CHAPTER 9.

A NEW DIFFERENTIAL URINARY FOLIC ACID EXCRETION TEST
FOR THE INVESTIGATION OF INTESTINAL MALABSORPTION
AS FOUND IN IDIOPATHIC STEATORRHOEA, TROPICAL
SPRUE, AND IN CONDITIONS WITH STRUCTURAL
CHANGES OF THE SMALL INTESTINE OR
MESENTERIC GLANDS.

In the course of the investigations described in this thesis many avenues have been explored, and more than one of them has proved to be a cul-de-sac.

There has, however, emerged from these studies one new diagnostic test which other workers do not appear to have considered. This is a "differential urinary folic acid excretion test" in which the patient is given 5 mg. of pteroylglutamic acid by subcutaneous injection, the urine being collected for twenty-four hours thereafter, and then a further 5 mg. of pteroylglutamic acid by mouth. A second twenty-four hour urinary collection is made and the folic acid output in the two collections is compared. As will be shown in the following pages, a significantly smaller output after the oral dose may be found in the conditions commonly grouped under the name "malabsorption syndrome". One complicating factor is that if a patient is severely depleted of folic acid, it may be necessary first to saturate the tissues with injections of pteroylglutamic acid. If this is not done there may be a negligible folic acid output in the urine, not only after the subcutaneous dose but also after the oral dose.

From the point of view of the hospital ward this is an easy/
easy test to carry out. Unfortunately, however, the estimation of the amount of folic acid in the urine, although easily performed in a laboratory where microbiological assays are being done daily, is not a suitable technique for most routine laboratories. On the other hand, provided the total volumes of the twenty-four hour urines are known, it is an easy matter to send small samples to a microbiological assay laboratory.

In most instances the diagnosis of 'sprue' is not a difficult matter. The chief application of the differential folic acid excretion test would seem to be in the investigation of cases of refractory megaloblastic anaemia without obvious steatorrhoea, in cases of refractory iron deficiency anaemia and perhaps some patients with troublesome glossitis for which no cause is obvious. It can also be used in hospitals where a fat balance test cannot be carried out as one test to confirm a diagnosis of intestinal malabsorption, provided aliquots of the urine specimens are sent to a microbiological assay laboratory. From the research point of view it would appear to be a useful tool to assist in the investigation of the metabolic changes in megaloblastic anaemia of pregnancy, in complex forms of nutritional megaloblastic anaemia as described in Chapter 2, and in megaloblastic anaemia associated with alimentary short circuits and blind loops. Particularly when used together with the measurement of the serum level of vitamin B12, the differential urinary folic acid excretion test can give a great deal of information very soon after the patient is admitted to hospital. Indeed it is frequently possible to carry out these tests at the patient's home.
In this chapter there are described the studies that led to the development of the test in the form mentioned above, and therefore some of the patients received doses of pteroylglutamic acid other than 5 mg. in the earlier stages of the investigation.

The results are given in tabular form in order to give a bird's eye view of the value of the test, but since further information about the cases is obviously required, they are considered in more detail at the end of the chapter. The results on control patients are shown only in tabular form, but the test has also been applied to certain other cases which will be referred to in later chapters.

**METHODS**

The pteroylglutamic acid used in these tests, whether it was given by injection or by mouth, was derived from ampoules of 'Folvite' (Lederle Laboratories Ltd.). The ampoules were taken from batches tested by us and found to contain 15 mg. of pteroylglutamic acid per ml. Certain batches were rejected because their content proved to be higher than that stated on the label. Most of the ampoules were, in fact, taken from one batch, supplied by Dr. A. T. Mennie, of Lederle Laboratories Ltd., London. In any one patient the same batch was used for all the tests.

On two occasions, where the intention was to load the tissues by large doses (Cases 37 and 41, Table 52) hospital stock ampoules were used.

The dose was accurately measured in a tuberculin syringe. For tests of excretion following oral therapy this test dose was diluted with a small quantity of water.
Twenty-four hour collections of urine were made in brown bottles containing toluene and a phosphate buffer of pH 6.8. The greatest possible care was taken to ensure that the urines were total twenty-four hour specimens. The urines were kept in a refrigerator at 4°C., and readings were usually made within 3 days. All the readings were made at least in duplicate.

Folic acid assays were by the method already described. No correction required to be made for the 'resting' urinary content of folic acid since, as we have seen in Chapter 7, the amount is so small. Urinary citrovorum factor was estimated in many instances since citrovorum factor is also a growth factor for S. faecalis. The urinary citrovorum factor content has not been corrected for its 'resting' value of citrovorum factor.

It will be seen that a small proportion of the folic acid administered in these investigations was excreted as citrovorum factor or as a substance with similar microbiological properties, but the figures given for folic acid excretion have not been corrected for this, because such a correction would in no way alter the conclusions.

Unless otherwise stated, the fat balance test was based on stool collections over two 4-day periods, a diet of 75 G. of fat being given daily. The cases of "sprue" were in many instances patients with advanced disease who had been known to us for several years and in whom the diagnosis was based on the general clinical picture together with an abnormal fat balance test and evidence of malabsorption of other factors, such/
such as calcium, glucose and vitamin K, but in some the clinical picture was not clear, and the first biochemical results available were those of the differential urinary folic acid excretion test.

The pernicious anaemia patients were untreated and were typical in every way, with a normal dietetic history, histamine-fast achlorhydria, no evidence of abnormal stools, and, in most instances, a megaloblastic marrow. Cases have, however, been included in Table 52 where there was subacute combined degeneration of the cord with a relatively high red cell count and transitional erythroblasts, rather than fully developed megaloblasts, in the marrow.

**CLINICAL APPLICATION OF DIFFERENTIAL FOLIC ACID EXCRETION TEST**

The first experiment consisted in the administration of 15 mg. of pteroylglutamic acid intravenously to a patient believed at the time to be suffering from idiopathic steatorrhoea (Case 1, Table 51). Later it was found that the malabsorption was due to extensive tuberculosis of the glands at the root of the mesentery: the bowel wall itself was normal in appearance. At the time there was mild iron deficiency anaemia (Hb. 10 G. per 100 ml., red cells 4,190,000 per c.mm.); a year previously a fat balance test had shown 77% absorption of fat, and the marrow had been normoblastic. No therapy with folic acid or with vitamin B₁₂ had been given. In the 24 hours following the test dose of 15 mg. pteroylglutamic acid the patient excreted only 0.112 mg. in the urine, compared with 1.73 - 3.8 mg. after a 5 mg. subcutaneous test dose that we have seen in control patients. There was no significant excretion of folic acid in

* p.329. There is a duplicate copy in a pocket at the end of this volume.
the urine of the patient in the subsequent 24 hours. Significant excretion for more than 24 hours has never been found in any patients or controls that we have investigated, except in the presence of a large effusion or severe renal impairment.

When 15 mg. of pteroylglutamic acid was given by mouth to Case 1 (Table 51) it led to a urinary excretion of 0.119 mg. At this time the patient was not available for further tests of this nature. The findings in this patient suggested that a mere comparison of the output of folic acid following an injected dose with the output following a similar oral test dose would be of little value as an indication of folic acid absorption in untreated cases of intestinal malabsorption. If the tissues were depleted of folic acid, the injected dose might only partially replace the tissue depletion, and it would not be clear whether a subsequent low excretion following an oral test dose indicated intestinal malabsorption or tissue depletion.

The possibility of first saturating the tissues with folic acid therefore required consideration.

It was found, moreover, that even the normal gut cannot absorb unlimited quantities of folic acid. Three non-anaemic controls were each given 105 mg. intramuscularly (in one subject intravenously) and then 105 mg. orally. The measurements suggested that almost all the injected dose was excreted in the urine within 24 hours, whereas the amounts excreted following the oral doses were respectively 28.0 mg., 29.7 mg. and 36.1 mg. It was decided finally that the basic test to be used in the investigations was a comparison of the urinary excretion of folic acid following a subcutaneous injection of 5 mg.
5 mg. of pteroylglutamic acid with that following a subsequent oral dose of 5 mg. A significantly smaller output of folic acid in the second collection of urine indicates malabsorption. Many of the cases of intestinal malabsorption had been treated with pteroylglutamic acid for long periods before this comparison was made, but Case 4, Table 51, showed clearly that in at least some cases it is necessary to give injections of pteroylglutamic acid before performing the test. Unfortunately we have not been able to trace any further patients suffering from untreated intestinal malabsorption who have required a saturating dose, so that it is not yet possible to say how much is needed to produce saturation. The findings in Case 4 suggest that 15 mg. daily intramuscularly for seven days will suffice, but whether one large injection, say of 60 mg., would give saturation cannot yet be stated.

In view of our findings in control patients, we have taken a urinary folic acid excretion of less than 1.5 mg. following the subcutaneous administration of 5 mg. to be an indication of gross tissue depletion. It follows that if attempts are first made to saturate the patient with folic acid, an excretion of less than 1.5 mg. following the oral administration of 5 mg. itself indicates severe malabsorption. If this part of the test is done alone, however, it is better to take a figure of 2 mg. rather than 1.5 mg. (see p.379).

RESULTS OF DIFFERENTIAL FOLIC ACID EXCRETION TESTS IN INTESTINAL MALABSORPTION

In Table 51 there are given the results of urinary folic acid/
<table>
<thead>
<tr>
<th>Case</th>
<th>Sex</th>
<th>Age Yr.</th>
<th>Diagnosis</th>
<th>Known Duration of illness (Yrs.)</th>
<th>&quot;Hb. (G./100 ml.)&quot;</th>
<th>&quot;WBCs mill/ c.mm.&quot;</th>
<th>Test meal</th>
<th>Absorption (%)</th>
<th>Split %</th>
<th>General Condition</th>
<th>Other evidence of malabsorption</th>
<th>Antimegaloblastic treatment given before folate acid test</th>
<th>Follic Acid Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>55</td>
<td>Tuberculous mesenteric glands</td>
<td>6</td>
<td>...</td>
<td>10</td>
<td>4.19</td>
<td>Free HCl</td>
<td>80.9</td>
<td>76</td>
<td>Poor Tetany, osteoporosis, low serum-sodium level, low prothrombin, X-ray pattern of malabsorption</td>
<td>None</td>
<td>15 IV 0.112</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>17</td>
<td>Adult coeliac disease</td>
<td>17</td>
<td>Normoblastic</td>
<td>7.4</td>
<td>5.32</td>
<td>Free HCl</td>
<td>88.0</td>
<td>61</td>
<td>Good Flat sugar curve, flat iron absorption curve</td>
<td>None</td>
<td>5 SC 2.42 5.2</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>44</td>
<td>Refractory iron deficiency anaemia</td>
<td>14</td>
<td>Normoblastic</td>
<td>8.7</td>
<td>4.54</td>
<td>Histine fast</td>
<td>96.1</td>
<td>71.5</td>
<td>Good Flat iron absorption curve, X-ray pattern normal</td>
<td>None</td>
<td>5 SC 0.07 4.7</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>36</td>
<td>Refractory one month megaloblastic anaemia</td>
<td>3.6</td>
<td>Megaloblastic</td>
<td>3.6</td>
<td>1.5</td>
<td>Free HCl</td>
<td>Not available; total stool fat 59 G./100 G. 80% of this was split</td>
<td>Poor</td>
<td>FA 15mg. Oral 37 days 5 SC 0.29 11.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>36</td>
<td>*Idiopathic steatorrhoea</td>
<td>11</td>
<td>Megaloblastic</td>
<td>11.5</td>
<td>3.1</td>
<td>Histine fast</td>
<td>Not available; total stool fat 49.5 G./100 G.</td>
<td>Poor</td>
<td>FA 60mg. Oral 14 days 5 SC 4.6 0.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>33</td>
<td>Adult coeliac disease</td>
<td>25</td>
<td>Megaloblastic</td>
<td>3.3</td>
<td>0.93</td>
<td>Histine fast</td>
<td>87</td>
<td>65</td>
<td>Poor Tetany, flat sugar curve, X-ray pattern of malabsorption</td>
<td>None</td>
<td>100 Oral 5.2</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>42</td>
<td>Idiopathic steatorrhoea</td>
<td>8</td>
<td>Megaloblastic</td>
<td>8.2</td>
<td>2.29</td>
<td>Free HCl</td>
<td>66</td>
<td>52</td>
<td>Poor Tetany, flat sugar curve, low serum-sodium level</td>
<td>None</td>
<td>13 Oral 0.005 0.3</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>36</td>
<td>Tropical sprue</td>
<td>6</td>
<td>Megaloblastic</td>
<td>14.0</td>
<td>4.39</td>
<td>Free HCl</td>
<td>81</td>
<td>72.5</td>
<td>Poor Flat sugar curve, X-ray pattern of malabsorption</td>
<td>FA 15mg. Oral 3 yrs. but none for 4 wks. 5 SC 3.1 6.7</td>
<td></td>
</tr>
</tbody>
</table>

Note: Please fold upwards before inwards.
acid excretion tests in 22 patients with evidence of small intestinal malabsorption. This is the total number of such patients that we have investigated. It was usually possible to carry out a fat balance test but in a few instances the patients were in distant peripheral hospitals where this was not possible. The marrow findings shown in Table 51 are those before any antimegaloblastic therapy had been given. This is also true of the blood counts except in Cases 8, 9, 10, 11, 12, 17, 19, 21 and 22 where, in view of the long period of treatment that had already been given, the counts shown are those done at the time of the folic acid excretion test.

The patients included in Table 51 will be referred to in detail on pp. 335 to 376. It will be seen from Table 51 that in these patients who had overt 'sprue' there was a much smaller urinary output of folic acid after the orally administered dose than after the injected dose. In several cases the urine was collected for more than 24 hours and it was shown that the difference was not due merely to delayed absorption from the small intestine.

The smallest difference between the injected and the oral test doses was in Case 19. This patient was one in whom a diagnosis of coeliac disease persisting into adult life had been made in 1947; she had no symptoms or signs when the folic acid balance test was done in 1952, and this was believed to be because of the treatment with pteroylglutamic acid and Anaheamin that she had received.

Apart then from this asymptomatic case in whom the excretion of folic acid after the oral dose was as much as 50% of/
of that excreted after the injected dose, there was a very marked difference between the urinary excretion after the two test doses in all the patients included in Table 51. The results differ greatly from what was found in the control cases.

The controls used in this investigation were patients with pernicious anaemia and persons with other forms of anaemia or with no anaemia at all. The results of these control studies are given in Tables 52 and 53. Cases of megaloblastic anaemia of pregnancy and other patients who had had partial or total gastrectomy operations will be considered separately in later chapters.

The general conclusion that seems likely from Table 52 is that most patients with pernicious anaemia have some defect as a result of vitamin B12 deficiency that leads to actual or apparent tissue depletion of folic acid. This has been shown previously (Bethell et al. 1947), but the results in Table 52 indicate that impaired absorption of folic acid from the small intestine is not a feature of pernicious anaemia that can be demonstrated by the present test. (This does not necessarily mean that absorption of the smaller quantities of folic acid or its conjugates in the diet is always normal in pernicious anaemia.) In untreated cases the excretion following the oral test dose may be greater than that following the subcutaneous dose, and the simplest explanation would seem to be that the subcutaneous dose partially corrects the desaturation and the oral dose is then well absorbed and more completely excreted. It will be seen from Table 52 that the administration of folic acid by mouth or by injection for 8 - 15 days did not interfere with further absorption of folic acid from the gut.
### TABLE 52.

**Differential Folic Acid excretion Tests in Pernicious Anaemia**

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Sex</th>
<th>Age (yr.)</th>
<th>Hb (g. per 100 ml.)</th>
<th>Red cells (million per c.mm.)</th>
<th>Absorption (%)</th>
<th>Split (%)</th>
<th>Drug</th>
<th>Dose daily unless stated</th>
<th>Route</th>
<th>Duration</th>
<th>Test dose of folic acid given (mg.)</th>
<th>Route</th>
<th>Urinary folic-acid activity in 24 hr after test dose (mg.)</th>
<th>Urinary cisorvamin-factor activity in 24 hr after test dose (µg.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>23 F</td>
<td>47</td>
<td>7.6</td>
<td>1.93</td>
<td>-</td>
<td>None</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5</td>
<td>SC</td>
<td>1.73</td>
<td>-</td>
</tr>
<tr>
<td>24 M</td>
<td>63</td>
<td>9.2</td>
<td>2.35</td>
<td>-</td>
<td>None</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5</td>
<td>SC</td>
<td>0.49</td>
<td>-</td>
</tr>
<tr>
<td>25 M</td>
<td>52</td>
<td>12.6</td>
<td>3.1</td>
<td>-</td>
<td>None</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5</td>
<td>SC</td>
<td>3.05</td>
<td>4.6</td>
</tr>
<tr>
<td>26 M</td>
<td>52</td>
<td>13.1</td>
<td>3.27</td>
<td>-</td>
<td>None</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5</td>
<td>SC</td>
<td>3.6</td>
<td>7.0</td>
</tr>
<tr>
<td>27 F</td>
<td>56</td>
<td>9.6</td>
<td>2.42</td>
<td>94.6</td>
<td>68</td>
<td>None</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5</td>
<td>SC</td>
<td>1.51</td>
<td>-</td>
</tr>
<tr>
<td>28 F</td>
<td>47</td>
<td>10.1</td>
<td>3.41</td>
<td>92.0</td>
<td>50</td>
<td>None</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5</td>
<td>SC</td>
<td>1.52</td>
<td>-</td>
</tr>
<tr>
<td>29 F</td>
<td>56</td>
<td>8.0</td>
<td>1.38</td>
<td>-</td>
<td>FA 20mg. Oral 14 days</td>
<td>5</td>
<td></td>
<td>SC 3.05</td>
<td>1.71</td>
<td>-</td>
<td>SC 2.94</td>
<td>34.8</td>
<td>-</td>
<td>SC 3.05</td>
</tr>
<tr>
<td>30 F</td>
<td>61</td>
<td>7.4</td>
<td>1.77</td>
<td>93.1</td>
<td>58</td>
<td>None</td>
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<td></td>
<td></td>
<td></td>
<td>5</td>
<td>SC</td>
<td>1.25</td>
<td>0.46</td>
</tr>
<tr>
<td>31 F</td>
<td>37</td>
<td>4.5</td>
<td>1.43</td>
<td>96.5</td>
<td>44</td>
<td>None</td>
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<td>5</td>
<td>SC</td>
<td>1.05</td>
<td>1.25</td>
</tr>
<tr>
<td>32 M</td>
<td>57</td>
<td>8.0</td>
<td>1.95</td>
<td>95.5</td>
<td>52</td>
<td>None</td>
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<td></td>
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<td></td>
<td>5</td>
<td>SC</td>
<td>0.666</td>
<td>1.23</td>
</tr>
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<td>33 F</td>
<td>64</td>
<td>5.6</td>
<td>1.78</td>
<td>93.3</td>
<td>36</td>
<td>None</td>
<td></td>
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<td></td>
<td></td>
<td>5</td>
<td>SC</td>
<td>0.122</td>
<td>1.39</td>
</tr>
<tr>
<td>34 M</td>
<td>51</td>
<td>6.0</td>
<td>1.63</td>
<td>92.0</td>
<td>42</td>
<td>None</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5</td>
<td>SC</td>
<td>2.02</td>
<td>12.7</td>
</tr>
<tr>
<td>35 M</td>
<td>69</td>
<td>5.3</td>
<td>1.72</td>
<td>-</td>
<td>FA 20mg. Oral 8 days</td>
<td>5</td>
<td></td>
<td>SC 3.08</td>
<td>17.1</td>
<td>-</td>
<td>SC 2.92</td>
<td>31.8</td>
<td>-</td>
<td>SC 3.02</td>
</tr>
<tr>
<td>36 M</td>
<td>44</td>
<td>8.2</td>
<td>2.03</td>
<td>96.2</td>
<td>51</td>
<td>FA 20mg. Oral 7 days</td>
<td>5</td>
<td></td>
<td>SC 2.43</td>
<td>10.5</td>
<td>-</td>
<td>SC 2.87</td>
<td>12.9</td>
<td>-</td>
</tr>
</tbody>
</table>

* Marrow contains "intermediate" ("transitional") erythroblasts.
RESULTS IN PEOPLE WITHOUT MEGALOBLASTIC ANAEMIA.

The results of differential urinary excretion tests in other patients suffering from a variety of conditions are given in Table 53. Cases with diarrhoea included in Table 53 had normal fat balance test results, and no other evidence of intestinal malabsorption, clinically, biochemically, haematologically or by X-ray examination.

It will be seen that, with the possible exception of Case 45 there was no significant diminution in urinary folic acid excretion when the test dose was given by mouth instead of by injection. In Case 45 the excretion after the oral dose was more than 50% of that after the injected dose. Indeed it is surprising that the body is able to deal so effectively and to such an equal extent with similar amounts of any substance given by such different routes.

As will be shown in Chapter 18 there may be a low output of folic acid following the test dose in widespread malignant disease, and it can be seen from Table 53 that in the cases of this type investigated by the differential urinary excretion technique, the folic acid depletion (if that is what is being measured) is not due to malabsorption that can be demonstrated in this way. The findings in Cases 37 - 44 show that intestinal absorption of folic acid is not impaired either by the administration of large doses by injection a few days before the oral test dose or by previous prolonged treatment by mouth.

FURTHER/
TABLE 53. DIFFERENTIAL URINARY FOLIC ACID EXCRETION TESTS IN PATIENTS WITHOUT MEgaloblastic ANAEMIA.
<table>
<thead>
<tr>
<th>Case</th>
<th>Sex</th>
<th>Age (yr)</th>
<th>Diagnosis</th>
<th>Blood findings before test</th>
<th>Antimegaloblastic substances given before folic acid test</th>
<th>Folic acid test</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Blood daily unless stated</td>
<td>Route</td>
</tr>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>57</td>
<td>F</td>
<td>34</td>
<td>Gastric Ulcer</td>
<td>14.0</td>
<td>None</td>
<td>5 Oral</td>
</tr>
<tr>
<td>58</td>
<td>F</td>
<td>9</td>
<td>Aplastic Anaemia</td>
<td>6.0</td>
<td>1.73 F.A.</td>
<td>15 mg.</td>
</tr>
<tr>
<td>59</td>
<td>F</td>
<td>45</td>
<td>Iron Deficiency Anaemia</td>
<td>8.9</td>
<td>3.70 F.A.</td>
<td>15 mg.</td>
</tr>
<tr>
<td>60</td>
<td>M</td>
<td>54</td>
<td>Carbohydrate Deficiency</td>
<td>34.9</td>
<td>5.2 F.A.</td>
<td>20 mg.</td>
</tr>
<tr>
<td>61</td>
<td>F</td>
<td>48</td>
<td>Pernicious</td>
<td>25.1</td>
<td>5.07 None</td>
<td>None</td>
</tr>
<tr>
<td>62</td>
<td>M</td>
<td>35</td>
<td>Normal</td>
<td>15.0</td>
<td>5.36 None</td>
<td>None</td>
</tr>
<tr>
<td>63</td>
<td>N</td>
<td>27</td>
<td>Normal</td>
<td>15.4</td>
<td>None</td>
<td>F.A. 20 mg.</td>
</tr>
<tr>
<td>64</td>
<td>N</td>
<td>27</td>
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<td>14.9</td>
<td>F.A. B12</td>
<td>100 µg.</td>
</tr>
<tr>
<td>65</td>
<td>M</td>
<td>26</td>
<td>Duodenal Ulcer</td>
<td>15.3</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>66</td>
<td>N</td>
<td>56</td>
<td>Duodenal Ulcer</td>
<td>9.7</td>
<td>4.91 None</td>
<td>None</td>
</tr>
<tr>
<td>67</td>
<td>F</td>
<td>42</td>
<td>Portal Cirrhosis</td>
<td>9.6</td>
<td>3.65 B12</td>
<td>100 µg.</td>
</tr>
<tr>
<td>68</td>
<td>F</td>
<td>49</td>
<td>Portal Cirrhosis</td>
<td>8.9</td>
<td>4.07 None</td>
<td>None</td>
</tr>
<tr>
<td>69</td>
<td>F</td>
<td>26</td>
<td>Tumours: no cause found</td>
<td>13.5</td>
<td>4.98 None</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total: no cause found</td>
<td>13.5</td>
<td>4.98 None</td>
<td>F.A. 60 mg.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Normal</td>
<td>14.0</td>
<td>None</td>
<td>Oily 3.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Normal</td>
<td>14.0</td>
<td>None</td>
<td>Oral 3.01</td>
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<tr>
<td>50</td>
<td>N</td>
<td>55</td>
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<td>14.8</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>51</td>
<td>F</td>
<td>42</td>
<td>Portal Cirrhosis</td>
<td>16.0</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>52</td>
<td>M</td>
<td>57</td>
<td>Portal Cirrhosis</td>
<td>57.0</td>
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<td>None</td>
</tr>
<tr>
<td>53</td>
<td>F</td>
<td>62</td>
<td>Portal Cirrhosis</td>
<td>8.6</td>
<td>4.3 None</td>
<td>None</td>
</tr>
<tr>
<td>54</td>
<td>M</td>
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<td>Portal Cirrhosis</td>
<td>22.1</td>
<td>4.92 None</td>
<td>None</td>
</tr>
<tr>
<td>55</td>
<td>M</td>
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<td>Portal Cirrhosis</td>
<td>22.2</td>
<td>3.96 None</td>
<td>None</td>
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<tr>
<td>56</td>
<td>F</td>
<td>65</td>
<td>Portal Cirrhosis</td>
<td>24.6</td>
<td>5.01 None</td>
<td>None</td>
</tr>
<tr>
<td>57</td>
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<td>Portal Cirrhosis</td>
<td>24.2</td>
<td>4.72 None</td>
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</tr>
<tr>
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<td>Portal Cirrhosis</td>
<td>22.5</td>
<td>4.01 None</td>
<td>None</td>
</tr>
<tr>
<td>59</td>
<td>F</td>
<td>42</td>
<td>Portal Cirrhosis</td>
<td>30.2</td>
<td>3.55 None</td>
<td>None</td>
</tr>
<tr>
<td>60</td>
<td>M</td>
<td>62</td>
<td>Portal Cirrhosis</td>
<td>5.0</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>61</td>
<td>M</td>
<td>43</td>
<td>Portal Cirrhosis</td>
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<td>1.6 None</td>
<td>None</td>
</tr>
<tr>
<td>62</td>
<td>M</td>
<td>46</td>
<td>Portal Cirrhosis</td>
<td>5.0</td>
<td>1.67 None</td>
<td>None</td>
</tr>
<tr>
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<td>Portal Cirrhosis</td>
<td>7.0</td>
<td>9.00 None</td>
<td>None</td>
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<td>64</td>
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<td>58</td>
<td>Portal Cirrhosis</td>
<td>7.0</td>
<td>1.67 None</td>
<td>None</td>
</tr>
<tr>
<td>65</td>
<td>M</td>
<td>47</td>
<td>Portal Cirrhosis</td>
<td>7.0</td>
<td>4.00 None</td>
<td>None</td>
</tr>
<tr>
<td>66</td>
<td>M</td>
<td>59</td>
<td>Portal Cirrhosis</td>
<td>4.0</td>
<td>4.5None</td>
<td>None</td>
</tr>
</tbody>
</table>

*For these injections hospital stock ampoules were used and the folic acid solution was not measured with a hypodermic syringe. It is likely that the patients received more than an accurate 105 µg.
FURTHER DETAILS OF THE CASES CONSIDERED IN TABLE 51.

The more important features of the case histories and various investigative findings are given in Table 51, but as this is a new test it is felt that fuller information is required about the cases of intestinal malabsorption, though not about the controls.

In each instance an indication will be given at the commencement of the history of the date of the differential urinary folic acid excretion test.

Case 1. Folic acid excretion tests in June & November 1952.

A female patient aged 55 years whose case has already been mentioned on p. 326. She had had symptoms of steatorrhoea with weight loss and low fever for about six years.

In the late stages of the illness marked hypotension, lethargy, and tetany developed, and there was little or no improvement with multivitamin therapy, together with the intravenous infusion of potassium, calcium and protein.

At post mortem, large tuberculous glands were found in the mesentery.

The patient had had pteroylglutamic acid for four months by mouth in a dosage of only 5 mg. daily up to the time that the differential folic acid excretion test was carried out, but this had been supplemented by monthly injections of 100 µg. of cyanocobalamin and 15 mg. of pteroylglutamic acid.
Folic acid excretion test

Urinary excretion:
in 24 hrs. after 5 mg. SC = 2.42 mg.
in 24 hrs. after 5 mg. Orally = 0.18 mg.

TEST POSITIVE.

Comment: A positive differential folic acid excretion test in a patient with steatorrhoea due to tuberculous mesenteric glands.

Case 2.

Folic acid excretion test in September 1952.

A female patient aged 17 years who had been treated for coeliac disease at the Royal Hospital for Sick Children, Edinburgh, at intervals from the age of 11 months. Up to the age of 11 years she had four bowel motions daily, the stools being pale and bulky, but thereafter there was some improvement in the number and appearance of the motions. For three years she had been treated for iron deficiency anaemia refractory to all preparations of iron given by mouth, but responding to intravenous saccharated iron oxide.

Thereafter she did not report to us for a period of three years and then was sent for admission on account of anaemia although her general health was good. The haemoglobin reading was now 7.4 G. per 100 ml. and the red cells were 5,320,000 per c.mm., C.I. 0.47. The marrow was normoblastic, there was a flat glucose absorption curve, and the fat balance test/
Folic acid excretion test

Urinary excretion:
in 24 hrs. after 5 mg. SC = 2.51 mg.
in 24 hrs. after 5 mg. Orally = 0.61 mg.

TEST POSITIVE.

Comment: Adult coeliac disease with iron deficiency anaemia and a positive differential folic acid excretion test.

Case 3 and Graph 14. Folic acid excretion test in December 1952.

A female patient aged 44 years who had suffered from tiredness and breathlessness for 14 years. She had not been abroad. The symptoms were due to iron deficiency anaemia which was refractory to treatment with iron by mouth. In 1948 an iron absorption curve showed impaired absorption of iron. (A rise of only 17 µg. per 100 ml. in the serum iron level after 18 grains of FeSO₄.) The haemoglobin level was 6.6 G. per 100 ml., red cells 3,580,000 per c.mm., and the marrow was normoblastic. A barium meal showed no abnormality; there was no diarrhoea. The haemoglobin and red cells counts were improved by the intravenous injection of saccharated oxide of iron to a total of 1.1 G. but after four months the haemoglobin level started to fall again, and a year later the haemoglobin reading was 10 G. per 100 ml. Despite the administration of iron by mouth, there was a further fall to a haemoglobin level of 7.2 G. per 100 ml. Intravenous iron was again given to a total of 1 G., and there was a rise of haemoglobin to 12.7 G. per 100 ml. Ferrous sulphate was administered by mouth but over the period of a year the haemoglobin/
CASE 3. REFRAC TORY HYPOCHROMIC ANAEMIA DUE TO MALABSORPTION.

GRAPH 14.

haemoglobin level fell again to 8.5 G. per 100 ml. Intravenous iron caused a further unsustained rise.

In November 1952 the patient was admitted to Ward 27, Royal Infirmary, Edinburgh, the haemoglobin reading being 9.8 G. per 100 ml., red cells 4,600,000 per c.mm. The marrow was normoblastic, and the test meal showed histamine fast achlorhydria. A fat balance test showed 96.1% absorption and there was a normal small intestinal X-ray pattern. A folic acid excretion test was carried out.

Folic acid excretion test

Urinary excretion:
in 24 hrs. after 5 mg. SC = 0.87 mg.
in 24 hrs. after 5 mg. Orally = 0.21 mg.

TEST POSITIVE.

This suggested both malabsorption of folic acid and tissue/
tissue depletion of the vitamin. The patient was given 2.6 G. of iron by intravenous injection over a period of three weeks, and the haemoglobin reading rose to 16.4 G. per 100 ml. The serum vitamin B12 was estimated in October 1953 at a time when the anaemia had not reappeared, and was found to be 230 µg./ml.

Comment: Refractory iron deficiency anaemia without diarrhoea, due to deficient absorption of iron. The fat balance test gave a normal result and the X-ray picture of the small intestine was normal, but the folic acid excretion test suggested malabsorption with tissue depletion. A further test after saturation with folic acid would be of value, but the patient does not wish to be readmitted and lives at a long distance from Edinburgh.

Case 4. Folic acid excretion test in November 1952.

A female patient aged 36 years. She was an in-patient at Peel Hospital, and was not seen by the present author who merely carried out folic acid balance tests to assist in the diagnosis of the cause of the megaloblastic anaemia from which she suffered. The history at the time of her admission in October 1952 was of tiredness, breathlessness on exertion and pallor. There had been no digestive upset or disorder of bowel function. A week before admission her practitioner had given four liver injections. There was no previous history of importance and the patient had not been abroad. No abnormality was found on physical examination other than the usual features of anaemia.

At the time of admission the haemoglobin level was recorded/
recorded as 3.7 G. per 100 ml., the red cells being 1,500,000 per c.mm. The marrow was megaloblastic but there was free hydrochloric acid in the test meal. The patient was treated by transfusion of a pint of blood and with cyanocobalamin by injection in three doses each of 100 µg. and pteroylglutamic acid by mouth in a dosage of 15 mg. daily for 37 days. The marrow became normoblastic but the haemoglobin reading was only 7.4 G. per 100 ml., red cells 2,800,000 per c.mm. A differential folic acid excretion test was now performed.

**Folic acid excretion test**

**Urinary excretion:**
- in 24 hrs. after 5 mg. SC = 0.29 mg.
- in 24 hrs. after 5 mg. Orally = 0.34 mg.

**Test:** Folic acid depletion too great for test to be of value.

The patient was now given 15 mg. of pteroylglutamic acid intramuscularly daily for 10 days and the test repeated.

**Folic acid excretion test**

**Urinary excretion:**
- in 24 hrs. after 5 mg. SC = 2.4 mg.
- in 24 hrs. after 5 mg. Orally = 0.08 mg.

**TEST POSITIVE.**

The patient improved greatly with this treatment and was allowed home, unfortunately without any further investigations being carried out. Her general practitioner kindly sent a specimen of stool for fat analysis, and it was found that 59% of the stool was fat.

**Comment:** A case of 'idiopathic refractory megaloblastic anaemia' without diarrhoea, in which there was a markedly positive/
positive differential folic acid excretion test indicating
deficient absorption of folic acid. The administration of
pteroylglutamic acid by mouth for 37 days in a dosage of 15 mg.
daily was not sufficient to saturate the depleted body stores,
if, in fact, it is the state of saturation of body stores that
is being measured by the injected dose.

Case 5. Folic acid excretion test in
October 1952.

A female patient aged 36 years, who was in the Eastern
General Hospital, Edinburgh. She had not been abroad. It
was reported that six months previously she had been in the
same hospital with a haemoglobin reading of 3 G. per 100 ml.
and a normoblastic marrow, but no further information about
the blood counts at that time was available. There was a
response to blood transfusion and pteroylglutamic acid by
mouth, but the treatment was not continued.

The patient was readmitted in September 1952 with weakness,
aanaemia, tetany and some diarrhoea. The tongue was red and
atrophic, there was a small intestinal X-ray pattern of mal-
absorption, a flat sugar absorption curve, and a serum calcium
level of 6.5 mg.%. There was histamine fast achlorhydria,
the marrow was definitely megaloblastic, and the blood counts
were haemoglobin 11.5 G. per 100 ml., red cells 3,100,000 per
c.mm. at the time of admission. The patient was therefore
considered to have idiopathic steatorrhoea, and showed a fair
clinical and haematological response to pteroylglutamic acid
by mouth in a daily dosage of 60 mg. for 14 days and then 20 mg.
for 4 days. It was at this stage that we were asked to do a
differential/
differential folic acid excretion test as fat balance tests could not be done at the hospital concerned. The general condition of the patient was very poor.

**Folic acid excretion test**

**Urinary excretion:**
- in 24 hrs. after 5 mg. SC = 4.6 mg.
- in 24 hrs. after 5 mg. Orally = 0.16 mg.

**TEST POSITIVE.**

The patient died soon afterwards, and no structural alteration of the small intestine was found at post mortem to disprove the diagnosis of idiopathic steatorrhoea.

**Comment:** Idiopathic steatorrhoea of severe degree with a positive differential folic acid excretion test.

**Case 6.**

A female patient aged 33 years. From the age of 8 years onwards there were attacks of diarrhoea of about two weeks duration, and at the time the stools were pale. Nothing is known of the patient's health before that age. In 1948 she was admitted to hospital with weakness, diarrhoea and cramps in the limbs. The haemoglobin level was 4.0 G. per 100 ml., red cells 810,000 per c.mm., C.I. 1.23. The marrow was megaloblastic and there was histamine fast achlorhydria, but a fat balance test showed 95% absorption. This was only one three-day collection. The serum calcium level was 7.9 mg.% and there was hypotension. There was a good response to pteroylglutamic acid therapy.

She was readmitted to hospital in November 1950 with paraesthesiae and cramps. This time the fat balance test showed/
showed 80% absorption. Folic acid therapy was continued. Tetany continued to be troublesome.

In March 1951 the patient was discharged from hospital but received no treatment until she was readmitted as an emergency case in June 1952. Now there was severe anaemia, the haemoglobin level being 3.3 G. per 100 ml., red cells 930,000 per c.mm.; the marrow was megaloblastic. Fat balance tests over three periods of three days showed 87%, 75.6% and 74.6% absorption. Experiments were done to see what proportion of an orally administered dose of 100 mg. of pteroylglutamic acid would be absorbed, and there was evidence of impaired absorption (Table 51). There was, however, a good response to pteroylglutamic acid therapy supplemented by calcium, vitamin B12 and other vitamins. About five months later a folic acid balance test was carried out at the patient's home. By now the patient was well and doing a full day's work as a weaver. There was slight diarrhoea but no tetany.

**Folic acid excretion test**

<table>
<thead>
<tr>
<th>Urinary excretion</th>
<th>in 24 hrs. after 5 mg. SC</th>
<th>4.18 mg.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>in 24 hrs. after 5 mg. Orally</td>
<td>0.009 mg.</td>
</tr>
</tbody>
</table>

**TEST POSITIVE.**

**Comment:** Coeliac disease continuing into adult life and resulting in megaloblastic anaemia and tetany. Response to polytherapy. Folic acid test strongly positive.

**Case 7.** Graphs 15 & 16.

Folic acid excretion tests in June 1952 & January 1953.

A female patient aged 42 years, who was first admitted to hospital/
hospital in March 1944 when she was 5 months pregnant. The haemoglobin level then was 8 G. per 100 ml., red cells 2,050,000 per c.mm., and the marrow was megaloblastic. A test meal showed the presence of free hydrochloric acid, and a diagnosis of megaloblastic anaemia of pregnancy was made. There was no response to Anahaemin, but a good response to proteolysed liver.

In April 1946 the patient was readmitted with a history of weakness and of intermittent diarrhoea of a fatty type. A fat balance test showed the absorption to be 75%. The patient had never been abroad, the dietetic history was good, and a diagnosis of idiopathic steatorrhoea was now made. The haemoglobin level was 6 G. per 100 ml., red cells 1,370,000 per c.mm., and the marrow was again megaloblastic. There was a/
a good clinical and haematological response to pteroylglutamic acid by mouth in a dosage of 20 mg. daily for 10 days and then 10 mg. daily for 28 days. With this treatment the haemoglobin and red cell levels rose respectively to 11.3 G. per 100 ml. and 3,640,000 per c.mm. A further rise occurred thereafter when other haematinics were given in addition.

The patient was readmitted to hospital twice in 1948 and once in 1949 as she repeatedly ceased taking pteroylglutamic acid, and then developed steatorrhoea and megaloblastic anaemia.

In March 1952 readmission was necessary on account of pain in the legs, possibly due to severe polyneuritis. There was no clinical or biochemical evidence of tetany. The blood counts were now haemoglobin 8.3 G. per 100 ml., red cells 2,290,000/
2,290,000 per c.mm., MCV 111.3 cu., and normoblasts were numerous in the peripheral blood. The marrow was frankly megaloblastic. A fat balance test showed 64% absorption.

A 15 mg. test dose of pteroylglutamic acid was given by mouth, but only 0.005 mg. was excreted: 12 mg. of citrovorum factor was then given intramuscularly, and only 0.86 µg. was excreted. For a month, treatment was with cyanocobalamin by injection in a dosage of 50 µg. thrice weekly, together with yeast tablets and various vitamin preparations other than pteroylglutamic acid. There ensued a slow rise in the haemoglobin level to 11.1 G. per 100 ml. and the red cell count to 3,300,000 per c.mm. The administration of pteroylglutamic acid, 10 mg. b.i.d. by mouth for two months caused a further rise to normal blood figures. At this time, however, a second oral test dose of 15 mg. of pteroylglutamic acid gave an excretion of only 0.027 mg. This is shown in the following figure.

**URINARY OUTPUT OF HAEMOPOIETIC FACTORS**

<table>
<thead>
<tr>
<th>L.C. IDIOPATHIC STEATORRHOEA</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 mg. PCA ORALLY</td>
</tr>
<tr>
<td>12 mg. CITROVORUM FACTOR I.M.</td>
</tr>
<tr>
<td>15 mg. PCA ORALLY</td>
</tr>
</tbody>
</table>

**20 mg. FOLIC ACID ORALLY DAILY FOR 8 WEEKS**

Fig. 16.
In June 1953 when the differential folic acid excretion test had been finally stabilised at two 5 mg. test doses, this investigation was carried out at the patient's house. It will be seen that, although therapy with pteroylglutamic acid by mouth in a dosage of 20 mg. daily had been continued for nine months, the excretion even after the injected dose was rather low.

**Folic acid excretion test**

Urinary excretion in 24 hrs. after 5 mg. SC = 1.95 mg.
Urinary excretion in 24 hrs. after 5 mg. Orally = 0.38 mg.

**TEST POSITIVE**

In October 1953, pteroylglutamic acid therapy having been continued by mouth, the blood levels were haemoglobin 12.7 G. per 100 ml., red cells 5,080,000 per c.mm. The serum level of vitamin B12 by the *L. leichmannii* technique was 370 µg. per ml., a figure well within the limits of normal. No injections of cyanocobalamin had been given for sixteen months.

Comment: A case of idiopathic steatorrhoea which had at first been considered to be megaloblastic anaemia of pregnancy.

Absorption of folic acid was markedly impaired.

**Case 8.**

Folic acid excretion test in September 1952.

A male patient aged 36 years. While in India in 1946 he developed diarrhoea which was thought to be due to bacillary dysentery. He returned to Britain that year and was treated first for duodenal ulcer and then for jaundice. In October 1948 he had abdominal pain, and a laparotomy was done: the appendix/
appendix was removed.

In April 1949 the patient was admitted to hospital in Liverpool with loss of weight, ulcerative stomatitis and dyspepsia. No diagnosis was made, but in October 1949 he was admitted to Edinburgh Royal Infirmary; by now he suffered again from diarrhoea. The haemoglobin level was 11.9 G. per 100 ml., red cells 4,300,000 per c.mm., MCV 81 cu., and the marrow contained intermediate erythroblasts. A test meal showed the presence of free hydrochloric acid, and fat absorption tests in two four-day collections showed 78% and 83% absorption. There was no response to cyanocobalamin therapy, but a response occurred to pteroylglutamic acid by mouth. He had relapses of diarrhoea in October 1951, February 1952 and May 1952. In August 1952 he was re-admitted to Edinburgh Royal Infirmary with vomiting and diarrhoea. No pteroylglutamic acid had been taken for a month. There was a flat oral glucose tolerance test, and an X-ray picture of intestinal malabsorption. The haemoglobin level was 14.0 G. per 100 ml., red cells 4,390,000 per c.mm., MCV 92 cu. A folic acid excretion test was carried out.

**Folic acid excretion test**

Urinary excretion

<table>
<thead>
<tr>
<th>Time after 5 mg. SC</th>
<th>Excretion (mg.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 hrs.</td>
<td>3.41</td>
</tr>
</tbody>
</table>

TEST POSITIVE.

Comment: Tropical sprue with a positive folic acid excretion test.

Follow up:
Follow up: There was distinct clinical improvement following treatment with pteroylglutamic acid, cyanocobalamin and a low gluten diet.

Case 2. Folic acid excretion test in August 1952.

A male patient aged 42 years who had served during the 1939-45 war in the Army in North Africa and Italy. In 1945 he reported sick on account of excessive fatigue, breathlessness on effort and alternating constipation and diarrhoea. He was sent home with a diagnosis of pernicious anaemia because he had a megaloblastic marrow with a haemoglobin level of 8.5 G. per 100 ml. and a red cell count of 1,640,000 per c.mm. There was no response to injections of liver extract, but the blood counts improved with the administration of liquid liver extract by mouth.

In 1946 a diagnosis of sprue was made by the Army authorities because of the continuation of weakness and flatulence, together with the presence of loose pale stools. The patient was discharged from the Services and treated as an out-patient at Edinburgh Royal Infirmary with pteroylglutamic acid by mouth. There was a good clinical and haematological response to continuation of this treatment together with liver injections. Symptoms recurred several times and the patient was treated at Edinburgh Royal Infirmary and at the Radcliffe Infirmary, Oxford. Pteroylglutamic acid therapy was continued for five years in a dosage of 15 mg. daily by mouth.

In August 1952 the patient was re-admitted to hospital with/
with weakness, tingling in the limbs, sore gums, diarrhoea and ankle swelling. There was latent tetany but the serum calcium level was 10.2 mg%, chlorides 561 mg%, potassium 16 mg%, sodium 329 mg%. Fat balance tests showed 51.2% and 56.2% absorption. (This was for two periods of three days on a 75 G. fat intake.) The haemoglobin level was 16.5 G. per 100 ml., red cells 5,330,000 per c.mm., the marrow was normoblastic and there was histamine fast achlorhydria. An X-ray pattern of intestinal malabsorption was present, and there was a flat sugar absorption curve.

**Folic acid excretion test**

Urinary excretion  
in 24 hrs. after 5 mg. SC = 2.02 mg.  
in 24 hrs. after 5 mg. Orally = 0.036 mg.

**TEST POSITIVE.**

Comment: A positive differential folic acid excretion test in a patient with tropical sprue.

**Follow up:** There was a slow improvement as a result of poly-therapy, including minerals and vitamins, and injections of pteroylglutamic acid and cyanocobalamin, together with pteroylglutamic acid by mouth.

**Case 10.**

A male patient aged 45 years who commenced having diarrhoea in Canada in 1946. A laparotomy was performed and a diagnosis of "chronic infective hypertrophic colitis" made. He returned to Britain and continued to have watery diarrhoea without/
without blood or mucus in the stools. In August 1948 and on six subsequent occasions he was admitted to Edinburgh Royal Infirmary. In 1948 he was found to have histamine fast achlorhydria, a fat balance test (6 day) showed 34% absorption, the stools were sprue like and there was a flat oral glucose tolerance curve, but a normal intravenous one. The plasma albumin level was 2.11 G. per 100 ml., globulin 1.32 G. per 100 ml., and the serum calcium level 7.8 mg. per 100 ml. The haemoglobin level was 10.4 G. per 100 ml., red cells 3,000,000 per c.mm., PCV 37 %, MCV 123.3 cu., MCHC 28.1 %. The marrow contained intermediate erythroblasts. A barium enema showed no abnormality, but a barium follow through examination was reported as showing the small intestinal pattern of 'sprue', there being 'bunching' of the barium and a delay in passage through the small intestine. He had osteoporosis and a calcified kidney but no evidence of renal tuberculosis. The function of the remaining kidney was good.

There was no response to liver injections but a good response first to proteolysed liver and later to folic acid given by mouth.

On subsequent admissions the patient suffered from tetany, peripheral neuritis, hypotension, and a low plasma prothrombin level.

In December 1952 a folic acid excretion test was carried out. The patient had been having polytherapy, including folic acid, for 3½ years.
Folic acid excretion test

Urinary excretion
in 24 hrs. after 5 mg. SC = 4.38 mg.
in 24 hrs. after 5 mg. Orally = 0.27 mg.

TEST POSITIVE.

Subsequently the patient developed ascites but it was never established that he had tuberculosis of the mesenteric glands. Two consultants in tuberculosis thought that this was unlikely.

Comment: A patient believed to have idiopathic steatorrhoea. The differential folic acid excretion test was markedly positive. A diagnosis of tuberculous adenitis of the mesenteric glands cannot be excluded.

Case 11.

Folic acid excretion test
in January 1953.

