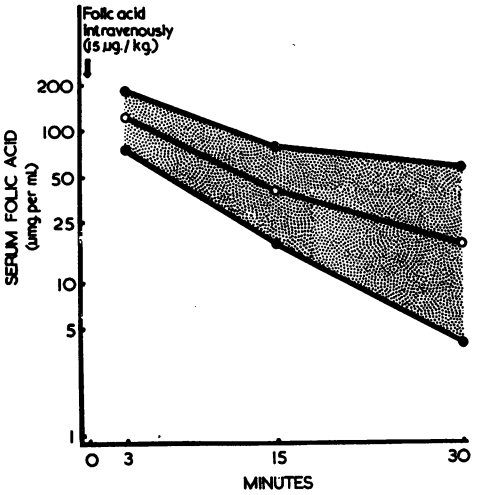

FIG. 1.—The plasma clearance of intravenous folic acid in 50 normal subjects.

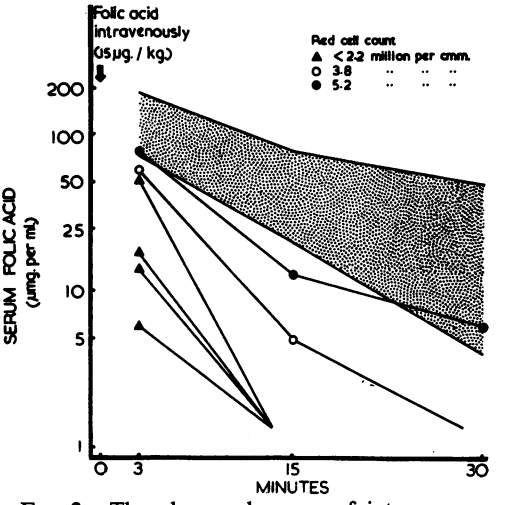

FIG. 2.—The plasma clearance of intravenous folic acid in 6 patients with untreated megaloblastic anæmia associated with idiopathic steatorrhœa and impaired absorption of folic acid. The stippled area represents the range of clearance in normal subjects.

The clearance in folic acid deficiency.—In patients with a megaloblastic anæmia in whom folic acid deficiency could be anticipated, i.e. patients with idiopathic steatorrhœa and impaired absorption of folic acid, a different pattern of folic acid clearance was found. This is shown in Fig. 2 which compares the clearance found in 50 normal subjects (stippled area) with the rate of clearance in 6 such patients.

The rate of clearance is more rapid than normal in all the patients irrespective of the degree of anæmia. In the most anæmic patients (those with red cell counts of less than 2·2 million per c.mm.) the serum folic acid concentration three minutes after the injection was lower than in normal subjects, and there was no measurable folic acid at fifteen minutes. In patients with very little anæmia and minimal megaloblastic change the rate of clearance may be less rapid. In these patients the three-minute serum folic acid level is usually either normal or slightly below the normal range; the fifteen-minute serum level is always reduced; and there is usually no folic acid in the thirty-minute sample.

These results indicate that this test can be used to detect folic acid deficiency. An increased rate of clearance of injected folic acid indicates an increase in the degree of "unsaturation" of the tissues for folic acid—in other words, folic acid deficiency.

The Absorption of Folic Acid

The absorption of folic acid has been assessed by giving a small oral dose of the vitamin and measuring the changes in serum concentration over the next few hours (Chanarin, Anderson and Mollin, 1958). The type of pattern found is shown in Fig. 3. As the folic acid is absorbed there is a rise in the serum level of the vitamin with a peak concentration at about one to two hours.

The results of such an absorption test in 55 normal subjects are shown in Fig. 4. The oral dose of folic acid used was 3·0 mg. In all subjects the peak serum level was greater than 40 μmg./ml. serum and the mean peak concentration in the whole group was 96 ± 4 μmg./ml. serum. Failure to attain a serum level of 40 μmg./ml. we regard as evidence of impaired absorption of folic acid.

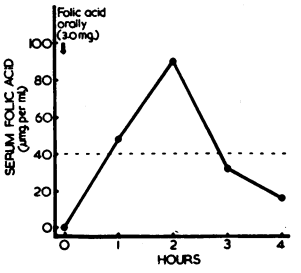

FIG. 3.—Serum folic acid concentration in a normal subject after an oral dose of 3·0 mg. of folic acid.

FIG. 3.