A male patient aged 49 years who, while in the Navy in 1943, developed diarrhoea. He was never abroad. The stools were pale and bulky and in 1944 he was admitted to a ward of Edinburgh Royal Infirmary where a diagnosis of idiopathic steatorrhoea was made. A sternal puncture then showed the marrow to be megaloblastic, but blood counts cannot be traced. He was treated with a low fat diet but continued to have diarrhoea and the tongue became painful. In May 1950 the haemoglobin level was 11.9 G. per 100 ml., red cells 4,400,000 per c.mm., and pteroylglutamic acid therapy was given in a dosage of 15 mg. daily.

He showed some improvement at first and was discharged, but/
but in June 1952 he was re-admitted to hospital. The stools were bulky and pale, the haemoglobin level was 9.8 G. per 100 ml., the red cell count 3,840,000 per c.mm., and the marrow was normoblastic. (The patient had been receiving pteroyl-glutamic acid up to the time of admission.) Two separate fat balance tests showed, respectively, 64.4% and 48.3% absorption. The serum calcium level was 7.9 mg. per 100 ml., serum sodium 217 mg. per 100 ml., serum potassium 12.2 mg. per 100 ml. There was latent tetany. An X-ray of the abdomen showed calcified glands. No improvement occurred with a gluten free diet, but there was a slow response to polytherapy with vitamins including pteroylglutamic acid and cyanocobalamin, together with minerals.

The patient was re-admitted to hospital in January 1953 and in addition to his former troubles he had tetany and five fractured ribs. The haemoglobin level was now 9.7 G. per 100 ml., red cells 3,840,000 per c.mm., MCV 86.6 cu. The marrow was normoblastic, but treatment with pteroylglutamic acid had continued up to the time of admission, the dosage being 5 mg. thrice daily by mouth.

**Folic acid excretion test**

Urinary excretion
in 24 hrs. after 5 mg. SC = 1.29 mg.
in 24 hrs. after 5 mg. Orally = 0.055 mg.

**TEST POSITIVE.**

Comment: Steatorrhoea with many features of malabsorption. Although the patient had been treated with pteroylglutamic acid by mouth there was evidence not only of severe impairment of absorption/
absorption of folic acid but also of some tissue depletion. It could not be ascertained whether the condition was idio-
pathic or due to tuberculous mesenteric glands.

Case 12. Graph 17.

A male patient of Polish birth aged 23 years, who first had diarrhoea in Siberia in 1940, shortly after he had been transported there by the Russians. In 1942, after the family had moved to South Russia he was diagnosed as having dysentery because of loose motions associated with the passage of blood and mucus. In the same year the family went to India, and a diagnosis of "tropical" sprue was made. In 1947 the patient moved to Britain; watery yellowish stools persisted.

In December 1948 he was admitted to Ward 29 of Edinburgh Royal Infirmary, and the diagnosis of tropical sprue was considered to be correct. The blood counts were haemoglobin 8.1 G. per 100 ml., red cells 2,160,000 per c.mmm., PCV 25%, MCV 115.7 cu., MCHC 32.4%. The marrow was frankly megaloblastic. A fat balance test showed 68% fat absorption, and there was histamine fast achlorhydria. The serum calcium level was 8.0 mg.% No organisms or cysts were found in the stools. There was a response to the proteolysed liver preparation "Hepamino" in a dosage of oz. ½ q.i.d., followed by the oral administration of pteroylglutamic acid in a dosage of 5 mg. daily.

This treatment was continued, but in May 1951 he was re-admitted to hospital with anaemia. The blood counts were haemoglobin/
haemoglobin 8 G. per 100 ml., red cells 2,310,000 per c.mm., PCV 22%, MCV 95.2 cu., and the marrow was megaloblastic although he had taken his pteroylglutamic acid regularly. A test dose of 5 mg. of pteroylglutamic acid given by mouth resulted in an excretion of only 0.015 mg. This indicated poor absorption of the substance. Accordingly 20 mg. were given daily by mouth for 10 days, but the marrow remained megaloblastic. A test dose of 5 mg. was given than by injection, but only 0.65 mg. was excreted, apparently confirming that absorption from the alimentary tract had been poor. There was no haematological response to 15 mg. of the substance daily intramuscularly, the marrow remaining megaloblastic. For this reason, 90 mg. were given intramuscularly daily for 14 days and this converted the marrow to the normoblastic state, and caused a rise in the haemoglobin and red cell levels. Thereafter 90 mg. were given intramuscularly weekly for 4½ months, but the marrow became megaloblastic again as shown when he was re-admitted in November 1951. At the time the bowels were moving up to five times per day.

The patient was admitted to hospital on this occasion in 1951 because of a pleural effusion, but no X-ray evidence of tuberculosis was found in the lungs or abdomen and no tubercle bacilli were isolated from the sputum or stool. The effusion was considered by the patient's physician to be non-tuberculous. The plasma albumin was 3.66 G. per 100 ml., globulin 1.30 G. per 100 ml., and the haemoglobin reading was 9.9 G. per 100 ml., red cells 2,480,000 per c.mm.

It/

* See photograph, p.359.
It was considered likely that the patient was now vitamin B₁₂ deficient and so 100 microgrammes of cyanocobalamin were given by intramuscular injection. Careful arrangements were made to ensure that no other therapy was given, but the physician on the ward who had undertaken to supervise this went off ill the same day and unfortunately another doctor gave the patient penicillin. (See Graph 17.)

The marrow was promptly converted to the normoblastic state, and the blood counts restored to normality, without further antianaemic therapy. At the time of discharge in January,
January 1952 the counts were haemoglobin 14.9 G. per 100 ml.,
red cells 5,530,000 per c.mm., and a month later there was a
further rise without treatment to haemoglobin 15.3 G. per 100
ml., red cells 6,040,000 per c.mm., PCV 48.5%, MCV 80.3 cu.
The patient continued without any therapy until May 1953, i.e.
for 18 months, when he was re-admitted to hospital with ankle
swelling, tetany and weight loss but no diarrhoea. He was
admitted to the metabolic ward where a seven day fat balance
test showed 95.3% absorption. (60 G. daily fat intake.)
The marrow contained megaloblasts and transitional erythro-
blasts, and the haemoglobin level was 12.9 G. per 100 ml., the
red cells being 3,340,000 per c.mm. A folic acid excretion
test was carried out.

Folic acid excretion test

Urinary excretion
in 24 hrs. after 5 mg. SC = 1.8 mg.
in 24 hrs. after 5 mg. Orally = 0.089 mg.

TEST POSITIVE.

Comment: This suggested malabsorption of folic acid and some
tissue depletion of the substance.

The patient was given 15 mg. of pteroylglutamic acid
intramuscularly daily for four days and this time it converted
the marrow to the normoblastic state, and led to a rise in the
blood counts. General improvement also resulted. The
patient who lived at a considerable distance from Edinburgh was
unwilling to report back as an outpatient and was anxious to
return to work. Accordingly he was given injections of
pteroylglutamic/
pteroylglutamic acid and cyanocobalamin and discharged.

**Comment:** The course of events in this interesting case seemed to be -

1. May 1951. Malabsorption of folic acid given by mouth.
   (It is not however quite certain that a low output of folic acid in the urine after an injected test dose may not be an indication of vitamin B₁₂ depletion in some instances.)
   Refractoriness to injections of pteroylglutamic acid which was overcome by giving 90 mg. daily by injection.

2. November 1951. Patient depleted of vitamin B₁₂, therefore responded well to one injection of this substance. The picture is confused by the injection of penicillin which was administered in error contrary to the present author's request.

3. May 1953. Patient now depleted of folic acid again to the extent of having megaloblastic anaemia that responded to pteroylglutamic acid therapy. No doubt if cyanocobalamin therapy had been withheld thereafter, vitamin B₁₂ deficiency would have developed again, but the patient had suffered enough. The *L. leichmannii* serum test for vitamin B₁₂ should answer the question in any future cases.

The case was diagnosed as being one of tropical sprue, but the diarrhoea commenced in Siberia. It cannot be said with certainty that tuberculous adenitis of the mesenteric glands was not present.

*Case 13.*
Case 12. Megaloblastic marrow despite injections of pteroylglutamic acid.

Case 12. Normoblastic marrow after an injection of cyanocobalamin.
Case 13. Folic acid excretion test in May 1953.

A male patient aged 52 years who was admitted to Dr. Gilchrist's ward of the Royal Infirmary of Edinburgh in May 1953 complaining of persistent pain in the left lower chest and upper abdomen for fifteen months.

In 1948 he had had two spontaneous fractures in the pelvis, involving the neck of the right femur and the superior ramus of the pubis on the right. These were treated in an orthopaedic ward, but the cause of the fractures was not investigated.

Fifteen months before the present admission there was a gradual onset of pain in the lower left chest. There was no history of diarrhoea or bulky stools. A detailed dietetic history gave no evidence of poor diet.

X-rays of the bones showed generalised osteoporosis with pseudo-fractures of the 8th and 9th right ribs posteriorly. The changes were reported as being 'almost certainly due to osteomalacia'. The pictures are not suitable for reproduction, but an X-ray photograph of the pelvis in May 1953 still gave evidence of a fracture of the right superior pubic ramus.

The/
The haemoglobin level was 15.2 G. per 100 ml; red cells 5,020,000 per c.mm. The marrow was normoblastic, no megaloblasts or intermediate erythroblasts being seen. There was free hydrochloric acid in the gastric juice. The serum calcium level was 8.3 mg. per 100 ml. and a fat balance test showed 85% and 74.6% absorption in two four-day collections.

Folic acid excretion test

Urinary excretion

in 24 hrs. after 5 mg. SC = 2.54 mg.
in 24 hrs. after 5 mg. Orally = 0.33 mg.

TEST POSITIVE

A therapeutic injection of 60 mg. pteroylglutamic acid was given intravenously, and two days later the above test was repeated.
Folic acid excretion test

Urinary excretion
in 24 hrs. after 5 mg. SC = 2.55 mg.
in 24 hrs. after 5 mg. Orally = 0.12 mg.

TEST POSITIVE.

The patient was treated with the emulsifying agent Sorlate by mouth in a dosage of 0.5 G. q.i.d. for a month. There was general improvement and gain in weight but an oral test dose of 5 mg. of pteroylglutamic acid then led to an excretion of only 0.048 mg. in the urine. The serum vitamin B12 level was 120 µg./ml.

Comment: Idiopathic steatorrhoea, presenting as osteomalacia. Although there was no anaemia, no megaloblastic changes in the marrow, and no evidence of tissue depletion of folic acid, there was impaired absorption of this substance. The serum vitamin B12 level also was low.

Case 14.

A female patient aged 60 years who had had diarrhoea with weight loss for two years. At first the motions were frequent but appeared normal. Later the bowels moved six or seven times daily and the stools were yellow and malodorous. No blood or mucus was seen. The diet was normal.

The patient had been in Canada for several years, but nowhere else outside Britain. There was a previous history of a prolapse operation, of removal of fibroids, and of acute rheumatism.
rheumatism.

In March 1950 she was diagnosed at Edinburgh Royal Infirmary Outpatient Department as having pernicious anaemia because the haemoglobin level was 9.8 G. per 100 ml., red cells 2,300,000 per c.mm., C.I. 1.47. Treatment at first was with Anahaemin, 2 ml. weekly. In March 1952 she was again seen at the Royal Infirmary for diarrhoea, and a barium enema was negative. The haemoglobin level was 14.2 G. per 100 ml., red cells 4,610,000 per c.mm., but the physician increased the frequency of the liver injections. Diarrhoea became worse and in April 1953 the haemoglobin level was 13.5 G. per 100 ml., red cells 4,080,000 per c.mm. Cyanocobalamin was therefore given in a dosage of 100 µg. fortnightly instead of the liver injections. This was continued up to the time of admission.

In June 1953 the patient was admitted to hospital. The haemoglobin level was 12.4 G. per 100 ml., red cells 4,170,000 per c.mm. The results of the main investigations were:

- **Stools**: Sprue like in appearance - pale and bulky.
- **Marrow**: Very megaloblastic (despite the relatively high red cell level).
- **Prothrombin activity**: 50%.
- **Fat balance tests**: 94.8% and 96.3% absorption.
- **Test meal**: High acid curve without histamine.
- **Barium follow through**: Negative (ordinary barium used).

**Folic acid excretion test**

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urinary excretion in 24 hrs. after 5 mg. SC</td>
<td>3.0 mg.</td>
</tr>
<tr>
<td>Urinary excretion in 24 hrs. after 5 mg. Orally</td>
<td>0.196 mg.</td>
</tr>
</tbody>
</table>

**TEST POSITIVE.**
Test repeated after 60 mg. of folic acid IM as therapy.

Urinary excretion
in 24 hrs. after 5 mg. SC = 4.5 mg.
in 24 hrs. after 5 mg. Orally = 0.60 mg.

TEST POSITIVE

Comment: "Refractory megaloblastic anaemia" due to malabsorption. Negative fat balance test and barium follow through, although the stools suggested 'sprue'. Note that although the marrow was megaloblastic and the patient was not deficient in vitamin B₁₂, the folic acid test did not give evidence of tissue depletion of folic acid.

Follow up: Treated with pteroylglutamic acid by mouth and cyanocobalamin by injection. Blood counts became normal and weight increased but some diarrhoea continued.

Case 15. Folic acid excretion test in September 1953.

A female patient aged 47 years who had been admitted to the Gastrointestinal Unit of the Western General Hospital, Edinburgh, with a complaint of tiredness, weight loss, pain in the back and three to four bowel motions daily.

In October 1951 she had been admitted to the same hospital with abdominal pain of eleven months duration, and vomiting for four days. At the time the haemoglobin reading was 13.1 G. per 100 ml., and white cell count 4,800. A laparotomy showed five areas of non-specific inflammation between the lower jejunum and the terminal ileum. The mesenteric glands were large and fleshy and a biopsy specimen showed/
showed non-specific inflammation and ulceration of the ileum, with chronic inflammatory reaction in the lymph node. In November 1951 a barium follow through gave results consistent with a diagnosis of regional jejunitis and ileitis.

In April 1953 the patient was re-admitted to hospital with pain and stiffness in the back and limbs. No clinical abnormality was found in the abdomen. The serum calcium level was 7.5 mg. per 100 ml., the haemoglobin level was 9.2 G. per 100 ml., and the red cell count was 4,350,000 per c.mm. A fat balance test (75 G. intake daily for two four-day periods) gave 87.2% absorption.

In September 1953 the patient was again re-admitted. The blood counts were haemoglobin 10.1 G. per 100 ml., red cells 3,710,000 per c.mm., PCV 38%, MCV 102 cu., MCHC 29%. The marrow was megaloblastic and there was histamine fast achlorhydria. There was a flat blood sugar curve following the oral administration of glucose, and a five-day fat balance test showed 66.8% absorption.

A barium follow through on 17/9/53 showed well marked dilatation of numerous coils in the lower ileum, with at least one organically narrowed segment showing a small ulcer crater. The terminal two inches of the ileum showed spasticity with mucosal atrophy and a scar of an old ulcer. Some spasticity was present in the caecum.

The/
The appearances were those of Crohn's disease.

The serum vitamin B₁₂ level (L. leichmannii assay) was 175 µg./ml.

Folic acid excretion test

Urinary excretion
- in 24 hrs. after 5 mg. SC = 2.69 mg.
- in 24 hrs. after 5 mg. Orally = 0.21 mg.

TEST POSITIVE.
Comment: Extensive Crohn's disease with evidence of intestinal malabsorption of fat, folic acid, glucose and calcium. Although the marrow was megaloblastic and the serum vitamin B12 level normal, the injected test dose of pteroylglutamic acid did not indicate folic acid deficiency.

Case 16.

Folic acid excretion test in November 1953.

A female patient aged 39 years. She was in Ward 27 of Edinburgh Royal Infirmary in May 1951 on account of pyelitis; there was also iron deficiency anaemia which had not responded to iron by mouth. She had no diarrhoea. There was a previous history of anaemia in pregnancy in 1935 and in 1943, but no details could be obtained.

At the time of this admission the haemoglobin level was 8 G. per 100 ml., red cells 4,060,000, MCV 71.4 c.i., MCHC 27.6%. A test meal showed free hydrochloric acid to be present, and the marrow was normoblastic. No abnormality was obvious on physical examination.

Treatment of the anaemia was by injections of saccharated oxide of iron intravenously and the haemoglobin level rose to 14.6 G. per 100 ml. The patient felt well and ceased attending the Blood Clinic.

In October 1953 Dr. J.H. Wright of Glasgow Royal Infirmary kindly wrote to say that the patient had been discharged from that hospital following admission for epigastric pain and anaemia. The haemoglobin level had been 8.2 G. per 100 ml. and/
and the red cell count 2,680,000 per c.mm. The marrow had contained cells showing megaloblastic change, but a fat balance test showed 92.6% absorption. The epigastric pain was due to a gastric ulcer. The serum vitamin B₁₂ level (Euglena assay) had been 255 µg./ml. (i.e. normal). Treatment had been with cyanocobalamin 100 µg. twice weekly and pteroylglutamic acid 20 mg. daily by mouth.

These were continued up to the time of admission to Edinburgh Royal Infirmary; with Dr. Wright's approval the patient consented to be admitted for 48 hours for a folic acid excretion test. By now she was complaining of slight diarrhoea but the stools appeared normal during the two days in the ward.

**Folic acid excretion test**

Urinary excretion
in 24 hrs. after 5 mg. SC = 3.21 mg.
in 24 hrs. after 5 mg. Orally = 0.021 mg.

**TEST POSITIVE.**

Comment: 'Refractory megaloblastic anaemia' without evidence of steatorrhoea. Markedly positive differential folic acid excretion test.

**Case 17.**

A female patient aged 72 years who had been having Anahaemin injections for several years for "anaemia". For about a year she had been feeling tired and there had been some/
some loss of weight, also pain in the back, the hips, and the knees. Diarrhoea was not a feature. The patient was admitted to the Rheumatic Unit at the Northern General Hospital and was found to have extensive decalcification of the bones with a fracture of the inferior ramus of the pubis on the right and a fracture of the left ulna. The haemoglobin level was 5.6 G. per 100 ml., and the red cell count was 3,280,000 per c.mm. The marrow contained megaloblasts. The serum calcium level was 7.2 mg. per 100 ml., and the alkaline phosphatase reading was 34 units. A test meal showed histamine fast achlorhydria.

The tongue was red and atrophic but a glucose tolerance curve showed a rise from a fasting blood sugar level of 80 mg. per 100 ml. to 142 mg. per 100 ml. one hour after 50 G. of glucose had been given by mouth. A barium follow through examination with ordinary flocculable barium showed no abnormality. Nevertheless a diagnosis of idiopathic steatorrhoea was suspected and a folic acid excretion test was requested.

**Folic acid excretion test**

<table>
<thead>
<tr>
<th>Urinary excretion</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>in 24 hrs. after 5 mg. SC</td>
<td>= 0.677 mg.</td>
</tr>
<tr>
<td>in 24 hrs. after 5 mg. Orally</td>
<td>= 0.051 mg.</td>
</tr>
</tbody>
</table>

**TEST POSITIVE.**

A fat balance test was done later (75 G. of fat for six days) and this showed 68.4% absorption. After three months treatment with pteroylglutamic acid the folic acid excretion test showed a 4.6 mg. output after the injected dose and 0.84 mg. after the oral dose.

Comment:
Comment: Idiopathic steatorrhoea with decalcification of the bones and fractures. Positive fat balance test but negative glucose absorption curve. The differential folic acid excretion test appeared to show tissue depletion of folic acid and malabsorption of the substance.

Case 18. Folic acid excretion test in December 1953.

A male patient aged 28 years, who had had diarrhoea for 2 months, loss of weight for two months and a cough for two weeks. The bowels moved about thrice daily and the stools were yellow-green and watery. There was no history of illness in childhood, the diet was normal and he had not been abroad. He was admitted to Dr. Gilchrist's ward of Edinburgh Royal Infirmary in October 1953. The haemoglobin level was 7.0 G. per 100 ml., red cells 3,070,000 per c.mm., C.I. 0.78, white cells 11,800 per c.mm. The blood film showed a dimorphic picture. A fat balance test was not permitted but in a 24 hour specimen 44% of the stool was fat. In another sample the fat content was 50.7%. A glucose absorption test gave a flat curve, the rise being from 73 mg.% to 96 mg.% after the 50 G. dose of glucose. The marrow was normoblastic, but intermediate erythroblasts were seen. A barium follow through examination with ordinary barium showed no flocculation pattern or other abnormality. A folic acid balance test was planned but owing to a misunderstanding 15 mg. were given in the oral test dose instead of 5 mg. The results were/
were as follows:

**Folic acid excretion test**

**Urinary excretion**

<table>
<thead>
<tr>
<th>Dosage</th>
<th>SC</th>
<th>Orally</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 mg.</td>
<td>3.12 mg.</td>
<td>3.01 mg.</td>
</tr>
<tr>
<td>15 mg.</td>
<td>4.98 mg.</td>
<td>0.79 mg.</td>
</tr>
</tbody>
</table>

Thereafter 15 mg. of pteroylglutamic acid were given intramuscularly daily for a week and the test repeated correctly. He improved a great deal in his general condition with this treatment, but there was no immediate haematological improvement. The serum vitamin B₁₂ level was 160 μg./ml.

**Folic acid excretion test**

<table>
<thead>
<tr>
<th>Dosage</th>
<th>SC</th>
<th>Orally</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 mg.</td>
<td>4.98 mg.</td>
<td>0.79 mg.</td>
</tr>
</tbody>
</table>

**TEST POSITIVE.**

**Comment:** Iron deficiency anaemia in a young man with intestinal malabsorption. When an oral test dose of 15 mg. of pteroylglutamic acid was given in place of the usual 5 mg. dose, the amount absorbed and excreted was similar to that of the normal person receiving 5 mg.

**Case 19.**

A female patient aged 56 years who had had bouts of diarrhoea from childhood up to the time of her admission to the Deaconess Hospital, Edinburgh, in August 1947. The diarrhoea/
diarrhoea was brought on by the eating of fat, greasy foods, or coarse vegetables, and when it was at its worst, the bowels moved eight or nine times daily. There was no koilonychia. At the time of admission to the Deaconess Hospital the haemoglobin level was 6.1 G. per 100 ml; red cells 3,270,000 per c.mm., 0.I. 0.64. The marrow was reported as being normoblastic. A test meal showed free hydrochloric acid to be present, and a fat balance test showed 85.3% absorption; this was only one collection although over a period of several days. Charcoal markers showed no evidence of intestinal hurry.

This was considered to be a case of adult coeliac disease, and the patient was treated with Anahaemin in a dosage of 2 ml. every 3 weeks up to the time of the folic acid balance test in November 1952, and with pteroylglutamic acid 5 mg. daily by mouth until April 1952.

The patient, who lived at a distance from Edinburgh, consented to come into hospital for two days for a folic acid balance test. At the time the haemoglobin level was 13.2 G. per 100 ml.; red cells 4,610,000 per c.mm. There had been no diarrhoea from the time of the commencement of treatment with liver injections and pteroylglutamic acid. The tongue was not painful, and the patient looked well.

Folic acid excretion test

Urinary excretion
in 24 hrs. after 5 mg. SC = 3.27 mg.
in 24 hrs. after 5 mg. Orally = 1.61 mg.

TEST: of doubtful significance.

Comment: A differential folic acid excretion test of doubtful significance in a patient who had been under treatment for adult/
adult coeliac disease and who had been free of symptoms for five years.

**Follow up:** A year after the pteroylglutamic acid therapy was stopped, diarrhoea recurred and the patient's doctor recommended this form of treatment with success.

**Case 20.**

Folic acid excretion test in November 1953.

A male patient aged 21 years who commenced to have diarrhoea at the age of 3 years. The stools were pale and bulky and he was admitted to the Royal Hospital for Sick Children at the age of six years. A diagnosis of coeliac disease was made. The diarrhoea continued and the boy was readmitted to the Children's Hospital at the age of 11 years.

His subsequent records are lost but it is known that he attended the Blood Clinic at Edinburgh Royal Infirmary in 1945 and that the haemoglobin level was then 4.8 G. per 100 ml. The anaemia was of an iron deficiency type. He was treated with diet, iron, liver injections and various vitamin preparations, and improved considerably.

He was seen again in June 1943 and apart from occasional diarrhoea he felt well. The haemoglobin level, without any therapy, was now 13 G. per 100 ml., and the red cell count was 4,290,000 per c.mm. In November 1953 diarrhoea was more troublesome but the general condition was good and he was working as a miner. The haemoglobin reading had fallen to 12.5 G. per 100 ml. and the red cell count to 3,670,000 per c.mm. The patient, who did not live in Edinburgh, agreed to come/
come into hospital for forty-eight hours for a folic acid excretion test.

**Folic acid excretion test**

- Urinary excretion in 24 hrs. after 5 mg. SC = 2.39 mg.
- Urinary excretion in 24 hrs. after 5 mg. Orally = 0.79 mg.

**TEST POSITIVE.**

The serum vitamin B₁₂ level (L.leichmannii assay) was 160 µg./ml.

**Comment:** Adult coeliac disease with minimal symptoms, but a positive differential urinary folic acid excretion test.

**Case 21.**

Folic acid excretion test in November 1953.

A male patient aged 42 years who had been in Edinburgh Royal Infirmary in 1950 for investigation because of a three years history of diarrhoea, the motions being as frequent as twenty times per day. The stools were thin, watery, pale and offensive, but contained no blood or mucus. Diarrhoea ceased on admission to hospital. The patient had not been abroad. Anaemia was present, and the marrow was megaloblastic. At the time the haemoglobin level was 6.2 G. per 100 ml., red cells 1,640,000 per c.mm., PCV 19.5%, MCV 108.2 µm., MCHC 31.8%. There was no response to an injection of 40 µg. of cyanocobalamin, but a good response to pteroylglutamic acid by mouth in a dosage of 20 mg. daily. This was later reduced to 15 mg. daily.

Over a period of eighteen days at the time of this admission/
admission to hospital six three-day stool collections were made. The patient was having a controlled diet of 50 G. of fat daily. It is of interest that in three three-day stool collections before pteroylglutamic acid therapy the fat absorption results were respectively 73.7%, 79.8% and 75.4%, whereas immediately after the commencement of such therapy they were 88.3%, 91.3% and 92.2%. Fat splitting varied from 48% to 80%.

The patient continued with folic acid by mouth as an outpatient, slight diarrhoea occurring from time to time. In November 1953 the haemoglobin level was 13.3 G. per 100 ml., red cells 5,340,000 per c.mm., and a folic acid excretion test was performed at the patient's house.

**Folic acid excretion test**

<table>
<thead>
<tr>
<th>Urinary excretion</th>
<th>SC</th>
<th>orally</th>
</tr>
</thead>
<tbody>
<tr>
<td>in 24 hrs. after 5 mg.</td>
<td>4.9 mg.</td>
<td>0.043 mg.</td>
</tr>
</tbody>
</table>

**TEST POSITIVE.**

The serum vitamin B₁₂ level (L.leichmannii assay) was 158 µg./ml.

**Comment:** Idiopathic steatorrhoea with a markedly positive differential folic acid excretion test.

**Case 22.**

A female patient aged 48 years who had been in one of the wards of Edinburgh Royal Infirmary in 1949. The records of that admission are lost but it is known that the bone marrow was megaloblastic and the patient was told that she had pernicious/
pernicious anaemia. She did not respond to liver injections, and was treated for three and a half years with pteroylglutamic acid alone, by mouth, in a dosage of 5 mg. daily. She was first seen at the Blood Clinic in November 1953. She had no very definite complaints and there was no diarrhoea. A test meal was done on an outpatient basis, and free hydrochloric acid was found to be present. A folic acid excretion test was done at the patient's home.

Folic acid excretion test

Urinary excretion
in 24 hrs. after 5 mg. SC = 4.95 mg.
in 24 hrs. after 5 mg. Orally = 1.30 mg.

TEST POSITIVE.

The serum vitamin B_{12} (L.leichmannii assay) was 314 µg./ml.

Comment: A patient wrongly diagnosed as having pernicious anaemia but treated with pteroylglutamic acid. There was free hydrochloric acid in the stomach and a normal serum vitamin B_{12} level. The folic acid excretion test was less positive than in many, but there were no symptoms or signs of steatorrhoea. The patient could not come into hospital for a fat balance test.

DISCUSSION.

The interpretation of these findings will be considered in Chapter 10.

In a recent review dealing with the diagnosis of idiopathic steatorrhoea, Cooke and his co-workers at Birmingham (1953) state that fat balance tests must remain the mainstay of diagnosis until more informative tests are devised. They say, further/
further, that usually a single three-day balance test is adequate for diagnosis but that when the defect is slight or the patient constipated, repeated three-day tests have to be carried out. Glucose absorption tests they dismiss because a flat tolerance curve is by no means invariable in idiopathic steatorrhoea. So far as our experience goes, Table 51 includes several patients with fat balance tests that show normal results, although there was undoubtedly other evidence of intestinal malabsorption. In Case 3 there was malabsorption of iron and folic acid but the fat balance test showed 96.1% absorption of fat. In Case 12 the diagnosis of steatorrhoea had been known for years and the patient was in poor condition with tetany, but a seven-day fat balance test carried out in the Metabolic Ward showed 95.8% absorption. In Case 14 the diagnosis was obvious clinically, but two four-day fat balance tests showed respectively 94.8% and 96.3% absorption.

In Case 3 the findings suggested tissue depletion of folic acid, and the ratio

\[ \frac{\text{excretion after oral test dose of pteroylglutamic acid}}{\text{excretion after subcut. test dose of pteroylglutamic acid}} \]

expressed as a percentage was 24.1%. In Case 12, despite the reported normal result for the fat balance test, the percentage folic acid excretion ratio calculated as above was 4.7%. In Case 14 the percentage folic acid excretion ratio was 6.5%. In the controls this ratio was usually in the region of 100% and the lowest ratio (Case 45) was 62.5%. It would/
would seem therefore that the differential folic acid excretion test is more satisfactory than the fat balance test in that the difference between a positive and a negative result is great.

We have seen that it is possible to have a positive folic acid excretion test with a negative fat balance test. No doubt the reverse may occur in some cases of intestinal malabsorption but no such patient has yet been encountered.

Cooke and his colleagues also state that radiography of the intestinal tract must be accepted as an essential diagnostic procedure. Using a flocculating barium emulsion they found a 'deficiency' pattern in all the 100 patients they examined. On the other hand they state that since regional ileitis and jejun-ileitis may closely resemble idiopathic steatorrhoea, additional information may be obtained by the use of a non-flocculating barium emulsion, by which strictures or abnormal mucosal patterns may be more clearly defined.

(Other papers on this subject are those by Snell & Cramp, 1934; Kantor, 1939; Golden, 1945; and Ardran, French & Mucklow, 1950).

It will be noted that in a number of instances the X-ray pattern of the small intestines of our patients was reported as being normal. It will be appreciated that the patients were in various hospitals in the region and that in some instances these X-ray examinations had been done before we had access to the patients for the folic acid excretion test. We were not, therefore, in a position to suggest to the radiologist/
radiologist what type of barium he should use. However, in
Cases 3, 18 and 21 the X-ray examinations were carried out in
Edinburgh Royal Infirmary by radiologists who were particularly
interested in this problem and no abnormal pattern was seen.
In Case 21 the radiologist, who had made a special study of
the subject, tried various barium emulsions and could not
demonstrate any abnormality. In Case 17 our first information
about the case came from the radiologist to the Northern
General Hospital who did not find any abnormality of the small
testine by X-ray examination.

Further Practical Details about the Test.

In those cases described in Table 51 where pteroylglutamic
cid therapy had been given for months or years, and even in
some cases where this had not been done, a mere comparison of
excretion following the oral dose with that following the sub-
cutaneous dose was sufficient.

In patients who have not had long-continued treatment with
olic acid, a preliminary test might be to measure the urinary
excretion following the administration of 5 mg. by mouth. If
the excretion were more than 2.0 mg. this would indicate that
there was unlikely to be severe malabsorption of folic acid,
and further investigations along these lines would probably not
be helpful. The figure of 2.0 mg. is taken rather than 1.5 mg.
because it will be recollected that Case 19 excreted 1.61 mg.
after the oral dose, and 3.27 mg. after the preceding subcutan-
ous dose.

If, however, the excretion were less than 2.0 mg. and time
permitted, it might be advisable to saturate the patient's
tissues
tissues by folic acid injections before doing the full differential urinary excretion test. The minimal dose required to saturate the tissues is not yet known, but it is suggested that 15 mg. of folic acid might be given subcutaneously daily for a week. Further work should show whether one single dose is effective. The experiments on control subjects give no reason for supposing that preliminary saturation with folic acid blocks further absorption from the alimentary tract.

Although the urinary excretion of administered folic acid occurs within a few hours, it is advisable to allow 48 hours to elapse between the administration of a saturating dose and the commencement of the differential excretion test. The folic acid for the test doses must be taken from ampoules of known potency, and ampoules from the same batch should be used for the subcutaneous and oral test doses. A tuberculin syringe should be used for measuring each dose.

No antibiotics, sulphonamides or other vitamin preparations should be given during the period of the test, as they would interfere with the microbiological assay. It is essential to ensure that all the urine passed during the 24 hours after each test dose is separately collected.

SUMMARY

A description is given of a new test for intestinal malabsorption consisting of a comparison of the urinary excretion of folic acid following subcutaneous and oral test doses of 5 mg. of pteroylglutamic acid.

The test has been applied to 22 cases of intestinal malabsorption/
malabsorption (including tropical sprue, idiopathic steatorrhoea and tuberculous adenitis of the mesenteric glands), 14 cases of pernicious anaemia and 30 controls of various types. Patients with megaloblastic anaemia of pregnancy and post-gastrectomy cases will be considered in later chapters.

Impairment of absorption of folic acid was found only in patients with features to suggest malabsorption of other substances, in vitamin B12 refractory megaloblastic anaemia or in refractory iron deficiency anaemia. A positive fat balance test or an abnormal X-ray pattern of the small intestine with a flocculable barium emulsion was not necessarily present.

The differential folic acid excretion test is easy to perform from the hospital ward viewpoint, but the folic acid assay can only be done in a microbiological assay laboratory. Domiciliary folic acid excretion tests are practicable.

In the normal person the ratio between the urinary excretion of folic acid after a 5 mg. oral test dose and that after a 5 mg. subcutaneous test dose is about 1 (a percentage ratio of 100%). In a patient with intestinal malabsorption the percentage ratio may be as low as 4.9% even when the fat balance test shows normal absorption of fat.
CHAPTER 10.

THE ABNORMALITIES OF METABOLISM OF HAEMOPOIETIC FACTORS IN THE CONDITIONS COMMONLY INCLUDED UNDER THE NAME "MALABSORPTION SYNDROME"

Folic Acid.

Anaemia is common in tropical sprue, idiopathic steatorrhoea, coeliac disease and in forms of intestinal malabsorption due to structural changes in the alimentary tract. In tropical sprue, idiopathic steatorrhoea or in coeliac disease persisting into adult life, the marrow is frequently megaloblastic or contains intermediate erythroblasts. In an infant or child with coeliac disease, iron deficiency anaemia is common.

The reason why iron deficiency anaemia rather than megaloblastic anaemia is typical of coeliac disease in the infant is not yet known. The factors to be considered include the extent of tissue storage of antimegaloblastic substances at birth, the time required for these stores to be used up, the degree to which folic acid and citrovorum factor are absorbed from the alimentary tract in coeliac infants, and the possibility that intestinal bacteria may supply antimegaloblastic substances by synthesising them in the small intestinal canal.

Since the relative importance of these various factors is not known, we need not consider them further. There is no doubt that a child with coeliac disease may later, as an adult develop/
develop megaloblastic anaemia, and it would appear reasonable to suggest that Case 2 in Table 51 will in due course develop this because her small intestine is unable to absorb folic acid normally. Absorption of folic acid from the large intestine is poor (Moore, 1947): with the kind co-operation of Professor A. R. Lowdon we have carried out an experiment to see to what extent pteroylglutamic acid can be absorbed from the human stomach. Laparotomy was carried out on an anaesthetised patient. The pylorus was occluded and 45 mg. of pteroylglutamic acid were injected into the stomach. The serum levels of folic acid were measured; they were as shown in the following illustration.

![Image of serum folic acid levels](image-url)
Thus although a very large amount of pteroylglutamic acid had been introduced into the stomach there was little evidence of absorption of the substance in the time during which it was possible to carry out the investigation. We have already seen that there is an appreciable rise in the serum level of folic acid at thirty minutes after its oral administration. It must be admitted, however, that a patient under a general anaesthetic is a very unphysiological subject for the above investigation.

The simplest explanation of our findings about folic acid absorption recorded in Chapter 9 would seem to be that in the conditions commonly known collectively as "the malabsorption syndrome", a substance that is not absorbed normally is pteroylglutamic acid (or a conjugate form of pteroylglutamic acid or some substance derived from these in the alimentary tract). Where there is megaloblastic anaemia in a case of this type, one would expect this anaemia to be an indication of depletion of the body stores of folic acid and related compounds (including citrovorum factor and its conjugates) or of vitamin B12, or of both these groups of substances. When the patient has been treated with cyanocobalamin and still has megaloblastic anaemia one would expect that biochemical tests would indicate folic acid depletion. Unfortunately, however, the data in Table 51 indicate that such is not necessarily the case. Patient No.14, who had a particularly megaloblastic marrow despite the relatively high red cell level and who had been/
been receiving adequate therapy first with liver injections and then with cyanocobalamin, excreted 3.0 mg. of an injected 5 mg. dose of pteroylglutamic acid. Patient No. 15 who had frankly megaloblastic anaemia associated with Crohn's disease, and who had received no treatment, excreted 2.69 mg. from the injected 5 mg. dose. Her serum vitamin B₁₂ level was at the lower limit of normal, being 170 µg./ml.

In both instances the proportion of the injected test dose of pteroylglutamic acid that was excreted in the urine was within normal limits and it can be stated with certainty that the explanation does not lie in any mistake in the administration of the dose or in the batch of pteroylglutamic acid used. The assay was repeated five times and no error had been made.

It will be noted further in Case 14 that when the test was repeated after the patient had been given, as treatment, 60 mg. of pteroylglutamic acid (and had ceased excreting from this injection), the urinary output after a 5 mg. injected test dose was 4.5 mg. In both patients the marrow was converted to the normoblastic state by pteroylglutamic acid. There was no biochemical or clinical evidence of liver disease.

It may therefore be said that although the differential folic acid excretion test is 'positive' in intestinal malabsorption, in that absorption of folic acid is impaired, it is not possible to conclude from a normal urinary output after an injected test dose that folic acid therapy is not required. (See also Chapter 14 dealing with Megaloblastic Anaemia of Pregnancy.)
Pregnancy.) It may be that although the body is depleted of folic acid compounds, under certain circumstances the excretion of these is so rapid that the organs and tissues do not have time to store them following their injection. It will be recollected that evidence has been given in Chapter 6 for the view that the storage form in the liver is largely citrovorum factor or its conjugates. We have inadequate knowledge of the mode of uptake of folic acid compounds by the body cells.

A similar explanation is put forward elsewhere (p. 467) to explain the urinary excretion of vitamin $B_{12}$ in pernicious anaemia when cyanocobalamin is injected. We have already seen (p. 292) that in vitamin $B_{12}$ deficiency there may be a low urinary output of folic acid following an injected test dose of pteroylglutamic acid. Now we are faced with the fact that in what seems to be a deficiency state involving folic acid the excretion of such a test dose may sometimes be normal. The most that can be said in the light of our findings is that if a patient has megaloblastic anaemia that is not due to vitamin $B_{12}$ deficiency, a low urinary excretion of folic acid following a 5 mg. injected test dose of pteroylglutamic acid suggests folic acid depletion but a normal output does not exclude it.

An alternative explanation for these unexpected findings would appear to be that although the anaemia responds to pteroylglutamic acid therapy, the deficiency is not always one of folic acid or of vitamin $B_{12}$ — a third substance is missing. Further evidence for this theory is the fact that combined/
combined therapy with pteroylglutamic acid and cyanocobalamin would not raise the red cell count of several of the cases of intestinal malabsorption to a completely normal level. Thus in Case 15 injections of cyanocobalamin, pteroylglutamic acid and saccharated oxide of iron failed to raise the red cell count to 4,000,000 per c.mm., despite the fact that the marrow was converted to the normoblastic state, and that there was no biochemical evidence of liver disease or uraemia and no clinical evidence of myxoedema or any other complicating condition outside the alimentary tract. On the other hand the patient was suffering from Crohn's disease and it is reasonable to consider that this condition itself might, like any chronic disease, cause anaemia.

The data in Table 51 may be explored further with a view to searching for evidence of another unknown haemopoietic factor. Case 8 was a man with tropical sprue who had been treated with pteroylglutamic acid by mouth for 3 years. The excretion of folic acid after a 5 mg. test dose was 3.41 mg. (normal). The red cell count was 4,390,000 per c.mm., and subsequent treatment with injections of cyanocobalamin, and with ascorbic acid and other vitamins, failed to restore the count to normal. Case 11 was a man who had had treatment with cyanocobalamin in addition to pteroylglutamic acid and various vitamin preparations. The red cell count was 3,840,000 per c.mm., but abdominal tuberculosis could not be excluded.

In summary, therefore, it may be said that although there is/
is some indirect clinical evidence that factors other than iron, ascorbic acid, vitamin $B_{12}$ and the folic acid compounds are lacking in patients with intestinal malabsorption, there is no direct proof of this. One clear finding is that folic acid absorption is impaired in the conditions known collectively as the malabsorption syndrome.

An alternative consideration might be that depletion of body stores of folic acid can be measured by estimating the excretion after a 5 mg. injected test dose and then repeating the test after a second such dose. If the amount retained after the second injection is considerably less than that after the first injection, this may itself indicate increased bodily requirements for folic acid. This matter will be referred to again later in Chapter 18 which deals with folic acid excretion in malignant disease. It should, however, be recalled that the data given in Table 53 show that even in a normal person who has had pteroylglutamic acid injections for several days, the excretion after a 5 mg. injected test dose is less than 5 mg. Either some of the folic acid is katabolised in the body or altered in some way so that, if excreted in the urine, it is no longer available for the growth of $S$.faecalis.

Citrovorum Factor.

As was seen in Chapter 7, the excretion after an oral test dose of citrovorum factor is, even in the normal person, considerably less than that after an injected dose of equal amount.
amount. This renders a differential urinary citrovorum factor excretion test difficult, but it would be instructive to carry out citrovorum factor excretion tests in cases of intestinal malabsorption (and malignant disease). Unfortunately no manufacturer has been able to supply us with any of this substance since our preliminary experiments were done. Dr. Frank Hartley of British Drug Houses Ltd. kindly looked into the possibility of producing citrovorum factor but found that it was impracticable, and Lederle & Co. Inc., who supplied it in the first instance, are no longer able to do so. One isolated observation that was made was that when Case 11, Table 51, who had been treated for idiopathic steatorrhoea with pteroylglutamic acid, was given 36 mg. of citrovorum factor by mouth, the serum level of total folic acid activity (with S. faecalis as test organism) rose to a maximum of only 10.3 µg/ml at half an hour. This is considerably less than the rise in the patients described in Chapter 7 and suggests that there was poor absorption of citrovorum factor or of pteroylglutamic acid or any intermediate compound into which the citrovorum factor might have been converted.

So far as the urinary excretion of citrovorum factor after the administration of pteroylglutamic acid is concerned, this did not appear to be giving us much information, so this investigation was not carried out in all cases. It is generally agreed, and it has been our experience, that when pteroylglutamic acid is given to patients a very small proportion is excreted as citrovorum factor, but that this excretion is/
is complete within eight hours. However in Cases 5 and 12 in Table 51 more citrovorum factor (though less folic acid) was excreted after the oral test dose of pteroylglutamic acid than after the injected dose.

Other Substances.

So far we have discussed the absorption of folic acid and of citrovorum factor. Some of the 'folic acid' in foodstuffs is the conjugate form pteroylhexaglutamyl glutamic acid but this substance has to our knowledge been prepared in any quantity only by workers at Parke Davis & Co.'s laboratories, Detroit, and they state that they cannot supply any at present. It is however unlikely that a patient with intestinal malabsorption could absorb this conjugate form well while absorbing the 'free' folic acid badly.


It is not the purpose of this thesis to attempt to add to the theories about the causation of 'sprue'. It is of interest however that Sir Philip Manson-Bahr (1953) has reverted to his original view that tropical sprue is caused by a virus that attacks the alimentary tract - a virus that can lie dormant for long periods. He comments on the fact that 'experiences of the armies in Burma and India during the late war afforded no support to the dietetic theory, for sprue was as common among troops on full rations as it was in the jungle Chindits on a bare subsistence'. This does not take account of the fact that the rations of many of the Chindits contained so little fat that steatorrhoea was unlikely, but nevertheless, apart from the Indian troops who have been considered in detail in/
in Chapter 2, our experiences in India and Burma would support Manson-Bahr's theory that the condition is not primarily a dietetic one. Leishman (1945) has recorded that in one R.A.F. Unit, within three weeks of its arrival in the Chittagong area, 10% of its personnel was ill with diarrhoea which rapidly developed into the full sprue syndrome. This certainly suggests that some infection or other irritative factor is affecting the intestine.

Further, Stefanini (1947) who carried out a careful investigation of sprue amongst Italian prisoners of war in India, stated that he observed 1069 cases amongst 12,000 prisoners, that the disease developed in only one camp which, unlike the others, was at an altitude of 4,000 feet, and that the prisoners subsisted on the same diet in each camp. Moreover, he states, without comment, that megaloblastic anaemia developed in about four to five weeks after the onset of the disease. Stefanini is a well known and an accurate observer and this statement must not be disregarded. There is no reason to believe that these Italians were malnourished before the disease developed and it seems most unlikely that the bodily stores of folic acid could be used up in as short a time as four or five weeks. The possibilities that require consideration are: that malabsorption of folic acid existed for several weeks or months before diarrhoea developed; that folic acid was excreted in some abnormal manner; that in sprue there is unduly rapid utilisation of folic acid compounds; or that there is some folic acid antimetabolite in the diet or the water, or produced by organisms in the intestine or even by viruses/
viruses in the tissues.

All this is extremely hypothetical and it is mentioned merely to indicate that our knowledge of the problem of megaloblastic anaemia in sprue is most incomplete. It would be of much interest to carry out differential folic acid excretion tests on sprue patients in India.

The Investigation of Suspected Cases of Intestinal Malabsorption: Difficulties of purely clinical Diagnosis.

We have already seen from Table 51 that the diagnosis of intestinal malabsorption is not necessarily easy in atypical cases. Case 1 was thought to have idiopathic steatorrhoea but really had tuberculosis of the mesenteric glands; Case 3 had iron deficiency anaemia that did not respond to iron by mouth; Case 4 was diagnosed as idiopathic refractory megaloblastic anaemia; Case 7 had been considered at one time to have megaloblastic anaemia of pregnancy; Case 13 had been treated for multiple fractures without any further investigation at the time; Case 14 had a normal fat balance test result but clinical features of steatorrhoea; Case 15, who had regional jejunitis and ileitis, was diagnosed by laparotomy; Case 16 had been treated for refractory iron deficiency anaemia and had a normal fat balance test result; Case 17 was first seen because of fractures in association with what was believed to be iron deficiency anaemia; Case 22 had been diagnosed elsewhere as having "pernicious anaemia refractory to liver injections". In all these instances the differential folic acid excretion test made the diagnosis clear.
On the other hand we have seen in Case 12 that it is possible to have vitamin B₁₂ deficiency superadded to that complex metabolic abnormality which we shall hereafter refer to as 'folic acid deficiency', and hence investigation of the serum vitamin B₁₂ level is of value in these patients. So far reference has been made to the differential urinary folic acid excretion test only in relation to the diagnosis of intestinal malabsorption. Under certain circumstances it is of value in suggesting that some other condition must be present for account for the clinical features present. For example, in Case 49, Table 53, the chief feature of the case was tetany - there was no diarrhoea, no anaemia, the serum calcium level was 9.9 mg. per 100 ml., and a fat balance test showed 97.4% absorption. Renal function was normal. The differential folic acid excretion test gave no evidence of malabsorption of folic acid. It was felt therefore that a diagnosis of idiopathic steatorrhoea was not tenable; there was no hyperpnoea and no organic cause for the tetany was found.

Case 38 was a child aged 9 years in whom a diagnosis of coeliac disease with macrocytic anaemia had been made at a hospital in the Border country, because a specimen of stool had been reported to contain 80% of fat. A folic acid excretion test was done by post and absorption was shown to be normal. The child was transferred to Edinburgh Royal Infirmary where the diagnosis of aplastic anaemia was unfortunately shown at post mortem to be correct.
It was thought that Case 55 might have steatorrhoea because he had had diarrhoea for seven years. However blood and mucus was present in the stool and although the man had not been out of Britain a diagnosis of amoebic dysentery was made by the isolation of the parasite *Entamoeba histolytica* from the stool and because of a favourable response to emetine therapy.

The use of the differential folic acid excretion test will be considered again in relation to the metabolic abnormalities of the megaloblastic anaemias of pregnancy, in cases with steatorrhoea or anaemia after complete or partial gastrectomy and in patients with intestinal short circuits.

**PRACTICAL POINTS ABOUT ANTIANAEMIAC THERAPY IN INTESTINAL MALABSORPTION.**

In the earlier stages of these investigations the value of pteroylglutamic acid therapy in various forms of intestinal malabsorption became apparent, and such treatment is now standard practice. (See Graph 18.) Occasional cases respond to cyanocobalamin therapy as is shown in Graph 19.

We have already examined evidence which suggests that a normal urinary output after an injected test dose does not necessarily exclude folic acid depletion. We shall see in later chapters that a poor output after an injected test dose may occur in malignant disease, very prolonged chronic infection, renal failure, or where there is a large effusion. If these conditions are not present it is reasonable to believe that in a patient who does not suffer from vitamin B12 deficiency, a low output (less than 1.5 mg.) after an injected test dose of 5 mg. is indicative of folic acid deficiency. In Table 51, Case/
FAILURE OF HAEMATOLOGICAL RESPONSE TO CYANOCOBALAMIN IN IDIOPATHIC STEATORRHOEA.

CYANOCOBALAMIN

PTERYLGLUTAMIC ACID
20 mg. b.i.d. ORALLY.

HAEMATOLOGICAL RESPONSE TO CYANOCOBALAMIN IN TROPICAL SPRUE.

GRAPH 18.

GRAPH 19.
Case 4 was severely depleted of folic acid despite the administration of 15 mg. of pteroylglutamic acid by mouth for 37 days, but the depletion was corrected by the injection of 15 mg. daily for ten days. In Case 11 the excretion after an injected test dose suggested folic acid depletion despite the fact that pteroylglutamic acid had been taken by mouth in a dosage of 15 mg. daily for 2½ years. After a 5 mg. oral test dose, Case 6 excreted only 9 μg., Case 9 excreted 36 μg. and Case 11 excreted 55 μg. It is suggested therefore that in the initial stages of treatment of megaloblastic anaemia associated with intestinal malabsorption the pteroylglutamic acid should be given by injection in a dosage say of 15 mg. daily for two weeks and that every month a further injection of 15 mg. should be given in addition to regular oral therapy. Whether or not folic acid deficiency itself can lead to atrophy of the small intestinal mucosa and thus lead to a vicious circle is unknown. Reference has already been made (p. 166) to claims that changes in the mucosa of the small intestine have been produced in animals by the administration of folic acid antagonists.

In Case 12 it seems likely that the patient became refractory to folic acid therapy because vitamin B₁₂ deficiency had developed, and for this reason it is suggested that patients with intestinal malabsorption should receive monthly injections of 100 μg. of cyanocobalamin.

**SUMMARY**
SUMMARY.

In two patients with megaloblastic anaemia associated with intestinal malabsorption, one of whom had been receiving injections of cyanocobalamin, there was megaloblastic anaemia associated with a normal urinary output of folic acid after an injected test dose of 5 mg. of pteroylglutamic acid, despite evidence of intestinal malabsorption of the substance. This suggests that although a low output after the injected dose in a case of megaloblastic anaemia not due to vitamin B₁₂ deficiency probably indicates folic acid desaturation, a normal output does not exclude it. Presumably the substance is excreted more rapidly than it can be taken up by the organs and tissues.

Comment is made on reports that megaloblastic anaemia may develop in tropical sprue within four to five weeks of the onset of the disease.

The diagnostic value of the differential urinary folic acid excretion test is discussed and it is suggested that patients with intestinal malabsorption should be treated not only with pteroylglutamic acid by mouth but also with regular injections of this substance and of cyanocobalamin.
CHAPTER 11.

CONSIDERATION OF A SELECTION OF CASES OF PERNICIOUS ANAEMIA AND A REPORT ON THE USE OF VARIOUS THERAPEUTIC AGENTS.

In the course of the researches recounted in this thesis many patients suffering from pernicious anaemia have been investigated and treated. It is now generally agreed that pernicious anaemia may be treated by the regular injection of cyanocobalamin or of a potent liver extract.

It may be added here that we have found one batch of high potency liver extract (20 µg./ml.) which almost completely lost its potency as judged by the \textit{L. leichmannii} assay over a period of six months, and that this finding was reported to the manufacturers who later confirmed that it was correct. The explanation for this change was not forthcoming but subsequent batches of this liver extract were entirely satisfactory. According to our findings most 'refined' liver extracts, unlike many of those produced during the war years, are now satisfactory as regards vitamin B\textsubscript{12} content, having 14 to 24 µg. of cyanocobalamin per ml. provided cyanide is added before the \textit{L. leichmannii} assay is performed in order to convert other cobalamin compounds into cyanocobalamin. "Crude" liver extracts contain 3 to 5 µg. of cyanocobalamin per ml. under these conditions. According to our assays the content of growth factors for \textit{S. faecalis} (as pteroylglutamic acid) is usually less than 500 µg./ml. in both 'crude' and 'refined' extracts.
extracts.

In the present chapter examples of cases of pernicious anaemia treated other than by routine methods will be considered. The biochemical implications will be discussed in Chapter 12.

To avoid frequent repetition it may be said now that unless otherwise stated the patients were typical cases of pernicious anaemia with a megaloblastic marrow, histamine fast achlorhydria, no clinical evidence of steatorrhoea or alimentary carcinoma, no history of operation on the alimentary tract, but with a normal dietetic history and, subsequently in most instances, a satisfactory maintained response to cyanocobalamin injections. Serum vitamin B\textsubscript{12} estimations were not technically possible when most of the cases were seen. Some atypical cases of pernicious anaemia will also be considered.

With regard to the haemoglobin readings, 100% should be regarded as equivalent to 14.8 G. per 100 ml. For the reader better accustomed to using the percentage scale, a conversion table is included in a pocket at the end of the thesis.

A short control period to exclude spontaneous remission preceded the use of each therapeutic agent.

**Mr. M.C. (Graph 20).**

A 46 year old man who was admitted in March 1947 to the Western General Hospital, Edinburgh, with pernicious anaemia. He was tired and listless and said that his arms and legs felt like/
like lumps of lead. In addition he had numbness and tingling in the hands and in the lower limbs below the knees.

It appeared that in 1943 he had been diagnosed at Edinburgh Royal Infirmary as having pernicious anaemia and that for about eighteen months he had received weekly injections of liver extract. Thereafter he had ceased visiting his doctor or the hospital.

In the Western General Hospital the patient was found to have histamine fast achlorhydria and megaloblastic anaemia without diarrhoea or sore tongue. There was loss of vibration sense in the legs extending as far up as the anterior superior spines, the knee jerks were sluggish and the ankle jerks were absent. The plantar responses were plantar flexor and no objective changes were found in the upper limbs. The diagnosis of pernicious anaemia was considered to be correct and the comment of Dr. Levin, the consultant neurologist, was "A very abortive case of subacute combined degeneration. Mainly long ascending tracts involved, and these only partially."

It was decided that the patient should be treated with pteroylglutamic acid given by mouth in a dosage of 1 mg. daily. The haematological results are shown in Graph 20.* At the commencement of therapy the levels were:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin</td>
<td>6.8 G. per 100 ml.</td>
</tr>
<tr>
<td>Red cells</td>
<td>1,550,000 per c.mm.</td>
</tr>
<tr>
<td>C.I.</td>
<td>1.5</td>
</tr>
<tr>
<td>PCV</td>
<td>19%</td>
</tr>
<tr>
<td>MCV</td>
<td>129.0</td>
</tr>
<tr>
<td>MCHC</td>
<td>35.8%</td>
</tr>
<tr>
<td>White cells</td>
<td>2,000 per c.mm.</td>
</tr>
<tr>
<td>Reticulocytes</td>
<td>&lt; 1%</td>
</tr>
</tbody>
</table>

After fourteen days of treatment the megaloblasts in the marrow showed/

* See p.402.
showed slight condensation of their nuclei and the blood levels were: haemoglobin 6.2 G. per 100 ml., red cells 1,560,000 per c.mm., C.I. 1.35, PCV 18%, MCV 115.4 cµ., MCHC 34.4%, reticulocytes 1.3%, white cells 4,600 per c.mm. The dose of pteroylglutamic acid was increased to 2.5 mg. daily and the blood levels rose to: haemoglobin 14.8 G. per 100 ml., red cells 4,190,000 per c.mm., C.I. 1.2, PCV 42.5%, MCV 101.4 cµ., MCHC 34.8%, reticulocytes < 1%, white cells 6,400 per c.mm.

By this time the symptoms of neurological involvement were more severe. Objectively there seemed to be some spasticity in the legs and there was tenderness in the calves in addition to the features that had been present before therapy commenced.

Treatment with Anahaemin was therefore given in a dosage of 4 ml. weekly but this preparation caused the patient to sneeze. The sneezing could be prevented by the giving of antihistamine drugs. After two months of Anahaemin therapy there was no neurological improvement, but the haemoglobin level was 15.0 G. per 100 ml., and the red cell count was 4,800,000 per c.mm.

A 'crude' liver preparation, 'Heparglandol' was next given in a dosage of 4 ml. weekly and subjective neurological improvement occurred after about two months. Vibration sense began to return to the thighs, but six years later, although the red cell count had never fallen below 4,800,000 cells per c.mm. and the patient had been having cyanocobalamin injections in a dosage of 50 µg. every two weeks for nearly three years, he still/
still had numbness and tingling in the legs. Objectively the gait was spastic, vibration sense was absent below the knees, the ankle jerks were absent, and the knee jerks could be elicited only with difficulty.

Comment. Pernicious anaemia with neurological degeneration affecting especially the posterior columns. This developed because of inadequate supervision of the patient after the diagnosis of pernicious anaemia had first been made. The neurological features became more severe when pteroylglutamic acid was given, and injections of liver extract and of cyanocobalamin failed to cause significant improvement.

When pteroylglutamic acid was given as initial treatment the dosage required to cause a haematological remission was in excess of 1 mg. daily.
Mrs. B.P. (Graph 21a)

A 49 year old patient who had been feeling generally unwell for three months and who was found to be suffering from uncomplicated pernicious anaemia.

At the time of admission to hospital the blood findings were:

- Haemoglobin: 6.1 G. per 100 ml.
- Red cells: 1,650,000 per c.mm.
- C.I.: 1.24
- PCV: 16%
- MCV: 97.0 µl.
- MCHC: 38.1%
- White cells: 4,200 per c.mm.
- Reticulocytes: 1.8%

The patient was given daily intramuscular injections of 5 mg. of pteroyldiglutamic acid (Diopterin) for eighteen days but, as can be seen from Graph 21a, this led only to partial conversion/
conversion of the marrow to the normoblastic state with a very slight rise in the haemoglobin and red cell levels. Pteroyldiglutamic acid is not active for the growth of S. faecalis, the test organism used in the folic acid assay, but in the first twenty-four hours of pteroyldiglutamic acid therapy, the urine contained 0.28 mg. of a growth factor for S. faecalis and in the next twenty-four hours 0.51 mg. of such a factor. This indicated at least partial conversion of pteroyldiglutamic acid to pteroylglutamic acid or some other compound.

Pteroylglutamic acid was then given by injection in the same dosage and as a result there was a second small reticulocyte peak, the marrow became normoblastic and the red cell count rose from 1,710,000 per c.mm. to 3,300,000 per c.mm. in eighteen days. Thereafter the patient was treated with 100 µg. of cyanocobalamin intramuscularly monthly, and the haemoglobin reading rose to a maximum of 14.7 G. per 100 ml. and the red cell count to 4,980,000 per c.mm.

Comment. Uncomplicated pernicious anaemia treated in the first instance with pteroyldiglutamic acid by injection in a dosage of 5 mg. daily. This halted the tendency to a fall in the blood count, whereas injections of 5 mg. of pteroylglutamic acid daily caused a distinct rise. Neurological complications did not supervene.

Mrs. J. Sm. (Graph 21b)

A 67 year old patient with a fifteen months history of general symptoms of anaemia and of paraesthesiae for a shorter period. The usual criteria for a diagnosis of pernicious anaemia/
anaemia were present and there was loss of vibration sense in the lower limbs extending to the iliac crests on both sides. In addition there was clinical evidence of early Parkinsonism. The BMR was + 5.

It was decided to treat the patient in the first instance with intramuscular injections of pteroyldiglutamic acid. The quantity of growth factors for S. faecalis that were excreted in the urine in the twenty-four hours after the first injection (as pteroylglutamic acid) was 0.012 mg.

At the commencement of treatment the blood levels were:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin</td>
<td>7.1 G. per 100 ml.</td>
</tr>
<tr>
<td>Red cells</td>
<td>1,870,000 per c.mm.</td>
</tr>
<tr>
<td>C.I.</td>
<td>1.4</td>
</tr>
<tr>
<td>MCV</td>
<td>119.7 µ.</td>
</tr>
<tr>
<td>MCHC</td>
<td>35.5%</td>
</tr>
<tr>
<td>White cells</td>
<td>1,400 per c.mm.</td>
</tr>
<tr>
<td>Reticulocytes</td>
<td>&lt; 1%</td>
</tr>
</tbody>
</table>

It will be seen from Graph 21b that the tendency for a fall in the blood counts was checked by daily intramuscular injections of 2.5 mg. of pteroyldiglutamic acid, that there was a slight reticulocytosis and then a rise in the haemoglobin and red cell levels, and that the marrow was converted to the normoblastic state. After 23 days of treatment with injections of pteroyldiglutamic acid the patient was discharged with a haemoglobin reading of 10.4 G. per 100 ml. and a red cell count of 2,600,000 per c.mm.

Thereafter pteroyldiglutamic acid was given by mouth in a dosage of 5 mg. daily. After a month of this treatment the haemoglobin reading was 13.8 G. per 100 ml., and the red cell count/
count was 4,330,000 per c.mm. There was marked general improvement and vibration sense was present though diminished in both lower limbs, vibration being felt even at the ankles. Subsequently there was further general, neurological and haematological improvement with cyanocobalamin therapy.

Comment. A case of pernicious anaemia with loss of vibration sense in the lower limbs. There was a response to small doses of pteroyldiglutamic acid given first by injection and later by mouth.

![Graph 21b.](image-url)
Mrs. M. H. (Graph 22)

A 56 year old patient with a six weeks history of vomiting and a nine months history of dyspnoea. This was found to be due to pernicious anaemia - the stool benzidine was negative and a barium meal showed no abnormality. The blood counts were:

- Hb: 5.8 G. per 100 ml.
- Red cells: 1,620,000 per c.mm.
- C.I: 1.2
- FCV: 16.5%
- MCV: 101.8 cu.
- MCHC: 35.1%

Treatment was with pteroyldiglutamic acid given by mouth. The response is shown in Graph 22. The amount of growth factors for S. faecalis excreted in the urine in the twenty-four hours after the first 5 mg. dose of pteroyldiglutamic acid/
acid was 0.27 mg. (as pteroylglutamic acid). Thereafter cyanocobalamin injections were given and the blood counts maintained at a normal level.

Comment. Uncomplicated pernicious anaemia responding to pteroylglutamic acid given by mouth.

Mrs. C. McI. (Graph 23)

A 64 year old patient with a six months history of symptoms of anaemia, who was found to have pernicious anaemia without neurological complications. At the time of admission the blood levels were:

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin</td>
<td>8.6 G. per 100 ml.</td>
</tr>
<tr>
<td>Red cells</td>
<td>2,400,000 per c.mm.</td>
</tr>
<tr>
<td>C.I.</td>
<td>1.2</td>
</tr>
<tr>
<td>PCV</td>
<td>23.3%</td>
</tr>
<tr>
<td>MCV</td>
<td>97.9 µm</td>
</tr>
<tr>
<td>MCHC</td>
<td>36.8%</td>
</tr>
<tr>
<td>White cells</td>
<td>5,200 per c.mm.</td>
</tr>
<tr>
<td>Reticulocytes</td>
<td>1.2%</td>
</tr>
</tbody>
</table>

The patient was treated in the first instance with pteroylglutamic acid (Teropterin) in a dosage of 10 mg. daily intramuscularly and as will be seen in Graph 23 the marrow became normoblastic and there was some improvement in the blood levels, the red cell count rising to 3,000,000 per c.mm. A further slight rise in the red cell level to 3,390,000 per c.mm. without a second reticulocyte response occurred when pteroylglutamic acid was given orally in the same dosage.

No neurological complications developed but it was considered advisable to treat the patient thereafter with injections of cyanocobalamin.

Comment. Uncomplicated pernicious anaemia showing a sub-optimal response to injections of pteroylglutamic acid.
MRS. C. McL. PERNICIOUS ANAEMIA WITH SUBOPTIMAL RESPONSE TO PTEROYLTRIGLUTAMIC ACID.

PTEROYLTRIGLUTAMIC ACID 10mg. i.m. D'LY
MEGALOBLASTIC MARROW
NORMOBLASTIC MARROW

Hb.
R.B.C.

REFER.

GRAPH 23.

MRS. T. R. PERNICIOUS ANAEMIA RESPONDING TO PTEROYLTRIGLUTAMIC ACID GIVEN BY MOUTH.

PTEROYLTRIGLUTAMIC ACID 2.5mg. DAILY BY MOUTH.

Hb.
R.B.C.

REFER.

GRAPH 24.
Mr. T. R. (Graph 24.)

A 75 year old man with a three months history of tiredness and weakness together with pain in the tongue and numbness and tingling in the limbs. A diagnosis of pernicious anaemia was made, the usual criteria being present. The stool benzidine was negative and a barium meal showed no abnormality.

At the time of admission the blood levels were:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb.</td>
<td>6.8 G. per 100 ml.</td>
</tr>
<tr>
<td>Red cells</td>
<td>2,030,000 per c.mm.</td>
</tr>
<tr>
<td>C.I.</td>
<td>1.1</td>
</tr>
<tr>
<td>PCV</td>
<td>19%</td>
</tr>
<tr>
<td>MCV</td>
<td>93.6 cu.</td>
</tr>
<tr>
<td>MCHC</td>
<td>35.9%</td>
</tr>
</tbody>
</table>

The patient was treated with pteroyltriglutamic acid given by mouth in a dosage of 2.5 mg. daily. The response is shown in Graph 24. No neurological complications developed, and the patient felt much improved. With subsequent cyanocobalamin injections the haemoglobin reading rose to 17 G. per 100 ml. and the red cell level to 6,800,000 per c.mm.

Comment. A patient with pernicious anaemia who showed a general and haematological response to pteroyltriglutamic acid given by mouth in a dosage of 2.5 mg. daily.

Mrs. M. S. (Graph 25.)

A 46 year old lady who was admitted to ward 30, Royal Infirmary of Edinburgh suffering with symptoms of anaemia of about two months duration. The criteria for a diagnosis of pernicious anaemia were present and the blood counts were:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin</td>
<td>7.4 G. per 100 ml.</td>
</tr>
<tr>
<td>Red cells</td>
<td>1,740,000 per c.mm.</td>
</tr>
<tr>
<td>C.I.</td>
<td>1.4</td>
</tr>
<tr>
<td>PCV</td>
<td>19%</td>
</tr>
<tr>
<td>MCV</td>
<td>119.5 cu.</td>
</tr>
<tr>
<td>MCHC</td>
<td>38.9%</td>
</tr>
</tbody>
</table>
As is shown in Graph 25 there was a satisfactory response to three injections of synthetic citrovorum factor, the haemoglobin level rising to 13.9 G. per 100 ml. and the red cells to 4,200,000 per c.mm. in the course of two months. The amount of citrovorum factor excreted in the urine after the first injection of 12 mg. was 0.79 mg. The paucity of blood counts in the later part of the graph is due to the fact that the patient had been discharged to her home in Berwick-on-Tweed.

Clinical improvement accompanied the haematological remission and there were no neurological complications.

Comment. Pernicious anaemia responding to injections of citrovorum factor.
Mrs. M. B. (Graph 26.)

A 70 year old patient who was admitted to hospital with a six years history of symptoms attributable to anaemia. For three years she had not been able to leave her house. The criteria for a diagnosis of pernicious anaemia were present and apart from some impairment of vibration sense around the left ankle there was no evidence of neurological complications.

The blood levels were:

- Haemoglobin: 6.2 G. per 100 ml.
- Red cells: 2,350,000 per c.mm.
- C.I.: 0.9
- PCV: 22%
- MCV: 83.7 cµ.
- MCHC: 32.2%
- White cells: 5,800 per c.mm.
- Reticulocytes: <1%

It was decided that the patient should be treated with citrovorum factor in a dosage of 3 mg. daily by mouth and, as will/
will be seen from Graph 26, there was a good response to this form of treatment. In 40 days the haemoglobin level rose to 13.2 G. per 100 ml. and the red cell count to 5,500,000 per c.mm. No neurological deterioration occurred and, indeed, vibration sense returned to normal.

Thereafter treatment was with injections of cyanocobalamin and two years later the patient was very well, apart from pains in the knees due to osteoarthritis.

Comment. Pernicious anaemia with a good response to citrovorum factor given by mouth.

Mrs. E. R. (Graph 27.)

A 56 year old patient with uncomplicated pernicious anaemia who was admitted to hospital in 1950 with the following blood findings:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin</td>
<td>10.1 G. per 100 ml.</td>
</tr>
<tr>
<td>Red cells</td>
<td>2,510,000 per c.mm.</td>
</tr>
<tr>
<td>C.I.</td>
<td>1.3</td>
</tr>
<tr>
<td>PCV</td>
<td>28%</td>
</tr>
<tr>
<td>ECV</td>
<td>111.5 cµ.</td>
</tr>
<tr>
<td>MCH</td>
<td>38.3%</td>
</tr>
<tr>
<td>White cells</td>
<td>2,600 per c.mm.</td>
</tr>
<tr>
<td>Reticulocytes</td>
<td>1%</td>
</tr>
</tbody>
</table>

The patient was given, on each of four successive days, 100 ml. of normal gastric juice together with 25 µg. of cyanocobalamin by enema. As will be seen in Graph 27 there was no haematological response.

The same mixture was then given for a period of four days by stomach tube. The marrow reverted to the normoblastic state and the haemoglobin level rose from 7.7 G. per 100 ml. to 11.2 G. per 100 ml., and the red cells from 2,200,000 per c.mm.
c.mm. to 3,170,000 per c.mm. The HCV was now 32.5% and the MCV was 102.5 µl.

Comment. A patient with pernicious anaemia who responded to a mixture of 25 µg. of cyanocobalamin and 100 ml. of normal gastric juice given by stomach tube on each of four successive days. There had previously been no response to a similar total of 100 µg. of cyanocobalamin and 400 ml. of normal gastric juice given by enema over a period of four days.
Mrs. J. B. (Graph 28.)

A 73 year old lady suffering from untreated pernicious anaemia who was under treatment for congestive cardiac failure seven months previously. There was no evidence of such disease while she was being investigated and treated for pernicious anaemia. The systolic blood pressure was now 160 mm., and the diastolic pressure 90 mm. The pulse rate, without digitalis therapy, remained steady at 70 to 80 beats per minute. A barium meal and enema were negative. The blood findings were:

Haemoglobin/
Haemoglobin 6.2 G. per 100 ml.
Red cells 1,650,000 per c.mm.
C.I. 1.27
HCV 19%
MCV 115.2 cµ.
MCHC 32.6%

As can be seen in Graph 28 there was a good response to the preparation Bifacton in a dosage of 6 tablets daily. Bifacton is a preparation of cyanocobalamin and intrinsic factor for oral administration. Six tablets contain 22.5 of cyanocobalamin together with intrinsic factor, and two tablets is considered to be one oral unit.

A month after she had been discharged from the Royal Infirmary the patient died in another hospital from congestive cardiac failure of sudden onset. Unfortunately the present author was not informed about this until several days later so that specimens of the organs were not obtained for microbiological assay of haemopoietic factors.

Comment. Pernicious anaemia responding to the preparation Bifacton which contains cyanocobalamin and intrinsic factor and is given by mouth.

Mrs. W. C. (Graph 29.)

A female patient who had first attended her doctor with symptoms of anaemia in 1946 when she was aged 39. No blood examination was done and the patient was given six injections of 2 ml. of liver extract together with iron by mouth. In 1951 the patient was referred to the Blood Clinic at Edinburgh Royal Infirmary with symptoms of anaemia. The doctor who referred/
referred her to the Clinic stated in his letter that he had
given the above treatment in 1946 and added - "She tells me
that on two other occasions (at possibly one year's interval)
I did likewise". A diagnosis of pernicious anaemia was made
at the Blood Clinic, the levels being haemoglobin 6.1 G. per
100 ml., red cells 1,600,000 per c.mm., C.I. 1.3. It was
suggested that the practitioner should give weekly injections
of 50 µg. of cyanocobalamin and after a month of treatment
the haemoglobin level was 11 G. per 100 ml. The patient's
doctor considered that this was satisfactory and stopped
treatment, but despite this the haemoglobin reading eight
weeks later was 15 G. per 100 ml. The practitioner was
asked to continue treatment, but did not do so. An occasion-
al injection of 50 µg. was given in 1952 but in January 1953
the patient, who had received no treatment for six months,
was admitted to Edinburgh Royal Infirmary with the following
blood levels:

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin</td>
<td>5.5 G. per 100 ml.</td>
</tr>
<tr>
<td>Red cells</td>
<td>1,470,000 per c.mm.</td>
</tr>
<tr>
<td>FGV</td>
<td>17%</td>
</tr>
<tr>
<td>MCV</td>
<td>115.6 µm.</td>
</tr>
<tr>
<td>MCHC</td>
<td>32.4%</td>
</tr>
</tbody>
</table>

Treatment with Bifacton tablets was commenced, the dosage
being 2 tablets daily (15 µg. of cyanocobalamin together with
intrinsic factor daily). The highest level reached was five
months after this treatment began, when the haemoglobin read-
ing was 17.0 G. per 100 ml., and the red cell count 5,380,000
per c.mm. Although the dosage was later increased to four
tablets daily, a normal count was not sustained, and after a
total/
total of eleven months of treatment with Bifacton the counts were - haemoglobin 12.3 G. per 100 ml., red cells 4,650,000 per c.mm., C.I. 0.89, MCV 93.5 cu., MCHC 28.3%. Iron therapy had not been given. The serum vitamin B₁₂ level (L. leichmanni assay) was 320 μg./ml. At no time was there any evidence of neurological complications.

Comment. A patient with pernicious anaemia who fared better on Bifacton tablets which she took regularly than on cyanocobalamin injections which her doctor repeatedly failed to administer.
Mrs. B. D. (Graph 30.)

A female patient aged 59 years who had anaemia complicated by tingling of the hands and forearm, and of the feet, legs and thighs, together with a feeling of weakness in the limbs. When she was referred to the Blood Clinic at Edinburgh Royal Infirmary in November 1952 these symptoms had been becoming progressively more severe and there was loss of vibration sense in the legs as far up as the anterior superior spines in both lower limbs. The reflexes were normal. The haemoglobin level was 10.5 G. per 100 ml., red cells 3,150,000 per c.mm.

It was found that the patient had been having daily for seven months, three 'plastules' containing folic acid, and that the neurological/
neurological features had developed while the patient was being treated for anaemia in this blunderbuss manner. The physician who saw her at the Blood Clinic suggested that treatment should be with iron alone, to enable a diagnosis to be made. Two months later the blood counts were haemoglobin 8 G. per 100 ml., red cells 2,500,000 per c.mm. The patient was admitted to hospital and a diagnosis of pernicious anaemia was made. The counts were now:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin</td>
<td>6.8 G. per 100 ml.</td>
</tr>
<tr>
<td>Red cells</td>
<td>1,940,000 per c.mm.</td>
</tr>
<tr>
<td>C.I.</td>
<td>1.18</td>
</tr>
<tr>
<td>PCV</td>
<td>20%</td>
</tr>
<tr>
<td>MCV</td>
<td>103.1 c.i.</td>
</tr>
<tr>
<td>MCHC</td>
<td>34%</td>
</tr>
<tr>
<td>White cells</td>
<td>3,000 per c.mm.</td>
</tr>
<tr>
<td>Reticulocytes</td>
<td>&lt; 1%</td>
</tr>
</tbody>
</table>

Vibration sense was absent in the lower limbs as before, and the ankle jerks were difficult to elicit. The neurological symptoms were unchanged.

The patient was treated with Bifacton as shown in Graph 30, the initial dosage being two tablets (15 µg. of cyanocobalamin together with intrinsic factor) daily. There was haematological improvement, but numbness and tingling in the limbs and loss of vibration sense were not improved and the treatment was changed five months later to injections of cyanocobalamin. The latter, in a dosage of 100 µg. intramuscularly weekly for five months, caused a further rise in the red cell level from 4,330,000 per c.mm. to 5,100,000 per c.mm., but no improvement in vibration sense or ankle jerks, although the paraesthesiae were lessened.

Comment. /
Comment. A patient who appeared to have developed degeneration of the posterior columns of the cord because her anaemia was treated blindly with plastules containing folic acid. There was incomplete haematological remission when the patient was treated with an oral vitamin B₁₂-intrinsic factor preparation and then maintained with it in the dosage recommended by the manufacturers for maintenance purposes. The neurological features were little improved by this therapy or by later cyanocobalamin injections.

In future cases of this type where blunderbuss therapy not including cyanocobalamin has been given, it should be possible to make a diagnosis quickly by estimating the serum vitamin B₁₂ level as described in Chapter 8.

Mrs. M. A. (Graph 31.)

A patient aged 72 years who had had symptoms of anaemia for about a year. There had been numbness and tingling in the hands and feet for six months. A diagnosis of pernicious anaemia was made, the usual criteria being present. There were no objective neurological abnormalities. Diarrhoea was not a feature and there was no history of poor diet. The blood findings were:

- Haemoglobin: 7.7 G. per 100 ml.
- Red cells: 2,220,000 per c.mm.
- Hb: 21.5%
- MCV: 98.8 µ.
- MCHC: 35.8%

It was decided to treat the patient in the first instance with one dose of 2 mg. of cyanocobalamin by mouth, and as can be seen in Graph 31 the haemoglobin level rose to 11.8 G. per 100 ml./
100 ml. and the red cell count to 3,650,000 per c.mm. The PCV was 34.5%, the MCV 94.5 µl. and the MCHC 34.2%. There was general improvement and the paraesthesiae ceased to be troublesome. Subsequently the patient showed further improvement with cyanocobalamin by injection.

Comment. Pernicious anaemia responding to 2,000 µg. of cyanocobalamin given by mouth in one dose.
Mrs. C. R. (Graph 32.)

A 67 year old patient who had had general symptoms of anaemia for about three years. She had all the features of pernicious anaemia and there was impairment of vibration sense throughout the lower limbs. At the time of admission the blood levels were:

- Haemoglobin 8.6 G. per 100 ml.
- Red cells 2,100,000 per c.mm.
- C.I. 1.38
- PCV 25.5%
- MCV 121.4 cu.
- MCHC 33.7%
- White cells 2,800 per c.mm.
- Reticulocytes < 1%

The marrow was megaloblastic.

It was decided to treat the patient with cyanocobalamin snuff/
snuff (prepared by Messrs. Paines & Byrne, Ltd.). Each day for nineteen days the patient broke the end off a capsule containing 100 µg. of cyanocobalamin and 'snuffed' it into the nose from the palm of the hand. As will be seen from Graph 32, although there was no real reticulocyte response the marrow became normoblastic and the haemoglobin level rose to 11.1 G. per 100 ml. and the red cells to 3,390,000 per c.mm.

Comment. Pernicious anaemia with response to nasal inhalation of cyanocobalamin. There is no need to comment on the lack of practical value of this form of treatment.

Mrs. I. K. (Graph 32.)

An old lady aged 73 years who was admitted to a surgical ward of Edinburgh Royal Infirmary in October 1953 with a fracture of the neck of the left femur which occurred as a result of a fall. There was a history of a fracture of the neck of the right femur in May of the same year.

While she was in the surgical ward it was noted that the patient was pale and the blood findings were found to be haemoglobin level 10.1 G. per 100 ml., red cells 2,700,000 per c.mm., C.I. 1.26. Two days later a more complete investigation showed that the haematocrit reading was 34.5% and the MCV 118.9 cu. The peripheral blood picture was consistent with a diagnosis of pernicious anaemia except that ovalocytosis and poikilocytosis were not marked. However, the patient's appearance suggested pernicious anaemia to the present author. There was atrophic glossitis, and a test meal showed histamine fast achlorhydria to be present. A sternal marrow puncture was done and the marrow found to be very cellular but completely/
completely normoblastic. The reticulocyte level was 2.8%.
The direct Coombs' test was negative, and there was no
evidence of abnormal haemolytic activity in the serum by an
acidified serum test. There was a slight excess of urobilinogen in the urine, but the patient's general condition was poor and it was not possible to do a 24 hour urine collection for a folic acid excretion test or a urobilinogen measurement. The blood urea nitrogen level was 11 mg. per 100 ml. A barium examination of the alimentary tract was not possible. The stool benzidine was negative.

The patient had lived in an old persons' home and enquiries made there indicated that her diet had been adequate. No folic acid or vitamin preparations had been taken and no parenteral therapy given except that nine million units of penicillin had been injected over a period of three days, finishing ten days before the above mentioned marrow puncture was done. The serum vitamin B₁₂ level was estimated by the _L.leichmannii_ method and found to be 50 µg./ml., a level consistent with a diagnosis of pernicious anaemia. A specimen of serum was sent to Dr. G.I.M. Ross at the Postgraduate Medical School of London. By the Euglena method of assay he found the serum vitamin B₁₂ level to be 50 µg./ml. No treatment was being given at the time that might interfere with these assays and liver function tests gave the following readings:

<table>
<thead>
<tr>
<th>Test</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum albumin</td>
<td>3.07 G. per 100 ml.</td>
</tr>
<tr>
<td>Globulin</td>
<td>1.88 G. per 100 ml.</td>
</tr>
<tr>
<td>M.P.N.</td>
<td>40 mg. per 100 ml.</td>
</tr>
<tr>
<td>Serum bilirubin</td>
<td>0.7 mg. per 100 ml.</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>198 mg. per 100 ml.</td>
</tr>
<tr>
<td>Free cholesterol</td>
<td>71 mg. per 100 ml.</td>
</tr>
</tbody>
</table>

*See p.427 for photograph.*
Alkaline phosphatase 6 units
Thymol turbidity 2 units
Thymol flocculation negative
Cephalin cholesterol \(+\) 1
Serum potassium 19.3 mg. per 100 ml.

There was thus no evidence of liver disease.

The patient was treated with cyanocobalamin in a dosage of 100 µg. twice weekly and demonstrated a haematological response, as shown in Graph 33, although the general condition remained poor.

Comment. An old lady with clinical features of pernicious anaemia, a blood count consistent with such a diagnosis, histamine fast achlorhydria and a serum vitamin B₁₂ level which by two methods was in the pernicious anaemia range. There was no history of malnutrition. The marrow was completely normoblastic but there was a haematological response to cyanocobalamin. The diagnosis seemed to be one of pernicious anaemia despite the marrow picture, but an absolute diagnosis could not be made since it was not possible to exclude carcinoma of the alimentary tract. No reason was found for the marrow picture not being in keeping with the other findings except that penicillin had been given ten days previously.
Marrow of Mrs. I.K. (page 424). Normoblastic.

Marrow of Mrs. M.M. (page 433). Normoblastic.
MRS. I.K. CLINICAL PICTURE AND BIOCHEMICAL EVIDENCE OF PERNICIOUS ANAEMIA BUT NORMOBLASTIC MARROW RESPONSE TO CYANOCOBALAMIN.

**CYANOCOBALAMIN 100 µg. i.m. TWICE WEEKLY**

### Serum Vitamin B₁₂
- Level 50 µg/ml.

### Normoblastic Marrow
- Hb.
- R.B.C.
- P.C.V.

### Graph 33.

(MRS. J.W.) PERNICIOUS ANAEMIA REFRACTORY TO PTEROYLGLUTAMIC ACID THERAPY, BUT RESPONDING TO CYANOCOBALAMIN.

**Pteroylglutamic Acid**
- Orally (MC)
- S.M.C.
- S.M.C.
- S.M.C.
- S.M.C.
- S.M.C.

**Cyanocobalamin**
- 100 µg. i.m.

### Graph 34.
The bone marrow of Mrs. J.W. (see page 430 and Graph 34) at the termination of pteroylglutamic acid therapy in hospital. This picture shows the megaloblastic change which was very evident despite this treatment.
Mrs. J. W. (Graph 34.)  
Marrow shown on p.429.

A 70 year old patient who was admitted to hospital with general clinical features of anaemia. Apart from some numbness in the legs there were no neurological features. The tongue showed atrophy of the papillae, the spleen was not palpable and no evidence of neoplasm was found in the alimentary tract. Barium enema, meal, and follow through were negative and the stool benzidine was repeatedly negative. There was histamine fast achlorhydria and the dietetic history was satisfactory. Diarrhoea was not a feature. The blood findings on admission were:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin</td>
<td>9.0 G. per 100 ml.</td>
</tr>
<tr>
<td>Red cells</td>
<td>2,680,000 per c.mm.</td>
</tr>
<tr>
<td>PCV</td>
<td>28%</td>
</tr>
<tr>
<td>HCV</td>
<td>104.4 qu.</td>
</tr>
<tr>
<td>MCHC</td>
<td>32.1%</td>
</tr>
</tbody>
</table>

The peripheral blood film was typically that of pernicious anaemia. The serum bilirubin level was 0.4 mg. per 100 ml. There was excess of urobilinogen in the urine.

The marrow was found to be frankly megaloblastic and for research purposes a differential urinary folic acid excretion test was done. Thereafter treatment with pteroylglutamic acid was given and the folic acid excretion test repeated. The results were as follows:

<table>
<thead>
<tr>
<th>Test Condition</th>
<th>FA</th>
<th>CF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urinary excretion in 24 hrs.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>after 5 mg. SC</td>
<td>0.28 mg.</td>
<td>0.77 µg.</td>
</tr>
<tr>
<td>in 24 hrs. after 5 mg. Orally</td>
<td>1.19 mg.</td>
<td>3.85 µg.</td>
</tr>
<tr>
<td>After treatment with 20 mg. of pteroylglutamic acid by mouth for 10 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urinary excretion in 24 hrs.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>after 5 mg. SC</td>
<td>0.45 mg.</td>
<td>4.4 µg.</td>
</tr>
<tr>
<td>in 24 hrs. after 5 mg. Orally</td>
<td>0.165 mg.</td>
<td>4.6 µg.</td>
</tr>
</tbody>
</table>

(Note FA = Folic Acid; CF = Citrovorum Factor)
It will be seen, however, from Graph 34 that the marrow was still frankly megaloblastic at the end of the period of pteroylglutamic acid therapy. In addition the patient's general condition was very much worse, and cyanocobalamin injections had to be given without further delay.

There was an excellent response to cyanocobalamin therapy, the blood counts being quite rapidly restored to normal figures. After five injections each of 100 µg. of cyanocobalamin had been given in the course of a month, the differential folic acid excretion test was repeated. The results were as follows:

<table>
<thead>
<tr>
<th>Urinary excretion</th>
<th>FA</th>
<th>CF</th>
</tr>
</thead>
<tbody>
<tr>
<td>in 24 hrs. after 5 mg. SC</td>
<td>1.56 mg.</td>
<td>5.7 µg.</td>
</tr>
<tr>
<td>in 24 hrs. after 5 mg. Orally</td>
<td>1.54 mg.</td>
<td>8.1 µg.</td>
</tr>
</tbody>
</table>

A fat balance test showed 95.6% absorption, and renal function as judged by a concentration and dilution test was normal. Liver function tests had been normal on admission and remained so. The BMR was +13. The blood urea nitrogen reading was 14 mg. per 100 ml. and the blood Wassermann reaction was negative.

This appeared at first to be a case of pernicious anaemia that was refractory to pteroylglutamic acid therapy. The first differential folic acid excretion test suggested that the tissues were "depleted of folic acid" to such an extent that only 0.28 mg. of the 5 mg. injected dose was available for excretion, and that the orally administered dose was absorbed satisfactorily and 1.19 mg. of it then excreted.

The second differential folic acid excretion test gave little information because even the 5 mg. injected dose was excreted to a lesser extent than the 5 mg. oral dose of the first/
first test. At the time the patient was critically ill and it was impossible to know whether there was a diminution in absorption, utilisation or excretion of folic acid, or whether all these metabolic processes were upset. The third differential folic acid excretion test indicated that malabsorption was not then a feature.

It will be noticed that the folic acid output after both the injected and the oral test doses was then only about 1.5 mg., a low figure (see also p.332). Since the patient had been having treatment with pteroylglutamic acid, albeit a month earlier, this may indicate that there had been excessive katabolism of folic acid in the tissues and organs. The precise meaning of the observation is not however clear.

At first it was understood that the patient had never received pteroylglutamic acid therapy before she was admitted to hospital. Enquiries about this were pursued and it was found that the "pills for anaemia" prescribed by the patient's doctor for two and a half years contained pteroylglutamic acid, iron and trace elements but no cyanocobalamin. These pills supplied 2 or 3 mg. of pteroylglutamic acid daily depending upon the number eaten. No such pills had been taken for six months. At no time was there evidence of subacute combined degeneration of the cord. The patient was seen before the serum vitamin B₁₂ test was available.

It seems therefore that this patient had pernicious anaemia and became refractory to folic acid therapy because of prolonged treatment with pteroylglutamic acid. (See also Mr./
Mr. R.R., with tropical sprue, p. 354. Folic acid could no longer substitute for vitamin B12 and while pteroylglutamic acid was being given the patient became seriously ill. Nevertheless it will be noted as a matter of some importance that clinical evidence of subacute combined degeneration of the cord did not appear.

Comment. A patient with pernicious anaemia who became refractory to treatment with pteroylglutamic acid and had to be given cyanocobalamin injections as a life saving measure. Neurological complications did not develop.

Mrs. M. M. (Graph 35.)

An intelligent 57 year old lady who was admitted to Ward 27 of Edinburgh Royal Infirmary in September 1953 with a history of having felt fatigued for eight months, and of having had numbness and tingling of the hands for two months with numbness of the feet and some difficulty in walking for a week. The general fatigue had been particularly bad for four months. At the time of admission the patient felt that she had "no feeling in her legs". The appetite was poor, but the diet had been satisfactory. Meat was eaten regularly. The bowels were regular. About two years previously the tongue had been sore but there had been no recurrence of such symptoms.

In 1946 the left breast had been removed for early carcinoma, and this was followed by routine therapy with X-rays, but no such therapy had been received since 1946. No evidence of recurrence had been demonstrated, and no secondary deposits were found clinically or by X-ray examination. There were no other/
other previous illnesses of note and the patient had not been abroad.

On examination the patient was seen to be pale and the tongue was red and somewhat atrophic. The edge of the liver was just palpable on inspiration and no abnormal glandular enlargement was found.

The objective neurological findings consisted of weakness of grip in the hands, possible weakness of hip flexors and extensors, slight impairment of the sensation of light touch in the hands, and complete loss of vibration sense in the legs extending up the trunk to the lower thoracic region. The only abnormality of reflexes was that the ankle jerks were diminished. No other neurological abnormality of sensation, motor function, coordination, reflexes or cranial nerves was found. A lumbar puncture showed no abnormality.

The clinical picture was one of pernicious anaemia complicated by degenerative changes particularly in the posterior columns of the spinal cord.

The blood findings appeared to be consistent with such a diagnosis. They were:

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin</td>
<td>10.2 G. per 100 ml.</td>
</tr>
<tr>
<td>Red cells</td>
<td>2,930,000 per c.mm.</td>
</tr>
<tr>
<td>C.I.</td>
<td>1.17</td>
</tr>
<tr>
<td>PCV</td>
<td>35.0%</td>
</tr>
<tr>
<td>MCV</td>
<td>119.4 f.l.</td>
</tr>
<tr>
<td>MCHC</td>
<td>29.1%</td>
</tr>
<tr>
<td>White cells</td>
<td>5,200 per c.mm.</td>
</tr>
<tr>
<td>Reticulocytes</td>
<td>1%</td>
</tr>
</tbody>
</table>

However, it was noticeable in the blood film that although macrocytes were present, ovalocytosis and poikilocytosis were not marked.
The marrow was completely normoblastic. (see p.427).

The serum vitamin B₁₂ level (estimated six times, from 2 separate samples of blood) was less than 50 µg./ml. (L. leichmannii assay). This was consistent with a diagnosis of pernicious anaemia.

A differential urinary folic acid excretion test gave the following findings:

Urinary excretion
in 24 hrs. after 5 mg. SC = 3.11 mg.
in 24 hrs. after 5 mg. Orally = 2.51 mg.

The test was therefore considered to be negative — it did not give evidence either of tissue depletion or malabsorption of folic acid.

There was histamine fast achlorhydria, the urine did not contain an excess of urobilinogen, the stool benzidine was negative on six occasions, there was no evidence of cold agglutinins, the Coombs' test was negative and abnormal porphyrin excretion was not detected in the urine. The blood and CSF Wassermann tests were negative.

The serum calcium level was 9.9 mg. per 100 ml.; and the blood chemistry findings were:

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum albumin</td>
<td>4.05 G. per 100 ml.</td>
</tr>
<tr>
<td>Serum globulin</td>
<td>1.58 G. per 100 ml.</td>
</tr>
<tr>
<td>Non protein nitrogen</td>
<td>28 mg. per 100 ml.</td>
</tr>
<tr>
<td>Serum bilirubin</td>
<td>0.3 mg. per 100 ml.</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>8 units</td>
</tr>
<tr>
<td>Cephalin flocculation</td>
<td>Negative</td>
</tr>
<tr>
<td>Thymol flocculation</td>
<td>1 unit</td>
</tr>
<tr>
<td>Thymol turbidity</td>
<td>Negative</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>161 mg. per 100 ml.</td>
</tr>
<tr>
<td>Free cholesterol</td>
<td>52 mg. per 100 ml.</td>
</tr>
</tbody>
</table>

The BMR was -12%; there was certainly no clinical suggestion of myxoedema.
The ESR on admission was 107 mm./hr. (Westergren). It slowly fell to 16 mm./hr. while the patient was under treatment.

Two three-day fat balance tests on a 75 G. fat intake showed, respectively, 94.7% and 97.7% absorption. Barium meal and 'follow through' examinations showed no abnormality, and X-rays of the chest, cervical, dorsal and lumbar spines and pelvis were also negative.

The clinical impression was that the patient had pernicious anaemia with neurological complications and the serum vitamin B12 findings seemed to confirm this. The patient's doctor affirmed with certainty that he had never given the patient any preparation containing folic acid, although he had used plasters containing no folic acid. Further enquiries from the patient's chemist revealed that in February or March 1953 he had supplied plasters and that they had contained folic acid. Forty-eight of these had been supplied and the patient confirmed that she had eaten them at that time.

Treatment with a high dosage of cyanocobalamin by injection was commenced, as shown in Graph 35. There was slow haematological and neurological improvement. Three months after the commencement of treatment the haemoglobin level was 15.0 G. per 100 ml., red cells 4,470,000 per c.mm., C.I. 1.1, PCV 43.0%, MCV 91.8 cu., MCHC 34.9%. Numbness persisted at the fingertips but walking was improved and vibration sense had returned in the legs to a level below the knees on each side.

Comment. A case with clinical features of pernicious anaemia and posterior column degeneration and a serum vitamin B12 level suggesting vitamin B12 deficiency. However, despite the anaemia/.
anaemia the marrow was normoblastic. No alternative cause for anaemia was found and there was a response to cyanocobalamin therapy. Folic acid had been given but not for six months. The patient's doctor may at that time have prescribed a preparation containing folic acid without being aware of the fact. An alternative explanation might be that the doctor merely indicated "---'s plastules" and that the pharmacist used his own discretion about which type he should supply.

The diagnosis of pernicious anaemia with a normoblastic marrow cannot be regarded as proved in this case. Time may show that there is an alternative explanation.

In this particular case a satisfactory microbiological test for intrinsic factor would be of value or, alternatively, tests of absorption of radioactive cyanocobalamin might aid the diagnosis.
Mrs. B. W. (GRAPH 36.)