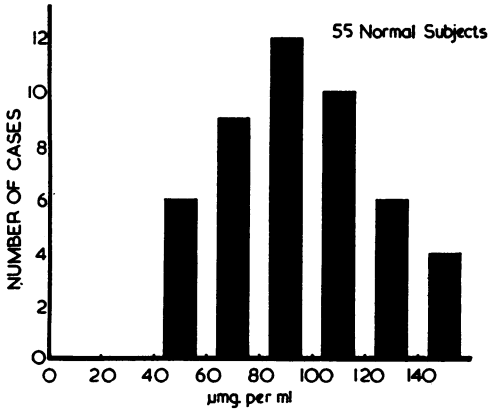

FIG. 4.—The peak serum folic acid concentration in 55 normal subjects after an oral dose of 3·0 mg. of folic acid.

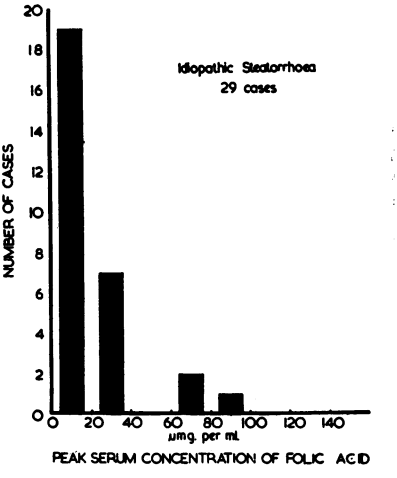

FIG. 5.—The peak serum folic acid concentration, in 29 patients with idiopathic steatorrhœa, after an oral dose of 3·0 mg. of folic acid.

RESULTS IN THE MEGALOBLASTIC ANÆMIAS

Megaloblastic Anæmia Associated with Idiopathic Steatorrhœa

The absorption of folic acid.—The results of a folic acid absorption test in 29 patients with idiopathic steatorrhœa, 19 of whom had a megaloblastic anæmia, are shown in Fig. 5. In 26 patients the peak serum level after the oral dose was subnormal (<40 μmg./ml.) suggesting that there was impaired absorption of the vitamin.

The other 3 patients had peak folic acid concentrations within the normal range and were therefore presumably able to absorb folic acid in a normal manner. All 3 had a mild megaloblastic anæmia which was not due to vitamin B_{12} deficiency.

These results either imply that the absorption test was insufficiently sensitive to detect impaired absorption of folic acid in these 3 patients, or that the megaloblastic anæmia in these patients was due to some cause other than deficiency of folic acid associated with malabsorption of the vitamin. The results of the folic acid clearance test in these 2 groups suggest that the latter alternative may be correct.

The clearance of folic acid.—The results of a folic acid clearance test in 6 patients who were unable to absorb folic acid normally are given in Fig. 2. The injected folic acid was cleared more rapidly than normal in all, irrespective of the degree of anæmia. These rapid clearances appear to confirm folic acid deficiency as the cause of the anæmia and this deficiency is presumably due to impaired absorption of the vitamin as suggested by Girdwood (1953).

On the other hand the folic acid clearance test was normal in 2 of the 3 patients who also absorbed folic acid normally. Nevertheless these 2 patients had a megaloblastic anæmia which required folic acid. It seems probable that this megaloblastic anæmia is not due to folic acid deficiency, but to an interference with its utilization, perhaps by antagonists. The abnormal intestinal bacterial flora often present in these patients may well provide a source for such folic acid antagonists.

Megaloblastic Anæmia Associated with the Use of Anticonvulsant Drugs

A group of megaloblastic anæmias which are undoubtedly caused by drugs used in the treatment of epilepsy (Badenoch, 1954; Hawkins and Meynell, 1954; Fuld and Moorhouse, 1956; Hobson *et al.*, 1956) might be considered at this point.

We have studied 2 patients with severe megaloblastic anæmia following the use of primidone (Chanarin, Elmes and Mollin, 1958), and one following barbiturate therapy. In all three the clearance of intravenous folic acid was normal. This would seem to exclude any significant degree of folic acid deficiency in these patients and suggests that the anæmia stems from interference by the drug with the utilization of available folic acid.

On the other hand, 2 patients who developed severe megaloblastic anæmia while receiving phenytoin sodium as well as 3 patients who received various combinations of these drugs cleared injected folic acid very rapidly. In these patients the anæmia was associated with folic acid deficiency. All, however, require treatment with folic acid.