A patient aged 42 years with uncomplicated pernicious anaemia. She had had general symptoms of anaemia for a period of six months.

In view of the fact that interest had been aroused in thymine as a possible antimegaloblastic substance, we had approached Messrs. Genatosan who kindly supplied a small quantity of the substance. It was decided to administer this to the patient under discussion. Before this was given the marrow was megaloblastic and the blood levels were:

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Haemoglobin</strong></td>
<td>9.2 G. per 100 ml.</td>
</tr>
<tr>
<td><strong>R.C.L.</strong></td>
<td>2,230,000 per c.mm.</td>
</tr>
<tr>
<td><strong>C.I.</strong></td>
<td>1.34</td>
</tr>
<tr>
<td><strong>White cells</strong></td>
<td>5,800 per c.mm.</td>
</tr>
<tr>
<td><strong>Reticulocytes</strong></td>
<td>&lt; 1%</td>
</tr>
</tbody>
</table>
After 100 G. of thymine had been given as shown in Graph 36 the figures were haemoglobin level 12.1 G. per 100 ml., red cells 3,060,000 per c.mm., C.I. 1.34, white cells 7,800 per c.mm., reticulocytes 1.2%, the marrow was normoblastic and there was some general improvement.

Comment. Uncomplicated pernicious anaemia with a response to thymine given by mouth.

Miss J. S. (Graph 37.)

A 71 year old patient who was seen at the Blood Clinic, Edinburgh Royal Infirmary, in August 1948. The symptoms were those of anaemia of five months duration and apart from paraesthesiae there was no evidence of any complications. The dietetic history was satisfactory and there had been no diarrhoea. The haemoglobin level was 5.9 G. per 100 ml., red cells 1,800,000 per c.mm., C.I. 1.1, and the peripheral blood film was typically that of pernicious anaemia. It was arranged that the patient should be admitted to hospital three days after her visit to the Clinic and that meantime no treatment should be given. On the day before admission, however, the patient ate approximately half a pound of lightly cooked liver and whether because of this or for some other reason, there was a reticulocyte response and a rise in the haemoglobin and red cell levels without any treatment. On admission the counts were:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin</td>
<td>5.3 G. per 100 ml.</td>
</tr>
<tr>
<td>Red cells</td>
<td>1,470,000 per c.mm.</td>
</tr>
<tr>
<td>C.I.</td>
<td>1.1</td>
</tr>
<tr>
<td>PCV</td>
<td>15%</td>
</tr>
<tr>
<td>MCV</td>
<td>102.0 µ.</td>
</tr>
<tr>
<td>MCHC</td>
<td>34.6%</td>
</tr>
<tr>
<td>White cells</td>
<td>2,000 per c.mm.</td>
</tr>
<tr>
<td>Reticulocytes</td>
<td>&lt; 1%</td>
</tr>
</tbody>
</table>
Twenty-eight days later the levels were haemoglobin 11.5 G. per 100 ml., red cells 3,010,000 per c.mm., C.I. 1.29, PCV 30.0%, MCV 99.7 µ., MCHC 38.3%, white cells 6,600, reticulocytes < 1%. No further response occurred until injections of liver extract were given and five years later the patient was still feeling very well and the blood counts were normal. Anahaemin in a dosage of 4 ml. every 2 weeks had been given throughout this period. It will be seen from Graph 37 that throughout the period of the "spontaneous" remission there was seen to be condensation of the nuclei of the megaloblasts in the marrow.

Comment. Pernicious anaemia with apparent spontaneous improvement which may have been due to the patient eating half a pound of lightly cooked liver on the day before admission to hospital.
Mrs. M. D. (Graph 38.)

A 56 year old patient with a three years history of general symptoms of anaemia, and a sore tongue for six months. She had slight symptoms of numbness in the lower limbs. She was first seen at the Blood Clinic, Royal Infirmary, Edinburgh, and was considered to have pernicious anaemia. The blood count was falling quite rapidly and at the time of admission the figures were:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin</td>
<td>7.3 G. per 100 ml.</td>
</tr>
<tr>
<td>Red cells</td>
<td>1,830,000 per c.mm.</td>
</tr>
<tr>
<td>C.I.</td>
<td>1.34</td>
</tr>
<tr>
<td>PCV</td>
<td>20%</td>
</tr>
<tr>
<td>MCV</td>
<td>109.3 µ.</td>
</tr>
<tr>
<td>MCHC</td>
<td>36.5%</td>
</tr>
<tr>
<td>White cells</td>
<td>3,200 per c.mm.</td>
</tr>
<tr>
<td>Reticulocytes</td>
<td>&lt; 1%</td>
</tr>
</tbody>
</table>

The criteria for a diagnosis of pernicious anaemia were present.
present. There was loss of vibration sense in the lower limbs to the anterior superior iliac spines, vibration sense was impaired in the arms and hands, the knee and ankle jerks were absent and the left plantar response was equivocal.

It was decided to try the effects of penicillin therapy and 200,000 units of crystalline penicillin were given twice daily intramuscularly. The patient's general condition deteriorated rapidly and there was no reticulocyte response. Mental confusion became severe and the treatment was hurriedly changed to liver extract by injection. There was a satisfactory response to this and after two such injections cyanocobalamin was given in a dosage of 40 µg. twice weekly for six months, then 50 µg. every two weeks. Eighteen months later the haemoglobin level was 15.4 G. per 100 ml. and the red cells 5,120,000 per c.mm. Paraesthesiae were not troublesome, vibration sense was present but not normally so in the lower limbs, the ankle jerks were absent but the knee jerks were present, and the plantar responses were normal. The patient felt very well.

Comment. Pernicious anaemia with failure of haematological response to penicillin injections.

Mrs. A. W. (Graph 39.)

A 67 year old patient with a years history of symptoms of anaemia. Two sisters had had pernicious anaemia, and the present patient fulfilled the criteria for such a diagnosis.

It was decided to treat the patient with 5 µg. of cyanocobalamin by mouth daily, together with the research preparation/
preparation 5:6 Dimethyl-benziminazole to which reference was made on page 254. It will be recollected that the hope was that this substance would prevent the utilisation of cyanocobalamin by intestinal organisms and thus make vitamin B₁₂ available to the patient. The results, which were negative, are shown in Graph 39. At the commencement of treatment the levels were:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin</td>
<td>8.8 G. per 100 ml.</td>
</tr>
<tr>
<td>Red cells</td>
<td>2,580,000 per c.mm.</td>
</tr>
<tr>
<td>C.I.</td>
<td>1.16</td>
</tr>
<tr>
<td>PCV</td>
<td>25.5%</td>
</tr>
<tr>
<td>MCV</td>
<td>98.8 µ₃</td>
</tr>
<tr>
<td>MCHC</td>
<td>34.5%</td>
</tr>
<tr>
<td>White cells</td>
<td>5,000 per c.mm.</td>
</tr>
<tr>
<td>Reticulocytes</td>
<td>1.4%</td>
</tr>
</tbody>
</table>

The marrow was megaloblastic. After sixteen days of this form of therapy the levels were haemoglobin 9.5 G. per 100 ml., red cells 2,470,000 per c.mm., C.I. 1.29, PCV 25%, MCV 101.2 µ₃, MCHC 38.0%, white cells 7,000 per c.mm., reticulocytes 3.1% and the marrow remained megaloblastic. For the first ten days the benziminazole compound was given in a dosage of 46 mg. daily and thereafter 92 mg. were given daily together with the 5 µg. of cyanocobalamin.

There was a subsequent response, without much evidence of reticulocytosis, to cyanocobalamin.

No more of the benziminazole compound was available for clinical trial.

Comment. A pernicious anaemia patient was given 5 µg. of cyanocobalamin daily by mouth, together with 5:6-Dimethyl-benziminazole in an attempt to prevent bacterial utilisation of vitamin B₁₂ in the small intestine. The experiment was unsuccessful/
unsuccessful in the dosage employed as regards producing a haematological remission.

MRS. A.W. FAILURE OF RESPONSE IN PERNICIOUS ANAEMIA TO SIMULTANEOUS ADMINISTRATION BY MOUTH OF CYANOCOBALAMIN AND 5:6-DIMETHYLBENZIMINAZOLE.

<table>
<thead>
<tr>
<th>RETICS%</th>
<th>R.B.C.</th>
<th>Hb. Gry (100 ml.)</th>
<th>W.B.C. M.I.L.S. comm.</th>
</tr>
</thead>
<tbody>
<tr>
<td>44-8</td>
<td>11-8</td>
<td>8-9 3</td>
<td>5-9 2</td>
</tr>
<tr>
<td>10</td>
<td>30</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

GRAPH 39.

MRS. E.M. PERNICIOUS ANAEMIA WITH A COLOUR INDEX OF 0.6.

<table>
<thead>
<tr>
<th>RETICS%</th>
<th>R.B.C.</th>
<th>Hb. Gry (100 ml.)</th>
<th>W.B.C. M.I.L.S. comm.</th>
</tr>
</thead>
<tbody>
<tr>
<td>48</td>
<td>11-8</td>
<td>8-9 3</td>
<td>5-9 2</td>
</tr>
<tr>
<td>10</td>
<td>30</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

GRAPH 40.
Mrs. E. M. (Graph 40.)

A 47 year old patient who was admitted to hospital with a history of having 'been anaemic' for fourteen years. Symptoms had been worse for seven months. Three weeks before admission she developed diarrhoea, and this lasted for two weeks. Vomiting had occurred for two weeks. The periods had never been heavy and they had ceased six months previously. No other source of blood loss was known and according to the information given by the patient the diet had been normal. The sedimentation rate (Westergren) was 30 mm./hr. The stool benzidine was repeatedly negative, and liver function tests revealed no abnormality. The blood urea nitrogen level was 5 mg. per 100 ml. There was no evidence of infection or of a collagen disease.

On 6.7.52, the blood levels were:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin</td>
<td>6.5 G. per 100 ml.</td>
</tr>
<tr>
<td>Red cells</td>
<td>3,590,000 per c.mm.</td>
</tr>
<tr>
<td>C.I.</td>
<td>0.6</td>
</tr>
<tr>
<td>PCV</td>
<td>24%</td>
</tr>
<tr>
<td>MCV</td>
<td>66.8 cµ.</td>
</tr>
<tr>
<td>MCHC</td>
<td>27.1%</td>
</tr>
</tbody>
</table>

There was histamine fast achlorhydria. The peripheral blood film was seen by several experienced observers, all of whom considered it to be typical of iron deficiency anaemia. The cause of the anaemia was not obvious: a barium meal and a chest X-ray were negative. The serum bilirubin level was 1.3 mg. per 100 ml. and the direct Coombs' test was negative. The urinary output of urobilinogen in 24 hours was 7.3 mg. The ankle jerks were absent, and the spleen could be palpated just below the costal margin.
A sternal puncture was attempted by the house physician on admission but no marrow was obtained. A second puncture was attempted eleven days after admission and the marrow was seen to be frankly megaloblastic. By now, however, the peripheral blood picture was more dimorphic in character and ovalocytosis was observed. The haemoglobin level was now 6.1 G. per 100 ml., the red cells 2,730,000 per c.mm., C.I. 0.75, PCV 21.0%, MCV 76.9 cu., MCHC 29.0%, white cells 2,800 per c.mm., reticulocytes 3.6%. It was considered that this was in fact a case of pernicious anaemia despite the low colour index, mean corpuscular volume and mean corpuscular haemoglobin concentration. The patient showed a good general and haematological response to injections of cyanocobalamin.

Comment. A patient with pernicious anaemia with a colour index of 0.6, a MCV of 66.8 cu. and a MCHC of 27.1%. The cause of the iron deficiency anaemia was not found. The patient was seen prior to the introduction of the serum vitamin B12 test which no doubt would have revealed the diagnosis more quickly.

DISCUSSION

In this chapter reference has been made to patients with pernicious anaemia who have been treated with cyanocobalamin by mouth, cyanocobalamin together with an intrinsic factor preparation by mouth, citrovorum factor by mouth and by injection, pteroylglutamic acid, pteroyldiglutamic acid, pteroyltriglutamic acid, thymine, penicillin, the substance 5:6-Dimethyl-benzimidazole which it was hoped would prevent bacterial/
bacterial utilisation of vitamin B₁₂, and with cyanocobalamin rectally and in the form of snuff.

Many of these preparations are of some research interest and the biochemical implications will be considered in Chapter 12. From the practical point of view, treatment of pernicious anaemia by the injection of cyanocobalamin in adequate dosage would seem to be the method of choice and none of our findings contradict this view. Details about the dosage of cyanocobalamin are considered in the next chapter.

As is well known, pteroylglutamic acid does not prevent the development of neurological complications in pernicious anaemia and, indeed, may precipitate them. For this reason, although it is usually safe to use pteroylglutamic acid for treatment in forms of megaloblastic anaemia other than pernicious anaemia, it should not be used alone in the latter condition. With the development of a serum vitamin B₁₂ assay technique it would appear reasonable now to extend this by making the statement that while pteroylglutamic acid therapy will be the method of choice in most cases of non-Addisonian megaloblastic anaemia, cyanocobalamin therapy should also be used where the serum vitamin B₁₂ level is below the lower limit of normal (140 µg./ml.).

In this chapter, we have seen that Mr. M.C. (Graph 20) developed subacute combined degeneration of the cord because he was not adequately supervised in the first instance, and that his condition deteriorated neurologically when pteroylglutamic acid therapy was given. Treatment with liver injections and later with cyanocobalamin did not lead to absolute improvement/
improvement and it must be taken that irreversible changes had occurred in the spinal cord. On the other hand, Mrs. J.W. (Graph 34) had become haematologically refractory to treatment with pteroylglutamic acid but had no evidence of neurological change in the spinal cord. Mrs. H.M. (Graph 35) had clinical features of pernicious anaemia with subacute combined degeneration of the cord and the serum vitamin B12 level was below the lower limit of normal. The marrow was normoblastic. In this instance pteroylglutamic acid had been taken but not in the six months before these investigations were carried out.

The interpretation of these cases is difficult, but one thing that is clearly brought out is the danger of polytherapy in uninvestigated cases of anaemia. We have seen that Mrs. W.C. (Graph 29) fared better on Bifacton tablets than on cyanocobalamin injections because her doctor did not give the latter, but in general we do not recommend such a form of treatment because of the danger of patients taking the tablets irregularly. Moreover in the five patients that we have treated with Bifacton the red cell level was not consistently maintained at a high level with two or four tablets daily.

In previous chapters reference has been made to the use of penicillin and other antibiotics in the treatment of megaloblastic anaemias. In this chapter Mrs. M.D. (Graph 38) did not respond to penicillin in the dosage used, whereas Mrs. I.K. (Graph 33) may conceivably have had the marrow converted from the megaloblastic to the normoblastic state by penicillin injections. The practical point that emerges is that/
that in patients with clinical features of pernicious anaemia this diagnosis should not immediately be abandoned because of a normoblastic marrow if the patient has recently received antibiotic therapy.

**SUMMARY**

A summary is given of twenty-two cases of pernicious anaemia treated by various therapeutic agents and the practical considerations that result are briefly discussed.
CHAPTER 12.

CONSIDERATION OF CERTAIN ASPECTS OF THE METABOLISM OF ANTlMEGALOBLASTIC SUBSTANCES, AND THE CHANGES IN THEIR METABOLISM THAT OCCUR IN PERNICIOUS ANAEMIA.

THERAPEUTIC CONSIDERATIONS.

Vitamin B₁₂, folic acid and citrovorum factor have many complex functions in the body. These will be considered in Chapter 13, and in the present chapter attention will be paid to certain aspects of the metabolism of these substances themselves. It is perhaps appropriate to commence with the food-stuffs and to follow the course of the haemopoietic factors from the mouth, through the stomach and beyond, gathering together as far as possible some of the threads from the previous chapters and from other published work.

THE OCCURRENCE OF ANTlMEGALOBLASTIC SUBSTANCES IN THE DIET.

In Chapter 2 we have already considered, with particular reference to megaloblastic anaemia in India, what is known about the content of vitamin B₁₂ and folic acid in the diet.

Foods rich in "folic acid" include liver, kidney and fresh green vegetables, while the content of folic acid in beans, potatoes, rice and milk is low. The findings given in Chapter 6 suggest that much of the apparent folic acid in liver is in fact citrovorum factor, but we have seen further (Chapter 7) that the normal gastric juice is able to convert part of the
citrovorum factor to folic acid, probably by virtue of the hydrochloric acid in the gastric secretion. Cooking makes much of the folic acid in the food unavailable as a growth factor for \textit{S. faecalis} or \textit{L. casei} but this does not necessarily mean that it is not available to the human body when ingested.

Vitamin B\textsubscript{12} is present particularly in liver and kidney and to a less extent in beef, pork, ham, mutton and veal. There is a small amount (less than 3 µg. per 100 G.) in cheese, egg yolk and milk powder, and a negligible quantity in potatoes, beans, green peas, cabbage and barley. As yet our knowledge of the vitamin B\textsubscript{12} content of foodstuffs is inadequate but reference may be made to articles by Lewis et al. (1949), Elvehjem (1950), Thompson et al. (1950), Peeler et al. (1951) and Collins et al. (1951).

There is no reason for thinking that pernicious anaemia is caused by any dietetic change, but there is the possibility that the anorexia which is commonly present in pernicious anaemia may make matters worse by leading to a deficient intake of antimegaloblastic substances. We do not know the daily requirements of these substances in normal persons, since calculations based on therapeutic dosage in pernicious anaemia patients are not valid, especially with relation to folic acid because we do not know for certain what is the precise disorder of folic acid metabolism in pernicious anaemia. Possible antimetabolites of antimegaloblastic substances have been mentioned on pages 54 and 391.

**INTRINSIC FACTOR.**

In the fundic portion of the stomach of man there is secreted the intrinsic factor (Fox & Castle, 1942). According/
According to Latner & McEvoy-Bowe (1953) this is mucoprotein in nature, and contains alanine, arginine, aspartic acid, cysteine or cystine, glutamic acid, glycine, leucine, methionine, proline, threonine and valine together with fucose, galactose and hexosamine. Prusoff et al. (1953) have endeavoured to concentrate intrinsic factor, and have shown that fractions with most intrinsic factor activity as estimated by haematological response in pernicious anaemia to oral administration with cyanocobalamin had least activity for preventing the growth of *L. leichmannii* in the presence of vitamin *B*₁₂. Spray (1952) had already shown that heating normal gastric juice did not impair its capacity to inhibit the response of *L. leichmannii* to cyanocobalamin but did impair its efficacy in causing a patient with pernicious anaemia to respond to its oral administration together with cyanocobalamin.

Burkholder (1952) has found that under certain environmental circumstances the ability of intestinal organisms to absorb vitamin *B*₁₂ may be inhibited by normal gastric juice, but we have not been able to confirm this. It is not yet possible to say with certainty whether intrinsic factor acts by combining with vitamin *B*₁₂ and promoting its absorption or by preventing intestinal bacteria from absorbing vitamin *B*₁₂. It seems however that the intrinsic factor derived from animal stomach, although itself thermolabile, becomes thermostable after interaction *in vitro* with cyanocobalamin, or that the product of this binding is thermostable. (Glass & Boyd, 1953). Presumably/
Presumably the resultant product might either not be available for the growth of intestinal bacteria and might then be absorbed through the small intestinal wall, or, alternatively, this product might be absorbable to a much greater extent than is cyanocobalamin itself, in which case it is not necessary to postulate any effect on intestinal bacteria.

**INTESTINAL BACTERIA AND THE ANTIMEGALOBLASTIC SUBSTANCES.**

This has already been considered on page 75 in relation to anaemia in Africa and in Chapter 5, which deals with our studies of the ability of intestinal organisms to produce or remove antimegaloblastic substances when growing in culture media. It seems that the capacity of organisms to do these things depends upon their environment and upon other experimental conditions. What is in doubt is the extent to which this is important in man. It is tempting, for instance, to suggest that subacute combined degeneration of the cord may develop more readily when organisms in the small intestine of a pernicious anaemia patient are vigorously producing folic acid and that this folic acid may, if absorbed in the absence of vitamin B₁₂, prevent haematological relapse without preventing neurological relapse. There is, however, no direct proof that this happens. We have been unable to demonstrate any consistent difference in the degree of excretion of folic acid following an injected test dose of pteroylglutamic acid in untreated pernicious anaemia patients with and without evidence of/
of neurological complications.

**THE ABSORPTION OF ANTIMEGALOBLASTIC SUBSTANCES.**

We have already seen that there is every reason to believe that these substances are absorbed in the small intestine, and not to any extent in the stomach or the large intestine (p.383). We have mentioned the fragmentary state of our knowledge of vitamin B12 absorption. In Table 52 we have presented certain evidence to show that the absorption of folic acid following the oral administration of relatively large doses of pteroylglutamic acid to pernicious anaemia patients is not impaired. This does not necessarily mean that the absorption of smaller quantities of folic acid or its conjugates in the diet is always normal in pernicious anaemia.

If citrovorum factor is given to normal persons by mouth, we have seen that it is absorbed and appears in the serum largely as folic acid (Chapter 7). If it be given by mouth to pernicious anaemia patients or to others with histamine fast achlorhydria it is absorbed and appears in the serum as citrovorum factor (or a substance with similar microbiological properties).

**THE OCCURRENCE OF ANTIMEGALOBLASTIC SUBSTANCES IN THE SERUM.**

This has been considered in Chapter 7, where we have seen that both in the normal person and in the patient with untreated pernicious anaemia or with another form of megaloblastic anaemia it is possible to demonstrate very small quantities of growth/
growth factors for *S. faecalis* and for *L. citrovorum* in the serum. The exact nature of such growth factors is, however, uncertain. The occurrence of growth factors for *L. leichmannii* is considered in Chapters 7 and 8.

It appears that unless the serum undergoes preliminary treatment (e.g. with acetate buffer at pH 4.6) the *L. leichmannii* assay measures substances other than vitamin B₁₂ in the heated serum of untreated pernicious anaemia patients and of controls. This is not due to precipitation of proteins by heating but is due to some unknown growth factor for the test organism. Preliminary treatment with acetate buffer makes it possible to use a *L. leichmannii* assay method for the serum.

Recently Mollin & Ross (1953) have published a further paper dealing with their results using the *Euglena* assay of Hutner et al. (1949). They found that pernicious anaemia patients treated with pteroylglutamic acid or citrovorum factor continued to have low readings for the serum level of vitamin B₁₂. Since introducing the modified *L. leichmannii* serum vitamin B₁₂ test we have been unable to trace any patients who have been treated for pernicious anaemia for a long period with either pteroylglutamic acid or citrovorum factor.

Mollin & Ross (1953) report, further, that the serum level of vitamin B₁₂ is normal in patients with megaloblastic anaemia that is refractory to cyanocobalamin therapy. In Chapter 8 we/
we have referred to some such cases where the vitamin B₁₂ level of the serum was normal by the *L. leichmannii* assay and one patient with idiopathic steatorrhoea whose serum vitamin B₁₂ level was low. The Hammersmith workers state that in patients with pernicious anaemia and neurological complications but little or no anaemia the marrow may show only early signs of megaloblastic change but there is a definitely low serum vitamin B₁₂ level. We have referred in Chapter 11 to two patients, Mrs. I.K. (Graph 33) and Mrs. M.M. (Graph 35) who had normoblastic marrows and a low serum vitamin B₁₂ level. In one of these cases Ross has with the Euglena assay confirmed our findings with the *L. leichmannii* assay. In the other case there was insufficient pre-treatment serum for this to be done.

The levels of folic acid, citrovorum factor (and perhaps related compounds) that are reached in the serum following the administration of pteroylglutamic acid, its synthetic conjugates and of citrovorum factor has already been recorded in Chapter 7 and will not be considered further.

The length of time that the serum level of vitamin B₁₂ is maintained within the range of normality in pernicious anaemia patients after treatment with cyanocobalamin injections is, however, of practical importance. This is a matter that Mollin & Ross (1953) have discussed. They report on the serum vitamin B₁₂ levels after single cyanocobalamin injections in thirty-seven patients who had pernicious anaemia in relapse, the marrows all being megaloblastic. In general, the greater the dose given, the longer was the serum vitamin B₁₂ level maintained within normal limits. It should however be noted that/
that these were patients whose tissue stores of vitamin B₁₂ were likely to be extremely low, and that it is possible that patients who have had cyanocobalamin therapy for long periods would maintain the serum vitamin B₁₂ at a normal level for longer periods. The data given by Mollin & Ross on their cases are not sufficiently detailed for us to make any satisfactory comparison with our own serum vitamin B₁₂ readings in patients on maintenance treatment for pernicious anaemia.

In discussing the changes in the serum in pernicious anaemia it is necessary to refer to the marrow culture experiments of Lajtha. In 1951 Callender & Lajtha showed that normal gastric juice together with cyanocobalamin formed a thermolabile substance which caused megaloblasts to ripen in vitro, whereas neither gastric juice nor cyanocobalamin was effective alone. This was taken (without any proof) as being due to the effect of intrinsic factor in the juice. Normal serum had a similar ripening activity, as had citrovorum factor and pteroylglutamic acid.

Lajtha (1952) claimed that cultures of normoblastic bone marrow become megaloblastic in pernicious anaemia serum, but megaloblastic marrow cells became normoblastic in normal serum. Lajtha considered that there was an inhibiting factor in pernicious anaemia serum because diluted pernicious anaemia serum enabled megaloblasts to ripen. (Lajtha, 1950). Fiehnann et al. (1952) and others have been unable to confirm this and there appears to be some divergence of views about the interpretation of the findings. Thompson (1952) found that folic acid by itself was able to effect maturation in an in vitro
in vitro culture of megaloblasts from pernicious anaemia patients, while liver extracts, cyanocobalamin and thymine could not do so. However, the terminology used by Thompson differed from the one commonly employed in Britain.

Callender & Lajtha (1951) considered that since cyanocobalamin by itself is haemopoietically inactive in tissue culture, it is necessary to postulate an extragastric intrinsic factor which potentiates cyanocobalamin when it is injected.

Horrigan et al. (1951), on the other hand, demonstrated that 1 µg. of cyanocobalamin injected into the iliac crest marrow cavity of patients with pernicious anaemia induced a change to normoblastic blood formation at the site of the injection but not on the opposite side. Pteroylglutamic acid or citrovorum factor in a dosage of 1 or 2 mg. did not cause local change. This may indicate that vitamin B₁₂ is active in the form in which it is injected while pteroylglutamic acid and citrovorum factor are not, or it may indicate that the latter two substances rapidly diffuse out of the marrow while the vitamin B₁₂ does not.

THE OCCURRENCE OF ANTIMEGALOBLASTIC SUBSTANCES IN THE TISSUES

In Chapter 6 there is given an account of the tissue content of vitamin B₁₂, folic acid and citrovorum factor in the human. Apart from the fact that one untreated pernicious anaemia patient was not found at post mortem to have any vitamin B₁₂ in the liver or other organs examined, our data about the tissue content of haemopoietic factors in pernicious anaemia are very scanty. It is not anticipated that such information/
information will readily become available since patients do not now die of untreated pernicious anaemia. Our early experiments with liver puncture biopsy specimens convinced us that the specimens were inadequate for satisfactory assays to be done although the French workers Drouett et al. (1951) subsequently reported results from this method of approach. As already stated our data suggest strongly that in the normal person much of the apparent folic acid in the liver is citrovorum factor or a conjugate form of this substance. We know of no other papers dealing with the folic acid content of human organs.

It will be noted that our figures for the vitamin B₁₂ content of the liver of a partially treated pernicious anaemia patient using a pancreatin method of extraction, gave a higher result than would be anticipated if the cyanocobalamin that had been administered were equally disseminated throughout a liver which had itself previously lacked vitamin B₁₂. This may indicate that contrary to what is known about the subject, there is in liver an unknown substance or substances that stimulate the growth of _L. leichmannii_ under the conditions of the assay despite the use of a technique that includes alkaline hydrolysis. If liver from another untreated or partially treated pernicious anaemia patient should become available for study, it is important that it should be assayed for vitamin B₁₂ by more than one technique and that, if there is any suggestion of other such substances being present, paper chromatography should be carried out.

It/
It may be, however, that in untreated pernicious anaemia the liver contains 'bound' forms of vitamin B₁₂ that are not available for haematopoiesis and that these cannot be utilised for the growth of L. leichmannii until pancreatin or other enzymes are used to release the active substance.

Subject to this provision it may be stated that our findings in normal persons suggest that the liver alone contains 500 µg. or more of vitamin B₁₂ and 4 mg. or more of folic acid (the term here being used to include pteroylglutamic acid and its conjugates together with citrovorum factor and its conjugates). These findings, especially as regards vitamin B₁₂, may be of importance in relation to the treatment of pernicious anaemia.

THE URINARY EXCRETION OF ANTMEGALOBLASTIC SUBSTANCES.

When we measure the urinary output of an antimegaloblastic substance that has been given by mouth, we are concerned with the extent of its absorption through the alimentary tract, the extent to which it is taken up by the tissues (this is believed to vary according to the degree of bodily depletion of the substance concerned), the extent to which it is metabolised, conjugated or otherwise converted or destroyed in the body, the question of whether it is excreted by any other route, the capacity of the kidneys to excrete the substance, the extent to which it breaks down or alters its structure in the urine, and the question of whether there is being excreted simultaneously some other substance that might affect the antimegaloblastic/
antimegaloblastic substance or interfere with the technique employed to measure it.

It may be stated here that recovery experiments in which cyanocobalamin, citrovorum factor or pteroylglutamic acid were added to the urine gave very satisfactory results, that with normal kidney function we have found, as have other workers, that test doses of these three substances are excreted within eight to ten hours (except with doses of 2 - 5 mg. of cyanocobalamin given by injection), that antibiotics, sulphonamides and possibly other vitamins interfere with the microbiological assays, and that in the presence of ascites, a pleural effusion or much oedema, the output of folic acid or citrovorum factor (and possibly vitamin B₁₂) in the urine may be low. We have been able to recover folic acid from the ascitic fluid of a patient with portal thrombosis who was given pteroylglutamic acid by mouth, and this patient continued to excrete folic acid in the urine over a period of three days. In chronic renal disease too, we have found impairment of folic acid excretion (see Chapter 17).

**Folic Acid.**

Attention has already been paid to the ability of the pernicious anaemia patient to excrete a substance with the microbiological properties of pteroylglutamic acid when the synthetic conjugates pteroyldiglutamic acid and pteroyltrimethyleneaminocacetic acid are given (Chapter 7). We have seen, too, that a test dose of 5 mg. of pteroylglutamic acid is well absorbed from the alimentary tract in pernicious anaemia in relapse,
and excreted in the urine, but that the mean quantity excreted in a series of cases of pernicious anaemia is considerably less than in a series of normal persons. The reason for this difference is not obvious, and no satisfactory explanation has yet been given. As will be seen in the chapter that follows, the metabolic functions of the antimegaloblastic substances are many and the subject is one of great complexity. It may be that folic acid or citrovorum factor acts by 'mass action' when the true deficiency is of vitamin B₁₂ and that when cell division is thus increased many enzyme systems utilising folic acid are involved, so that an abnormally large amount of pteroylglutamic acid is required. The results in Table 52 indicate that it is possible to have untreated pernicious anaemia with a relatively high red cell level and a low output of folic acid following a 5 mg. test dose, or to have a relatively low red cell level and a high output of folic acid. In addition one patient with transitional erythroblasts rather than true megaloblasts in the marrow excreted 3.05 mg. of the test dose (a normal amount), whereas another such patient excreted 1.51 mg. (a low output). It is not possible therefore to relate the results of a urinary folic acid excretion test in individual cases to the red cell level or the degree of megaloblastic change in the marrow, and it will be seen in Chapter 18 which deals with the output in cases of malignant disease, that such patients may have an extremely low output after the injected test dose in the absence of any megaloblastosis. To add to
the complexity it should be recalled that two patients with intestinal malabsorption and megaloblastic anaemia which was not due to vitamin B12 deficiency, excreted a normal amount of folic acid following an injected test dose, and yet responded to pteroylglutamic acid therapy, and it will be seen in Chapter 14 that a similar state of affairs may operate in megaloblastic anaemia of pregnancy. It may be that the deficient substance in these cases is not pteroylglutamic acid itself but something else that can be derived from pteroylglutamic acid or replaced by it, or it may be that the urinary excretion of folic acid is so rapid that the tissues, although deficient in the substance, do not have sufficient time to hold it. The problem in pregnancy will be considered further in the relevant chapter. It should be added that the results in Table 53 indicate that even the normal person who has been saturated with folic acid does not excrete the whole of an injected 5 mg. test dose, so that some metabolism of the substance must occur. This was shown further by the increased amount of citrovorum factor that was excreted in these cases.

**Citrovorum Factor.**

Further observations that may be mentioned here concern the relative extent to which citrovorum factor and folic acid are excreted by the same patient after a test dose. The data that we have are few because we have been unable to obtain any further supplies of citrovorum factor.

We have seen (p.292) that on the average the normal person excretes/
excretes about 56 per cent of an injected 5 mg. test dose of pteroylglutamic acid, whereas on the average the pernicious anaemia patient excretes about 31 per cent. The data in Chapter 9 indicate that the absorption of a 5 mg. test dose of pteroylglutamic acid is satisfactory in pernicious anaemia patients as in controls. On the other hand we have seen (p. 299) that a normal healthy person excreted 16.6 per cent of an injected dose of 18 mg. of citrovorum factor, but that when 36 mg. of this substance was given by mouth only 1.246 mg. of folic acid and 0.207 mg. of citrovorum factor were excreted in the urine. This low output was not due to any delay in absorption, as urine collections were continued for several days and tests made. It was not due to quantitative change as a result of conversion of citrovorum factor to pteroylglutamic acid, since 1 mg. of the former gives 0.87 mg. of the latter.

These are not isolated examples. Two other normal persons given injections of 12 mg. of citrovorum factor excreted, respectively, 1.82 mg. and 1.10 mg. of citrovorum factor. When 36 mg. was then given by mouth the urinary output of substances with growth activity for S. faecalis in the first case was as shown in Fig.17, which also indicates the extent of excretion after test doses of pteroylglutamic acid.
In the second normal person the urinary excretion of citrovorum factor after a 12 mg. oral test dose of citrovorum factor was 75 µg. There was also excreted 45 µg. of folic acid.

In two untreated pernicious anaemia patients there were obtained the results shown in Figs. 18a and 18b.
URINARY EXCRETION - CITROVORUM FACTOR AND FOLIC ACID IN UNTREATED PERNICIOUS ANAEMIA.

**Fig. 18a.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>CF (mg)</th>
<th>Folic Acid (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12 mg CF i.m.</td>
<td>0.57</td>
<td>1.46</td>
</tr>
<tr>
<td>12 mg CF orally</td>
<td>0.033</td>
<td>1.46</td>
</tr>
<tr>
<td>5 mg PGA s.c.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 mg PGA orally</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

3 mg Folic Acid

**Fig. 18b.**
Because of lack of citrovorum factor for investigative purposes our data are too scanty for any conclusions to be drawn about the extent to which pernicious anaemia patients differ from controls in relation to urinary output of citrovorum factor after an injected test dose. It is obvious from Figs.13a and 13b however that in pernicious anaemia, when citrovorum factor is administered, unlike what happens when pteroylglutamic acid is given, there is, as in normal persons, a considerably smaller output after the injected dose than after a similar dose given by mouth. This may indicate that citrovorum factor is destroyed or in some way altered in the alimentary tract or that it is not absorbed satisfactorily.

If citrovorum factor is the 'biologically active' form of folic acid it is not clear why such a small proportion of an injected dose is excreted in normal persons.

_Vitamin B12._

Although we have already seen that the liver and other organs and the tissues of one untreated pernicious anaemia patient were completely or almost completely devoid of vitamin B12, we have also seen (p.290) that the pernicious anaemia patient, like the normal person, excretes about a third of an injected test dose of 100μg. of cyanocobalamin, and therefore it is not possible to devise a balance experiment based on urinary excretion.

The fact that the pernicious anaemia patient in relapse excretes such a high proportion of an injected dose must surely indicate that the kidneys excrete vitamin B12 faster than the tissues/
tissues can 'fix' it. It is, however, surprising that the normal person retains just as much, but this is a finding reported by many workers. The amount of cyanocobalamin required to produce a haemopoietic response is very small. In one case of pernicious anaemia in relapse there was a rise of red cells of 670,000 per c.mm. in 13 days and a partial conversion of the marrow towards the normoblastic state following a single injection of 400 mg. of cyanocobalamin, and it would seem likely therefore that the blood forming tissue has the first call upon cyanocobalamin when it is administered. Since the normal person only excretes a proportion of the injected dose of cyanocobalamin the remainder must be metabolised or conjugated in some way, and presumably this occurs equally in the pernicious anaemia patient. It seems that the degree of this change is such as to overshadow the small amount of vitamin B12 taken up in pernicious anaemia for haemopoiesis.

**THERAPEUTIC CONSIDERATIONS**

So far we have neither produced nor quoted any evidence to refute the view of most haematologists that the treatment of pernicious anaemia by repeated injections of cyanocobalamin is a satisfactory method of treatment. In Table 54 there are given the blood counts of a random sample of patients with pernicious anaemia most of whom had been treated in this way: we were rather surprised at the number of patients with red cell levels of less than 5,000,000 per c.mm. However, cyanocobalamin therapy was as effective as, or more effective than liver injections.

Conley et al. (1952) had rather unsatisfactory results with cyanocobalamin for maintenance therapy, but they used only 45
### TABLE 54.

Results of Maintenance Therapy in Cases of Pernicious Anaemia seen during the Period September, 1953 - February, 1954.

<table>
<thead>
<tr>
<th>Case</th>
<th>Sex</th>
<th>Age</th>
<th>Hb.</th>
<th>RBCs</th>
<th>Dose of Vit.B12</th>
<th>Duration</th>
<th>Total Duration</th>
<th>Previous Therapy and whether blood levels now Same (S) Better (B) or Worse (W)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>70</td>
<td>14.1</td>
<td>4.59</td>
<td>40 µg.</td>
<td>4</td>
<td>3 1/2</td>
<td>11</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>44</td>
<td>13.5</td>
<td>4.93</td>
<td>40 µg.</td>
<td>3</td>
<td>3 1/2</td>
<td>6</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>49</td>
<td>15.8</td>
<td>5.11</td>
<td>40 µg.</td>
<td>3</td>
<td>3</td>
<td>13</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>46</td>
<td>12.6</td>
<td>5.03</td>
<td>40 µg.</td>
<td>4</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>42</td>
<td>16.6</td>
<td>5.30</td>
<td>50 µg.</td>
<td>3</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>52</td>
<td>13.0</td>
<td>4.10</td>
<td>50 µg.</td>
<td>3</td>
<td>1 1/2</td>
<td>8</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>78</td>
<td>12.4</td>
<td>4.27</td>
<td>50 µg.</td>
<td>4</td>
<td>1 1/2</td>
<td>7</td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>60</td>
<td>12.4</td>
<td>4.40</td>
<td>50 µg.</td>
<td>3</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>9</td>
<td>F</td>
<td>47</td>
<td>12.6</td>
<td>4.47</td>
<td>50 µg.</td>
<td>3</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>10</td>
<td>F</td>
<td>68</td>
<td>12.4</td>
<td>4.63</td>
<td>50 µg.</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>11</td>
<td>F</td>
<td>67</td>
<td>13.2</td>
<td>4.68</td>
<td>50 µg.</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>12</td>
<td>F</td>
<td>62</td>
<td>15.5</td>
<td>4.98</td>
<td>50 µg.</td>
<td>3</td>
<td>3 1/2</td>
<td>3</td>
</tr>
<tr>
<td>13</td>
<td>M</td>
<td>77</td>
<td>13.2</td>
<td>5.10</td>
<td>50 µg.</td>
<td>3</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>14</td>
<td>F</td>
<td>51</td>
<td>13.9</td>
<td>5.11</td>
<td>50 µg.</td>
<td>3</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>15</td>
<td>F</td>
<td>46</td>
<td>13.3</td>
<td>5.29</td>
<td>50 µg.</td>
<td>3</td>
<td>2 1/2</td>
<td>2</td>
</tr>
<tr>
<td>16</td>
<td>F</td>
<td>59</td>
<td>15.4</td>
<td>5.30</td>
<td>50 µg.</td>
<td>3</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>17</td>
<td>F</td>
<td>71</td>
<td>16.7</td>
<td>5.52</td>
<td>50 µg.</td>
<td>3</td>
<td>2 1/2</td>
<td>2</td>
</tr>
<tr>
<td>18</td>
<td>M</td>
<td>46</td>
<td>12.1</td>
<td>4.54</td>
<td>50 µg.</td>
<td>3</td>
<td>2 1/2</td>
<td>2</td>
</tr>
<tr>
<td>19</td>
<td>F</td>
<td>58</td>
<td>12.4</td>
<td>4.53</td>
<td>50 µg.</td>
<td>2</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>20</td>
<td>M</td>
<td>69</td>
<td>15.1</td>
<td>5.00</td>
<td>50 µg.</td>
<td>3</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>21</td>
<td>M</td>
<td>53</td>
<td>12.6</td>
<td>5.22</td>
<td>50 µg.</td>
<td>2</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>22</td>
<td>F</td>
<td>49</td>
<td>13.6</td>
<td>4.63</td>
<td>100 µg.</td>
<td>4</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>23</td>
<td>F</td>
<td>51</td>
<td>12.4</td>
<td>4.20</td>
<td>100 µg.</td>
<td>3</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>24</td>
<td>M</td>
<td>80</td>
<td>13.6</td>
<td>4.67</td>
<td>100 µg.</td>
<td>3</td>
<td>3 1/2</td>
<td>7</td>
</tr>
<tr>
<td>25</td>
<td>F</td>
<td>55</td>
<td>15.2</td>
<td>5.10</td>
<td>100 µg.</td>
<td>3</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>26</td>
<td>F</td>
<td>60</td>
<td>15.3</td>
<td>5.15</td>
<td>100 µg.</td>
<td>2</td>
<td>3 1/2</td>
<td>4</td>
</tr>
<tr>
<td>27</td>
<td>M</td>
<td>53</td>
<td>15.5</td>
<td>5.24</td>
<td>100 µg.</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>28</td>
<td>F</td>
<td>63</td>
<td>15.7</td>
<td>5.50</td>
<td>100 µg.</td>
<td>1</td>
<td>1 1/2</td>
<td>4</td>
</tr>
</tbody>
</table>

**Anahaemin**

<table>
<thead>
<tr>
<th>Case</th>
<th>Sex</th>
<th>Age</th>
<th>Hb.</th>
<th>RBCs</th>
<th>Dose of Vit.B12</th>
<th>Duration</th>
<th>Total Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>29</td>
<td>F</td>
<td>69</td>
<td>13.6</td>
<td>4.59</td>
<td>4 ml.</td>
<td>3</td>
<td>1 1/2</td>
</tr>
<tr>
<td>30</td>
<td>F</td>
<td>71</td>
<td>15.7</td>
<td>5.13</td>
<td>4 ml.</td>
<td>3</td>
<td>3 1/2</td>
</tr>
<tr>
<td>31</td>
<td>F</td>
<td>63</td>
<td>9.3</td>
<td>4.03</td>
<td>4 ml.</td>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td>32</td>
<td>F</td>
<td>47</td>
<td>13.6</td>
<td>4.48</td>
<td>4 ml.</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>33</td>
<td>M</td>
<td>57</td>
<td>15.5</td>
<td>5.0</td>
<td>2 ml.</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>34</td>
<td>F</td>
<td>68</td>
<td>10.1</td>
<td>4.97</td>
<td>2 ml.</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>35</td>
<td>F</td>
<td>44</td>
<td>14.1</td>
<td>4.63</td>
<td>2 ml.</td>
<td>1</td>
<td>3</td>
</tr>
</tbody>
</table>

**Previous Therapy and whether blood levels now Same (S) Better (B) or Worse (W)**

- Anahaemin (S)
- Anahaemin (B)
- Anahaemin (S)
- Anahaemin (B)
- Anahaemin (S)
- Anahaemin (B)
- Anahaemin (S)
- Anahaemin (B)
- Anahaemin (S)
- Anahaemin (B)
- Anahaemin (S)
45 \mu g. every six weeks. Blackburn et al. (1952) who advise 50 \mu g. of cyanocobalamin every two weeks for maintenance, found that cyanocobalamin was quite satisfactory and superior to liver injections for keeping the blood counts at a normal level. The cyanocobalamin had been administered by them for at least two years and it may be that the liver extracts that had been used before that on these patients did not contain as much vitamin B_{12} as do those now on sale. In most instances it seems that those haematologists who do not find cyanocobalamin therapy to be satisfactory are using inadequate dosage. This is the case with the series reported by Meacham et al. (1950) and Beard et al. (1950). Other claims about the inability of cyanocobalamin to give entirely satisfactory results are those of Owren (1950, 1951) and Larsen (1951) who found a low prothrombin content of the blood and macrocytosis on cyanocobalamin therapy. This has not been confirmed by other workers.

Our findings on the content of vitamin B_{12} in the normal liver and other organs have been taken, together with other evidence, by Mollin & Ross (1953) to indicate that it is advisable to treat pernicious anaemia in relapse in the first week of therapy with five injections each of 1000 \mu g. of cyanocobalamin. Of this 5000 \mu g. about 4000 \mu g. will be excreted in the urine. This form of therapy may be theoretically correct but patients progress very satisfactorily on much smaller dosage. Since cyanocobalamin is relatively inexpensive and does not have toxic effects it is perhaps desirable to give a large "saturating" dose of 500 \mu g. on the first day of treatment, then 100 \mu g. weekly until the blood counts are normal; certainly/
certainly not less than 100 µg. should be given every three or four weeks for maintenance. If there is evidence of neurological change in the spinal cord it is better to give 100 µg. twice or thrice weekly till the blood counts are normal and for six months thereafter, the dose then being reduced to 100 µg. weekly for at least a further six months. After that it is unlikely that further neurological improvement will occur.

Whether or not pteroylglutamic acid therapy should supplement cyanocobalamin injections in pernicious anaemia is uncertain. Certainly pteroylglutamic acid should not be given alone because of the danger of precipitating neurological complications. We have found neurological deterioration in eight out of twenty pernicious anaemia patients treated with pteroylglutamic acid alone. Admittedly in most of these we did not allow the changes to become sufficiently marked for us to be certain that the tracts in the spinal cord rather than the peripheral nerves were involved. Chodos & Ross (1951) found that neurological features developing during pteroylglutamic acid therapy did not progress when liver extract or cyanocobalamin was administered, even although pteroylglutamic acid treatment was continued. The exact metabolic interrelationships of folic acid and vitamin B12 are uncertain (see Chapter 13). In many cases of pernicious anaemia in relapse a urinary folic acid excretion test following a 5 mg. test dose of pteroylglutamic acid suggests that there is bodily depletion of this substance. If this interpretation of the experimental findings/
findings is the correct one, then possibly there is some reason for giving 15 mg. of pteroylglutamic acid by mouth in the first week of therapy, but anything that encourages manufacturers to market preparations containing a mixture of all known haematinics is to be deprecated.

The metabolic changes that lead to subacute combined degeneration of the cord are unknown, and it is not certain why pteroylglutamic acid therapy appears not only to fail to prevent neurological complications but even to precipitate them. It may be that when pteroylglutamic acid is given in relatively large amounts to pernicious anaemia patients in relapse the metabolic processes that result utilise rapidly any traces of vitamin B₁₂ that remain in the body. It has been shown by Richards (1945) that a state of pyridoxine deficiency can be induced in rats by the administration of excessive quantities of thiamine, and it is claimed that pellagrins treated with nicotinic acid may develop beri beri or riboflavin deficiency (Spies et al., 1939; Sebrell & Butler, 1938, 1939; Sydénstricker et al., 1940). Other workers have noted the development of pellagra after large doses of riboflavin or of thiamine (Bichel & Meulengracht, 1941; Salvesen, 1940; Braandstrup, 1940; Lehmann & Nielson, 1939).

In Table 55 there is given a list of some of the substances that have been used in the treatment of pernicious anaemia, but most of these are of theoretical interest rather than practical value.
### Table 55.

**Substances with Antileukoblastic Activity in Pernicious Anemia.**

<table>
<thead>
<tr>
<th>Substance</th>
<th>Reason for Haemopoietic Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh liver - raw or lightly cooked</td>
<td>Folic acid and its conjugates, Citrovorum factor and its conjugates, Vitamin B₁₂ and B₁₂b</td>
</tr>
<tr>
<td>Liquid extract of liver</td>
<td>As fresh liver</td>
</tr>
<tr>
<td>Proteolysed liver</td>
<td>As fresh liver. Proteolysis may liberate folic acid, citrovorum factor and vitamin B₁₂ from &quot;bound&quot; forms, i.e. linked with peptides and proteins</td>
</tr>
<tr>
<td>Liver extract for parenteral use - crude</td>
<td>Vitamin B₁₂, B₁₂b. Present day batches contain very little folic acid.</td>
</tr>
<tr>
<td>Liver extract for parenteral use - refined</td>
<td>Vitamin B₁₂, B₁₂b</td>
</tr>
<tr>
<td>Vitamin B₁₂ (from liver, culture broth of streptomycetes, etc.)</td>
<td>Cyanocobalamin</td>
</tr>
<tr>
<td>Vitamin B₁₂b (from liver, culture broth of streptomycetes, etc.)</td>
<td>Hydroxocobalamin</td>
</tr>
<tr>
<td>Vitamin B₁₂c (from culture broth of streptomycetes)</td>
<td>Nitrocobalamin</td>
</tr>
<tr>
<td>Other analogues of cyanocobalamin have been produced artificially</td>
<td></td>
</tr>
<tr>
<td>Hogs' stomach preparations</td>
<td>Vitamin B₁₂ + intrinsic factor</td>
</tr>
<tr>
<td>Vitamin B₁₂ + hogs' duodenal extract</td>
<td>Vitamin B₁₂ + intrinsic factor</td>
</tr>
<tr>
<td>Autolysed yeast (marmite)</td>
<td>? Folic acid</td>
</tr>
<tr>
<td>Folic acid</td>
<td>This term may include other related substances but is usually intended to mean pteroylglutamic acid</td>
</tr>
</tbody>
</table>
Pteroylglutamic acid
Pteroyldiglutamic acid  ? Converted to PGA
Pteroyltriglutamic acid  ? Converted to PGA
Pteroylhexaglutamyl-glutamic acid  Trials insufficient to say whether it is active
Animal protein factor  Chiefly vitamin $B_{12}$
Thymine  See Chapter 13
Thymidine  See Chapter 13
Uracil  See Chapter 13
Citrovorum Factor  Usually the term is used for 5-formyl,5,6,7,8 tetrahydro PGA
Folinic acid  Usually taken to be the same as citrovorum factor but there are possibly several natural citrovorum factors
Preparations of kidney, brain (oral)  PGA  ? Vitamin $B_{12}$
Antibiotics  Probably by effect on intestinal bacteria that produce or remove haemopoietic factors

**SUMMARY**

Present knowledge of the metabolism of the antimegaloblastic substances is considered and an attempt is made to integrate this knowledge with our experimental findings described in other chapters. The practical treatment of pernicious anaemia is considered.
FUNCTIONS OF THE ANTEMEGALOBlastic SUBSTANCES IN THE
BODY AND SOME ASPECTS OF THEIR METABOLIC
INTERRELATIONSHIPS

Throughout this thesis the metabolism of folic acid,
vitamin B₁₂ and related substances has been referred to repeat-
edly but as yet little has been said about their functions in
the body. It is to repair this omission that the present
chapter, dealing largely with the work of others, is included.

NUCLEIC ACID METABOLISM.

In the metabolism of living cells nucleoproteins play a
vital part; they are built up of protein together with histone
or protamine and nucleic acid.

Schlenk (1949) has given an account of our knowledge of
the nucleic acids. They are polynucleotides containing the
purine bases adenine and guanine, the pyrimidine bases cytosine
and either thymine or uracil each attached by a glucoside
linkage to a pentose sugar which may be ribose or deoxyribose.

\[
\text{Adenine} \quad \text{Uracil}
\]

\[
\begin{align*}
&\text{N} \quad \text{C} \quad \text{NH}_2 \\
&\text{H} \quad \text{C} \quad \text{N} \quad \text{OH} \\
&\text{N} \quad \text{C} \quad \text{NH} \\
&\text{Adenine}
\end{align*}
\]

\[
\begin{align*}
&\text{HN} \quad \text{C} \quad \text{O} \\
&\text{O} \quad \text{C} \quad \text{H} \\
&\text{HN} \quad \text{C} \quad \text{H} \\
&\text{Uracil}
\end{align*}
\]
Ribosides and deoxyribosides of the purines or pyrimidines are known as nucleosides, and when the nucleosides combine with phosphate (by an ester linkage) they are called nucleotides. Thus:

Thymine + deoxyribose

\[ \text{Thymine deoxyribose (also known as thymine nucleoside or thymidine)} \]
+ phosphate

\[ \text{Thymine nucleotide (also known as thymidilic acid)} \]

Both ribonucleic acids (RNA) and deoxyribonucleic acids (DNA) contain guanine, adenine, cytosine and phosphoric acid. DNA contains thymine and deoxyribose, while RNA contains uracil.
uracil and ribose. It may be that further research will 
require modification of this generalisation.

In the blood forming organs ribonucleic acids in associa-
tion with protein are found principally in the cytoplasm of the 
cells, but also occur in the nucleoli. The reticular material 
of the reticulocytes contains ribonucleic acids which disappear 
as the reticulum is lost (Davidson et al., 1951). London et 
al. (1950) report that the reticulocyte can synthesise haem 
until all the RNA has disappeared. It should be noted that it 
is the RNA that gives the erythrocyte precursors the blue colour 
seen with Romanowsky stains before haemoglobinisation is com-
plete. Thorell (1947 ) states that during the period of 
maturity of the normal red cell precursors there is first a 
phase of growth when the concentration of cytoplasmic RNA is 
maximal. As growth declines this concentration diminishes and 
there occurs synthesis of cellular proteins, such as globin. 
Differentiation thereafter occurs with rapid formation of 
haemoglobin and disappearance of RNA.

Ribonucleic acids in the nucleoli and cytoplasm appear to 
be concerned with proliferation of the young cells but they 
also play an important role in the synthesis of haemoglobin in 
the red cells and of enzyme systems in the specific granules 
of the white cells (White, 1947). Unfortunately there is no 
specific stain for RNA. According to Kunitz (1940) ribo-
uclease can be used to digest ribonucleoprotein thereby 
abolishing basophilia; Thorell (1947) and Caspersson (1946, 
1950) have described a complex method of study of RNA that 
involves ultra violet light absorption.

Desoxyribonucleic/
Desoxyribonucleic acids occur only in the chromosomes of the nucleus, and are combined with a histone or protamine. They appear to be responsible for cell mitosis, and division. Recently an important correlation between the chromosome number and the DNA content of the nucleus has been established (Swift, 1950; Alfert, 1950; Ris & Mirsky, 1949; Leuchtenberger et al., 1951, 1952). The quantity of DNA present in the chromosomes is believed to be a constant value in the interphase diploid somatic nuclei of all tissues within a species and is twice the amount of DNA present in the haploid germ cell nuclei. Moreover it has been shown (Swift, 1950; Pollister et al., 1951; Howard & Peel, 1951; Price & Laird, 1950) that a build up of DNA occurs in a cell during the preprophase of mitosis, until at the onset of prophase the DNA content is double that of the intermitotic value.

The desoxyribonucleoproteins may be stained in the manner described by Feulgen & Rossenbeck (1924) or desoxyribonuclease may be used to digest these nucleoproteins and abolish their staining reactions (Kunitz, 1950).

In the cells of the blood forming organs there is constant breakdown and synthesis of cytoplasmic RNA and to a lesser extent of the nuclear DNA, and it is known that DNA can be formed from RNA (Mitchell, 1942). Mitotic division requires constant production of nucleoproteins, porphyrins, lipids and other substances.

Investigations involving the use of radioactive Cs in animals have suggested that the nucleoproteins are built up in the body from simple substances such as/
glycine \[ \text{CH}_2\text{COOH} \]
\[ \text{NH}_2 \]

formate \[ \text{HCOOH} \]
carbon dioxide \[ \text{CO}_2 \]

and rather than from purines and pyrimidines in the food. (Elwyn & Sprinson, 1950; Plaut et al., 1950).

This is obviously a very complex matter involving many enzymes and coenzymes. For example the bone marrow cells contain phosphatases (nucleases, nucleotidases and nucleosidases) nuclease inhibitors (Henstell & Freedman, 1952), phosphorylases, peptidases, carbonic anhydrase, amylase, lysozyme, catalase, cytochrome oxidase, lipase, cathepsin, lecithinase, beta-glucuronidase, oxidases, peroxidases and proteolytic enzymes. (For a review of this see Rebuck, 1947.) Alkaline phosphatase is believed to be concerned with the destruction of nuclear material.

In addition to the enzyme systems mentioned above, folic acid and vitamin \( B_12 \) require consideration. Folic acid occurs throughout the cell; vitamin \( B_12 \) is found particularly in the mitochondria; it may be that citrovorum factor is present rather than pteroylglutamic acid (Swendseid et al., 1951).

**THE PLACE OF THE ANTIMICROBIAL SUBSTANCES IN NUCLEIC ACID METABOLISM.**

**FOLIC ACID AND CITROVORUM FACTOR.**

Much of our knowledge of the biochemical actions of folic acid/
acid and vitamin B12 has been derived from investigations involving the use of experimental animals or of microorganisms.

In 1941 Snell & Mitchell and also Stokstad showed that thymine and purines together would replace folic acid for the growth of *S. faecalis* and *L. casei* in a medium containing amino acids. Thymine (which is 5-methyl uracil) could not be replaced by the pyrimidines uracil or cytosine, but the purine requirements could be supplied by adenine, guanine, hypoxanthine or xanthine. Stokes (1944) who could not demonstrate folic acid in a medium containing thymine in place of folic acid suggested that folic acid functions as a co-enzyme for the enzyme system responsible for the synthesis of thymine or a thymine-like compound which, in turn, is used by bacteria to form nucleic acid. In the presence of thymine, folic acid is not necessary for growth, the synthesis of the former being no longer required when it has been supplied from an extraneous source. Thus

\[
\text{NH}_2 \text{C} \quad \text{Thymine} + \text{Desoxyribose} + \text{Phosphate} \quad \text{Nucleic acid}
\]

\[
\text{Folic acid} \quad \text{Purines}
\]

Other investigators suggested that folic acid is required not only for the synthesis of thymine, but also for the conversion of thymine to thymidine. (Welch & Heinle, 1951).

\[
\text{NH}_2 \text{C} \quad \text{Thymine} + \text{Desoxyribose}
\]

\[
\text{Folic acid} \quad \text{Thymidine} + \text{phosphate} \quad \text{Nucleic acid} + \text{purine}
\]

Rogers & Shive (1948) found that, in the presence of purines, thymine prevented the growth inhibiting effect of x-methyl folic acid.
acid on *L. casei* and agreed with Stokes that folic acid functions in the biosynthesis of thymine and possibly purines.

Citrovorum factor is 5-formyl 5,6,7,8-tetrahydropteroylglutamic acid, which is believed to be the biologically active form of pteroylglutamic acid in the body. (Nichol & Welch, 1950a). It is logical to assume that the citrovorum factor transfers a formyl group to the carbon in position -5 of uracil, possibly to form a 5-hydroxymethyluracil which is subsequently reduced to 5-methyluracil (thymine), but there is no concrete evidence to support the hypothesis (Welch & Heinle, 1951). Moreover it is now considered to be likely that folic acid (or citrovorum factor) is concerned with the transfer of the necessary single carbon units to the 2 and 3 positions in the biological formation of the purine ring. (Jukes, 1953).

In animal experiments it should however be noted that Petering & Delor (1947, 1949) could not substitute thymine or thymine together with adenine or adenine nucleoside (adenosine) for folic acid in promoting growth and a rise in the white cell count in folic acid deficient rats, and that Daniel et al. (1948) could not substitute thymine for folic acid in chick growth experiments.

We have already seen (p. 438) that it is possible to cause a haematological remission in untreated pernicious anaemia by administering thymine by mouth in large doses, and this has been the experience of other workers.

In 1948 Wright et al. in a paper dealing with the ability of thymidine to replace vitamin B12 as a growth factor for certain lactobacilli carried the theories a stage further by suggesting/
suggesting that vitamin B₁₂ may function as a co-enzyme in carrying out reactions concerned with the conversion of thymine to thymidine. This has been elaborated further by Vilter et al. (1950).

These various views may be combined thus:

\[ \text{NH}_2 \text{C sources} \rightarrow \text{Purines and Pyrimidines (particularly thymine or related substances)} \]
\[ \text{Citrovorum factor} \]
\[ \text{Folic acid} \]
\[ \text{Ribose and Desoxyribose} \]
\[ \text{Nucleosides (e.g. thymidine)} \]
\[ \text{Vitamin B₁₂ (? also citrovorum factor)} \]
\[ \text{Nucleotides} \]
\[ \text{Nucleic acid} \]

We have been unable to obtain sufficient thymidine to carry out therapeutic experiments but Hausmann (1949) has reported responses in pernicious anaemia to doses of 1 to 2 G. of the substance.

The necessity for administering such large doses of thymine or thymidine in pernicious anaemia does not invalidate the above theories, since in normal metabolism these substances might be released in small quantities where they are required, whereas when they are given by mouth or by injection they are disseminated throughout the body as a whole. Folic acid and vitamin B₁₂ appear to be concerned in an enzyme system whereas thymine and thymidine are actual building stones in nucleic acid formation.

Reference has been made above to the possible role of folic/
Folic acid in reactions involving single carbon units. It has been shown further that folic acid is also concerned in a similar way in reactions involving the single carbon unit as in the reversible formation of serine from glycine and formate, and in the formation of choline, creatine and histidine. These relationships have been illustrated diagrammatically by Jukes in the following manner:

A very detailed review of animal studies of the biochemical
actions of folic acid has been made by Welch & Nichol (1952).

It is sufficient here to summarise by stating that the published evidence suggests -

1. that the 'biologically active form' of folic acid is citrovorum factor and that the latter functions in reactions through which the formyl group is alternately gained and lost. Broquist, Fehrenbach et al. (1951) have shown that 5,6,7,8-tetrahydropteroylglutamic acid itself has citrovorum factor activity. Although we shall continue to use the term folic acid in relation to its functions in various enzyme systems, it may be that the active substance is citrovorum factor alternately losing and gaining its 5-formyl group. The reduction of pteroylglutamic acid to the 5,6,7,8-tetrahydro form appears to be mediated by ascorbic acid. Synthetic citrovorum factor is about half as active biologically as the natural substance, and this may be because the latter is a racemic mixture of dextro and laevo compounds, only one of which is active. (May, Bardos et al., 1951). Welch & Nichol (1952) suggest further that there may also be metabolic participation of a hypothetical compound, a N⁵-hydroxymethyl derivative of tetrahydropteroylglutamic acid.

2. Folic acid is concerned with the formation of pyrimidines (Snell & Mitchell, 1941; Stokes, 1944; Rogers & Shive, 1948).

3. Folic acid is concerned with the transfer of carbon for the synthesis of the labile methyl groups in the formation of substances such as methionine and choline. (Sakami/)
(Sakami & Welch, 1950; Stekol et al., 1951a,b; du Vigneaud et al., 1951).

4. Folic acid also appears to be concerned in the enzymatic oxidation of choline by homogenates of liver in certain species (Dinning et al., 1949, 1950, 1951; Williams, 1951a,b,c,d). It will be recalled that Muntz (1950) considers that choline oxidase is required for transmethylation reactions from choline, and Williams has shown that folic acid antagonists inhibit choline oxidase activity.

5. Folic acid is concerned with the transfer of methyl groups in the formation of pyrimidine ribosides. (Hammarsten et al., 1950). There is evidence that one function of folic acid (by means of citrovorum factor) in lactic acid organisms is the formation of thymidine, and this reaction is inhibited by 4-aminopteroylglutamic acid. The inhibition is overcome by thymidine. (Shive et al., 1948b; Broquist et al., 1949, 1950; Jukes et al., 1950).

6. Folic acid probably facilitates the incorporation of formate and glycine into the purine ring and is probably concerned with the closure of this ring. (Shive et al., 1947; Skipper et al., 1950; Drysdale et al., 1951).

Sulphonamides act by interfering with the utilisation by bacteria of para-aminobenzoic acid, which itself is an essential constituent of the pteroylglutamic acid molecule. When certain microorganisms are cultivated under the influence of sulphonamides, or are treated under such circumstances/
circumstances with folic acid antagonists, there accumulates an intermediate product 4-amino-5 carbonamidomidazole, and this is a purine precursor. (Shive et al., 1947; Stetten & Fox, 1945; Woolley & Pringle, 1950; Shive, 1953).

7. Folic acid activates processes concerning the formation of non-essential amino acids. (Woods, 1951; Lascelles et al., 1951).

8. Folic acid appears to be concerned in the synthesis of porphyrins.

Haemoglobin is a chromoprotein made up of a combination of haem with the protein globin, and haem is formed by the linkage of ferrous iron to protoporphyrin.

The pyrrole nucleus of protoporphyrin in turn can be made by the body from compounds such as glycine and acetate. It is believed that folic acid is one of the catalysts that assists in this reaction. (Fleut et al., 1950).

**VITAMIN B₁₂.**

The metabolic functions of vitamin B₁₂, too, are complex. It will be remembered that, in addition to cyanocobalamin (vitamin B₁₂), there are the forms hydroxocobalamin (vitamin B₁₂a or B₁₂b) and nitrocobalamin (vitamin B₁₂c). A deficiency of inorganic cobalt in the soil produces a wasting disease in ruminants which can be treated by large doses of vitamin B₁₂, but this is probably replacement therapy. (Smith & Koch, 1951).

In association with proteins of animal origin and stimulating the growth of animals restricted to certain diets are
factors known as the animal protein factors. (Cary et al., 1946). The principal, but not the only animal protein factor is vitamin B₁₂. (Stokstad et al., 1949). Like folic acid, vitamin B₁₂ takes part in the utilisation of proteins and in the formation and transfer of one-carbon intermediates. If anything, less is known about the functions of vitamin B₁₂ than of folic acid.

1. The addition of thyroid hormone to the diet increases the requirement for vitamin B₁₂ by rats, mice and chicks, and measurement of the growth of animals on diets supplemented in this way has been used as an assay method for vitamin B₁₂. (Bosshardt et al., 1941; Betheil & Lardy 1949; Register et al., 1949; Emerson, 1949). The mechanism of this action is not known, and in any event Ershoff (1951) has reported that liver residue contains a factor other than vitamin B₁₂ with similar effects.

2. In pernicious anaemia there is a disorder of tyrosine metabolism leading to an increased urinary excretion of total phenolic compounds. This may be corrected by vitamin B₁₂ therapy. (Swendseid et al., 1947).

3. We have already seen that Wright et al. (1948) have suggested that vitamin B₁₂ may function as a co-enzyme in carrying out reactions concerned with the conversion of thymine to thymidine. Shive et al. (1948) have shown that thymidine can be substituted for vitamin B₁₂ in the promotion of growth of Lactobacillus lactis Dorner when purines are supplied. It may therefore be the case that vitamin B₁₂ is of importance in relation to pyrimidine metabolism.
metabolism in man, in that it acts in the enzyme system concerned with the synthesis of their nucleosides. The role of vitamin $\text{B}_{12}$ in the synthesis of purine nucleosides is uncertain. (Welch & Michol, 1952).

4. Many workers (e.g. Stekol et al., 1951a) have claimed that vitamin $\text{B}_{12}$ is concerned in the transfer of a methyl group to homocysteine to form methionine

\[
\text{HS(CH$_2$)$_2$ CH(NH$_2$)COOH} \rightarrow \text{CH$_3$S(CH$_2$)$_2$ CH(NH$_2$)COOH}
\]

\[
\text{Vitamin B$_{12}$}
\]

The methyl group may be derived from choline, $\left[\text{HO(CH$_2$)$_2$N(CH$_3$)$_3$}\right]\text{OH}$ or betaine, $(\text{CH$_3$})$_3\text{N(OH)CH$_2$ COOH}.$

This complex subject of the transfer of labile methyl groups has been reviewed by Jukes & Stokstad (1951).

5. It has been shown by Stekol et al. (1951a,b) and by Arnstein & Neuberger (1951) that the utilisation of glycine for the synthesis of serine depends upon vitamin $\text{B}_{12}$.

6. It has been suggested by Dubnoff (1950a,b, 1951) that vitamin $\text{B}_{12}$ is involved in reactions that lead to the formation of sulphydryl-containing compounds (containing $-\text{SH}$ groups) from their disulphide precursors (which contain $\text{S-S}$ groups).

7. Vitamin $\text{B}_{12}$ inhibits the development of histological changes following the administration of carbon tetrachloride to rats (Popper et al., 1943), and vitamin $\text{B}_{12}$ has/
has a lipotropic effect in rats (Gyorgy & Rose, 1950). This may be related to the effects of vitamin B12 in the metabolism of choline and methionine.

**ASCORBIC ACID.**

We have already seen (p. 17) that megaloblastic anaemia in infants is related to ascorbic acid deficiency and that although it does not usually respond to the administration of vitamin C it may be prevented by supplementing the diet with ascorbic acid or cured by the administration of pteroylglutamic acid. It would therefore be reasonable to consider the hypothesis that ascorbic acid deficiency diminishes the ability of the tissues to convert pteroylglutamic acid to citrovorum factor. If there is a nutritional megaloblastic anaemia in the infant from folic acid deficiency this will become more severe if there is associated ascorbic acid deficiency. Some of the many papers written by May and his colleagues in relation to megaloblastic anaemia in infancy have been referred to on pp. 16 - 18. The same workers (May et al., 1951; Sundberg et al., 1952) found that megaloblastic anaemia could be more easily produced in monkeys if ascorbic acid deficiency was produced in addition to folic acid deficiency. Ascorbic acid deficiency would not itself produce megaloblastic anaemia. When megaloblastic anaemia did develop, citrovorum factor was more active as a therapeutic agent than was pteroylglutamic acid, and the latter was more active than ascorbic acid which could, however, itself convert the marrow to the normoblastic state.

Nichol/
Nichol & Welch (1950a) have shown that ascorbic acid accelerates the conversion of folic acid to citrovorum factor in liver slices and in humans and laboratory animals. Broquist and his co-workers at Pearl River (1951b) found that when normal humans were given ascorbic acid, it did not increase the urinary output of citrovorum factor when a standard test dose of the latter was given to them. Welch et al. (1951) and Gabuzda et al. (1951) have demonstrated that the urinary output of folic acid following a test dose of pteroylglutamic acid is diminished in persons with scurvy.

**The Interrelationships of Factors That Influence The Megaloblastic Anemias.**

Unfortunately it is not possible to summarise the work that has been done by so many investigators by means of a simple diagram illustrating with any degree of certainty the metabolic interrelationships of vitamin B₁₂, pteroylglutamic acid, citrovorum factor, ascorbic acid and the other substances that may be involved.

In 1946 Bethell and his co-workers at Ann Arbor and the group of workers at Cleveland suggested that vitamin B₁₂ (at that time the liver factor) might activate a conjugase system or play a part in the removal of an inhibitor of such a system so that free pteroylglutamic acid was thus released from a conjugate form in which it occurred in foods (Bethell et al., 1946; Welch et al., 1946). We have already seen in Chapters 7 and 11 that pernicious anaemia patients are able to convert the synthetic folic acid conjugates into pteroylglutamic acid.
pteroylglutamic acid or some substance with similar micro-
biological properties, and that a haematological response
occurs. In the food, however, the chief naturally occurring
conjugate as yet identified is pteroylhexaglutamylglutamic
acid and it is difficult to obtain this free from conjugase
inhibitors, (Bethell et al., 1947; Swendseid, Bird et al.,
1947), so that the experiments of the Ann Arbor and Cleveland
workers have not been repeated. Objections to their theory
based on haematological responses in pernicious anaemia to
other, synthetic, folic acid conjugates are not valid and the
possibility that one function of vitamin B₁₂ is to release
pteroylglutamic acid from its conjugates cannot be excluded.
Moreover, although there is no experimental proof of the theory,
there is nothing to exclude the suggestion of Jukes (1952) that
vitamin B₁₂ plays a part in the formation of citrovorum factor
from folic acid. We know nothing about citrovorum factor con-
jugates.

Vilter & Mueller (1952) have selected certain of the above
findings that were based on animal or microbiological investi-
gations and have used them to build up a theory about the
metabolic functions of vitamin B₁₂ and folic acid, and have
attempted to confirm their views by therapeutic experiments in
man. They state that in pernicious anaemia there was no
haemopoietic response to adenine 5 G., guanine 2.5 G., cytidine
150 mg., uridine 150 mg., adenosine 150 mg. or adenylic acid
50 mg. (It is not stated whether these were daily doses.)
On the other hand, as has already been mentioned, there was a
response to larger doses of thymine or thymidine, and Vilter &
Mueller/
Mueller add that they have had a response to an even larger dose of uracil. This is taken to mean that the major defect in pernicious anaemia resides in the formation of pyrimidines of the thymine type. Against this the same workers have had no response to RNA or DNA in doses of 10 G. daily by mouth. This they consider to be due to these substances being split into many smaller molecules during digestion.

In Fig. 20 we have incorporated the experimental results of various workers in connection with the metabolic functions of the various haemopoietic factors. It must however be stressed that much of the work has been carried out on animals or on microorganisms and that it is by no means always correct to transport such findings directly to problems of metabolism in the human. Some of this, particularly the view that citrovorum factor alternately loses and gains a formyl group, as yet lacks experimental proof.
Fig. 20. A Diagram incorporating Various Views about
the Metabolic Functions of Folic Acid, Citrovorum
Factor, Vitamin B₁₂, and Ascorbic Acid in the Body.

Citrovorum factor
conjugates

Conjugases

Folic acid conjugates

? Vitamin B₁₂

Citrovorum
in stomach

Folic acid

Ascorbic acid

5-formyl-5,6,7,8-tetrahydropteroylglutamic
acid (and possibly other forms)

Vitamin B₁₂

Glycine → Serine →

Methyl,
Formate &
amino groups

Pyrimidines,
eg. thymine

Ribose,
Desoxyribose

Purines

Nucleosides,
eg. thymidine

Vitamin B₁₂

Other simple
substances,
including choline
& methionine

Nucleotides

Nucleic Acid

* Free purines and pyrimidines may not themselves be formed,
the nucleoside or nucleotide resulting when the purine or
pyrimidine ring is formed later. (Greenberg, 1950, 1951).
We have seen that Vilter & Mueller (1952) consider that in pernicious anaemia there is some abnormality in pyrimidine metabolism, particularly affecting thymine. This view arose in the first instance from the bacteriological findings of Snell & Mitchell, 1941, Stokes, 1944 and of Wright, 1948.

There is confusion as to the occurrence of RNA and DNA in the erythroblasts in pernicious anaemia. According to Davidson (1947) and Davidson et al. (1951) the content of both is high until treatment is given. Horrigan et al. (1951) state that in pernicious anaemia the RNA in the red cell precursors is clumped, but that vitamin B₁₂ or folic acid treatment causes it to become normally dispersed through the cytoplasm.

These last workers were unable to show any abnormality of DNA in pernicious anaemia by cytochemical methods. Moreover Reisner & Korson (1951), who thought it likely that the "paler staining properties of the megaloblast nucleus in pernicious anaemia" might be due to a decrease in the amount of DNA, were unable to show any significant deviation from the normal amounts of total or polymerized DNA in the nuclei of the red cell precursors in pernicious anaemia throughout the maturation process.

We have seen (p. 478) that it is now considered that there is a doubling of the DNA in the cell at the end of the pre-prophase of mitosis. It is understandable therefore that the bodily requirements of folic acid and of many other of the substances/
substances that we have considered may be much increased in widespread malignancy. Experiments on folic acid excretion in malignant disease will be described in Chapter 18.

**SUMMARY**

Various investigations dealing with the metabolic functions of folic acid, citrovorum factor, vitamin B12, ascorbic acid and related substances are briefly considered. Since much of this work deals with bacteriological or animal investigations and as it has not been found possible to devise suitable human experiments, any metabolic scheme must be regarded as very hypothetical.
CHAPTER 14.

THE MEGALOBLASTIC ANAEAMIAS OF PREGNANCY.

Attention has already been paid (pp. 18 - 23) to some of the published work dealing with megaloblastic anaemia in pregnancy, and in Chapter 2 it has been seen that nutritional megaloblastic anaemia and megaloblastic anaemia of pregnancy are closely associated in countries where malnutrition is rife. Reference has been made to the claims of Bethell & Blecha (1942) of Michigan that there is a definite relationship between the incidence of macrocytic anaemia in pregnancy and the animal protein content of the diet, and to the view of Kothari & Bhende (1950) that there is no difference between nutritional megaloblastic anaemia as seen in India and the megaloblastic anaemia of pregnancy that occurs in temperate climates. We have no hesitation in saying that we have seen megaloblastic anaemia occurring temporarily during pregnancy in well nourished patients who had taken a normal diet before and during the pregnancy and that we do not agree with this view of the Indian workers.

The purpose of the present chapter is to give examples of various forms of megaloblastic anaemia that we have seen in pregnancy and to see to what extent our biochemical investigations may help in classifying the cases.

Amongst the first specimens tested were sera and urines kindly supplied by Dr. C. C. Ungley from two patients with megaloblastic/
megaloblastic anaemia of pregnancy who had red cell counts of less than 2,500,000 per c.mm. and who had been given 15 mg. of pteroylglutamic acid intravenously for other research purposes. We have not carried out an intensive study of the urinary output of folic acid in normal persons given 15 mg. of pteroylglutamic acid intravenously, but in four normal controls the outputs were respectively 7.5 mg., 7.3 mg., 6.5 mg. and 5.1 mg. In the two cases of megaloblastic anaemia of pregnancy referred to above the outputs were 1.58 mg. and 1.75 mg. There was no increase above the resting urinary folic acid level in the following two days in each case. It was considered that these findings indicated folic acid depletion, but subsequently we have had cases in which the amount excreted after a test dose was normal.

In Table 56 there is given a summary of the findings in seventeen cases of anaemia in pregnancy that we have encountered since introducing the folic acid excretion test. Some of these cases were not diagnosed until the puerperium. In the earlier cases measurement was made of the excretion after the injection of 5 mg. of pteroylglutamic acid. In the later cases, an oral test dose was also given. Recently it has been possible to include in the investigations a measurement of the serum vitamin B12 level, carried out by the L. leichmannii method.

(Note: In the column dealing with the bone marrow in Table 56, M = megaloblastic, N = normoblastic and I indicates that intermediate erythroblasts were present.)
## Table 56. Biochemical Findings in Patients with Anaemia in Pregnancy.
<table>
<thead>
<tr>
<th>Case</th>
<th>Age (yrs)</th>
<th>Stage of pregnancy or puerperium</th>
<th>Gastric juice Free HCl.</th>
<th>Haematological state before test</th>
<th>Antimegaloblastic substances before folic acid test</th>
<th>Folic acid test</th>
<th>Serum Vit.B12 level µg. per ml.</th>
<th>Response to FGA therapy</th>
<th>Diet, etc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>33</td>
<td>37th week of 2nd pregnancy; also 2 miscarriages</td>
<td>Yes</td>
<td>4.2 1.02 M</td>
<td>None</td>
<td>5 SC 2.66</td>
<td>160</td>
<td>Yes Diet satisfactory</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>27</td>
<td>11 days after birth of second child; also 1 miscarriage</td>
<td>No</td>
<td>4.3 1.48 M</td>
<td>FGA 15mg. Oral</td>
<td>5 SC 0.47 3.9</td>
<td>220</td>
<td>Yes Diet satisfactory</td>
<td>Vomiting throughout</td>
</tr>
<tr>
<td>3</td>
<td>35</td>
<td>37th week of 10th pregnancy</td>
<td>Yes</td>
<td>5.9 1.50 M</td>
<td>None</td>
<td>5 SC 1.69 6.5</td>
<td>254</td>
<td>Yes Diet poor</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>25</td>
<td>3 months after 4th pregnancy</td>
<td>No</td>
<td>5.3 1.64 M</td>
<td>None</td>
<td>15 IV 3.84 -</td>
<td>Yes Diet poor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>35</td>
<td>37th week of 2nd pregnancy</td>
<td>Yes</td>
<td>5.5 1.95 M</td>
<td>None</td>
<td>5 SC 1.33 -</td>
<td>Yes Diet poor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>36</td>
<td>11th week of 3rd pregnancy; also 2 miscarriages</td>
<td>Yes</td>
<td>4.9 1.96 M</td>
<td>None</td>
<td>5 SC 2.59 -</td>
<td>Yes Diet satisfactory</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>33</td>
<td>38th week of 10th pregnancy; also 2 miscarriages</td>
<td>Yes</td>
<td>8.2 2.12 M</td>
<td>None</td>
<td>5 SC 2.61 -</td>
<td>172</td>
<td>Yes Diet poor</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>30</td>
<td>25th week of 2nd pregnancy</td>
<td>Yes</td>
<td>9.9 2.52 M</td>
<td>None</td>
<td>5 SC 3.06 -</td>
<td>258</td>
<td>Yes Morning sickness till 22nd week</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>25</td>
<td>4 days after spontaneous delivery, 5th pregnancy</td>
<td>Yes</td>
<td>4.6 2.6 M</td>
<td>FGA 20mg. Oral</td>
<td>5 SC 3.22 42.1</td>
<td>Yes Diet satisfactory</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>29</td>
<td>14 days after evacuation of uterus incomplete abortion in the 7th pregnancy</td>
<td>Yes</td>
<td>6.0 2.87 I</td>
<td>None</td>
<td>5 SC 2.38 2.0</td>
<td>Yes Diet poor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>42</td>
<td>31st week of 10th pregnancy</td>
<td>No</td>
<td>9.5 3.23 I</td>
<td>None</td>
<td>5 SC 1.27 -</td>
<td>Not given Diet poor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>32</td>
<td>36th week of 4th pregnancy</td>
<td>No</td>
<td>7.4 3.50 N</td>
<td>None</td>
<td>5 SC 3.30 5.9</td>
<td>Yes Diet satisfactory</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>37</td>
<td>Twin pregnancy 34th week of 7th pregnancy</td>
<td>Yes</td>
<td>8.6 3.81 M</td>
<td>FGA 20mg. Oral</td>
<td>5 SC 3.26 50.7</td>
<td>Yes Diet satisfactory</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>27</td>
<td>28th week of 1st pregnancy; 2 previous miscarriages</td>
<td>No</td>
<td>11.8 3.85 M</td>
<td>None</td>
<td>5 SC 0.25 15.3</td>
<td>Yes Diet normal; vomiting throughout pregnancy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>22</td>
<td>38th week of 1st pregnancy; 1 miscarriage</td>
<td>Yes</td>
<td>7.4 4.18 I</td>
<td>None</td>
<td>5 SC 1.62 2.5</td>
<td>Yes Appetite poor throughout pregnancy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>25</td>
<td>6 weeks after delivery of twins</td>
<td>Yes</td>
<td>11.0 4.13 N</td>
<td>None</td>
<td>5 SC 1.50 -</td>
<td>Not tried Diet satisfactory</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>31</td>
<td>22nd week of 2nd pregnancy</td>
<td>Yes</td>
<td>9.2 4.47 I</td>
<td>None</td>
<td>5 SC 2.65 5.1</td>
<td>Polytherapy given</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Most of the cases included in Table 56 were in various maternity hospitals in Edinburgh and district and it was found impracticable to do more than the minimum of investigative procedures.

In carrying out urinary folic acid excretion studies on several hundred persons after the subcutaneous administration of 5 mg. of pteroylglutamic acid, we have never found the output to be less than 1.7 mg. except in those suffering from intestinal malabsorption, pernicious anaemia, malignancy, chronic renal disease, very prolonged infections or wasting diseases, or in the presence of a large effusion. It will be seen that of the eleven patients in Table 56 who had frankly megaloblastic bone marrows, there was evidence of folic acid depletion by this test in only five. Two patients with intermediate erythroblasts in the marrow (Cases 11 & 15) and one with a normoblastic marrow (Case 16) had a low output after the test dose.

Where it was possible to carry out the differential urinary folic acid excretion test there was no definite evidence of malabsorption in any. In Case 10 the difference between the excretion after the injected dose and that after the oral dose was not sufficient for a diagnosis of intestinal malabsorption to be made.

It is obvious that patients with megaloblastic anaemia of pregnancy subsequently responding to pteroylglutamic acid therapy frequently do not have biochemical evidence by this test of folic acid depletion. We have already seen (pp. 362 and/
and 364) that such was the case in two patients with intestinal malabsorption, and the suggestion has been made that this may indicate that either the true deficiency is not of pteroylglutamic acid itself but of something that can be formed from it or replaced by it (hence the therapeutic effect of pteroylglutamic acid), or alternatively that the tissues that are deficient in folic acid do not have time to conserve the administered pteroylglutamic acid because of its rapid excretion by the kidneys. In the two patients with megaloblastic anaemia of pregnancy whose sera were sent to us by Dr. Ungley there was an appreciable rise in the serum level of folic acid for seven hours after the injection of pteroylglutamic acid.

It seems more likely, however, that there are various types of megaloblastic anaemia of pregnancy and that interference with folic acid metabolism rather than true folic acid deficiency may be the cause in some cases and may act as a contributory factor in others. If there is any truth in this hypothesis then it is possible that some antagonist or inhibitor may arise during pregnancy and in thinking of a possible source of this one naturally turns to the sex hormones. It is probable that in some cases true folic acid deficiency occurs because of faulty diet together with the increased demands of pregnancy, and this can obviously be made worse by vomiting during pregnancy.

No doubt in some instances, particularly in the tropics, there is a predominant deficiency of vitamin B12 rather than of folic acid in the diet.

Holly/
Holly (1951) has claimed therapeutic success in megaloblastic anaemia of pregnancy from combined treatment with cyanocobalamin and high dosage of ascorbic acid, but we have seen no reports to confirm this. It was not possible to obtain detailed information about the diets of many of the cases in Table 56; it will be seen however that the patients with biochemical evidence of folic acid depletion had had poor diet, multiple pregnancies, a twin pregnancy or vomiting or diarrhoea during pregnancy. On the other hand some patients had had such difficulties without developing biochemical evidence of folic acid deficiency.

In the five patients in whom it was measured the vitamin B12 level was normal.

Cell survival studies involving transfused blood were not carried out on these cases, but Thompson & Ungley (1951) have shown that excessive destruction of donated blood is a feature of some cases of megaloblastic anaemia of pregnancy.

In the following pages a brief account is given of various cases to illustrate the types of megaloblastic anaemia of pregnancy that we have encountered during the years of preparation of this thesis, and their response to various therapeutic agents.

Case 100. (Graph 41.)

Spontaneous remission.

Prior to the introduction of proteolysed liver and later of pteroylglutamic acid to our therapeutic armamentarium, we saw a few sad cases of death from megaloblastic anaemia of pregnancy.
pregnancy. Case 100 is the only patient that we saw with an apparently spontaneous remission.

This was a 42 year old patient who was seen nine days after the spontaneous delivery of her fourth child. She had symptoms of anaemia in pregnancy but no investigations were done. Vomiting had not been a feature of pregnancy, but little meat or vegetables had been taken. There was no history of anaemia in previous pregnancies or of any previous illness of importance.

When the patient was first seen at the Blood Clinic, Edinburgh Royal Infirmary, her haemoglobin level was 7.4 G. per 100 ml., red cells 3,350,000 per c.mm., C.I. 0.75, FCV 27%, MCV 80.6 cµ., MCHC 27.4%, white cells 10,600 per c.mm. The peripheral blood presented a dimorphic picture. Numerous nucleated red cells were seen in the peripheral blood. The marrow was megaloblastic but some of the cells showed condensation of the nuclear chromatin. No treatment was given but haematological improvement occurred and the patient ceased to report.
CASE 100. MEGALOBLASTIC ANAEMIA OF PREGNANCY — SPONTANEOUS REMISSION.

CASE 101. MEGALOBLASTIC ANAEMIA OF PREGNANCY RESPONDING TO PROTEOLYSED LIVER.
Case 101. (Graph 42.)

Response to Proteolysed Liver.

During the ten years of preparation of this thesis the author has never seen a case of megaloblastic anaemia of pregnancy that responded to injections of cyanocobalamin or liver extract. Cases responding to liver injections have been reported from Edinburgh (Davidson et al., 1942) but it is not intended that this thesis should include patients that the present author has not seen personally. There is no doubt that proteolysed liver given by mouth is effective in the treatment of megaloblastic anaemia of pregnancy, as exemplified by the following case.

A 41 year old doctor's wife was found to be anaemic in the 30th week of her third pregnancy. The diet had been normal and neither vomiting nor diarrhoea had been troublesome. There was no previous illness of importance. The haemoglobin level was 7.7 G. per 100 ml., red cells 2,760,000 per c.mm., C.I.O. 94, PCV 29%, Hb 105.1 gr., MCH 26.6%, white cells 6,200 per c.mm., and the marrow was megaloblastic. There was no improvement with two injections of Campolon, but the response to proteolysed liver given by mouth was entirely satisfactory, the red cell count rising to 5,270,000 per c.mm. at the time of delivery.

No further treatment was given, and three and a half years later the blood counts were found to be normal.

Case 102. (Graph 43.)

Response/
Case 102. (Graph 43.)

**Response to Pteroylglutamic Acid.**

A 35 year old patient who developed diarrhoea in the 32nd week of her third pregnancy. The diet had been poor because of nausea. There was no previous history of importance. Before delivery it was found that the blood levels were—haemoglobin 5.5 G. per 100 ml., red cells 1,950,000 per c.mm., C.I. 0.94, PCV 13%, MCV 86.6 cµ., MCHC 27.4%. The marrow was megaloblastic.

Spontaneous delivery occurred prematurely, and although bleeding was not heavy transfusion was required. Cyanocobalamin was given, but the marrow remained megaloblastic. A 5 mg. test dose of pteroylglutamic acid was given by injection and of this 1.33 mg. were excreted in the urine, suggesting folic acid depletion. A test meal revealed free hydrochloric acid to be present in the gastric juice. Therapy with pteroylglutamic acid was continued. The patient at this stage became severely ill with a urinary infection, unexplained hepatomegaly and high fever, possibly due to septicaemia. Fortunately the infection responded to antibiotics and the haematological response was entirely satisfactory. Three months after delivery all haematinics were withdrawn and a year later there was no evidence of relapse.
CASE 102. MEGALOBLASTIC ANAEMIA OF PREGNANCY RESPONDING TO PTEROYLGLUTAMIC ACID.

CHLORAMPHENICOL 0-25 G. 4-HOURLY.
PENCILLIN 500000 UNITS 4-HOURLY.
FERROUS SULPHATE 10 ml.
PTEROYLGLUTAMIC ACID 20 mg. DAILY ORALLY.

CASE 103. MEGALOBLASTIC ANAEMIA OF PREGNANCY RESPONDING TO CITROVORUM FACTOR.

PTEROYLGLUTAMIC ACID 10 mg. DAILY ORALLY.
Case 103. (Graph 44.)

Response to citrovorum factor given by injection.

The patient who was aged 36, had had symptoms of anaemia during pregnancy and was first seen by us two weeks after she had given birth to a healthy male child, without much loss of blood. Her previous medical history revealed no features of note. There had been four earlier pregnancies, but there was no history of megaloblastic anaemia before or after the previous births. There was no history of diarrhoea or steatorrhoea. The patient normally partook of a good diet, except that she never ate fish. During pregnancy, however, she had much sickness even in the later months and did not eat meat or vegetables. When we first saw her, she was pale but had no oedema. A test meal showed free hydrochloric acid to be present in the stomach; the bone marrow was megaloblastic, and examination of the blood showed haemoglobin 5.03 G. per 100 ml., red cells 2,050,000 per c.mm., C.I. 0.83, FGV 16.1%, MCV 78.5 cµ., MOHO 31.2%, white cells 5,000 per c.mm.

Graph 44 shows that there was a moderate reticulocyte response to intramuscular cyanocobalamin 80 µg, but no change in the marrow picture. There were fewer red cells on the twelfth day than when treatment started. There was an excellent response to citrovorum factor given by intramuscular injection, the red cells increasing from 1,560,000 to 4,060,000 per c.mm. in 27 days. Thereafter pteroylglutamic acid therapy was given.

Case 104. (Graph 45.)

Response/
CASE 104. MEGALOBLASTIC ANAEMIA OF PREGNANCY RESPONDING TO CITROVORUM FACTOR GIVEN BY MOUTH.

CITROVORUM FACTOR 12 mg.
BY MOUTH

MEGALOBLASTIC MARROW

NORMOBLASTIC MARROW

DELIVERY

DAYS.

GRAPH 45.

CASE 105. MEGALOBLASTIC ANAEMIA OF PREGNANCY WITH BIOCHEMICAL EVIDENCE OF DEFICIENCY OF FOLIC ACID.

PTEROYGLUTAMIC ACID 5 mg. t.d. ORALLY
FERROUS SULPHATE gr. VI t.d.
FERROUS SULPHATE gr. IV t.d.

PGA R.B. TOLIC ACID EXCRETION AFTER 5 mg. TEST DOSE
= 0.47 mg.

MEGALOBLASTIC MARROW

DAYS.

GRAPH 46.
Case 105. (Graph 46.)

Response to pteroylglutamic acid in a patient with biochemical evidence suggesting folic acid deficiency.

This was a 27 year old patient who was found to be anaemic eleven days after the spontaneous birth of her second child. There was no undue blood loss at delivery. No previous serious illnesses had occurred although the patient had had a miscarriage in the second month of pregnancy five years previously. Normal delivery of a healthy child had occurred the following year. There was no previous history of anaemia or of diarrhoea. The diet was usually satisfactory but the patient had vomited two or three times a day throughout pregnancy.

At the time of admission the blood levels were haemoglobin 4.3 G. per 100 ml., red cells 1,480,000 per c.mm., C.I. 1.0, PCV 13%, MCV 87.8 c.µ., MCHC 33.1%, white cells 4,800, reticulocytes < 1%. The peripheral blood film was similar to what is found in pernicious anaemia and a megaloblast was seen in the peripheral blood. The marrow was frankly megaloblastic, and there was no hydrochloric acid in the gastric juice after histamine. The serum vitamin B₁₂ level, however, was 240 µg./ml. The amount of folic acid excreted after a 5 mg. test dose was 0.47 mg., suggesting folic acid depletion. The patient responded well to pteroylglutamic acid treatment by mouth and after 14 days a differential urinary folic acid excretion test showed the excretion after an injected 5 mg. test dose to be 2.04 mg., and after a 5 mg. oral test dose to be/
be 2.34 mg. There was thus no evidence of intestinal mal-absorption, and a fat balance test showed 97.2% absorption of fat.

Three months after all treatment was discontinued, the patient felt very well and the haemoglobin level was 13.8 G. per 100 ml. and the red cell count 4,650,000 per c.mm.

It seems likely that this patient had a tendency to megaloblastic anaemia in pregnancy and that this was aggravated by vomiting which led to folic acid depletion.

Case 106. (Graph 47.)

Response to pteroylglutamic acid in a patient without biochemical evidence of folic acid deficiency.

A 33 year old patient who had already had nine pregnancies and two miscarriages. The family lived in an abandoned Army hut and although the patient claimed that her diet was satisfactory except that she did not like meat, it is likely that her food intake was below standard. There were no previous illnesses of note and no history of anaemia in pregnancy, of diarrhoea, or of vomiting. The general condition was good, the patient being obviously anaemic but not wasted; indeed she was rather plump. At the time of admission in the 38th week of pregnancy the haemoglobin level was 8.2 G. per 100 ml., red cells 2,120,000 per c.mm., C.I. 1.3, PCV 23%, MCV 108 cu., MCHG 35%, reticulocytes < 1%. There was free hydrochloric acid in the gastric juice. The serum vitamin B₁₂ level was 172 μg. per ml., and when a differential folic acid excretion test was performed, the excretion after the 5 mg. subcutaneous dose/
dose was 2.40 mg., and after the 5 mg. oral dose it was 2.61 mg. There was thus no evidence of folic acid depletion. Nevertheless the anaemia responded to treatment with pteroyl-glutamic acid.

**CASE 106. MEGALOBLASTIC ANAEMIA OF PREGNANCY WITHOUT BIOCHEMICAL EVIDENCE OF DEFICIENCY OF FOLIC ACID.**

PGA IBM  20 mg. DAILY ORALLY

SERUM VIT.B12 LEVEL +750 μg/ml.

MEGALOBLASTIC MARROW

DELIVERY

**CASE 107. IRON DEFICIENCY ANAEMIA IN PREGNANCY REQUIRING PTEROYGLUTAMIC ACID THERAPY.**

FERROUS SULPHATE gr. m. t. d. g.

PTEROYGLUTAMIC ACID 5 mg. b.i.d. BY MOUTH

GRAPH 47.