Megaloblastic Anæmia of Pregnancy

Megaloblastic anæmia associated with pregnancy is probably the commonest form of megaloblastic anæmia requiring treatment with folic acid. The data provided by Goodall (1957) suggests that its incidence in this country may even be as high as 1% of all pregnancies.

The folic acid clearance was extremely rapid in all the patients we have studied, being as rapid as in severe megaloblastic anæmia associated with malabsorption of folic acid. The anæmia is therefore a folic acid deficiency state. At least three factors should be considered: (1) the absorption of folic acid, (2) the folic acid requirements of the fœtus, and (3) the dietary intake of folic acid.

(1) *The absorption of folic acid.*—Pregnant patients do not absorb folic acid as well as control subjects (Chanarin *et al.*, 1959). The mean peak serum folic acid concentration in 23 pregnant subjects after an oral dose of folic acid was 48 μmg./ml. as compared with 96 μmg./ml. in the normal control group (Fig. 6). However, the absorption of folic acid in 7 patients with treated megaloblastic anæmia of pregnancy was no different from that found in the pregnant controls, so that this factor alone does not produce the anæmia.

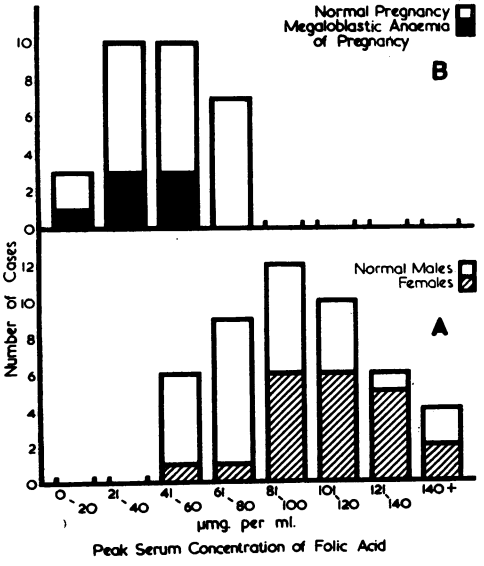

FIG. 6.—The peak serum folic acid concentration in 23 normal pregnant subjects and 7 patients with megaloblastic anæmia of pregnancy who had been treated with folic acid. The results in 55 control subjects are also given.

(2) *The folic acid requirements of the growing fœtus.*—Megaloblastic anæmia appears most frequently during the last two months of pregnancy. This is also the time of most rapid growth of the fœtus. Furthermore in twin pregnancy the incidence of megaloblastic anæmia is ten times as high as in single pregnancy.

Folic acid requirements in normal pregnancy were studied by following the clearance of injected folic acid in these patients (Chanarin, MacGibbon, Mollin and O'Sullivan, 1959). A moderately rapid clearance of injected folic acid is common in the last two months of pregnancy. The rate of clearance is most rapid in patients with twin pregnancy where it may be similar to the clearance in patients with megaloblastic anæmia due to folic acid deficiency. It seems reasonable to attribute these results to the increased requirements for folic acid by the growing fœtus.

(3) *The dietary intake of folic acid in pregnancy.*—Thompson (1957) found that megaloblastic anæmia of pregnancy in this country was more frequent in the winter months, and this, he suggested, was due to a diminished availability of green vegetables which are a source of folic acid.

However, a seasonal incidence of megaloblastic anæmia need not necessarily be associated with changes in diet. Rifkin (1958) found that of 396 cases of megaloblastic anæmia seen in a large hospital in Durban, South Africa, over a three-year period 140 (37%) occurred in

the hot months of the year, i.e. November, December and January, as compared with 75 cases (19%) in May, June and July. As with the patients described by Wills (1932) and Manson-Bahr (1951), a very large proportion of the patients were pregnant women. There was no evidence that the diet was particularly defective during the hot months. On the other hand, this was the time of the year when gastro-intestinal infection was prevalent.

The increased demand for folic acid by the growing fœtus, coupled with relatively poor absorption of this vitamin, tends to the production of folic acid deficiency in pregnancy. Possibly under these circumstances a good intake of folic acid may prevent the onset of megaloblastic change, and a poor diet may precipitate it.

Megaloblastic Anæmia Due to an Increased Demand for Folic Acid

Another group of megaloblastic anæmias, which has not received adequate recognition, also appears to be caused by an increased demand for folic acid. This is the group of megaloblastic anæmias associated with leukæmia and related disorders and with hæmolytic anæmia. The presence of megaloblastic change in leukæmia was recognized by Von Leube (1902), and in carcinomatosis by Ehrlich and Lazarus (1900). An example of this type of case is illustrated in Fig. 7.