GRAPH 48.
Case 107. (Graph 48.)

A patient with iron deficiency anaemia in pregnancy who did not respond haematologically until pteroylglutamic acid was given.

A 22 year old patient who was first seen at the Blood Clinic, Edinburgh Royal Infirmary, ten weeks before the expected date of birth of her first child. A previous pregnancy had ended in a miscarriage. There was no previous history of note, except that she had been having ferrous sulphate gr. 3, t.i.d. for four months and yet, according to the blood counts done at the Simpson Memorial Pavilion of Edinburgh Royal Infirmary, the haemoglobin level had fallen in that period from 7.1 G. per 100 ml. to 6.8 G. per 100 ml. It was now found at the Blood Clinic that the haemoglobin level was 7.8 G. per 100 ml. and the red cell count 4,830,000 per c.mm., giving a colour index of 0.55. The film was that of iron deficiency anaemia.

It was recommended that a preparation of saccharated oxide of iron should be given intravenously, but neither the patient’s general practitioner nor the staff of the Ante-Natal Clinic were willing to administer it, so ferrous sulphate was continued by mouth for a month without benefit. Thereafter 1.5 G. of saccharated iron oxide were given intravenously at the Blood Clinic over a period of eleven days. No haematological or general improvement occurred, the blood levels thereafter being haemoglobin 7.4 G. per 100 ml., red cells 4,180,000 per c.mm., C.I. 0.59. The peripheral blood film now showed the presence of macrocytes in addition to the hypochromic cells and there was a shift to the right in the Arneth count./*
The patient was at once admitted to hospital and the marrow found to show transitional erythroblasts. The gastric contents contained free hydrochloric acid after histamine had been injected. A differential folic acid excretion test showed an excretion of 1.62 mg. after the injected 5 mg. dose and 2.63 mg. after the oral dose. This suggested folic acid depletion without malabsorption.

There was a very satisfactory haematological response to pteroylglutamic acid therapy, and it seems possible that it was the lack of folic acid in the body that prevented a response to treatment with iron even when it was given intravenously.

Case 108. (Graph 42.)

A patient with iron deficiency anaemia in pregnancy who did not appear to respond to ferrous sulphate until pteroylglutamic acid was given.

This 30 year old patient was first seen at the Blood Clinic, Edinburgh Royal Infirmary, in the 18th week of her second pregnancy. The first had ended in a stillbirth. There was no previous history of note and the patient was in good circumstances, and partook of a normal diet. The haemoglobin level was 6.8 G. per 100 ml., red cells 4,010,000 per c.mm., C.I. 0.57. The film was typical of iron deficiency anaemia. The treatment advised was ferrous sulphate gr. 6, t.i.d. and ascorbic acid 75 mg. daily. It was established with reasonable certainty that the treatment was taken regularly. No improvement occurred in the course of a month, and accordingly
this treatment was supplemented with pteroylglutamic acid 15 mg. daily by mouth. There was thereafter rapid improvement in general wellbeing and a satisfactory rise in the haemoglobin and red cell levels.

At the time of writing the expected date of delivery has not been reached.

This patient appears to be similar to the previous one.

CASE 108. ANAEMIA IN PREGNANCY WHICH DID NOT APPEAR TO RESPOND TO IRON UNTIL PTEROYLGLUTAMIC ACID WAS GIVEN.

| FERROUS SULPHATE gr. v t.i.d. + ASCORBIC ACID 75 mg. DAILY |
| PTEROYLGLUTAMIC ACID 15 mg. DAILY ORALLY |

GRAPH 49.
Case 109. (Graph 50.)

Pernicious anaemia complicated by pregnancy.

This patient, an unmarried woman aged 38, was in the 6th month of pregnancy when she developed severe anaemia which failed to respond to treatment with iron and various preparations of liver.

In 1940, at the age of 30, she had been admitted to the Royal Infirmary of Edinburgh suffering from severe anaemia. The following information was obtained from her case records. Before admission she had been getting increasingly pale, easily tired, and breathless, and vomiting and diarrhoea had occurred.
There was oedema of the ankles, the skin had a lemon-yellow colour, and the conjunctival mucous membrane was very pale. Apart from atrophic glossitis, anaemia, and achlorhydria, no abnormality was noted in any system. Her blood count on April 15th 1940 was: red cells 1,720,000 per c.mm., haemoglobin 6.7 G. per 100 ml., C.I. 1.3. Intensive treatment with parenteral liver extract was given. She received an intramuscular injection of 5 ml. of "pernaemon" daily. As the response was poor, "campolon", one ampoule daily, was substituted. The response continued to be poor, and despite this intensive treatment the gain in red cells and haemoglobin over a period of two and a half months was only 1,500,000 red cells and 3.7 G. per 100 ml. (25%) haemoglobin. After discharge from hospital the patient continued to have injections of liver extract from her doctor, at first daily and then weekly for the next two years. In 1942 she had two severe reactions following the injection of liver extract. When anahaemin was substituted for the previous liver extract no reactions followed. The doctor states, however, that the patient attended for treatment irregularly and at long intervals during the next five years. Throughout this period she was pale, breathless, and easily tired, but continued working most of the time. In July 1947, she reported to her doctor because she was pregnant, and was admitted to the Royal Infirmary on December 22nd, 1947 because of severe anaemia.

The patient was extremely pale but showed no obvious loss of weight. She stated that her appetite was poor and that she frequently suffered from flatulent dyspepsia and had occasional attacks of diarrhoea. A dietary history indicated that her intake/
intake of animal protein and vitamin C in particular was very unsatisfactory. Glossitis and koilonychia were absent. There was no enlargement of liver, spleen, or lymph nodes. A test meal revealed histamine fast achlorhydria. During her stay in hospital she had no diarrhoea and a fat balance test gave no evidence of malabsorption of fat. In view of her irregular and insufficient treatment with liver extract, particular attention was paid to examination of the nervous system. Although she stated that she had never had any paraesthesiae or ataxia, indubitable signs of involvement of the spinal cord were found—namely, grossly exaggerated reflexes in both lower limbs and a bilateral extensor plantar response. Apart from a mild degree of incoordination no evidence was obtained of involvement of the peripheral nerves or posterior columns of the cord.

Her blood count was: red cells 1,200,000 per c.mm., haemoglobin 4.1 G. per 100 ml., white cells 3,600; the MCV on two occasions was 93.9 cµ. and 92.7 cµ.; the blood picture was typical of pernicious anaemia, and her bone marrow was megaloblastic. In view of her grave clinical state she was given a transfusion of blood. During the next 14 days she received 3 gr. of ferrous sulphate three times a day, and 32 ml. of anaehaemin, 6 ml. of "plexan" and $\frac{3}{2}$ oz. of proteolysed liver ("hepamino"). As no clinical or haematological improvement resulted a further transfusion of 4 pints of blood was given, and this raised her red cell and haemoglobin levels to 2,980,000 per c.mm. and 10.1 G. per 100 ml. respectively. A second sternal puncture indicated that the marrow was still megaloblastic.
megaloblastic. After a control period of six days, during which no therapy was given, the red cells had fallen to 2,480,000 per c.mm., and the haemoglobin to 8.6 G. per 100 ml. The reticulocyte count never exceeded 1%.

Accordingly, treatment with folic acid was started, a single injection of 60 mg. being given intramuscularly. A third sternal puncture carried out 36 hours later showed transformation of the marrow to the normoblastic state. This was followed by a rise in the reticulocyte count, the peak of 15.6% occurring on the eighth day, and a dramatic improvement in the patient's clinical condition. A gain of 600,000 red cells and 1.8 G. (12%) haemoglobin occurred in ten days. On the fourteenth day after the first injection of folic acid labour was induced. A second injection of 60 mg. of folic acid was given next day, and a third injection of the same quantity seven days later. Thereafter she received 10 mg. daily by mouth, and on the 59th day after the initial injection of folic acid her red cells amounted to 4,630,000 and haemoglobin to 13.3 G. per 100 ml.

It is of interest to note that the patient's father had suffered from pernicious anaemia for nine years.

This case was taken to be one of pernicious anaemia complicated by pregnancy and certainly the neurological findings support this view. It is however surprising that the response to liver injections was so poor in 1940 since the liver extracts on the market then were usually satisfactory. It has not been possible to trace the patient for further investigation.
The normal result of the fat balance test which was carried out over a period of only three days was not sufficient to exclude intestinal malabsorption. To-day it would be easy to investigate this case more satisfactorily by means of a serum vitamin B12 estimation and a differential urinary folic acid excretion test.

Case 110. (Graphs 51a & b.)

**Congenital haemolytic anaemia complicated by megaloblastic anaemia of pregnancy.**

This 21 year old patient was admitted to hospital on 3.11.49. on account of anaemia in the fifth month of pregnancy. The story was that she had had recurrent attacks of jaundice since the age of four years and that liver injections (Examem) had been given since the age of nine years in a dosage of 1 ml. weekly up to the time of admission. There was no family history of anaemia. The patient was in good circumstances and had had no diarrhoea or any serious illness. A blood film showed the presence of microspherocytes, and red cell fragility was increased, haemolysis commencing in 0.65% saline. The haemoglobin level was 11.1 G. per 100 ml., red cells 2,910,000 per c.mm., C.I. 1.2%, PCV 28%, MCV 96.2 cu., MCCH 39.6%, white cells 11,200, reticulocytes 7.8%. The marrow was hyperplastic and normoblastic. The urine contained an excess of urobilinogen, there was no biochemical evidence of liver disease, and the Coombs' test was negative. No abnormal agglutinins were detected. A diagnosis of congenital haemolytic anaemia was made, and the patient sent home. However her doctor continued to/
to give liver injections (Examen 1½ ml. weekly).

The intention was to readmit the patient when labour was due, but on December 1st 1949 she came back to hospital seriously ill, the blood levels being haemoglobin 3.1 G. per 100 ml., red cells 1,180,000 per c.mm., PCV 10%, MCV 84.7 µ., MCHC 31%, white cells 6,000 per c.mm., reticulocytes 5%. It was thought that a haemolytic crisis had occurred but a sternal puncture showed that the marrow was frankly megaloblastic. The patient was transfused and treated with pteroylglutamic acid by mouth as shown in Graph 51a. The response was very good and the patient was discharged on 30th December 1949.

Her doctor continued to give pteroylglutamic acid and at the time of readmission on 13th February 1950, the blood levels were haemoglobin 12.3 G. per 100 ml., red cells 3,880,000 per c.mm., C.I. 1.07, PCV 31.5%, MCV 81.0 µ., MCHC 39.0%, white cells 10,600 per c.mm., reticulocytes 9.4%. A healthy baby was born without any complications on 8th April 1950.

Thereafter no treatment was given and it was arranged that splenectomy should be carried out at a later date.

The patient continued to suffer from haemolytic anaemia, and on December 6th of that year she was readmitted to hospital in order that ACTH therapy might be tried.

The patient felt well, but had slight clinical icterus. The spleen was enlarged 4½ ins. below the costal margin and the urine contained much urobilinogen. The marrow was hyperplastic and normoblastic. The blood figures were: haemoglobin 11.8 G. per 100 ml., red cells 3,810,000 per c.mm., PCV 29.5%, MCV/
MCV 77 cµ., white cells 8,200 per c.mm., reticulocytes 5.8%. Microspherocytes were present, and haemolysis began in 0.6% saline. Free acid was present in the gastric juice, and there was X-ray evidence of the presence of a gallstone. The blood was Rh-positive, and, as on previous admissions, the direct Coombs' test was negative. There was no evidence of "cold" or other abnormal agglutinins. The plasma proteins were normal and the Kahn test was negative.

It was decided to treat the patient with ACTH, and 25 mg. was given six-hourly for six days, to a total dosage of 600 mg. The various findings before and after treatment are given in Graph 51b.

The spleen became 2 ins. smaller at its tip within 24 hours of the beginning of treatment, and at the end of therapy it had become in all 3½ ins. smaller.

The effect of ACTH on the blood picture may be summarised as follows. There was no change in the haemoglobin or erythrocyte level. There was a fall in the eosinophil count from 175 per c.mm. to 31 per c.mm. and a rise in the total white cell count from 12,400 to 16,800 per c.mm. The persistent reticulocytosis was not reduced nor was the osmotic fragility of the erythrocytes decreased, but there appeared to be a significant reduction of the serum bilirubin level from an average of 3.4 to 2.5 mg. per 100 ml. Within a few days of the cessation of treatment all figures had returned to the pre-treatment level. Accordingly, six weeks later splenectomy was successfully undertaken by Sir James Learmonth, and within a few days there was a fall in the reticulocyte count and serum bilirubin level.
The patient lived at a considerable distance from Edinburgh and so it was not possible to do further blood counts. A follow up letter was sent to the patient two years later but was returned marked 'addressee unknown'.

Comment. This patient had been receiving injections of liver extract for many years before megaloblastic anaemia developed so it is unlikely that vitamin B12 deficiency played a part. The likelihood is that megaloblastic anaemia of pregnancy due to deficiency of folic acid or interference with folic acid metabolism was superadded upon congenital haemolytic anaemia. A possible association between haemolytic processes and megaloblastic anaemia has already been discussed on page 121.
CASE 110. HAEMOLYTIC ANAEMIA COMPLICATED BY MEGALOBLASTIC ANAEMIA OF PREGNANCY.

Liver Extract

PTEROYLGLUTAMIC ACID ORALLY.

RETICS / 6 Hb. Gm./100 c.c. R.B.C. M.L.S./c.mm. P.C.V. %

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DELIVERY

GRAPH 51A.

CASE 110. HAEMOLYTIC ANAEMIA THAT HAD BEEN MEGALOBLASTIC DURING PREGNANCY.

ACTH

SPLENECTOMY

RETICS %

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FEB. MARCH.

GRAPH 51B.
Case 111. (Graphs 15 & 16.)

Megaloblastic anaemia of intestinal malabsorption complicated by pregnancy.

An example of this has been considered on p. 343. The relevant graphs are reproduced below.
THERAPEUTIC CONSIDERATIONS

The evidence presented here suggests that in temperate climates megaloblastic anaemia of pregnancy may be due to folic acid deficiency or to interference with folic acid metabolism or both. Very rarely the condition present may be pernicious anaemia or intestinal malabsorption complicated by megaloblastic anaemia of pregnancy. There are grounds for suspecting that some cases of apparent iron deficiency in pregnancy will be refractory to treatment unless pteroylglutamic acid is given. It is, of course, a well known fact that in many instances megaloblastic anaemia of pregnancy may occur with a low colour index, mean corpuscular volume and mean corpuscular haemoglobin concentration.

The present author is much opposed to blunderbuss therapy in the treatment of the anaemias, but feels that more benefit than harm would come from treating all patients with anaemia in pregnancy during the pregnancy with a combination of iron and ascorbic acid together with pteroylglutamic acid in a dosage of 5 mg. daily. There is the theoretical danger that an occasional case of true pernicious anaemia might be missed but such cases are rarely encountered in this age group. If there is any reason for suspecting pernicious anaemia, it is now possible to measure the serum vitamin B12 level. Possibly all pregnant women (excluding any suffering from untreated true pernicious anaemia) would benefit from the administration of the above combination of therapeutic agents.

SUMMARY.

In/
SUMMARY.

In this chapter an attempt is made to show that the term megaloblastic anaemia of pregnancy is used to include a variety of conditions. There is nutritional megaloblastic anaemia of pregnancy which may be due to deficiency of vitamin B₁₂, folic acid, or both: the type seen in Britain is likely to be due frequently to some unknown agent interfering with folic acid metabolism. There is no evidence of tissue depletion of folic acid in the latter cases and absorption of pteroylglutamic acid is normal as is the serum vitamin B₁₂ level. Megaloblastic anaemia of pregnancy may complicate pernicious anaemia, intestinal malabsorption or haemolytic anaemia.

Various therapeutic agents are considered and it is suggested that iron deficiency anaemia in pregnancy may occasionally become refractory to treatment because of lack of available folic acid.
MEGALOBLASTIC ANAEMIAS ASSOCIATED WITH OPERATIONS,
SHORT CIRCUITS AND OTHER STRUCTURAL CHANGES
IN THE ALIMENTARY TRACT.

Brief reference has been made in pages 25 to 30 to the
literature dealing with the megaloblastic anaemias that occur
after operations on the alimentary tract or in association with
'blind loops' and other abnormalities. More recently Naish &
Capper (1953) have reported five further cases. One of these
had megaloblastic anaemia following ileo-transverse colostomy
for chromaffinoma of the terminal ileum. In this case there
was a blind loop: the anaemia first responded to an injection
of cyanocobalamin; later there was a spontaneous remission;
after this no response occurred to cyanocobalamin by mouth,
but there was said to be a response to the oral administration
of aureomycin and later pteroylglutamic acid. A second
patient developed peripheral neuritis, subacute combined degen-
eration of the cord and macrocytic anaemia some thirty years
after gastro-enterostomy, and at autopsy a carcinoma was found
to be present at the stoma. There was histamine fast achlor-
hydria but the authors state that the anaemia was not caused
by the same mechanism as in Addisonian anaemia. It is not
clear why they make this statement but presumably they base it
on the fact that the fundus of the stomach was normal histol-
logically at post mortem. The third case had severe steatorr-
hoea/
steatorrhoea and protein deficiency five years after a Polya gastrectomy. If a long afferent loop is left in the Polya operation it would appear to provide a stagnant area for the growth of bacteria which can at least theoretically have devastating effects on the metabolism of antimegaloblastic substances. (See Chapter 5.) There is however need of further research on the bacterial content, if any, of the afferent loop after this operation. The fourth patient of Naish & Capper's series had an apparently normal gastro-enterostomy except that there was enormous dilatation of the duodenum. Steatorrhoea and hypoprotinaemic oedema were the main features of this case. The fifth patient had first a gastro-enterostomy and then an antecolic Polya gastrectomy done. There resulted a megaloblastic form of anaemia that responded to injections of cyanocobalamin. Unfortunately it is not possible to be certain of the mechanism of production of anaemia in these patients since in any individual case it is necessary to perform biochemical tests for antimegaloblastic substances, to obtain any sort of answer.

In this chapter a brief account will be given of a few cases that we have investigated and the possible mechanisms of production of anaemia in this group of conditions will thereafter be reconsidered.

Case 200. (Graph 52.)

Megaloblastic anaemia developing after total gastrectomy.

This 48 year old female patient was first seen at Ward 27, Edinburgh/
Edinburgh Royal Infirmary, seven years after she had had a total gastrectomy operation for a gastric ulcer which was thought to be malignant, but which on section proved to be simple. Three years after the operation the patient developed flatulence and diarrhoea and from time to time passed loose motions containing undigested food. Eight months before the date of admission to Ward 27 she had been in another hospital ward because of this diarrhoea together with glossitis, symptoms of anaemia and weight loss. She improved on treatment with iron, ascorbic acid, 'Sorlate' (an emulsifying agent) and pteroylglutamic acid, but soon after discharge ceased taking this treatment.

At the time of admission to Ward 27 the blood levels were
haemoglobin 7.8 G. per 100 ml., red cells 2,010,000 per c.mm.,
C.I. 1.31, PCV 25%, MCV 124.3 cµ., MCHC 31.2%, white cells
8,000 per c.mm., reticulocytes < 1%. The marrow was frankly
megaloblastic.

The weight was 5 st. 7 lbs., and the tongue was inflamed,
with atrophy of the papillae. A barium follow through examination showed no abnormality of the intestine other than hurry,
and the stool benzidine was negative. A four day fat balance test on a 75 G. fat intake showed 82.3% absorption, 57% of the
fat being unsplit.

At the time serum vitamin B12 examinations were not
possible but repeated differential urinary folic acid excretion
tests were carried out as follows.

Test 1./
Test 1.

Urinary excretion:
- after 5 mg. PGA subcutaneously
- after 5 mg. PGA orally

15 mg. PGA then injected IM on 7 consecutive days. Marrow now normoblastic.

<table>
<thead>
<tr>
<th>Test 1</th>
<th>Folic acid</th>
<th>Cit. Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg.</td>
<td>µg.</td>
</tr>
<tr>
<td>after 5 mg. PGA subcutaneously</td>
<td>2.59</td>
<td>4.8</td>
</tr>
<tr>
<td>after 5 mg. PGA orally</td>
<td>2.83</td>
<td>3.6</td>
</tr>
</tbody>
</table>

Test 2.

Urinary excretion:
- after 5 mg. PGA subcutaneously
- after 5 mg. PGA orally

<table>
<thead>
<tr>
<th>Test 2</th>
<th>Folic acid</th>
<th>Cit. Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg.</td>
<td>µg.</td>
</tr>
<tr>
<td>after 5 mg. PGA subcutaneously</td>
<td>2.37</td>
<td>7.2</td>
</tr>
<tr>
<td>after 5 mg. PGA orally</td>
<td>2.24</td>
<td>9.2</td>
</tr>
</tbody>
</table>

Test 3.

Urinary excretion:
- after 15 mg. PGA subcutaneously
- after 15 mg. PGA orally

<table>
<thead>
<tr>
<th>Test 3</th>
<th>Folic acid</th>
<th>Cit. Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg.</td>
<td>µg.</td>
</tr>
<tr>
<td>after 15 mg. PGA subcutaneously</td>
<td>6.7</td>
<td>31.9</td>
</tr>
<tr>
<td>after 15 mg. PGA orally</td>
<td>8.2</td>
<td>21.3</td>
</tr>
</tbody>
</table>

Test 4.

Urinary excretion of antimegaloblastic substances
after 100 µg. of cyanocobalamin IM

<table>
<thead>
<tr>
<th>Test 4</th>
<th>Vitamin B₁₂</th>
<th>Folic acid</th>
<th>Citrovorum Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>21.9 µg.</td>
<td>0.77 µg.</td>
<td>1.5 µg.</td>
</tr>
</tbody>
</table>

These results are interpreted as giving no proof of folic acid depletion (it will be recollected that pteroylglutamic acid therapy had been given several months before) and as indicating that the anaemia was not due to malabsorption of folic acid. The measurements of antimegaloblastic substances in the urine after 100 µg. of cyanocobalamin by injection gave no information of value — such results might equally well be found in a normal person or in one with pernicious anaemia.

As sometimes occurs in pernicious anaemia cases, this patient showed a haematological response to pteroylglutamic acid therapy although there was no biochemical evidence of folic acid depletion. It is commonly considered that in such cases/
cases folic acid acts by "mass action" where the substance lacking is really vitamin B₁₂.

In this case the patient's complaints about her painful tongue were so frequent and vigorous that cyanocobalamin therapy was soon given in addition to the pteroylglutamic acid. The glossitis became less troublesome after the injections of cyanocobalamin and meantime the diarrhoea had ceased. Unfortunately the pain in the tongue returned later despite combined therapy with pteroylglutamic acid, cyanocobalamin and a multivitamin preparation.

It seems likely that the anaemia in this patient was due not to folic acid depletion associated with the steatorrhoea, but to deficiency of vitamin B₁₂ as a result of lack of intrinsic factor.
Case 201. (Graph 53.)

Macrocytic anaemia developing after partial gastrectomy.

This 51 year old female patient was admitted to Ward 27, Edinburgh Royal Infirmary, in July 1946 suffering from anaemia. A partial gastrectomy operation for a gastric ulcer had been performed in 1943, but operative details could not be obtained. The haemoglobin level was now 4.4 G. per 100 ml., red cells 2,700,000 per c.mm., and the colour index was 0.55. The film was typical of iron deficiency anaemia, and the stool benzidine reaction was positive on one occasion, but negative twice. There was histamine fast achlorhydria. Treatment with ferrous sulphate gr. 3 t.i.d. and ascorbic acid 50 mg. b.i.d. was given for five weeks in hospital and the blood counts rose to a haemoglobin level of 9.8 G. per 100 ml. and a red cell count of 3,750,000 per c.mm. The colour index was now 0.9.

The patient was discharged to her home which was a considerable distance from Edinburgh and was not seen again until September 1952. In the intervening period no treatment had been taken and the blood levels had fallen to haemoglobin 4.4 G. per 100 ml., red cells 2,910,000 per c.mm., C.I. 0.5.

Ferrous sulphate was given in a dosage of gr. 6 t.i.d. and the blood counts rose to 7.3 G. haemoglobin per 100 ml., red cells 3,310,000 per c.mm., C.I. 0.74, but then the patient ceased taking her tablets. She was persuaded to take them again, and further improvement occurred. In April 1953, however, despite the fact that the patient was now taking ferrous sulphate regularly, the haemoglobin level had risen only to 9.9 G.
per 100 ml. The tongue was painful and red with atrophy of
the papillae, and the nails were flat. There was no diarr-
hea. The red cell count, however, had fallen to 2,280,000
per c.mm. and the colour index had risen to 1.25. The
peripheral blood film now showed some macrocytes and some
anisocytosis and poikilocytosis.

The patient was therefore readmitted to hospital. A
more complete blood investigation gave the following figures:
haemoglobin level 8.1 G. per 100 ml., red cells 2,320,000 per
c.mm., C.I. 1.18, PCV 29%, MCV 125 cu., MCHC 27.9%, white
cells 8,800 per c.mm., reticulocytes < 1%. The sternal
marrow did not show a frankly megaloblastic appearance, but
intermediate (transitional) erythroblasts were present. There
was histamine fast achlorhydria and a barium follow through
examination showed a normally functioning stoma and no evidence
of disease of the small intestine. A fat balance test demon-
strated 90% fat absorption, 71.5% of the fat being unsplit.
The stool benzidine reaction was negative.

A differential urinary folic acid excretion test was
carried out and the results were as follows.

<table>
<thead>
<tr>
<th>Urinary excretion:</th>
<th>Folic acid</th>
<th>Cit.Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>in 24 hrs. after 5 mg. SC</td>
<td>1.75</td>
<td>12.0</td>
</tr>
<tr>
<td>in 24 hrs. after 5 mg. Orally</td>
<td>2.92</td>
<td>10.9</td>
</tr>
</tbody>
</table>

There was thus no evidence of malabsorption of folic acid, but
the amount excreted after the injected dose was at about the
lowest level of normality. The amount of folic acid convert-
ed to citrovorum factor appeared to be unduly high.

It/
It was thought likely that the anaemia was due to vitamin B12 deficiency, either as a result of lack of intrinsic factor or because of destruction of vitamin B12 in a blind afferent loop, but at the time this could not be proved. There was no evidence of neurological complications and for research purposes it was decided to try the effect of treatment with pteroylglutamic acid alone. The haemoglobin level rose to a maximum of 11.1 G. per 100 ml. and the red cell count to 4,490,000 per c.mm. and then showed a slight regression, despite the administration of iron. In November 1953 the serum vitamin B12 level was estimated and found to be 113 µµg. per ml. - an abnormally low level. In January 1954 the level was 75µµg./ml.

This would appear to have been macrocytic anaemia due to deficiency of vitamin B12.

Case 201. Megaloblastic anaemia developing after partial gastrectomy.

Graph 53.
Megaloblastic anaemia after gastro-enterostomy.

A 63 year old man who developed symptoms of anaemia and vague epigastric pains. The history was that he had had an operation for a perforated peptic ulcer fourteen years previously and a further operation for a duodenal ulcer four years after that. Details of the operations could not be traced. Seven months before he was admitted for the anaemia he had had a two stage operation for benign prostatic hypertrophy. There was no elevation of the blood urea nitrogen level.

Since the details of the stomach operation were not available, a barium meal was done to see what information could be obtained. The report was as follows:

Barium Meal. Oesophagus, negative.
Stomach. A stoma was present at the most dependent point of the stomach and through this barium began to flow immediately. After the stomach had been filled up to some extent it was seen that this stoma appeared to be due to a gastroenterostomy. The antral part of the stomach was deformed and the lesser curvature much shorter. There was some pre-pyloric narrowing and the duodenal cap was partly outlined and appeared to be somewhat deformed. The stomach appeared to be practically empty in two hours and the head of the meal was in the proximal colon.
The appearances described could be due to old ulceration and to operative interference, but with so much deformity it is difficult to exclude the presence of neoplasm.

The haemoglobin level was 6.1 G. per 100 ml.; red cells 1,600,000 per c.mm., C.I. 1.24, POV 16.5%, MCV 103.1 cu., MCHC 37.0%, white cells 1,800 per c.mm., reticulocytes < 1%. The marrow was megaloblastic, and there was histamine fast achlorhydria. At this time no biochemical tests for anti-megaloblastic/
antimegaloblastic substances were available and when it had been established that there was no biochemical evidence of hepatic or renal disease, treatment with cyanocobalamin was commenced. To this there was a rather delayed response, and iron deficiency anaemia developed, as is shown in Graph 54.

The patient has now been seen regularly as an outpatient for a further four years, and with regular cyanocobalamin therapy he is perfectly fit and has a normal blood count.

One cannot be certain of the mechanism of the anaemia in this case, but it would seem that either the patient has a blind loop in which ingested vitamin B₁₂ is being destroyed or absorbed by bacteria, or in which vitamin B₁₂ antagonists are being produced, or alternatively, that there is sufficient atrophic gastritis or other pathological change in the stomach to abolish the cells that normally secrete intrinsic factor.
Case 203.

Post-gastrectomy syndrome without anaemia.

A male patient aged 47 years was admitted to hospital on account of tiredness, anorexia and cold sweats. He had had a partial gastrectomy (Polya) performed eighteen months previously. The tongue had been painful on occasions for four months, but his dentures were ill-fitting. There was no diarrhoea and the haemoglobin level was 15.1 G. per 100 ml., red cells 5,220,000 per c.mm. An X-ray of the alimentary tract showed a normally functioning stoma and no abnormality of the small bowel or of its function. The serum vitamin B12 level was normal, being 250 μg./ml. A differential urinary folic acid excretion test also showed no abnormality, the figures being:

<table>
<thead>
<tr>
<th>Urinary excretion:</th>
<th>Folic acid</th>
<th>Cit. Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>in 24 hrs. after 5 mg. SC</td>
<td>2.17</td>
<td>8.95</td>
</tr>
<tr>
<td>in 24 hrs. after 5 mg. Orally</td>
<td>2.89</td>
<td>7.70</td>
</tr>
</tbody>
</table>

Comment. This was a patient with post-gastrectomy symptoms. While he was in hospital the opportunity was taken to establish that there was no detectable abnormality of metabolism of antimegaloblastic substances at a period of eighteen months after operation.

Case 204.

Glossitis after partial gastrectomy.

A 67 year old female patient who had had a partial gastrectomy operation in 1950 for a gastric ulcer with recurrent haemorrhage. She was referred to the Blood Clinic at Edinburgh Royal Infirmary three years later because of a sore tongue/
tongue, difficulty in swallowing and loss of weight. There was no diarrhoea. She wore dentures, but they had not caused her any trouble and she had had them for several years.

The blood levels were haemoglobin 13.6 G. per 100 ml., red cells 4,910,000 per c.mm., C.I. 0.93. There was no abnormality in the levels of serum electrolytes. Histamine fast achlorhydria was present. Two three day fat balance tests on a 75 G. daily intake showed, respectively, 96.6% and 94.0% absorption. An oral glucose tolerance test in which blood specimens were taken at half hourly intervals after 50 G. of glucose given by mouth showed a rise to 206 mg. per 100 ml. in half an hour, from a fasting level of 107 mg. per 100 ml. There was an equally rapid fall.

The report on a barium meal and follow through examination was as follows.

"There is a normally functioning partial gastrectomy. No filling of the afferent loop was noted. Although the stomach emptied fairly rapidly there was no overloading of the proximal jejunum, which showed a normal mucosal pattern. A follow through examination after one and a half hours showed most of the barium in the ileum. The mucosal pattern was within normal limits. A further film taken four hours from the beginning of the meal showed practically all the barium within the colon: some had even been excreted."

The serum vitamin B₁₂ level was found to be 140 µg./ml., the lower limit of normality.

A differential urinary folic acid excretion test gave the following results.
Urinary excretion:
in 24 hrs. after 5 mg. SC  4.29 mg.
in 24 hrs. after 5 mg. Orally  4.26 mg.
There was thus no evidence of folic acid depletion or of malabsorption of folic acid. Some improvement in the symptoms followed injections of cyanocobalamin, but the appearance of the tongue did not alter, and the lingual pain did not disappear completely.

Comment. A patient with glossitis and difficulty in swallowing coming on three years after partial gastrectomy. There was no evidence of folic acid deficiency and the vitamin B\textsubscript{12} level of the serum was just within normal limits. There was thus no evidence that the symptoms were due to deficiency of folic acid or vitamin B\textsubscript{12}.

Case 205.

A 53 year old female patient who was seen at the Blood Clinic, Edinburgh Royal Infirmary three years after she had had a partial gastrectomy operation. Her chief complaints were of weakness, loss of weight and a painful tongue of six months duration. New dentures had been provided after the symptoms commenced and no benefit had resulted. There was evidence of old fibroid tuberculosis in both lungs, but the ESR was only 10 mm. per hour (Westergren) and the sputa were repeatedly negative. The tongue was red and atrophic. The haemoglobin level was 11.8 G. per 100 ml., red cells 3,800,000 per c.mm., C.I. 1.05. There was histamine fast achlorhydria. The serum vitamin B\textsubscript{12} level was found to be 250 \(\mu\text{g.}/\text{ml.}\) (normal).
The patient was treated as an outpatient with cyanocobalamin 100 µg weekly, since previous treatment with multivitamin preparations by injection together with iron, yeast and ascorbic acid had given no benefit. No haematological or clinical benefit followed the cyanocobalamin therapy, and pteroylglutamic acid was then given, in addition, by mouth in a dosage of 5 mg thrice daily. Five weeks later there was still no improvement in the lingual symptoms, and the blood levels were haemoglobin 11.0 G per 100 ml., red cells 4,280,000 per c.mm., C.I. 0.86.

Comment. Glossitis and mild anaemia after partial gastrectomy. Normal serum vitamin B12 level and no significant response to any haematinic or to multivitamin therapy.

In two other patients with steatorrhoea occurring several years after operations on the alimentary tract we were fortunate in that the physicians looking after the patients carried out differential urinary folic acid excretion tests before giving any pteroylglutamic acid therapy.

Case 206.

Diarrhoea after vagotomy.

A 69 year old female patient who had a gastro-enterostomy operation performed in 1924 for chronic duodenal ulcer. She suffered from Raynaud's phenomenon, and had right and left lumbar ganglionectomy operations performed in 1938 and again in 1942. Paravertebral injections were tried in 1947 for Raynaud's phenomenon affecting the upper limb.

In May 1948 the patient was found to have an active stomal/
stomal ulcer, and vagotomy was performed. In October of that year she began to have diarrhoea and a stool fat examination showed 39.3% of fat to be present, 53.9% of this being unsplit. The patient continued to attend Edinburgh Royal Infirmary, and from 1948 to 1953 she was seen frequently at the Dietetic Outpatient Department. The tongue was painful and diarrhoea was frequent. In May 1953 the patient was admitted to Ward 21 for a short period. Diarrhoea was troublesome and the weight was 6 st. 11 lbs. No fat balance test was done, but a differential urinary folic acid excretion test was permitted. The results were:

<table>
<thead>
<tr>
<th>Urinary excretion:</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>In 24 hours after 5 mg. SC</td>
<td>4.81 mg.</td>
</tr>
<tr>
<td>In 24 hours after 5 mg. Orally</td>
<td>4.62 mg.</td>
</tr>
</tbody>
</table>

Comment. Diarrhoea for 5 years after vagotomy, but no evidence of folic acid depletion or malabsorption.

Case 207.

Duodeno-jejunostomy followed by partial gastrectomy.

A 63 year old male patient who had a duodeno-jejunostomy carried out in Edinburgh Royal Infirmary in 1930 after 20 years of dyspepsia. He continued to have symptoms and in June 1952 was admitted to the Gastro-Intestinal Unit of the Western General Hospital for partial gastrectomy (Folya). Before the operation a barium meal showed that there was dilatation of the second and third parts of the duodenum. A few weeks after the operation the patient started to have 4 to 8 loose watery stools daily, and developed cramps in the limbs with stiffness of the fingers and hands. Swelling of the/
the feet and ankles also occurred.

The patient was readmitted in December 1952. A barium meal showed that the stomach emptied rapidly, filling both the afferent and efferent loops. The duodenum was filled and its second part was dilated. Two fat balance tests (4 and 3 day tests respectively) showed 87.4% and 76% absorption. The stools contained striated and partially digested muscle fibres. The serum sodium level was 300 mg. per 100 ml., chlorides 618 mg. per 100 ml., albumin 2.2 G. per 100 ml., globulin 2.17 G. per 100 ml., calcium 8.7 mg. per 100 ml., urea nitrogen 7 mg. per 100 ml. The prothrombin activity was 72%, and the serum bilirubin level was 0.3 mg. per 100 ml. The bone marrow was normoblastic, and the haematological findings were:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin</td>
<td>10.2 G. per 100 ml.</td>
</tr>
<tr>
<td>Red cells</td>
<td>2,970,000 per c.mm.</td>
</tr>
<tr>
<td>C.I.</td>
<td>1.16</td>
</tr>
<tr>
<td>PCSV</td>
<td>33%</td>
</tr>
<tr>
<td>MCV</td>
<td>111 cu.</td>
</tr>
<tr>
<td>MCHC</td>
<td>30.9%</td>
</tr>
</tbody>
</table>

The peripheral blood film showed anisocytosis and poikilocytosis.

The serum vitamin B12 level was found to be 165 µg./ml. The results of the differential urinary folic acid excretion test were

Urinary excretion:
- in 24 hours after 5 mg. SC 3.53 mg.
- in 24 hours after 5 mg. Orally 4.30 mg.

In so far as the folic acid output after an injected dose is a measure of bodily stores of folic acid the test did not/
not indicate depletion of the substance, and certainly there was no evidence of malabsorption of folic acid. When a random urine taken before this test was used in place of water to dilute the standard in a folic acid assay there was no inhibition of growth of \textit{S. faecalis}. Therefore, in so far as this is a test for folic acid antagonists, there was no evidence of their presence in the urine.

Treatment with cyanocobalamin was tried. This was given by injection, 400 µg. being given in 28 days. At the end of this period the red cell level showed no rise, and there was no improvement in the general condition.

Comment. This patient who had already had a duodeno-jejunostomy performed, later had a partial gastrectomy done. When the stomach emptied, both the afferent and efferent loops filled, and the duodenum, which was in the afferent loop, was dilated. There was thus a blind afferent loop which was not however necessarily stagnant.

Neither the serum vitamin B12 level nor the differential urinary folic acid excretion test showed the reason for the macrocytic anaemia (with normoblastic marrow) that was present.

Cases of gastric carcinoma will be referred to in Chapter 16.

Case 208./
A 49 year old patient who was offered to us in December 1952 as a case of idiopathic steatorrhoea that might be suitable for a differential urinary folic acid excretion test.

In brief the history was that she had had an appendix operation as a child and thereafter appears to have been well until she was admitted to a ward of Edinburgh Royal Infirmary in 1929 at the age of 25 with loss of weight, diarrhoea and weakness. A diagnosis of pernicious anaemia was made and liver injections were given for three weeks in hospital but not subsequently.

She felt fairly well for three years and then in 1932 was admitted to another ward of the same hospital. A diagnosis of/
of pernicious anaemia was again made and liver injections were
given for a year. No treatment was given thereafter for five
years and then, in 1938 she was again admitted twice. In 1940
she was readmitted in relapse and treated for pernicious
anaemia for three weeks with liver injections. At the time
she had severe diarrhoea and oedema. Two weeks after discharge
she had a relapse and was treated at Seafied Hospital as an in-
patient for a year with liver injections and vitamin therapy.
The notes of this time are no longer available.

In 1949 she reappeared in Edinburgh Royal Infirmary with
symptoms of anaemia, swelling of the ankles, much diarrhoea and
a painful tongue. No significant treatment had been given in
the intervening period. This time a diagnosis of idiopathic
steatorrhoea was considered. There was histamine fast achlor-
ydria, the marrow contained intermediate erythroblasts rather
than megaloblasts, and two three-day fat balance tests showed
86% and 75% absorption. The blood levels were haemoglobin
11.0 G. per 100 ml., red cells 2,900,000 per c.mm., PCV 29%,
MCH 100 qo., MCHC 37.9%, white cells 5,000 per c.mm., reticulo-
cytes < 1%.

Treatment now was with pteroylglutamic acid by mouth
together with nicotinic acid, riboflavin and liver injections.
Therapy was continued for about a year.

In November 1952 the patient was again readmitted with
anorexia, loss of weight, general features of anaemia and
bulky stools, the bowels moving three or four times daily.
The blood levels were estimated to be haemoglobin 7.4 G. per
100 ml., red cells 1,720,000 per c.mm., C.I. 1.47. Treatment with pteroylglutamic acid was commenced in a dosage of 10 mg. twice daily by mouth. After this had been given for three days we were invited to see the patient. The results of the urinary folic acid excretion test were:

<table>
<thead>
<tr>
<th>Urinary excretion:</th>
<th>Folic acid</th>
<th>Git. Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>in 24 hrs. after 5 mg. SC</td>
<td>2.75 mg.</td>
<td>3.12 µg.</td>
</tr>
<tr>
<td>in 24 hrs. after 5 mg. Orally</td>
<td>1.92 mg.</td>
<td>4.11 µg.</td>
</tr>
</tbody>
</table>

At the time serum vitamin B₁₂ measurements were not possible.

The results of the folic acid excretion test were disturbing in that the patient certainly had steatorrhoea with anaemia, but the difference in folic acid output in the two 24 hour specimens was not as great as we expected to find in a patient with idiopathic steatorrhoea in relapse. Indeed, the urinary output of folic acid after the 5 mg. oral test dose was within normal limits.

A glucose tolerance test and a barium follow through examination were therefore suggested, and meantime pteroylglutamic acid therapy was continued in a dosage of 10 mg. twice daily for a further nine days.

The oral glucose tolerance test gave no evidence of mal-absorption, since the blood sugar level after 50 G. of glucose rose from 82 mg. per 100 ml. to 144 mg. per 100 ml. in an hour.

The barium follow through examination was done both with flocculable and on a second occasion with non-flocculable barium, and briefly the following were the findings.

There/
There was no abnormality in the oesophagus, stomach or duodenum. The jejunum showed no abnormality other than a few diverticulae. There was difficulty in demonstrating the ileum and barium appeared in the rectum after 90 minutes. An ileo-rectal fistula was suspected.

Accordingly a barium enema examination was carried out and there was reported to be an ileo-colic fistula approximately five centimetres above the pelvi-rectal junction. There was distortion of the mucosal pattern in the transverse colon particularly.

The following are three of the pictures obtained.

Barium meal and follow through at 2½ hours.
Barium enema.

Barium enema.
The patient was referred to Sir James Learmonth who agreed that operative interference was indicated. While the patient was awaiting admission to hospital for the operation, the treatment given was cyanocobalamin alone by injection in a dosage of 100 µg. every two weeks. Pteroylglutamic acid therapy itself had brought the haemoglobin level to 11.3 G. per 100 ml., and the red cell count to 3,180,000 per c.mm., giving a colour index of 1.19. The subsequent levels on cyanocobalamin treatment are shown in Graph 55.

It will be seen that on the day of operation the marrow was normoblastic, but it must be stated that both streptomycin and sulphasuccidine were given immediately the patient was admitted to the surgical ward and for four days prior to operation.
operation. At operation an ileo-sigmoid fistula was indeed found to be present, and there was no evidence of any disease such as regional ileitis. It was thought that the fistula might have been associated with the previous appendicitis.

It was felt that the patient who had suffered for so long should be given every chance and in any event intensive treatment with terramycin had been given after operation. Accordingly both cyanocobalamin and pteroylglutamic acid were now administered. The red cell count rose to 5,240,000 per c.mm., a level that was considerably higher than had ever been reached before as a result of therapy with liver injections or cyanocobalamin, or with liver injections and pteroylglutamic acid together. The mean cell volume which had commonly been 100 cu. or more fell to 76.3 cu. Moreover the patient felt much better, and the diarrhoea ceased. Two three day fat balance tests after operation on a 50 G. daily fat intake showed 98.2% absorption with 88% splitting of fat and 98.4% absorption with 86% splitting.

At operation a sample of the contents of the stagnant loop of the small intestine was obtained. The predominant organism was found to be B. coli, intermediate type I. This organism did not absorb, destroy, inactivate or produce vitamin B₁₂ or folic acid when tested for these properties under aerobic or anaerobic cultural conditions. It should be remembered however that streptomycin and sulphasuccidine were given prior to operation.

Comment. The mechanism of production of anaemia in this patient is not clear. Although the absorption of the orally administered/
administered dose of 5 mg. of pteroylglutamic acid in the original differential folic acid excretion test was 1.92 mg., this does not prove that the smaller amounts of folic acid substances (i.e. pteroylglutamic acid and its conjugates plus citrovorum factors and their conjugates) were being absorbed normally. The haemoglobin and red cell levels were maintained reasonably well and the marrow remained normoblastic with cyanocobalamin therapy alone, and it is possible that the patient's dietary vitamin B12 (in the presence of intrinsic factor) was not being absorbed normally. To complicate matters further, however, the patient had histamine fast achlorhydria and therefore it is not certain whether there was any intrinsic factor: its absence would mean that pernicious anaemia was a complicating factor - either true Addisonian pernicious anaemia or intrinsic factor loss because of damage to the gastric mucous membrane from bacterial spread up from the large bowel and consequent gastritis.

We do not know what proportion of ingested substances passed through the length of the small intestine and what proportion passed through the fistula. It was not possible to show that the organisms in the stagnant loop could destroy or absorb vitamin B12 or folic acid, but it is likely that there was an abnormal flora extending up above the fistula and that this varied in character from day to day. Our investigations did not therefore by any means prove that the anaemia was not due to bacterial action on antimegaloblastic substances, particularly since sulphasuccidine and streptomycin/
streptomycin had been given immediately the patient was admitted to the surgical ward for operation.

The investigation of any future case similar to this would ideally include:

1) Serum vitamin B₁₂ level
2) Differential urinary folic acid excretion test
3) Gastric biopsy
4) Oral administration and measurement of the faecal excretion of radioactive vitamin B₁₂ by the patient
5) Measurement of intrinsic factor. At present the only reasonably certain way to do this is to give the gastric juice together with radioactive vitamin B₁₂ to a pernicious anaemia patient and measure the absorption of vitamin B₁₂ indirectly by calculating its output in the faeces.

Case 209.

Measurement of levels of antimegaloblastic substances in a patient with multiple pathology of the alimentary tract.

A 44 year old man who was admitted to the Gastro-Intestinal Unit of the Western General Hospital, Edinburgh in October 1953. Seven months previously he had been operated upon in Edinburgh Royal Infirmary for an appendix abscess. Thirteen days after appendicectomy he developed intestinal obstruction and an ileo-transverse colostomy was done. Thereafter he remained well for several months.

When he was admitted to the Western General Hospital he was believed to have an acute intestinal obstruction. A laparotomy was performed and there was found to be a partial volvulus involving a portion of ileum. This was reduced by operation and the adhesions divided. It is of interest that there was diarrhoea at the time of the obstruction because the foodstuffs/
foodstuffs passed through the ileo-transverse colostomy opening. (See Fig. 29, p. 567.)

Our investigations were carried out about a month after the operation for volvulus. The patient was much improved, and a barium enema confirmed that there was an ileo-transverse colostomy opening.

The marrow was normoblastic and the blood levels were:

- Haemoglobin: 13.3 G. per 100 ml.
- Red cells: 4,820,000 per c.mm.
- Colour Index: 0.93
- PCV: 45%
- MCV: 93 cµ.
- MCHC: 29%
- White cells: 13,000 per c.mm.

The serum vitamin B\textsubscript{12} level was found to be 315 µg./ml.

The differential urinary folic acid excretion findings were as follows.

**Urinary excretion:**
- in 24 hours after 5 mg. SC: 0.04 mg.
- in 24 hours after 5 mg. Orally: 0.173 mg.
- in 24 hours after a further 5 mg. Orally: 2.69 mg.

A random sample of urine was obtained from the patient prior to the carrying out of this test. When a folic acid assay was set up with \textit{S. faecalis}, the urine of this patient being used instead of water to dilute the standard, there was no inhibition of growth of the test organism. Therefore, in so far as this is a test for folic acid antagonists there was no evidence of their presence in the urine.

It would seem that in this case there was folic acid depletion which was not corrected until 15 mg. of pteroylglutamic acid had been administered. Absorption of folic acid appeared to be satisfactory as judged by the excretion after the/
the administration of the second oral test dose of pteroylglutamic acid. Delayed absorption was not the explanation since several days elapsed between the giving of the two oral test doses.

Although there was evidence of folic acid depletion, there was no anaemia and the marrow was normoblastic. It may be that anaemia was about to develop because folic acid substances were passing from the ileum to the transverse colon without having time to be absorbed, or it may be that there was some unknown metabolic upset produced by the volvulus. Any explanation of the findings would, however, be entirely hypothetical and present knowledge is insufficient for the matter to be pursued further with advantage.

Case 210.

Amoebiasis with mild anaemia.

A 48 year old patient who had never been abroad was admitted to the Gastro-Intestinal Unit of the Western General Hospital, Edinburgh, with a seven years history of diarrhoea with blood and mucus in the stools. There was associated abdominal pain. The appendix had been removed ten years before the patient came into the Western General Hospital, and at that time there had been suspicion that he had tuberculosis of the caecum. The next year a barium enema examination had shown ulcers of the rectum. On two occasions the patient had been admitted to a sanatorium for diarrhoea and poor general health; it was thought that he had abdominal tuberculosis.
In the Western General Hospital barium examinations of the alimentary tract suggested ulcerative colitis complicated by tuberculosis of the caecum, but a definite diagnosis of amoebiasis of the colon was made, vegetative forms of Entamoeba coli being isolated. From our point of view the patient was a control subject.

The blood findings were:

- Haemoglobin: 11.2 G. per 100 ml.
- Red cells: 3,980,000 per c.mm.
- Colour Index: 0.95
- PCV: 34%
- MCV: 85 cµ.
- MCHC: 33%

The serum vitamin B₁₂ level was 438 µµg./ml.

The results of the differential urinary folic acid excretion test were:

- Urinary excretion:
  - in 24 hours after 5 mg. SC: 2.96 mg.
  - in 24 hours after 5 mg. Orally: 3.08 mg.

The test was negative.

Case 211.

Reticulosarcoma of the ileum.

A man aged 43 was well until December 1949, when he first noticed swelling of both ankles. This subsided spontaneously during the next few weeks. In October 1950 he first complained of diarrhoea, characterised by four or five frothy, yellowish, watery stools a day. This continued intermittently for eleven months, and he was admitted to the Royal Infirmary, Edinburgh, on September 14th 1951, for investigation. During the preceding...
preceding months he had lost 42 lb. in weight in spite of a reasonably good appetite. There was no history of abdominal pain. The family, social, occupational and past histories revealed no information of diagnostic value.

On examination he was seen to be a thin, pale, middle-aged man. The tongue was atrophic. The respiratory, nervous, and cardiovascular systems were normal except for the presence of hypotension; his blood pressure was 90/60. The liver and spleen were not palpable and no abdominal masses were felt. He had gross clubbing of the fingers. There was minimal sacral oedema, but a moderate degree of bilateral ankle swelling. X-ray examination of the chest showed no abnormality. The stool benzidine reaction was negative on the two occasions that it was tested. The urine contained no albumin, sugar or excess of urobilinogen. Liver function tests were negative. The Robinson-Kepler-Power water test gave a positive result. The glucose tolerance curve was flat. 17-ketosteroid excretion was 8.4 mg. per 24 hours. A three day fat balance study showed that there was only 66% fat absorption and a barium meal gave evidence of a "deficiency" pattern.

The present author was on holiday when the patient was admitted, but it appears that a diagnosis of idiopathic steatorrhoea was made and pteroylglutamic acid therapy started. There was certainly nothing to suggest that this was not the correct diagnosis. The blood levels before therapy were:

| Haemoglobin | 14.2 G. per 100 ml. |
| Red cells   | 4,600,000 per c.mm. |
| Colour Index| 1.04                |
| PCV         | 40%                 |
| MCV         | 86.9 cm.            |
| MCHC        | 35.5%               |
The ESR was 3 mm./hr. (Westergren).

Treatment with pteroylglutamic acid given by mouth for a fortnight in a dosage of 20 mg. daily caused no change in the red cell count. The haemoglobin level fell to 10.2 G. per 100 ml. and the PCV to 33%.

About a month after admission the patient developed frank tetany with carpal spasm. This was relieved with antitetanic therapy, including calcium gluconate, "para-thor-mine", and vitamin D. The Sulkowitch test consistently revealed the absence of calcium from the urine.

After the patient had been given five weeks of treatment with 20 mg. of pteroylglutamic acid daily by mouth, supplemented by numerous other vitamins, ferrous sulphate, added protein (Casinal) and sodium chloride, a test dose of 5 mg. of pteroylglutamic acid was given by mouth. The result was:

<table>
<thead>
<tr>
<th>Urinary excretion:</th>
<th>Folic acid Cit.Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>in 24 hrs. after 5 mg. Orally 0.057 mg. 1.56 µg.</td>
<td></td>
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When 12 mg. of citrovorum factor was given by mouth there was excreted only 23 µg.

There was thus evidence of malabsorption of folic acid and citrovorum factor. The urinary collections had been continued for 48 hours to enable it to be ascertained with certainty that the low outputs were not merely due to delayed absorption.

The patient was then given five injections of 15 mg. of pteroylglutamic acid and 30 mg. of the substance daily by mouth for six days. Thereafter a test dose of 5 mg. was injected, but the amount excreted was only 1.59 mg., a low figure considering/
considering the treatment that had been given.

The patient was discharged on December 11th, subjectively much improved. He had gained 28 lb. in weight, the blood pressure had risen to 100/74 and the serum calcium level was 11.3 mg. per 100 ml. The haemoglobin reading was 10.4 G. per 100 ml. but the red cell count had fallen to 4,280,000 per c.mm. There were only one or two stools a day, and oedema had disappeared.

The patient returned to work during January and February 1952, but had to stop because of weakness. In May he developed colicky abdominal pain which gradually increased in severity. Laparotomy was carried out in another centre, and a tumour of the ileum was found completely obstructing the intestinal lumen. Microscopically this was a reticulosarcoma which had invaded the adjacent mesenteric glands. The tumour was resected. Although relieved of his obstructive symptoms the patient continued to feel tired and was readmitted to the Royal Infirmary, Edinburgh, in September of the same year. The blood levels were haemoglobin 11.4 G. per 100 ml., red cells 4,440,000 per c.mm., C.I. 0.86, PCV 37%, MCV 83.3 µ., MCHC 30.9%, but the patient had been having continuous treatment with pteroylglutamic acid. The liver was now palpable three fingerbreadths below the right mid-costa! margin and a chest X-ray film showed consolidation in the left lower lobe with a large hilar shadow on the left side, suggesting metastatic tumour formation. The patient continued to become progressively/
progressively weaker, and was discharged to die at his home.

Comment. When the patient was first admitted to hospital it would appear that the diagnosis of idiopathic steatorrhoea was a reasonable one. The stool benzidine reaction was negative and the sedimentation rate was not raised. It cannot be said that the folic acid excretion test was of diagnostic assistance since it merely showed malabsorption to be present without indicating to what it was due. In retrospect, however, the presence of some complicating factor might have been considered because the output after an injected test dose was only 1.59 mg. even when therapeutic injections of pteroylglutamic acid had been given. It is possible that folic acid depletion was present not only because of malabsorption but because of increased requirements by neoplastic cells.

***************

Our biochemical approach to the problems of the megaloblastic anaemias and allied deficiency states associated with structural changes in the alimentary tract due to operation or disease has given some information, and in at least one instance (Case 208) the results obtained led to a correct diagnosis being made. However the biochemical problems are complex and not likely to be solved fully until we have more knowledge of the activities of intestinal organisms in their natural environment in producing, destroying, or modifying haemopoietic factors. In Case 200 there was megaloblastic anaemia/
anaemia and steatorrhoea after total gastrectomy, and our results suggest that the anaemia was due to vitamin $B_12$ deficiency rather than to malabsorption of folic acid.

This would also appear to be true in at least certain cases of macrocytic anaemia after partial gastrectomy (e.g. Case 201 where, however, intermediate erythroblasts rather than true megaloblasts were found in the marrow). In Case 202, a man with megaloblastic anaemia after gastroenterostomy, the results of therapy indicate that vitamin $B_12$ deficiency was the cause of the anaemia.

So far as glossitis is concerned, it seems that this troublesome symptom can persist after stomach operations despite the administration of all known vitamin preparations. From time to time, however, we see patients at the Blood Clinic with equally troublesome glossitis and no history of operation. In a proportion of these cases, there is no improvement following the replacement of the dentures or the giving of cyanocobalamin, pteroylglutamic acid or multivitamin preparations, and fat balance tests show no abnormality.

It is in relation to abnormalities involving the small intestine that the pathogenesis of megaloblastic anaemia is most difficult to explain. Reference is made in the literature to blind loops and stagnant loops, but it must be remembered that the terms are not synonymous. A blind loop is not necessarily stagnant, and a stagnant loop is not necessarily blind.

In a stagnant loop it is possible that bacteria may destroy/
destroy, absorb or in some way modify antimegaloblastic substances, or that antagonists to them or to some part of the biochemical cycle of haemopoiesis may be produced.

The main mechanisms that may be involved in the production of megaloblastic anaemia in the group of conditions under consideration therefore are:

(1) Loss of intrinsic factor bearing area.

(2) Malabsorption due to structural change in the intestinal wall or mesenteric glands.

(3) Rapid passage of antimegaloblastic substances from stomach, duodenum or jejunum to ileum or large intestine without sufficient time being available for their absorption.

(4) Stagnant loops.

(5) Possibly malignant tissues requiring an increased supply of the antimegaloblastic substances, which have many functions in the body other than catalyzing the production of red cells.

(6) A combination of factors.

The chief abnormalities that occur are shown in Figs. 20 to 29.

SUMMARY

An account is given of a series of patients with abnormalities of the alimentary tract that produced or might produce megaloblastic anaemia. Where it was possible, differential folic acid excretion tests were carried out and serum vitamin B₁₂ levels were estimated. The possible mechanisms of production/
production of the anaemia are discussed.

**FIGURE 20.** TOTAL GASTRECTOMY, EXTENSIVE PARTIAL GASTRECTOMY OR NEOPLASM, etc., DESTROYING STOMACH FUNDAL TISSUE.

**FIGURE 21.** PARTIAL GASTRECTOMY WITH DILATED LOOP
FIGURE 22. POLYA GASTRECTOMY WITH LONG AFFERENT LOOP.

FIGURE 23. Y SHAPED ANASTOMOSIS (see Brit. M.J. 1959, 2, 598)
Possible stagnant loop

Possible gastritis

Possible hurry from stomach to lower intestine

Figure 24: Gastroenterostomy.

Possible stagnant loop
Antimegaloblastic substances passing direct into large intestine

Possible fundal gastritis

Possible stagnant loop

Widespread malignancy may increase requirements for antimegaloblastic substances

Malabsorption due to disease or resection

FIGURE 26
GASTRO-COLIC FISTULA

FIGURE 27
NEOPLASM OF SMALL INTESTINE, REGIONAL ARATHERties, etc.
Malabsorption due to disease

FIGURE 28 EXTENSIVE TUBERCULOSIS OF MENSEROID GLANDS.

FIGURE 29 COMBINATION OF ILEO - TRANSVERSE COLONIC AND VULVULAR.
CHAPTER 16.

REFRACTORY MEGALOBLASTIC ANAEMIA.

The term achrestic anaemia, from the Greek words meaning failure to utilise ($\gamma \rho \nu \tau \iota \sigma \theta \alpha i$, to utilise) was introduced in 1935 by Wilkinson & Israels to describe a group of cases that were similar to those of pernicious anaemia as regards the bone marrow and peripheral blood picture but which showed a failure in response to liver injections. It was stated that free hydrochloric acid was present in the gastric secretions, and that the "antianaemic principle" was present in the liver. This last claim was based on the fact that a suitable extract for parenteral injection was prepared from the liver of some of these cases at post mortem and was shown to produce a haemopoietic response in pernicious anaemia patients in relapse. We have already seen, however, (p.261) that a patient with no vitamin B12 in the liver at autopsy may still have folic acid substances present, and conversely a patient deficient in folic acid may have vitamin B12 present. Therefore it is difficult to interpret the results of experiments of this nature. The word "achrestic" has been changed to achrestic by subsequent writers, including Wilkinson himself (1949). The word "achrestic" is meaningless.

In 1941 Bomford & Rhoads applied the term "refractory anaemia" to a wide variety of types of anaemia that were refractory.
refractory either temporarily or permanently to the haematinics then available. In 1943 Davidson et al. published a series of papers entitled "Studies in Refractory Anaemia" and divided the anaemias that are refractory to liver extracts into two main groups, viz. refractory anaemias with hypocellular normoblastic marrows and those with hypercellular megaloblastic marrows. Thereafter the term "idiopathic refractory megaloblastic anaemia" was used widely to mean a form of megaloblastic anaemia with no obvious cause which differed from pernicious anaemia in that there was no response to injections of liver extract that had produced haematological remission in patients with true pernicious anaemia. Such cases might or might not have histamine fast achlorhydria and so it will be seen that cases of "achrestic anaemia" would be included. Later Wilkinson (1949) extended his definition to include patients with histamine fast achlorhydria. He also considered at this time that most of the cases of so called 'refractory pernicious anaemia' were patients with pernicious anaemia who had been treated with non-potent liver extracts.

The term achrestic anaemia is an unsatisfactory one in that it assumes that the cause is failure to utilise something. It would be better to use the term "idiopathic refractory megaloblastic anaemia", but in view of our advances in knowledge the unwieldy term "idiopathic vitamin B12-refractory megaloblastic anaemia" or "idiopathic cyanocobalamin-refractory megaloblastic anaemia" would be more accurate since such cases respond to pteroylglutamic acid therapy. (See also p.432.)

Obviously/
Obviously it would be of considerable importance to apply biochemical tests for antimegaloblastic substances to patients suffering from idiopathic refractory megaloblastic anaemia. The introduction of the fat balance test for the investigation of suspected cases of idiopathic steatorrhoea, the realisation that diarrhoea is not necessarily a very troublesome feature in this disease, and now the introduction of the differential urinary folic acid excretion test and methods for the assay of the serum levels of vitamin B₁₂ give us a number of diagnostic weapons for the investigation of these cases. Moreover the use of cyanocobalamin for the treatment of suspected cases removes any possibility that the investigator is merely treating pernicious anaemia with non-potent extracts.

The following case would at first sight appear to be a good example of "idiopathic refractory megaloblastic anaemia". In this instance we have departed from our rule of including only cases seen personally — the case records of the patient are unsigned and we do not know who investigated the case. It is given here because the case was quoted by the present author as one of idiopathic refractory megaloblastic anaemia in lectures in the United States in 1948 since the haematological changes were so striking.

Case 300. (Graph 56.)

A 56 year old female patient was admitted to a ward of Edinburgh Royal Infirmary in 1944 on account of weakness and breathlessness. Two years previously she had had an operation for uterine prolapse and had not felt well thereafter. While
in hospital there was no diarrhoea and there is no record of her ever having suffered from this.

It was, however, noted that her general state of nutrition was poor and that she suffered from ulcers on the floor of the mouth.

The haemoglobin level was 3.3 G. per 100 ml., red cells 840,000 per c.mm., C.I. 1.3, white cells 4,800 per c.mm. and reticulocytes < 1%. The blood film was reported as showing anisocytosis, poikilocytosis and numerous macrocytes. The stool benzidine reaction was negative and a single specimen of stool contained 12 G.% of fat, of which 80.3% was split fat.

There was histamine fast achlorhydria, and a barium meal examination was negative.

Graph 56.

This graph is reproduced exactly as prepared for us at the University of Michigan.
The patient was given a transfusion of a pint of whole blood and received "Anahaemin" by injection in a dosage of approximately 2 ml. twice weekly, a total of 28 ml. being given in five weeks. This was supplemented by ferri et ammon cit. gr. 20, t.i.d. and ascorbic acid 50 mg. t.i.d. There was no significant improvement.

At the end of five weeks the blood levels were:

- Haemoglobin: 4.4 G. per 100 ml.
- Red cells: 1,180,000 per c.mm.
- PCV: 12.5%
- MCV: 108 cµ.
- MCHC: 35.2%
- White cells: 3,000 per c.mm.
- Reticulocytes: < 1%

The marrow was megaloblastic.

Treatment with liver injections was stopped and proteolysed liver was given by mouth as shown in Graph 56. There was an immediate response to this treatment and the patient was discharged from hospital thirty-four days later with a haemoglobin reading of 11.5 G. per 100 ml. and a red cell count of 4,180,000 per c.mm. Two weeks later she was seen as an out-patient and found to have no change in the haemoglobin level but the red cell count had risen to 4,440,000 per c.mm.

There can be no doubt that this patient had "refractory megaloblastic anaemia". The word "idiopathic" means "primary", "not depending on or preceded by another disease". In 1944 it was fair to use this term in relation to this case in the light of available knowledge. The fat balance test had not been introduced, and it was not possible to assay the Anahaemin microbiologically for its potency.

In/
In the notes of this case there is a summary by some anonymous doctor which includes the statement — "The interest of this case lies in the immediate and marked response to some as yet unidentified fraction present in the cruder preparation (i.e. proteolysed liver) and presumably absent as such or neutralised in Anahaemin, so effective in most cases of primary anaemia. It may be noteworthy that skiagrams would hint at disturbance of calcium metabolism in early life."

The first sentence is prophetic. The second is based on an X-ray picture of the wrists and knees which is reported upon as follows:

"There are some transverse sclerotic lines in the left femur and both tibiae, suggestive of nutritional disturbance in the early years."

The notes indicate that the patient's height was 5 feet 1 inch and the weight 6 st. 2 lbs. The serum electrolytes were not measured, but the blood pressure was low, the three readings that were taken in the first week being 105/45, 100/50 and 110/50.

It seems very likely that this patient was suffering from intestinal malabsorption, probably arising from coeliac disease, and that the anaemia was due to folic acid deficiency. Proteolysed liver contains a variable amount of folic acid in different batches, but the content is about 1 mg. per ounce, (together with about 300 µg. of vitamin B₁₂).

This patient who lived in lodgings in Fife, did not report again to Edinburgh Royal Infirmary. We have endeavoured without success to trace the patient and, unfortunately, her general/
general practitioner has died.

It might perhaps be added that a differential urinary folic acid absorption test would have been of particular interest in this case. By chance, in the week that we were trying to trace the above patient we were asked by Dr. J.G.M. Hamilton to investigate "by post" a case in Fife suspected of having coeliac disease. The patient who was aged 15 years was given 5 mg. of pteroylglutamic acid subcutaneously and a 24 hour urine collected, then 5 mg. of the same batch by mouth and a second 24 hour urine collected. A 4 oz. aliquot of each collection was sent to us together with a note of the total volume.

The results were as follows-

Urinary excretion:
in 24 hours after 5 mg. SC = 1.59 mg.
in 24 hours after 5 mg. Orally = 0.14 mg.

It was therefore possible to say that, subject to the collections having been carried out correctly and the correct doses administered, the results were consistent with a diagnosis of intestinal malabsorption.

It is unfortunate that the patient shown in Graph 56 could not be traced in order to have this test carried out, "by post" if necessary.

In many cases of suspected "idiopathic refractory megaloblastic anaemia" the diagnosis becomes obvious with the passage of time. In any individual case possible explanations that have to be eliminated are:

1. that the patient has intestinal malabsorption without/
without diarrhoea;

2. that there is carcinoma of the alimentary tract, particularly the stomach;

3. that the liver extract used is not potent.
   There have been no reports as yet of non-potent cyanocobalamin preparations.

The following cases may be cited. They are not selected but consist of a group of recent cases of refractory anaemia about which we have a considerable amount of data.

**Case 301. (Graph 57.)**

*Idiopathic steatorrhoea presenting as idiopathic refractory megaloblastic anaemia.*

A man aged 61 years was admitted to the Royal Infirmary of Edinburgh six weeks after a diagnosis of pernicious anaemia had been made. He had been given a total of 24 ml. of Anahaemin before we saw him, but no response had occurred.

The blood levels were falling and the bone marrow was frankly megaloblastic. The history was of two years of breathlessness and weakness. The patient suffered from haemorrhoids which had bled intermittently during the preceding six years.

In hospital, however, the stool benzidine reaction was persistently negative. The dietetic history was normal, and there had been no diarrhoea. There were no abnormal neurological signs, the liver and spleen were not palpable, and there was no abnormal glandular enlargement. There was no history of haematemesis and no visible enlargement of veins on the abdominal wall. There was no albuminuria. It is noteworthy that the patient's daughter had died in the Royal Infirmary of Edinburgh/
Edinburgh a year previously with a severe macrocytic form of anaemia and had been found at autopsy to have cirrhosis of the liver. Liver function tests were performed on the patient at present under consideration. The serum bilirubin level was 0.65 mg. per 100 ml., the blood cholesterol level was 167 mg. per 100 ml. and the cephalin cholesterol test was negative. The serum alkaline phosphatase was 7 units per 100 ml; serum albumin 3.06 G. per 100 ml.; serum globulin 2.15 G. per 100 ml. The laevulose tolerance test and hippuric acid test were both normal. In fact there was no clinical or biochemical evidence of the hepatic dysfunction that was suspected.

The blood levels were:

- Haemoglobin 3.9 G. per 100 ml.
- Red cells 350,000 per c.mm.
- Colour Index 1.4
- PCV 13.0%
- MCV 136.8 cµ.
- MCHC 30.0%
- White cells 5,200 per c.mm.
- Reticulocytes 1%

Graph 57.
As is shown in Graph 57 there was a 38.6% reticulocyte response to pteroylglutamic acid given by mouth in a dosage of 20 mg. daily, and this was followed by a rise in the red cell level. The marrow was rapidly converted to the normoblastic state.

The graph shown here is the one prepared at the University of Michigan from the data that we took with us for lecturing purposes, and it demonstrates the initial response to pteroylglutamic acid therapy. It will be seen that there was a tendency for the red cell response to cease to be satisfactory, and thereafter proteolyzed liver was given by mouth with success. Later, however, further treatment with pteroylglutamic acid was given and the patient's red cell count rose to 4,990,000 per c.mm. seven months after our treatment began.

This patient was first seen before the fat balance test was in general use. The subsequent history soon made the diagnosis obvious. The patient developed diarrhoea with pale bulky stools four months after he had been discharged from hospital and, later, tetany and peripheral neuritis became severe.

The anaemia and the diarrhoea were controlled with pteroylglutamic acid by mouth except that evidence of iron deficiency developed. When the red cell count was 4,990,000 per c.mm., the other levels were haemoglobin 9.5 G. per 100 ml., C.I. 0.64, P.C.V. 35.5%, M.C.V. 71.2 cµ., M.C.H.C. 26.3%. Combined therapy with ferrous sulphate and pteroylglutamic acid by mouth and other vitamin preparations by mouth and by injection maintained/
maintained the red cell level between 4,110,000 per c.mm. and 5,060,000 per c.mm. and the colour index at about 0.9 during the further seven months in which the patient continued to report to us. The neuritis and tetany continued to be troublesome, and the patient was readmitted to hospital several times. While he was in hospital again it was found possible to investigate his condition more fully and eighteen months after his first admission a fat balance test done over a period of eight days showed 77.8% absorption of fat, 59% of this being split. There was a flat oral glucose tolerance curve, the serum calcium level was frequently about 8 mg. per 100 ml. and an X-ray of the bones showed evidence of osteoporosis.

The patient expressed a desire to attend an hospital nearer to his home and consequently became lost to us.

This was obviously a case of steatorrhoea, probably idiopathic. The rather poor rise in the red cell count after the initial response was possibly due to malabsorption of the orally administered pteroylglutamic acid.

Case 302. (Graph 58.)

Refractory iron deficiency anaemia followed by megaloblastic anaemia - probably due to idiopathic steatorrhoea.

This 60 year old female patient was admitted to Ward 27 of the Royal Infirmary of Edinburgh twice for anaemia in 1946 and once in 1947 and was seen at the Blood Clinic thereafter. She lived at a distance from Edinburgh and her attendances at the Clinic were very irregular.

At/
At all times when symptoms were present they were the general ones of anaemia, except that in 1947, after she had been given treatment with pteroylglutamic acid, troublesome peripheral neuritis developed. At the commencement of the illness there was difficulty in swallowing and marked koilonychia. Analysis of the gastric contents showed that there was histamine fast achlorhydria, the stool benzidine reaction was persistently negative, the patient did not suffer from haemorrhoids or any other source of bleeding, and X-rays of the whole alimentary tract revealed no abnormality. There was never diarrhoea. The blood urea nitrogen level was normal, and liver function tests revealed no abnormality. In relation to the blood levels it should be noted that the patient’s doctor had empirically given her eight injections of liver extract before sending her for admission but that the last of these injections was given nine months before she was admitted.

In hospital it was found that the blood levels were:

<table>
<thead>
<tr>
<th>Test</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin</td>
<td>6.8 G. per 100 ml.</td>
</tr>
<tr>
<td>Red cells</td>
<td>2,930,000 per c.mm.</td>
</tr>
<tr>
<td>C.I.</td>
<td>0.8</td>
</tr>
<tr>
<td>FCV</td>
<td>25.5%</td>
</tr>
<tr>
<td>MCV</td>
<td>87 cμ.</td>
</tr>
<tr>
<td>MCHC</td>
<td>26.7%</td>
</tr>
<tr>
<td>White cells</td>
<td>3,200 per c.mm.</td>
</tr>
<tr>
<td>Reticulocytes</td>
<td>1%</td>
</tr>
</tbody>
</table>

The marrow was normoblastic and contained many of the poorly stained late normoblasts such as are found in iron deficiency.
It will be seen from Graph 58 that the response to iron therapy was inadequate. In July 1947 the patient became very much more ill and in the course of a month there was a sudden drop in the red cell count from 3,560,000 per c.mm. to 1,810,000 per c.mm. It was found that the bone marrow picture was now frankly megaloblastic.

An injection of 4 ml. of Anaibamin was given but 21 hours later, apart from possible very slight condensation of the nuclei of the megaloblasts, there was no change in the marrow picture. (It should be noted that the marrows have been re-examined at the time of writing.) The blood levels were now haemoglobin 7.1 G. per 100 ml., red cells 2,010,000 per c.mm., C.I. 1.19, FCV 22%, MCV 109.4 cu., MCHC 32.3%. Since 4 ml. of/
of this batch of Anahaemin was capable of producing remission in untreated pernicious anaemia and there was no diarrhoea, a diagnosis of idiopathic refractory megaloblastic anaemia was considered. According to the records a fat balance test was carried out just before the patient was discharged to her home and a normal degree of absorption shown to be present, but the actual figures cannot be traced. It is now known, however, that some patients with pernicious anaemia will respond to very small quantities of cyanocobalamin given by injection (see p. 488), while others require large amounts, and so the Anahaemin was not necessarily fully potent. Moreover this patient did have a certain degree of response to Anahaemin, the red cell count rising from 1,930,000 per c.mm. to 2,580,000 per c.mm. during the period of administration of 12 ml. of Anahaemin, and a further sternal puncture showed the marrow to contain intermediate erythroblasts which were more normoblastic than megaloblastic. A further fallacy in the investigations, that was not known at the time, is that the fat balance test was carried out over one single period of three days and as we have already seen in the chapter dealing with intestinal malabsorption, and as Cooke (personal communication) agrees, a normal result after a single three day test does not exclude a diagnosis of steatorrhoea.

The patient was treated thereafter with pteroylglutamic acid given by mouth and the red cell level rose to 5,020,000 per c.mm. in three months. By this time evidence of iron deficiency/
deficiency had reappeared for the colour index was now 0.6. The response to iron given by mouth was poor, so saccharated oxide of iron was given intravenously and the haemoglobin level rose from 9.2 G. per 100 ml. to 14.8 G. per 100 ml. Pteroylglutamic acid therapy was stopped just after the injections of saccharated iron oxide were begun and Anahaemin injections were given in a dosage of 4 ml. monthly instead.

The patient became so well that she was reluctant to travel to Edinburgh for further investigations, but in April 1951 the haemoglobin level was 11.7 G. per 100 ml. and the red cell count 4,490,000 per c.mm. In March 1953 the patient was persuaded to come for a blood count and re-examination as an outpatient. She had no symptoms or abnormal signs and was apparently in the best of health. The blood levels were:

<table>
<thead>
<tr>
<th>Component</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin</td>
<td>10.4 G. per 100 ml.</td>
</tr>
<tr>
<td>Red cells</td>
<td>4,700,000 per c.mm.</td>
</tr>
<tr>
<td>C.I.</td>
<td>0.74</td>
</tr>
<tr>
<td>PCV</td>
<td>37.5%</td>
</tr>
<tr>
<td>MCV</td>
<td>79.7 µ</td>
</tr>
<tr>
<td>MCHC</td>
<td>27.7%</td>
</tr>
</tbody>
</table>

Treatment with Anahaemin injections alone had been continued for nearly five years.

Comment. It is possible that a final diagnosis could be made in this case by a differential urinary folic acid excretion test, and it is unfortunate that the patient is unwilling to have it done. The test could be carried out "by post", but the patient is not sufficiently intelligent for one to be certain that the urinary collections would be complete or the second bottle uncontaminated with urine from the first twenty-four hour collection.
The most likely possibility is that the patient has idiopathic steatorrhoea and that the capacity of the small intestine to absorb haemopoietic factors varies from time to time. Hence the iron deficiency was persistent but the red cell level fell considerably only at a time when there was malabsorption of folic acid substances in the food, and perhaps also of dietetic vitamin B_{12}. Alternatively there might have been temporarily a marked change in the intestinal flora that led to depletion of antimegaloblastic substances. It is possible, but unlikely, that the patient had a combination of 'refractory iron deficiency anaemia' with true pernicious anaemia or of idiopathic steatorrhoea with the latter.

**Case 303.** (Graphs 59a,b,c,d.)

**Idiopathic steatorrhoea showing a varying response to treatment.**

A man born in 1878 who served in the Regular Army from 1896 to 1920 and was in India in 1901-1906 where he had an attack of acute bacillary dysentery but no other diarrhoeal disease. In 1914, when in Britain, he developed diarrhoea with loose pale stools. Thereafter he had frequent exacerbation of the symptoms, sometimes associated with sore tongue. He was admitted to hospital in 1940 with megaloblastic anaemia and a diagnosis of pernicious anaemia was made. There was considered to be histamine fast achlorhydria. The blood levels were:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin</td>
<td>9.6 G. per 100 ml.</td>
</tr>
<tr>
<td>Red cells</td>
<td>2,550,000 per c.mm.</td>
</tr>
<tr>
<td>C.I.</td>
<td>1.28</td>
</tr>
<tr>
<td>PCV</td>
<td>29%</td>
</tr>
<tr>
<td>MCV</td>
<td>113.7 cu.</td>
</tr>
<tr>
<td>MCHC</td>
<td>33.1%</td>
</tr>
<tr>
<td>White cells</td>
<td>2,800 per c.mm.</td>
</tr>
<tr>
<td>Reticulocytes</td>
<td>&lt; 1%</td>
</tr>
</tbody>
</table>
As can be seen in Graph 59a there was a response to treatment with Anahaemin and the patient was discharged from hospital.

During the next four years he continued to have 4 ml. of Anahaemin intramuscularly weekly but diarrhoea continued. The general condition was poor and in March 1944 the patient was readmitted to hospital. Free hydrochloric acid was now shown to be present in the gastric juice and the blood levels at the time of readmission were haemoglobin 8.8 G. per 100 ml., red cells 2,530,000 per c.mm., C.I. 1.18, white cells 5,200 per c.mm., reticulocytes < 1%. A diagnosis of steatorrhoea was made but in view of the history it was uncertain whether or not it should be considered to be tropical sprue.
As can be seen in Graph 59b there was a haematological response to the treatment given, which consisted of dieto-therapy together with Anahaemin injections and vitamin preparations given by mouth. The patient was discharged in April 1944 on weekly injections of Anahaemin but was readmitted in October of the same year in relapse. The marrow was normoblastic.

He improved with treatment in hospital, was discharged in November 1944, and continued to have 4 ml. of Anahaemin weekly.

In July 1945 he came to hospital again with diarrhoea and general features of anaemia. The blood levels were haemoglobin 8.6 G. per 100 ml.; red cells 2,450,000 per c.mm., C.I. 1.18.
It will be seen from Graph 59c that three injections of Campolon had little effect, but that there was a good response to treatment with proteolysed liver. A return to liver injections resulted in a relapse but there was improvement again when a liquid liver preparation was given by mouth.

Once more Anahaemin was tried and relapse occurred. In January 1947 the patient was readmitted with diarrhoea and anaemia. He was having two or three pale loose bulky stools daily. A fat balance test was now possible and it showed 78.3% absorption. The marrow was essentially normoblastic but proerythroblasts and early normoblasts were numerous. The blood levels were haemoglobin 10.7 G. per 100 ml., red cells 2,680,000 per c.mm., C.I. 1.33, PCV 32.5%, MCV 121.3 cu., MCHC 32.9%, white cells 7,200 per c.mm., reticulocytes < 1%. On this/
This occasion the treatment was with pteroylglutamic acid by mouth.

It will be seen from Graph 59d that there was an excellent and sustained haematological response to treatment, the red cell count rising to 5,400,000 per c.mm. Diarrhoea ceased and the patient felt very well. Unfortunately, however, he died suddenly of a cerebral haemorrhage in May 1948. Just prior to his death the blood levels were haemoglobin 18.1 G. per 100 ml., red cells 5,440,000 per c.mm., PCV 52%, MCV 95.5 cμ., MCHC 34.5%.

Comment. A patient with idiopathic steatorrhoea (or, possibly, tropical sprue) who showed successive responses to Anahaemin, proteolysed liver, a liquid liver preparation taken by mouth and pteroylglutamic acid taken by mouth.

Case/
Case 304. (Graph 60)

**Idiopathic Steatorrhoea, believed at first to be Idiopathic Refractory Megaloblastic Anaemia**

This was a 63 year old female patient who was admitted to hospital in November 1946 complaining of general weakness of nearly two years' duration. For about a year she had had shooting pains in the back of the neck, the chest, the shoulders and, to a lesser extent, the arms. The patient had had diarrhoea off and on for two years. The stools had not been pale, but she had been taking an iron preparation regularly.

The general state of nutrition of the patient was poor and the tongue was smooth, shiny and atrophic. The stool was pale and watery. Apart from evidence of Parkinsonism no other clinical abnormality was found. The patient weighed 80 lbs. The blood urea level was normal. A test meal showed hydrochloric acid to be present in the gastric contents after the administration of histamine. The stool benzidine reaction was usually negative, but was positive on one occasion.

At the time of admission the bone marrow was megaloblastic and the blood levels were:

- **Haemoglobin**: 5.6 G. per 100 ml.
- **Red cells**: 1,450,000 per c.mm.
- **C.I.**: 1.31
- **PCV**: 16%
- **MCV**: 110.3 cu.
- **MCHC**: 35%
- **White cells**: 3,000 per c.mm.
- **Reticulocytes**: < 1%

The/
Graph 60.

The graph included here as Graph 60 was the one used by the present author in the United States for lecturing purposes. It will be seen that there was a reticulocyte response on two occasions to injections of liver extract (2 ml. of NeoHepatex and 2 ml. of a B.D.H. liver extract respectively), but that no significant haematological change occurred apart from this. There was, however, a good response to pteroylglutamic acid given by mouth. Diarrhoea persisted, and a fat balance test done over a period of three days on a 50 G. daily intake showed 21.4% total fat and 89.7% absorption. It was considered that there was therefore no definite evidence of poor absorption of fat, and a diagnosis of idiopathic refractory megaloblastic anaemia was made. According to the records the present/
present author was of that opinion at the time.

It seems obvious now that the true diagnosis was steatorrhoea, probably idiopathic and indeed a subsequent fat balance test showed 85.4% fat absorption. Possibly our knowledge of the interpretation of a fat balance test was inadequate in 1946.

Comment. Steatorrhoea with megaloblastic anaemia, believed at the time during which the patient was in hospital to be idiopathic refractory megaloblastic anaemia. It will be noted that liver injections produced a reticulocyte response without any change in the marrow picture. This may conceivably have been because of a dual deficiency of vitamin B₁₂ and folic acid. Vitamin B₁₂ may perhaps have been able to produce some reticulocytes without, in the absence of folic acid, being able to cause the marrow to become normoblastic.

Case 305. (Graph 61).

Refractory Megaloblastic Anaemia with Gastric Carcinoma

This female patient was first admitted to a ward of the Royal Infirmary of Edinburgh in November 1945 at the age of 61. The story was that seven years previously she had begun to have frequent fainting attacks and that her doctor had given her liver injections (NeoHepatex) for bloodlessness once every three or four weeks, but that she did not improve. Accordingly the doctor gave injections of Hepalon, an allegedly "crude" liver extract, for about a year at three weekly intervals. Just before admission the patient was receiving daily injections of this liver preparation, but they had no beneficial effect. The appetite was poor and tongue was red and sore. There was no/
no diarrhea, and loss of weight was not a feature of note. There is no record of a stool benzidine test having been done, and it seems that a test meal was not carried out.

The blood levels on admission, despite the therapy referred to above, were

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin</td>
<td>3.5 G. per 100 ml.</td>
</tr>
<tr>
<td>Red cells</td>
<td>860,000 per c.mm.</td>
</tr>
<tr>
<td>C.I.</td>
<td>1.28</td>
</tr>
<tr>
<td>PCV</td>
<td>9%</td>
</tr>
<tr>
<td>MCV</td>
<td>104.7 cu.</td>
</tr>
<tr>
<td>MCHC</td>
<td>36.4%</td>
</tr>
<tr>
<td>White cells</td>
<td>2,800 per c.mm.</td>
</tr>
<tr>
<td>Reticulocytes</td>
<td>&lt; 1%</td>
</tr>
</tbody>
</table>

The marrow was megaloblastic.

It will be seen from Graph 61 that three pints of blood were transfused and that this together with three injections
of 4 ml. of Anahaemin raised the blood levels to haemoglobin 6.2 per 100 ml., red cells 1,740,000 per c.mm. The marrow remained megaloblastic although there was some nuclear condensation. There was, however, a good response to the liquid liver extract Hepatex Oral given by mouth.

The patient was discharged on 1.1.46, having Anahaemin by injection in a dosage of 2 ml. weekly and this was later supplemented by ferrous sulphate given by mouth. She complained of a feeling of sickness in 1946 and continued to do so throughout the follow up period at the Blood Clinic. An abstract of the main subsequent findings and treatment is perhaps best recorded in tabular form (Table 57).
TABLE 57.

Abstract of Follow up of Case 305.

<table>
<thead>
<tr>
<th>Date</th>
<th>Hb. G/100</th>
<th>RBC 100 Mills/ c.mm.</th>
<th>Treatment</th>
<th>Date of such Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>3. 7.46</td>
<td>10.4 1428</td>
<td>Anahaemin 2 ml. weekly</td>
<td>1. 1.46 - 5. 3.48</td>
<td></td>
</tr>
<tr>
<td>4.12.46</td>
<td>13.0 3.76</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. 3.47</td>
<td>12.1 3.96</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24. 7.47</td>
<td>12.1 3.89</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. 3.48</td>
<td>12.1 1428</td>
<td>Plexan 4 ml. 2wkly.</td>
<td>5. 3.48 - 13. 1.49</td>
<td></td>
</tr>
<tr>
<td>16. 3.48</td>
<td>Barium enema negative. (diarrhoea)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>29. 9.48</td>
<td>8.3 2.21</td>
<td>No diarrhoea but petechiae. Platelets 120,000 Bleeding time 1 minute. No subsequent petechiae noted.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18.10.48</td>
<td>9.2</td>
<td>Anahaemin 4 ml. 3wkly.</td>
<td>13. 1.49 - 24. 4.50</td>
<td></td>
</tr>
<tr>
<td>13. 1.49</td>
<td>16.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>31.10.49</td>
<td>13.3</td>
<td>24. 4.50 Barium meal negative Anahaemin 4 ml. 2wkly.</td>
<td>24. 4.50 - 7.10.50</td>
<td></td>
</tr>
<tr>
<td>10.11.49</td>
<td>Barium meal negative</td>
<td>Cyanocobalamin 40 µg. 2 wkly.</td>
<td>7.10.50 - 15. 1.53</td>
<td></td>
</tr>
<tr>
<td>24. 4.50</td>
<td>11.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.10.50</td>
<td>12.3 4.77</td>
<td>Cyanocobalamin 50 µg. 2 wkly.</td>
<td>15. 1.53 - 31. 10.53</td>
<td></td>
</tr>
<tr>
<td>11. 4.51</td>
<td>15.4 5.12</td>
<td>Ankle jerks absent. PCV 43%, MCV 84 cu.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30.10.51</td>
<td>15.0 4.77</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. 2.52</td>
<td>Barium meal negative.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8. 9.52</td>
<td>17.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. 1.53</td>
<td>12.7 5.32</td>
<td>Cyanocobalamin 50 µg. 2 wkly.</td>
<td>15. 1.53 - 31. 10.53</td>
<td></td>
</tr>
<tr>
<td>25. 3.53</td>
<td>Barium meal negative</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15. 6.53</td>
<td>12.6 4.71</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9. 9.53</td>
<td>10.4 4.05</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.11.53</td>
<td>Laparotomy; inoperable gastric adenocarcinoma with widespread secondary spread, forming a large mass involving particularly the omentum.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
It will be seen from Table 57 that on one occasion there was diarrhoea but this lasted for only one or two weeks. At the next visit there were petechiae and thrombocytopenia, but no further evidence of petechiae was noted at later visits. Altogether this is a most unusual case, and perusal of the records suggest that although the patient was seen many times in the course of eight years by at least twelve physicians and examined by most of them, certain elementary side-room investigations were not done. The case was taken over by the Blood Clinic from one of the hospital wards and it was assumed that a test meal had been done there, when in fact, it had not. When the patient was in hospital the first time, a stool benzidine examination was not carried out. It was not done when the patient was attending the Blood Clinic because X-rays of the alimentary tract revealed no lesion. In the light of later developments it seems that stool benzidine examinations should have been done and that there are times when this examination may be more helpful than a series of X-rays of the alimentary tract. It may be added that when the patient was admitted to Ward 27 for transfer to a Surgical Ward, the stool benzidine reaction was strongly positive and there was histamine fast achlorhydria.

It will be noted from Table 57 that in 1943 the patient developed first diarrhoea and then purpura with a low platelet count and should have been admitted then, particularly since there was a marked fall in the blood counts at the time. The present author was in America at the time and does not know exactly what happened, but it may be that, as on a later occasion/
occasion, the patient refused to be admitted. The clinical features in 1948 were such that a fat balance test, stool benzidine test, barium follow through, and sternal marrow examination were indicated. It should be noted that the general condition was good even as late as June 1953, and that although a mass was palpable in the left hypochondrium in October 1953, it was not felt in June. It is true that sickness was a feature for several years but although the barium meals were always negative as regards carcinoma, there was a large duodenal diverticulum shown each time, and this was thought to be responsible for the symptoms. There is no mention in the operation notes as to whether this was present. Indeed the opening sentence of the history in the notes made in the Surgical Ward begins "Patient's history goes back four weeks . . . . ."

It is difficult to make any definite statement about the cause of this patient's anaemia. In 1945 there was megaloblastic anaemia which seemed to be refractory to both Hepalon and Anahaemin but the vitamin B12 potency of the former is very doubtful and there were many batches of Anahaemin of low potency about that time. The alternatives in 1945 would seem to be (1) that the patient had pernicious anaemia that was being treated with non-potent liver extracts, or (2) that she had idiopathic refractory megaloblastic anaemia or even idiopathic steatorrhoea and the gastric carcinoma was incidental, or (3) that a gastric carcinoma was then present. If there was a gastric carcinoma present then, it must be taken that it was/
was present for at least eight years and that despite this, the anaemia later responded to Anahaemin and to cyanocobalamin. In Chapter 18 an account is given of apparent folic acid depletion of the body in malignant disease, but when such depletion occurs from this cause it is advanced malignancy that is present. It is not obvious why a gastric carcinoma that was capable of continuing to grow for a further eight years without leading to obvious deterioration in the general condition should have caused the patient to be refractory to Anahaemin then or why the patient should later have responded to the same form of treatment and to cyanocobalamin. If gastric carcinoma ever causes megaloblastic anaemia by destroying the intrinsic factor bearing area, then at a time when it is localised to the stomach the patient should theoretically respond to parenteral cyanocobalamin therapy in the same way as does a patient with uncomplicated pernicious anaemia. In this present case it seems possible that the course of events was:

In 1945 the patient had uncomplicated pernicious anaemia. This was first treated with injections of non-potent liver extracts, but there was later a response to oral Hepatex, possibly because of its content of folic acid substances. Anahaemin which was more potent than the previous Hepalon was then given by injection and the patient's condition remained good until a less potent batch of Plexan was used. A return to Anahaemin therapy resulted in a remission and then cyanocobalamin injections were given with success, but gastric carcinoma developed.

Comment.
Comment. A puzzling case of refractory megaloblastic anaemia in which gastric carcinoma was eventually shown to be present. The mechanism of production of the anaemia is uncertain, but a hypothesis about this is put forward for consideration.

Case 306. (Graph 62)

Refractory Megaloblastic Anaemia due to Gastric Carcinoma

This 42 year old female patient was admitted to hospital in June 1950 because she was believed to have pernicious anaemia that had become refractory to treatment with liver injections. The original diagnosis had been made in Ward 27 of Edinburgh Royal Infirmary in February 1944. At the time there was histamine fast achlorhydria, a megaloblastic marrow and a haemoglobin level of 5.3 G. per 100 ml. with a red cell count of 1,600,000 per c.mm. At the time before any treatment was given, the patient developed an anginal condition of the throat with high fever and leucopenia. Despite this there was a good general and haematological response to treatment with blood transfusions and NeoHepatex. Thereafter treatment was with Anahaemin first in a dosage of 4 ml. every three weeks, then, because of reactions, in a dosage of 2 ml. every three weeks. This was later increased to 2 ml. every two weeks.
As can be seen from Graph 62 the blood levels six months before the second admission to hospital were haemoglobin 12.9 G. per 100 ml., red cells 4,360,000 per c.mm. At this time there was rather vague epigastric pain and a barium meal showed some deformity of the duodenal cap but no definite ulcer. Some irregularity was noted in the pyloric region. Treatment with Anahaemin was continued, but the general condition deteriorated and it was for this reason that the patient was admitted again on 14/6/50. The blood levels three days later were:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin</td>
<td>10.5 G. per 100 ml.</td>
</tr>
<tr>
<td>Red cells</td>
<td>3,340,000 per c.mm.</td>
</tr>
<tr>
<td>C.I.</td>
<td>1.22</td>
</tr>
<tr>
<td>PCV</td>
<td>31.5%</td>
</tr>
<tr>
<td>MCV</td>
<td>94 cu.</td>
</tr>
<tr>
<td>MCHC</td>
<td>33.3%</td>
</tr>
<tr>
<td>White cells</td>
<td>4,800 per c.mm.</td>
</tr>
<tr>
<td>Reticulocytes</td>
<td>&lt; 1%</td>
</tr>
</tbody>
</table>

The marrow was megaloblastic.
There was no response to two injections of cyanocobalamin. The marrow remained megaloblastic. A test dose of 5 mg. of pteroylglutamic acid was given, and this resulted in the excretion of 1.16 mg. of folic acid in the urine, an abnormally low figure for a patient with pernicious anaemia under adequate treatment.

There was a strongly positive stool benzidine reaction and an X-ray of the stomach suggested a scirrhous type of carcinoma of the stomach. The patient was transferred to a Surgical Ward and died two days after a partial gastrectomy operation had been done. The carcinoma had spread throughout the peritoneum and into the liver.

A brother of the patient who had pernicious anaemia developed gastric carcinoma and died seven days after a partial gastrectomy operation.

Comment. A patient who probably developed gastric carcinoma while under treatment for pernicious anaemia rather than megaloblastic anaemia because of gastric carcinoma, since the neoplasm was antral in origin. The refractoriness to Ananaemin or cyanocobalamin therapy may have been due to folic acid deficiency.