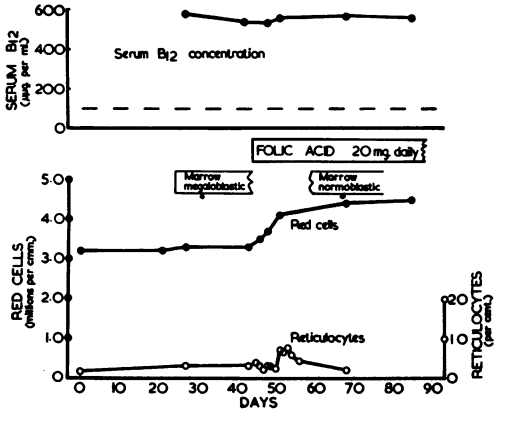

FIG. 7.—The hæmatological response to treatment of a patient with megaloblastic anæmia and chronic myelofibrosis.

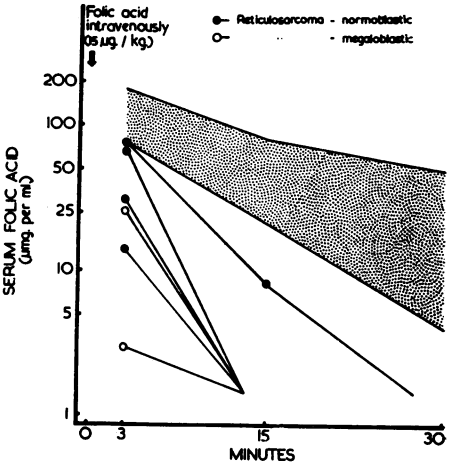

FIG. 8.—The plasma clearance of intravenous folic acid in 6 patients with reticulosarcoma. In 2 patients whose clearances are indicated by open circles, there was a severe degree of megaloblastic erythropoiesis.

The patient was a woman of 72 with chronic myelofibrosis. The red cell count was 3·2 million/c.mm., the blood film was macrocytic and marrow aspiration yielded rather scanty fragments which showed megaloblastic erythropoiesis. There was no vitamin B_{12} deficiency because serum B_{12} concentration was normal (590 $\mu\mu$g./ml.). Folic acid deficiency was confirmed by an extremely rapid clearance of injected folic acid from the plasma. In fact, the clearance was complete at the time of taking the first blood sample three minutes after the injection. The absorption of folic acid was normal. She responded to oral folic acid with a return of normoblastic erythropoiesis and a sustained rise in the red cell count.

This increased rate of clearance of injected folic acid is not confined to those patients with myelofibrosis who have a megaloblastic anæmia. 4 other patients with chronic myelofibrosis we have studied had a rapid clearance of injected folic acid.

Chronic myelofibrosis is one of a group of conditions in which there is a very large demand for folic acid. Other conditions in this category are leukæmia, hæmolytic anæmia, reticulosarcoma, myeloma and carcinomatosis.

The clearance of injected folic acid in 6 patients with reticulosarcoma is shown in Fig. 8. In all there is an abnormally fast clearance of injected folic acid suggesting folic acid deficiency. In 2 patients there was a severe degree of megaloblastic erythropoiesis. This increased demand for folic acid then forms the background for a megaloblastic anæmia which may arise.

The increased demand for folic acid may not be the only factor in causing the anæmia in these conditions. Malabsorption of fat, glucose, vitamin B_{12} as well as folic acid, may occur in a surprising number of these patients.

These observations were all made in untreated patients, but, of course, treatment with folic acid antagonists and 6-mercaptopurine may produce megaloblastic change (Berman *et al.*, 1949).

Vitamin B_{12} Deficiency

The classical vitamin B_{12} deficiency state occurs in Addisonian pernicious anæmia.

The clearance of injected folic acid in 12 patients with untreated Addisonian pernicious anæmia is shown in Fig. 9. Rapid clearances were found in all the patients with an initial red cell count of less than 2·0 million/c.mm. and the clearances were most rapid in those whose red cell counts were less than 1·7 million/c.mm. The clearances were normal in patients with red cell counts of more than 3·0 million/c.mm. Thus in pernicious anæmia folic acid deficiency is present when the degree of anæmia has become moderately severe.

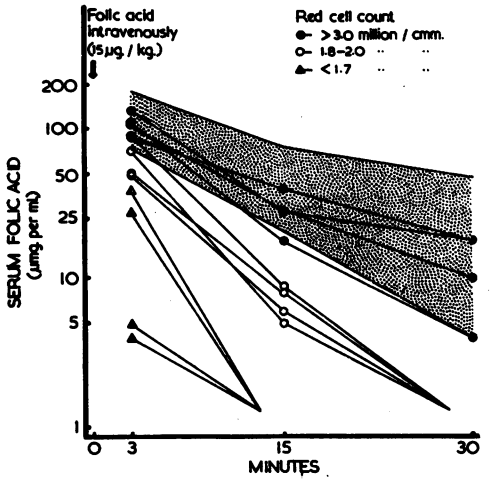

FIG. 9.—The plasma clearance of intravenous folic acid in 12 patients with untreated Addisonian pernicious anæmia.

Although in general there is a fair correlation between the severity of the anæmia in this condition and the serum B_{12} level—very low serum B_{12} levels being found in the most anæmic patients (Mollin and Ross, 1952)—there are many exceptions. In particular, patients with subacute combined degeneration of the cord may have little or no anæmia but invariably have very low serum B_{12} levels (Mollin and Ross, 1954). It is not, therefore, the degree of vitamin B_{12} depletion alone which determines the development of anæmia in patients with Addisonian pernicious anæmia.

On the other hand there is a very clear relationship between the degree of anæmia and the rate of clearance of injected folic acid, and this suggests that anæmia in these patients is due to the development of a secondary folic acid deficiency.

In spite of the presence of folic acid deficiency as indicated by the rapid clearance, patients with primary B_{12} deficiency do not respond to the dose of folic acid used in the clearance test. This is in contrast to the result often found in uncomplicated folic acid deficiency where there may be a significant reticulocyte response with a return towards normoblastic erythropoiesis in the marrow.

SUMMARY

Table II summarizes our views on the pathogenesis of these megaloblastic anæmias.

TABLE II.—FOLIC ACID AND THE MEGALOBLASTIC ANÆMIAS

Folic Acid Deficiency	*Impaired Utilization of Folic Acid*
Malabsorption:	Drugs:
Idiopathic steatorrhœa	Antagonists
Tropical sprue	Anticonvulsants
Other malabsorption syndromes	Vitamin B_{12} deficiency
Increased demand:	*Uncertain Factors*
Fœtus in pregnancy	Abnormal intestinal flora
Leukæmia	
Reticulosarcoma	
Myelofibrosis	
Hæmolytic anæmia	
(Dietary deficiency)	

There are 2 major groups. The first is folic acid deficiency; the second is impaired utilization of the vitamin. Folic acid deficiency may arise from impaired absorption; but it may also arise from the presence of conditions which produce an increased demand for folic acid. Reduced dietary intake may be a factor.

Impaired utilization may be due to folic acid antagonists such as aminopterin and amethopterin, and also to the anticonvulsant drugs used in the treatment of epilepsy. Impaired utilization may also result from a primary vitamin B_{12} deficiency.

The role of bacteria in producing megaloblastic anæmia is uncertain. They may interfere with the absorption of folic acid, as in tropical sprue, and with the absorption of vitamin B_{12}, as in patients with anatomical lesions of the small intestine. Whether these bacteria can also function by producing substances which act as folic acid antagonists has not been demonstrated.

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Hypoplastic Anæmia in Infancy

By JOHN L. STAFFORD, M.B.

London

SIGNIFICANT anæmia, resulting from marrow hypoplasia, is uncommon in infancy yet it is seen sufficiently frequently for its diagnosis to present a problem. Many papers on this topic have been written during the twenty years since Josephs (1936) and Diamond and Blackfan (1938) initially described their cases of familial hypoplasia, and classification has become diffuse with the recording of previously undescribed features, many being but variations on the same theme. This complexity is probably unnecessary and an attempt will be made to simplify the various categories into three main groups.

Table I shows the gradation of effect between the various types of marrow hypoplasia.

TABLE I.—THE GRADATION OF EFFECT IN MARROW FAILURE IN CHILDHOOD

1. Total (pancytopenic) marrow failure
2. Failure of erythropoiesis possibly associated with agranulocytosis or pancytopenia
3. Isolated failure of erythropoiesis (pure red cell aplasia)

Marrow response may be limited. In the majority of instances the organ has failed as a whole and all cellular products are diminished. Some patients, however, suffer preponder-