Case 307. (Graph 63)

Refractory Megaloblastic Anaemia due to Gastric Carcinoma

A 70 year old female patient who was admitted to Ward 27 of the Royal Infirmary of Edinburgh in March 1947 with a four years history of diminution of strength and energy, with loss of appetite and pain in the tongue. Her general practitioner diagnosed pernicious anaemia and treated the patient first with/
with injections of Hepalon and later with Anahaemin. This caused a distinct improvement but four months prior to admission the general condition deteriorated and the patient obviously began to lose weight. A barium meal examination carried out privately at this stage suggested gastric carcinoma. The patient was referred to the Blood Clinic in February 1947 and a barium meal carried out then did not give evidence of carcinoma. The weight was 88 lbs. and the patient was in very poor general condition. No mass could be palpated. The blood levels were:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin</td>
<td>6.8 G. per 100 ml.</td>
</tr>
<tr>
<td>Red cells</td>
<td>1,830,000 per c.mm.</td>
</tr>
<tr>
<td>C.I.</td>
<td>1.26</td>
</tr>
<tr>
<td>PCV</td>
<td>19%</td>
</tr>
<tr>
<td>MCV</td>
<td>103.8 cp.</td>
</tr>
<tr>
<td>MCHC</td>
<td>35.8%</td>
</tr>
<tr>
<td>White cells</td>
<td>2,000 per c.mm.</td>
</tr>
<tr>
<td>Reticulocytes</td>
<td>&lt; 1%</td>
</tr>
</tbody>
</table>

The patient's doctor continued to give Anahaemin injections, and on 7th March admission to Ward 27 took place. The haemoglobin level was 4.7 G. per 100 ml. and the red cell count had fallen to 1,400,000 per c.mm., the PCV now being 15%. The marrow was megaloblastic.

The subsequent course of events is shown in Graph 63.
601.

Case 307. Refractory Megaloblastic Anaemia due to gastric carcinoma.

Graph 63.

It will be seen from Graph 63 that there was a slight response to an injection of 4 ml. of a batch of Anaemia that had been successfully used in the ward for the treatment of uncomplicated pernicious anaemia. Meantime it was established that there was histamine fast achlorhydria, and that the stool benzidine reaction was strongly positive. There was some doubt as to whether or not a mass could be felt in the epigastrium, but the patient was seen by a surgeon who considered that, despite the negative barium meal examination, the patient had inoperable carcinoma of the stomach. It will be seen from Graph 63, however, that there was a response to pteroylglutamic acid given by mouth and that thereafter the blood levels were maintained by liver injections until the patient died of her gastric carcinoma in February, 1950.

Comment. It seems likely that patients with gastric carcinoma/
carcinoma become refractory to liver therapy because they develop folic acid deficiency. The alternative and less likely explanation in this case would seem to be that there was a combination of vitamin B<sub>12</sub> depletion and increased requirements for vitamin B<sub>12</sub> and that this could not be satisfied by the amount of vitamin B<sub>12</sub> in Anahaemin treatment in the war years, but pteroylglutamic acid could compensate for vitamin B<sub>12</sub> depletion by "mass action".

Case 308. (Graph 64)

**Refractory Megaloblastic Anaemia which may have been Pernicious Anaemia that was being treated with Non-Potent Liver Extracts**

A 53 year old male patient who was admitted to Ward 26, Royal Infirmary of Edinburgh in March 1947, with severe anaemia.

The story was that he had been diagnosed in 1931 by his general practitioner as having pernicious anaemia at the age of 37 and was treated first with liver by mouth but when Anahaemin became available he was given this by injection in a dosage of 2 ml. once every two or three weeks.

About eight weeks prior to admission the patient became easily tired and felt very breathless despite his liver injections and was referred to the Blood Clinic. It should, however, be stated that a month before he was seen there, the patient had been discharged to his home after spending a month in a Surgical Ward of Edinburgh Royal Infirmary with an abscess of the buttock and that he was given penicillin but not his liver injections in hospital.

When the patient was first seen at the Blood Clinic on 5th February, 1947, the haemoglobin level was 11.8 G. per 100 ml. and the red cell count was 3,510,000 per c.mm., the colour/
Anæmia was then given in a dosage of 4 ml. weekly. Graph 64 was used to illustrate a lecture given by the author in the United States of America, and it will be seen that this was then considered to mean that the patient received 60 units of liver extract intramuscularly each week. The patient's condition deteriorated rapidly and when he was admitted to hospital on 29th March, 1947, the blood levels were:

- Haemoglobin: 8.0 G. per 100 ml.
- Red cells: 2,030,000 per c.mm.
- C.I.: 1.3
- PCV: 23%
- MCV: 113.3 cµ.
- MCHC: 34.8%
- White cells: 3,800 per c.mm.
- Reticulocytes: < 1%

The marrow was megaloblastic. There was histamine fast achlorhydria.
achlorhydria and the stool benzidine reaction was negative. The patient was given 4 ml. of a batch of Anaheimin that had been shown to induce a remission in a patient with uncomplicated pernicious anaemia but no improvement resulted. Indeed a blood transfusion was required. There was, however, a dramatic response to treatment with pteroylglutamic acid given by mouth following a dose of 30 mg. given by intramuscular injection on the first day of this form of therapy.

While the patient was in hospital and under this treatment he developed what was diagnosed as mild peripheral neuritis with paraesthesiae and loss of knee jerks. A three day fat balance test on a 50 G. intake showed 97% absorption of fat.

The subsequent cause of the illness may perhaps best be summarised in tabular form (Table 58).
Abstract of Follow up of Case 308.

<table>
<thead>
<tr>
<th>Date</th>
<th>Hb.</th>
<th>RBC</th>
<th>Treatment</th>
<th>Date of such Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>24.</td>
<td>7.47</td>
<td>15.7</td>
<td>Pteroylglutamic acid 5 mg. daily by mouth</td>
<td>3. 5.47 - 16. 1.48</td>
</tr>
<tr>
<td>16.</td>
<td>1.48</td>
<td>17.8</td>
<td>Pteroylglutamic acid 30 mg. daily by mouth</td>
<td>17. 1.48 - 16. 2.48</td>
</tr>
<tr>
<td>16.</td>
<td>2.48</td>
<td>16.8</td>
<td>Peripheral neuritis very troublesome</td>
<td>17. 2.48 - 23. 2.49</td>
</tr>
<tr>
<td>16.10.48</td>
<td></td>
<td>16.3</td>
<td>Peripheral neuritis worse</td>
<td>20 mg. daily by mouth</td>
</tr>
<tr>
<td>23.</td>
<td>2.49</td>
<td>16.6</td>
<td>Paraesthesiae troublesome. Loss of vibration sense up to D8. Ankle jerks still absent. Extensive litigation - received compensation.</td>
<td>23. 2.49 - 28. 9.50</td>
</tr>
<tr>
<td>3.8.49</td>
<td>16.6</td>
<td></td>
<td>Bilateral extensor plantar responses. Campolon 4 ml.</td>
<td>23. 2.49 - 23. 3.49</td>
</tr>
<tr>
<td>28.</td>
<td>9.50</td>
<td>16.7</td>
<td>Campolon Forte 2 ml. weekly</td>
<td>28. 9.50 - 22. 3.51</td>
</tr>
<tr>
<td>22.</td>
<td>3.51</td>
<td>15.1</td>
<td>Cyanocobalamin 100 µg. weekly</td>
<td>22. 3.51 - 18. 7.51</td>
</tr>
<tr>
<td>13.</td>
<td>7.51</td>
<td>15.8</td>
<td>Bilateral plantar flexor plantar responses. Vibration sense absent up to knees.</td>
<td>18. 7.51 - 23.11.53</td>
</tr>
<tr>
<td>16.</td>
<td>5.52</td>
<td>16.7</td>
<td>Cyanocobalamin 100 µg. 3 wkly.</td>
<td>18. 7.51 - 23.11.53</td>
</tr>
<tr>
<td>23.11.53</td>
<td>14.2</td>
<td>5.38</td>
<td></td>
<td>23.11.53 - 28. 9.50</td>
</tr>
</tbody>
</table>
The patient was seen by various doctors at the Blood Clinic after the present author sailed for the United States in 1948 and was not seen by him again until March 1951. Meantime there had been extensive litigation which sought to prove that the neurological complications that developed in 1947 and which were very severe in 1948 were the result of an accident that led to the admission of the patient to a Surgical Ward in 1946 for treatment of an abscess following a haematoma of the buttocks. Apparently it was agreed that the patient suffered psychological trauma as a result of the accident and compensation was paid. A note from a relative in November 1948 states "We have to sit with him night and day and the nerves in his legs are now affected. He is unable to feed himself as he has not the use of his hands and cannot bear anyone touching them. It is, in fact, a case of being helpless, everything having to be done for him, his whole body being affected. He is unable to get out of bed".

It was considered that this was due to mild peripheral neuritis but that there was marked superadded psychological overlay. In February 1949, however, there was definite clinical evidence of changes in the posterior and lateral columns of the cord. Intensive treatment with liver injections and later with cyanocobalamin was given and in July 1951 the patient was working seven shifts a week as a miner and walking two miles each way to and from work. In 1954 there is no complaint of paraesthesiae, vibration sense is absent below the knees, the ankle jerks are absent and there is a suggestion of spasticity in the gait. The plantar responses are bilaterally plantar flexor. The patient is having an anti-obesity/
anti-obesity diet.

Comment. The main problem here concerns the correct diagnosis. Idiopathic steatorrhoea has not been satisfactorily excluded since only a three day fat balance test was done. It is unlikely, however, that the patient suffers from this. Apart from the absence of diarrhoea, there are the further points that the patient developed subacute combined degeneration of the cord and that he has been maintained satisfactorily on cyanocobalamin injections for several years. It is difficult to suggest any diagnosis other than Addisonian pernicious anaemia. This would mean that in 1946 and 1947 the patient was being treated with non-potent Anahaemin. One batch of Anahaemin marketed in 1947 that was tested by us contained only 0.9 µg. of vitamin B\textsubscript{12} per ml. (L.\textit{leichmannii} assay). If such a batch was being given the patient would in fact be receiving only 3.6 µg. weekly from his 4 ml. weekly injection and the test dose of 4 ml. given in Ward 27 after admission may not have been sufficient to induce remission.

Case 309. (Graph 66)

Refractory Megaloblastic Anaemia requiring further investigation

A 34 year old female patient was admitted to a ward of Edinburgh Royal Infirmary in August 1947 eleven weeks after the birth of her first child. The story was that she had been found to be anaemic at the time of labour and had remained so despite liver injections given empirically in pregnancy. Transfusions totalling four pints of blood were given at the time/
time of the birth.

On the subsequent admission to Edinburgh Royal Infirmary in August, 1947, an immediate transfusion of two pints of blood was given because the haemoglobin level was only 3 G. per 100 ml. Three days later the blood levels were found to be:

- Haemoglobin: 3 G. per 100 ml.
- Red cells: 730,000 per c.mm.
- C.I.: 1.37
- White cells: 4,000 per c.mm.
- Reticulocytes: 5.2%
- Platelets: 120,000 per c.mm.

The marrow was reported by Miss Lindsay, Professor Davidson's chief haematological technician, as being completely normoblastic. No further information is available to enable a decision to be made as to whether or not there was evidence of haemolysis at that time.
A rapid haematological remission coincided with the administration of pteroylglutamic acid as shown in Graph 65, and a diagnosis of megaloblastic anaemia of pregnancy was made.

All therapy was stopped on 1st September, 1947, and the patient was well when she was seen as an outpatient in February 1948. In February 1949 when the present author was in the United States the patient was admitted to Ward 27 with severe anaemia and a megaloblastic marrow. The blood levels were:

| Haemoglobin | 3.6 G. per 100 ml. |
| Red cells   | 880,000 per c.mm. |
| C.I.        | 1.36              |
| PCV         | 9%                |
| MCV         | 102.3 cu.         |
| MCHC        | 40%               |
| White cells | 4,200 per c.mm.   |
| Reticulocytes | 2.4%            |

No spherocytes were seen, and the blood film had characteristics similar to those found in megaloblastic anaemia in relapse. A blood transfusion was given, and this was followed first by 4 ml. of Anahaemin by injection, then 4 ml. of Neo-Hepatex. The marrow remained megaloblastic. By microbiological assay (L. leichmannii) we have found that 4 ml. of the batch of Anahaemin used supplied 16.0 µg. of vitamin B₁₂ (including B₁₂β) and that 4 ml. of the batch of NeoHepatex used supplied 10 µg. of vitamin B₁₂ (including B₁₂β). This would be expected to produce a remission in uncomplicated pernicious anaemia but, in fact, the haemoglobin level fell to 2.3 G. per 100 ml. and the red cell count to 900,000 per c.mm. There was, however, a good response to pteroylglutamic acid given by mouth after transfusion. The opportunity was taken to perform a three day fat balance test on a 50 G. intake and this showed 92% absorption of fat. A test meal showed histamine fast achlorhydria.
achlorhydria, and the stool benzidine reaction was repeatedly negative.

In December 1951 the patient was readmitted to hospital on account of pyelitis. There was no evidence of impaired renal function.

The blood counts had been reasonably well maintained with pteroylglutamic acid therapy (although it must be noted that the physician supervising the case was usually content to have only a haemoglobin estimation carried out). The levels were now:

<p>| | | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin</td>
<td>13.5 G. per 100 ml.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Red cells</td>
<td>4,980,000 per c.mm.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C.I.</td>
<td>0.91</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCV</td>
<td>37%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCV</td>
<td>74.2 fl.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCHC</td>
<td>30.5 fl.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White cells</td>
<td>5,000 per c.mm.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reticulocytes</td>
<td>&lt; 1%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The blood film was normal.

There was no diarrhoea, the red cell fragility was normal and the Coombs' test (direct) was negative. There was no reticulocytosis.

Treatment was changed to cyanocobalamin by injection and two years later the patient was well and the blood counts were: haemoglobin 12.3 G. per 100 ml., red cells 4,420,000 per c.mm.

Comment. This is a difficult case to interpret, and it is one that requires further investigation. Unfortunately, however, the patient has already been under treatment for an anxiety state and is anxious not to be readmitted to hospital. The diagnosis seems to rest between haemolytic anaemia that cannot/
cannot be shown by the usual, simpler tests, pernicious anaemia, idiopathic steatorrhoea without diarrhoea and idiopathic vitamin B₁₂-refractory megaloblastic anaemia that ceased to be refractory in 1951. The objection to a diagnosis of pernicious anaemia would seem to be that in February and March 1949, no response occurred to two liver injections which according to our calculations supplied 26 µg. of vitamin B₁₂. In any future case of this type, a much larger dose of cyanocobalamin could and should be administered. An extended fat balance test, a glucose absorption test and a differential urinary folic acid absorption test might be of help in the diagnosis of this case, but as the blood counts have remained satisfactory for two years with injections of cyanocobalamin, this suggests that folic acid substances are now being absorbed satisfactorily. If all these tests could be done and if they proved negative, investigations for the more atypical forms of haemolytic anaemia with, for instance, tests for warm and cold agglutinins and haemolysins might be done. If these were negative, cross transfusion experiments with cell survival studies might be considered. All this, however, is theoretical. The patient has been under treatment for an anxiety state at Jordanburn Nerve Hospital, now feels very well and would certainly not benefit from the further extensive investigations listed above.

Case 310. (Graph 66)

Refractory Megaloblastic Anaemia possibly due to Idiopathic Steatorrhoea

This 44 year old female patient was seen by Dr. J.G.M. Hamilton at Ward 27, Royal Infirmary of Edinburgh, on 13th June/
June, 1952, on account of anaemia associated with recurrent epistaxes. Dr. Hamilton noted that the tongue was smooth and the nails flat. The blood levels were:

- Haemoglobin: 4.4 G. per 100 ml.
- Red cells: 2,700,000 per c.mm.
- C.I.: 0.55
- White cells: 6,600 per c.mm.
- Reticulocytes: < 1%

The film showed that the red cells were hypochromic, that the white cells were normal and that platelets were plentiful.

The menopause had occurred two years previously and there was no known source of blood loss. An oral iron preparation had been taken for a month and this treatment was continued.

Exactly two weeks later the patient was sent for admission with a haemoglobin reading of 3.0 G. per 100 ml. and a red cell count of 1,020,000 per c.mm. The colour index was now 1.0. The marrow was megaloblastic.

There was histamine fast achlorhydria. The patient had not suffered from diarrhoea, and has not done so subsequently. Liver function tests did not give evidence of hepatic disease. It is noteworthy, however, that a test of prothrombin activity was not done. The diet had been very bad for six months before admission and poor even before that. A typical day's diet just before admission was:

- **Breakfast:** Slice of toast; cup of tea.
- **Lunch:** Soup, potatoes, pudding.
- **Tea:** Tomatoes, bread.
- **Supper:** Cup of cocoa.
Marrow of Case 310 after cyanocobalamin therapy.
(Megaloblastic)

Marrow of Case 311 after cyanocobalamin therapy.
(Megaloblastic)
The diagnosis was not obvious and, after blood transfusion, treatment with injections of cyanocobalamin was tried without success, the marrow remaining megaloblastic.

A differential urinary folic acid excretion test was carried out. The results were as follows:

Urinary excretion
in 24 hours after 5 mg. subcutaneously 1.92 mg.
in 24 hours after 5 mg. orally 1.23 mg.

This was interpreted as being negative, in that the excretion after the dose given orally, although less than the normal output after a 5 mg. test dose, was not so very different from that after the subcutaneous dose. It was thought at the time that/
that megaloblastic anaemia associated with folic acid deficiency would not occur when there was a normal output after 5 mg. given subcutaneously. Later work showed, however, that this premise is not necessarily true (p. 384).

At this stage the present author went on holiday and apparently further investigations for intestinal malabsorption were not carried out. There was a good response to treatment with pteroylglutamic acid and the patient was discharged from hospital. The blood counts remained high with pteroylglutamic acid therapy.

In February 1953, however, it was felt that, although there was no evidence of neurological disturbance, fuller investigations would be helpful. The patient who was feeling in the best of health was willing to be re-admitted, provided it was only for a very few days, for further tests of intestinal absorption. The results were:

**Fat Balance Test.** (3 days on 75 G. intake).

98.3% absorption. 35% of the fat was split.

**Oral Glucose Tolerance Test.**

The blood sugar level rose from a fasting level of 79 mg. per 100 ml. to a peak of 145 mg. per 100 ml. one hour after the oral dose of 50 G. of glucose.

**Differential Urinary Folic Acid Excretion Test.**

<table>
<thead>
<tr>
<th>Urinary Excretion</th>
<th>PGA</th>
<th>CF</th>
</tr>
</thead>
<tbody>
<tr>
<td>in 24 hours after 5 mg. subcutaneously</td>
<td>2.55</td>
<td>10.3</td>
</tr>
<tr>
<td>in 24 hours after 5 mg. orally</td>
<td>2.99</td>
<td>19.8</td>
</tr>
</tbody>
</table>

There was thus no evidence of intestinal malabsorption by the tests employed. The stool benzidine reaction was again negative and there was histamine fast achlorhydria. Treatment was/
was changed to injections of cyanocobalamin, and a deterioration occurred in the haemoglobin reading and red cell count. The serum vitamin B₁₂ level was checked from time to time. It remained high.

Comment. The only suggestion of intestinal malabsorption in this case arises from an equivocal result of the first differential urinary folic acid excretion test. Nevertheless the sudden change from refractory iron deficiency anaemia to refractory megaloblastic anaemia suggests that this was the correct diagnosis, and possibly the recurrent epistaxes were due to a low prothrombin content of the blood. Although there was no history of diarrhoea or of coeliac disease, the patient who weighed 104 lbs. at her first admission was only 4 ft. 11½ ins. in height. If this diagnosis is correct, then the results of the second differential urinary folic acid excretion test suggest that it is possible for a patient to have megaloblastic anaemia due to malabsorption of folic acid substances in the food and yet be able to absorb normally a test dose of 5 mg. of pteroylglutamic acid. It may be, however, that a remission induced by pteroylglutamic acid may lead to improved absorption of many substances including folic acid itself. Although a short spell of treatment with pteroylglutamic acid does not improve fat absorption, it has been shown by Badenoch (1952) that longer periods of such therapy may cause improvement in this.

Case 311. (Graph 67)

Refractory Megaloblastic Anaemia possibly due to Megaloblastic Anaemia of Pregnancy followed by Malnutrition

This 41 year old female patient was admitted to Ward 27 of/
of Edinburgh Royal Infirmary in July 1953, when the present author was on holiday and discharged on the day of his return, but was subsequently seen as an outpatient.

The complaint was of loss of weight, anorexia, pallor and dyspnoea following a miscarriage in May 1953. The patient had one son, aged 17 years, but had had five miscarriages in ten years. The blood Wassermann reaction was negative. The miscarriage in May 1953 occurred in the third month of pregnancy. Thereafter the patient, who was an epileptic and mentally rather odd, spent her days sitting in a chair or lying in bed, quite unable to do any cooking or other work. There was no diarrhoea then or at any other time. As far as could be ascertained the miscarriage was a spontaneous one. The patient was in the habit of taking phenobarbitone and from January 1953 she had also been having Mysaline (5-Phenyl-5-Ethyl-hexo-hydro-propanidine 4:6 dione).

A dietetic history was taken and it appears that after the miscarriage the diet contained on an average 177 G. of carbohydrate, 32 G. of protein and 49 G. of fat, giving a daily intake of 1,277 Calories, with 0.92 mg. of iron and 37 mg. of ascorbic acid. There was inadequate intake of niacin and nicotinic acid. The folic acid and vitamin B12 intake was not calculated. Before the abortion the patient was eating 3,566 Calories daily, according to the calculations, with 104 G. of protein. The weight had fallen from 154 lbs. to 122 lbs. in seven months.

The blood levels at the time of admission were:

Haemoglobin/
There was found to be hydrochloric acid in the gastric juice after histamine had been given but a trial of cyanocobalamin therapy was made. It will be seen from Graph 67 that no response occurred and a blood transfusion was required.

A differential urinary folic acid excretion test was carried out and the results were:

<table>
<thead>
<tr>
<th>Urinary excretion</th>
<th>FA</th>
<th>CF</th>
</tr>
</thead>
<tbody>
<tr>
<td>in 24 hours after 5 mg. subcutaneously</td>
<td>1.93</td>
<td>4.01</td>
</tr>
<tr>
<td>in 24 hours after 5 mg. orally</td>
<td>2.54</td>
<td>2.48</td>
</tr>
</tbody>
</table>
The test was repeated after eight injections of 15 mg. of pteroylglutamic acid had been given for treatment. The results now were:

**Urinary excretion**

<table>
<thead>
<tr>
<th></th>
<th>FA</th>
<th>CF</th>
</tr>
</thead>
<tbody>
<tr>
<td>in 24 hours after 5 mg. subcutaneously</td>
<td>2.52</td>
<td>6.79</td>
</tr>
<tr>
<td>in 24 hours after 5 mg. orally</td>
<td>2.85</td>
<td>13.07</td>
</tr>
</tbody>
</table>

Neither test gave evidence of malabsorption of folic acid but the excretion of folic acid after the first dose (on excretion of 1.93 mg.) was rather low, and may be indicative of depletion since there was a greater output than this after the subsequent oral test dose.

An oral glucose tolerance test was done, and was considered to be flat, but it seems likely that the peak was missed. The fasting level was 82 mg. per 100 ml., and the level both at half-an-hour and at one hour was 103 mg. per 100 ml. A fat balance test was not done, possibly because the patient would not co-operate in taking the diet for this. The patient took pteroylglutamic acid tablets for about a month after discharge, but then ceased to take them. No general or haematological deterioration has occurred in the following three months.

**Comment.** It seems likely that this patient had megaloblastic anaemia of pregnancy and that subsequent malnourishment led to the continuation of megaloblastic anaemia.

**Case 312.** (Graph 68)

**Refractory Macrocytic Anaemia of Unknown Origin**

It is well known that macrocytic anaemia may occur in a variety of conditions, including, for instance, myxoedema, uraemia, aplastic anaemia and leukaemia. Consideration of such/
such forms of anaemia is outwith the scope of this thesis, but the following case is included because intermediate (transitional) erythroblasts were found in the bone marrow.

A 77 year old female patient who had had no previous serious illness was admitted to hospital in November 1952 with a two years' history of general symptoms of anaemia. There were no localising features and apart from the anaemia no abnormality was found on physical examination. The mother, two sisters and two brothers had died of gastric carcinoma or other malignant diseases in the abdomen. The diet had been good and there had been no constipation or diarrhoea.

The patient had been seen at the Blood Clinic two weeks prior to admission and at that time the blood counts were haemoglobin 9.9 G. per 100 ml., red cells 3,140,000 per c.mm., white cells 8,200 per c.mm., polymorphs 40%, lymphocytes 54%, monocytes 4%, myelocytes 2%. There were three normoblasts per 100 white cells.

On admission the blood counts were found to be haemoglobin 9.9 G. per 100 ml., red cells 2,740,000 per c.mm., C.I. 1.22. The PCV was 27.0%, the MCV 98.6 cu., the MCHC 36.7% and the platelet count was 260,000 per c.mm.

Intermediate erythroblasts were present in the marrow, and persisted despite treatment with pteroylglutamic acid.
It will be seen from Graph 68 that there was no response to treatment with pteroylglutamic acid or cyanocobalamin and the patient was discharged from hospital on 9/12/52 without having shown any clinical or haematological improvement.

Thyroid extract was given, not because there was any suggestion of myxoedema but because nothing else had produced benefit and no cause for the anaemia had been found. It was suggested to the patient's doctor that he should continue with thyroid extract gr. 1 b.i.d., cyanocobalamin 100 µg. weekly, and ferrous sulphate.

The investigations carried out in the ward included:

E.S.R./
E.S.R. readings
Stool benzidine reaction
Liver function tests
Urinary urobilinogen
Red cell fragility
Non-protein nitrogen
Test meal
X-rays of bones
X-ray of chest
Barium meal and follow through
Urinary folic acid excretion test
E.N.T. examination
Rectal examination

70 - 110 mm./hr.
repeatedly negative.
completely normal.
No excess.
Normal.
30 mg. per 100 ml.
Free hydrochloric acid.
No evidence of malignancy or of myelosclerosis.
Normal.
Negative.

2.3 mg. excreted after 5 mg. test done (normal).

Normal.
No abnormality.

The temperature never exceeded 98° and the white cell count only once exceeded 6,000 per c.mm. in the ward.

It was thought that the patient must have hidden malignant disease with secondary deposits.

A month after discharge from hospital the patient was admitted to a Surgical Ward with a five day's history suggesting a large bowel obstruction. Laparotomy showed that there was a mass in the pelvis, and this was thought to be a carcinoma of the pelvic-rectal junction. An inguinal colostomy was performed, but the patient died two days later.

At post-mortem it was found that a diverticulum of the pelvic colon had perforated and caused an abscess measuring 10 cm. across. It was situated behind the rectum, and around it/
it there was dense fibrous tissue.

No other significant abnormality was found in any organ and the bones and their marrow were normal.

Comment. It is not obvious why this patient developed anaemia with the appearance of normoblasts in the peripheral blood.

The pelvic abscess was surrounded by fibrous tissue - the patient did not suffer from constipation or diarrhoea while in the Medical side of the hospital and rectal examination there revealed no abnormality. If, however, the abscess was present all the time it is surprising that a localised abscess which gave no evidence of toxaemia was able to produce such severe anaemia with the presence of transitional erythroblasts in the marrow.

DISCUSSION

An attempt has been made to follow up all the cases of idiopathic refractory megaloblastic anaemia seen in Edinburgh since the condition was first recognised as an entity. Many of the patients could not be traced, as might be expected in a series of cases first seen during the war years and the immediate post-war period. In addition to the above patients, we have traced four others. One had a domiciliary differential urinary folic acid excretion test carried out and the result indicated intestinal malabsorption. Another, who had histamine fast achlorhydria, had received only a 2 ml. test dose of Anahaemin in hospital and thereafter was treated with pteroylglutamic acid by mouth. It was found, however, that the patient could be satisfactorily maintained in remission with cyanocobalamin therapy. In the other two instances the patients/
patients had been sent into hospital with a diagnosis of refractory megaloblastic anaemia but there was a satisfactory response in hospital to Anaheamin therapy. The cause of the apparent refractory state in these two instances was that the liver extracts used by the practitioners were not potent, and this was realised when the patients were treated in hospital. One of the extracts employed was found by us to contain only 0.25 μg of vitamin B₁₂ per ml. This was marketed in 1948. Since the introduction of microbiological assay methods to supplement the clinical testing of liver extracts there has been a marked improvement in their potency.

Most of the patients referred to above were considered at some stage to be cases of idiopathic vitamin B₁₂-refractory megaloblastic anaemia. It will be recollected, however, that an account has already been given of two patients with folic acid-refractory megaloblastic anaemia (p. 354 and Graph 17; p. 430 and Graph 34). These were, respectively, a case of intestinal malabsorption and one of pernicious anaemia previously treated for long periods with pteroylglutamic acid.

From the data presented it is clear that a diagnosis of idiopathic vitamin B₁₂-refractory megaloblastic anaemia must not be made lightly, and indeed it is felt that none of the cases followed up can now be classified as truly idiopathic.

The minimum investigations that are required before such a diagnosis is made in this centre where special techniques are available would seem to be:

1. A careful history, taken by a skilled observer.
2. A most thorough physical examination.
3/
(3) A marrow puncture and full blood examination.

(4) Repeated stool benzidine tests.

(5) A carefully done fat balance test, at least two separate four day collections being made without the use of purgatives.

(6) A differential urinary folic acid excretion test, repeated if necessary after saturation with folic acid.

(7) Liver function tests and blood urea estimation.

(8) Serial estimations of the sedimentation rate.

(9) A careful assessment of the response to two injections of 100 µg. of cyanocobalamin, preferably from different batches.

(10) Radiological examination of the alimentary tract, including a barium meal and follow through examination. Carcinoma of the large intestine is not in itself likely to produce megaloblastic anaemia, but the possibility of an entero-colic fistula may indicate the necessity for a barium enema being done. Occasionally laparotomy will require consideration.

It is assumed that the patient has been considered to be refractory because of failure in response to treatment and therefore the only value of a serum vitamin B₁₂ estimation would be as a check on the potency of preparations already used, or as a purely experimental investigation where there is the theoretical suspicion that malignancy or other metabolic disturbance may be increasing the requirements of vitamin B₁₂.

Additional investigations that are of interest and should be carried out include the test meal and various tests of intestinal absorption such as the glucose tolerance test. In a case of suspected pernicious anaemia, the test meal if of course essential but an adequate dose of histamine must be given (0.01 mg./Kilo of body weight), it must be ensured, by fluoroscopy if necessary, that the tube is in the stomach, and Gunzberg's/
Gunzberg's reagent must be used. In a case of suspected refractory megaloblastic anaemia the test meal does not assist in diagnosis in that histamine fast achlorhydria may or may not be present, and it is unlikely that carcinoma of the stomach would be suspected from a test meal when all the other tests were negative. Nevertheless the case is not fully investigated unless a test meal is done.

It is conceivable that a patient with megaloblastic anaemia from some other cause, complicated by myxoedema might not respond to antimegaloblastic therapy until thyroid preparations were given, but the author has not seen such a case.

In cases of apparent folic acid-refractory megaloblastic anaemia it is important to establish with certainty what previous therapy has been given, if necessary from the patient's pharmacist if the practitioner is in doubt.

**SUMMARY**

An account is given of a series of cases of vitamin B12-refractory megaloblastic anaemia. The main causes of this condition are

1. The administration of non-potent liver preparations or inadequate dosage of these. This problem is seldom encountered since the introduction of cyanocobalamin therapy and of microbiological assay methods for testing the potency of liver extracts.

2. Intestinal malabsorption without diarrhoea. In most instances this may be demonstrated by a differential urinary folic acid excretion test if not by a fat balance estimation. Some of the cases included in Table 51 as examples of/
of intestinal malabsorption would formerly have been classified as idiopathic refractory megaloblastic anaemia. The steatorrhoea is idiopathic but the anaemia is not.

(3) Gastric or small intestinal carcinoma which is not necessarily demonstrable by X-ray examination.

Reference is made to the minimal investigations that are now required before a diagnosis of idiopathic refractory megaloblastic anaemia can be entertained.
MEGALOBLASTIC ANAEMIA ASSOCIATED WITH CHRONIC HEPATIC DISEASE

Brief reference to the published work dealing with megaloblastic anaemia in chronic hepatic disease has been made on pp. 30-33, and it is pointed out that although macrocytic anaemia is common, the differences in terminology employed by various authors cause difficulty in interpreting their bone marrow findings. In a paper by Movitt (1950), however, there are described three patients with true megaloblastic anaemia associated with hepatic cirrhosis. One of these responded to cyanocobalamin therapy, and two to injections of liver extracts.

In the course of the present investigations it was possible to obtain samples of liver and of kidney from a patient dying of chronic liver failure, and two cases of megaloblastic anaemia associated with possible liver disease were encountered. The second of these was admitted to hospital while this section of the thesis was being written.

Measurement of the Content of Antimegaloblastic Substances in the Liver and Kidney of a Patient dying of Chronic Liver Disease

In September 1947 a 34 year old patient was admitted to Ward 27, Edinburgh Royal Infirmary, 11 days after the premature birth of her fourth child. She had developed jaundice with vomiting and diarrhoea six weeks before admission. The liver and spleen were palpably enlarged, the haemoglobin level was 10.7 G. per 100 ml. with a red cell count of 4,210,000 per c.mm.
and a white cell count of 2,200 per c.mm.; the marrow was normoblastic. Liver function tests were done on seven occasions during the six weeks in hospital. At the time of discharge the findings were:

Plasma albumin 1.9 G. per 100 ml.
Plasma globulin 3.2 G. per 100 ml.
Serum bilirubin 3.5 mg. per 100 ml.
Alkaline phosphatase 12 units
Cephalin cholesterol (+ 1)

The urine no longer contained bile. A diagnosis of subacute yellow atrophy was made.

The patient was not seen again until her readmission in January 1951. Apparently she had continued to have a "nagging" pain in the upper abdomen for three years and it had been worse for four weeks. For six weeks she had had difficulty in walking due to a feeling of loss of power in the legs.

On admission the patient was restless and had a "muddy" complexion. The breath smelt of mercaptan. The liver could not be felt, but the tip of the spleen was palpable. There was a bilateral extensor plantar response and knee and ankle clonus, with spasticity of the lower limbs. The blood pressure was 130/90.

The biochemical and haematological findings were:

Plasma albumin 2.63 G.\%
Plasma globulin 3.40 G.\%
Serum alkaline phosphatase 40 units
Serum potassium 20 mg. per 100 ml.
Serum sodium 306 mg. per 100 ml.
Serum chlorides 604 mg. per 100 ml. (as NaCl)
Serum bilirubin 6.0 mg. per 100 ml.
Serum total base 140 m. Equiv. per 1,000 ml.
N.P.N. 44 mg. per 100 ml.
Cephalin Cholesterol Flocc. + 4
CO₂ combining power 60 vols.\%
Urinary urobilinogen Excess present.
Urinary bile Nil.
Urinary albumin Nil.
Urinary/
Urinary sp.gr. 1.012
Urinary deposit Granular and hyaline casts.
No pus cells. Culture negative.
Marrow Normoblastic.
Haemoglobin 12.3 G. per 100 ml.
Red cells 3,830,000 per c.mm.
C.I. 1.08
HCV 38%
HGV 99.2 µl.
MCHG 32.4
White cells 9,200 per c.mm.
Reticulocytes < 1%

The patient rapidly became comatose. A lumbar puncture showed no abnormality. Four days after admission the patient died. At no time had the patient received injections of liver, cyanocobalamin, pteroylglutamic acid, or antibiotics, or any of these preparations by mouth.

At post-mortem there was evidence of bronchopneumonia. The liver weighed 690 G. It was very small and the surface was granular, the nodules being of varying size up to about 1 cm. in diameter. These nodules were yellowish in colour and the intervening tissue was pink. On the cut surface the normal architecture was completely destroyed. The tissue was broken up into nodules of hyperplastic liver tissue yellow in colour, separated by bands of pinkish connective tissue. The picture was that of a post-necrotic scarring.

The mid-femoral marrow was red and reactive. The spleen was large, weighing 770 G. No other significant abnormality was found.

On microscopic examination no significant abnormality was discovered in the brain.

The liver tissue was found on microscopic examination to be broken up into nodules of varying size by bands of connective tissue.
In some places, wide areas of liver had apparently been destroyed, and replaced by connective tissue which was infiltrated by lymphocytes, plasma cells, and occasionally polymorphs. There was moderate hyperplasia of bile ducts in the connective tissue. In the nodules of liver parenchyma much autolysis was present. Rather infrequently the semblance of normal lobular structure was seen, but for the most part the architecture was replaced by hyperplasia. A moderate amount of fatty change was demonstrable. There was no recent massive necrosis, though one or two small foci of hepatic necrosis were present, mainly around interlobular veins. Round most of the latter, congestion was marked.

The diagnosis of death from liver failure was considered to be correct.

We estimated the content of haemopoietic factors in the liver and kidney. The results were:

<table>
<thead>
<tr>
<th>Organ</th>
<th>Vit. B₁₂ Content</th>
<th>Apparent Folic Acid Activity (L. leichmannii)</th>
<th>Citrovorum Factor Activity (S. faecalis)</th>
<th>True Folic Acid Activity (L. citrovorum)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>40.6 µg./100 G.</td>
<td>244 µg./100 G.</td>
<td>266 µg./100 G.</td>
<td>49 µg./100 G.</td>
</tr>
<tr>
<td>Kidney</td>
<td>13.8 µg./100 G.</td>
<td>48 µg./100 G.</td>
<td>68 µg./100 G.</td>
<td>negl.</td>
</tr>
</tbody>
</table>

This was a pancreatin extraction method, sodium cyanide being added for the L. leichmannii assay.

This did not indicate any depletion of vitamin B₁₂, folic acid, or citrovorum factor.
Megaloblastic Anaemia associated with Chronic Hepatic Disease in a Complex Case with Multiple Pathology

A 32 year old male was admitted to Ward 26 of Edinburgh Royal Infirmary in April 1950 with a four months' history of tiredness, pallor, palpitations and dyspnoea. The stools had been tarry for a month, but this might have been due to the iron tablets that his doctor had prescribed for him. He had had nausea and a feeling of fullness in the stomach, also for about four months. There was no previous history of illness, except that he had had phlebitis of the left leg two years before.

The patient was very pale though not shocked at the time of admission. The blood pressure was 90/48, and it was decided that an immediate blood transfusion was required. It was, however, first established that the haemoglobin level was 4.1 G. per 100 ml. and the red cell count 1,020,000 per c.mm., giving a colour index of 1.37. The peripheral blood film was consistent with a diagnosis of pernicious anaemia and the white cell count was 700 per c.mm. In the course of the first five days in hospital four pints of blood were given by transfusion. It was then established that the marrow was frankly megaloblastic, the picture being similar to that found in pernicious anaemia. It was not macronormoblastic. The stool benzidine reaction was strongly positive, the platelet count was 50,000 per c.mm. and the bleeding time 15 minutes. The E.S.R. (Westergren) on admission was 158 mm. per hour.

It was noted that abdominal veins were prominent, and that
there was brown pigmentation of the skin. A barium swallow and meal showed no evidence of peptic ulcer, carcinoma, oesophagael varices or any other abnormality. There was histamine fast achlorhydria.

After blood transfusion the blood levels were found to be:

- Haemoglobin: 5.6 G. per 100 ml.
- Red cells: 1,410,000 per c.mm.
- C.I.: 1.35
- PCV: 16.5%
- MCV: 117 cµ.
- MCHC: 31.9%
- White cells: 3,000 per c.mm.
- Reticulocytes: < 1%

Treatment at first was with ferrous sulphate and ascorbic acid but then cyanocobalamin was given by injection, and the marrow was converted to the normoblastic state. The response is shown in Graph 69a.
No enlargement of the liver or spleen could be detected and there was no neurological changes. The presence of cirrhosis of the liver was suspected. The biochemical findings in relation to this were:

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin</td>
<td>3.62 G. per 100 ml.</td>
</tr>
<tr>
<td>Globulin</td>
<td>1.65 G. per 100 ml.</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>8 units.</td>
</tr>
<tr>
<td>Cephalin Flocculation</td>
<td>(+ 4)</td>
</tr>
<tr>
<td>Serum bilirubin</td>
<td>0.3 mg. per 100 ml.</td>
</tr>
<tr>
<td>Non-protein nitrogen</td>
<td>42 mg. per 100 ml.</td>
</tr>
</tbody>
</table>

There was a trace of albumin in the urine and an increase of urobilinogen but no other abnormality. The specific gravity was 1018.

A liver biopsy was carried out and was reported to show "common cirrhosis" of the liver.

A fat balance test showed 96.4% absorption of fat (53.9% of the fat being unsplit) and when this was repeated for a second period of three days it showed 90.4% absorption (54.5% of the fat being unsplit).

It was considered that the patient had megaloblastic anaemia due to hepatic cirrhosis although the possibility of pernicious anaemia complicated by hepatic cirrhosis could not be ruled out. As can be seen from Graph 69a there was a slow response to treatment with various antimegaloblastic substances.

The patient ceased attending the Blood Clinic in October 1950 and no significant treatment was given thereafter until he was readmitted.

In April 1953 he was sent into Ward 26 of the same hospital again with a history of a week of vomiting, bright red/
red blood being seen in the vomitus. From the time of discharge he had had nausea and a feeling of fullness. His stools had not been black, but he had been breathless on exertion. His general condition was good apart from anaemia, and he had never been jaundiced. The veins were prominent on the lower abdominal wall (see picture). The patient was not shocked and the blood pressure was 120/80.
An immediate transfusion of two pints of blood was given and then the blood levels were found to be:

- **Haemoglobin**: 5.3 G. per 100 ml.
- **Red cells**: 1,710,000 per c.mm.
- **C.I.**: 1.05
- **PCV**: 16.5%
- **MCV**: 96.5 cµ.
- **MCHC**: 32.1%
- **White cells**: 3,600 per c.mm.

Serum vitamin B12 assays were not possible at the time.

Two further pints of blood were given and this caused a rise in the haemoglobin level to 8.3 G. per 100 ml. and in the red cell count to 2,480,000 per c.mm. The platelet count was 50,000 per c.mm. The marrow was megaloblastic. A barium swallow did not reveal oesophageal varices, but this examination suggested that varices might be present in the stomach.

The biochemical findings now were:

- **Albumin**: 3.96 G. per 100 ml.
- **Globulin**: 2.63 G. per 100 ml.
- **Total cholesterol**: 81 mg. per 100 ml.
- **Free cholesterol**: 20 mg. per 100 ml.
- **Alkaline phosphatase**: 5 units.
- **Serum bilirubin**: 0.3 mg. per 100 ml.
- **Thymol turbidity**: 3 units.
- **Thymol flocculation**: + 1
- **Cephalin Cholesterol Flocculation Test**: + 3
- The prothrombin activity was 24% of normal.

The abnormalities were confirmed by repetition of the test, and a fat balance test showed 97.3% absorption, 26% of the fat being unsplit.

There was about 0.5 G. of albumin per litre present in the urine, but no bile and no excess of urobilinogen. Microscopically there was an occasional granular cast. During the nine weeks in hospital there was a short period during which a urinary/
urinary infection developed, and this responded to a four day course of streptomycin injections. Before this it had been established that the urea clearance was only about 20% of the average normal. The direction of flow of blood in the veins of the abdominal wall had meantime suggested inferior vena caval block, and a venogram appeared to confirm this. Whether or not the renal functional impairment was associated with this was uncertain. A retrograde pyelogram showed no abnormality of the right kidney and ureter, but the position of the ureter was such that the catheter could not be passed into it on the left.

Here then was a patient who appeared to have inferior vena caval obstruction, hepatic cirrhosis and renal functional impairment together with megaloblastic anaemia. A differential urinary folic acid excretion test was attempted before the urinary investigations were complete, and, at first, therapy with pteroylglutamic acid was attempted with some success, as shown in Graph 69b. Thereafter cyanocobalamin was injected, and after discharge polytherapy led to substantial haematological improvement.
CASE 313. MEGALOBLASTIC ANAEMIA ASSOCIATED WITH HEPATIC CIRRHOSIS.

PROTEOLED LIVER 1/2 oz. DAILY.
100 μg CYANOCOBALAMIN i.m. MONTHLY.
15 mg PTEROGLUTAMIC AC IDU AC I. MONTHLY.

The results of the differential urinary folic acid excretion test were:

<table>
<thead>
<tr>
<th>Urinary excretion</th>
<th>FA</th>
<th>CF</th>
</tr>
</thead>
<tbody>
<tr>
<td>in 24 hours after 5 mg. subcutaneously</td>
<td>0.645</td>
<td>0.645</td>
</tr>
<tr>
<td>in 24 hours after 5 mg. orally</td>
<td>0.156</td>
<td>0.390</td>
</tr>
</tbody>
</table>

This was done before the extent of renal impairment was known and at first sight it would appear to indicate not only folic acid depletion, but also malabsorption. After the therapeutic administration of 220 mg. of pteroylglutamic acid (150 mg. of this by injection) over a period of several days, the test was repeated with the following results:

Urinary/
Urinary excretion

<table>
<thead>
<tr>
<th></th>
<th>FA</th>
<th>CF</th>
</tr>
</thead>
<tbody>
<tr>
<td>in 24 hours after 5 mg. subcutaneously</td>
<td>1.46</td>
<td>14.7</td>
</tr>
<tr>
<td>in 24 hours after 5 mg. orally</td>
<td>1.64</td>
<td>6.5</td>
</tr>
</tbody>
</table>

This did not suggest malabsorption. However, the results are difficult to interpret as it was subsequently found that after a 15 mg. dose given intramuscularly significant excretion in the urine continued for 48 hours.

Comment. A complex case of megaloblastic anaemia associated with hepatic cirrhosis (diagnosed by punch biopsy and by liver function tests), renal functional impairment and possible inferior vena caval obstruction. It seems likely that the megaloblastic anaemia was due to cirrhosis and there was a response to cyanocobalamin on the first admission and to pteroylglutamic acid on the second. On this second occasion cyanocobalamin therapy was not tried before the pteroylglutamic acid was given.

Case 315.

Megaloblastic Anaemia following Infective Hepatitis

as a Problem in Diagnosis

A 51 year old male patient was admitted to one of the wards of Edinburgh Royal Infirmary in April 1952 with a history that he had had pale stools and dark urine for six weeks, and jaundice for ten days. The man was a labourer and there was no known contact with drugs or chemicals, exposure to leptospiro infection or history of injections. There was no previous history of note, except that he had had osteomyelitis of the right ankle and the hips in 1937.

*No abnormality was found on examination, the liver and spleen/

* Information obtained from the case notes of the ward to which the patient was admitted in 1952.
spleen not being palpable. There was bile and urobilinogen in the urine and a diagnosis of infective hepatitis was made. The patient spent five weeks in hospital, but unfortunately no haemoglobin estimation or blood cell count was done.

The laboratory investigations were as follows:

**Liver Function Tests**

<table>
<thead>
<tr>
<th>Date</th>
<th>9/4</th>
<th>18/4</th>
<th>25/4</th>
<th>1952</th>
<th>2/5</th>
<th>9/5</th>
<th>26/5</th>
<th>23/6</th>
<th>21/1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin G.%</td>
<td>4.3</td>
<td>2.5</td>
<td>2.9</td>
<td>3.2</td>
<td>2.8</td>
<td>3.2</td>
<td>2.8</td>
<td>3.2</td>
<td>3.0</td>
</tr>
<tr>
<td>Globulin G.%</td>
<td>1.4</td>
<td>3.2</td>
<td>2.7</td>
<td>3.4</td>
<td>2.6</td>
<td>2.6</td>
<td>2.6</td>
<td>2.6</td>
<td>2.6</td>
</tr>
<tr>
<td>NPN mg.%</td>
<td>31</td>
<td>31</td>
<td>37</td>
<td>29</td>
<td>32</td>
<td>32</td>
<td>32</td>
<td>32</td>
<td>40</td>
</tr>
<tr>
<td>S.Bilirubin mg.%</td>
<td>10.5</td>
<td>3.7</td>
<td>1.7</td>
<td>3.0</td>
<td>2.5</td>
<td>0.8</td>
<td>1.1</td>
<td>1.1</td>
<td>1.1</td>
</tr>
<tr>
<td>Alk.Phosphatase Units</td>
<td>16</td>
<td>13</td>
<td>15</td>
<td>10</td>
<td>9</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td>Ceph.Cholesterol Flocc.</td>
<td>+4</td>
<td>+4</td>
<td>+4</td>
<td>+4</td>
<td>+4</td>
<td>+3</td>
<td>+3</td>
<td>+3</td>
<td>+3</td>
</tr>
<tr>
<td>Thymol Turbidity Units</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thymol Floccul.</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Cholesterol mg.%</td>
<td>220</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Free Cholesterol mg.%</td>
<td>75</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Urine

- Bile: ++
- Urobilinogen: +

The stool benzidine reaction was negative.

The patient never really felt well after this attack of hepatitis but returned to light work. About October 1953 he became breathless on exertion and easily tired. His appetite deteriorated and he noticed that his stools were light but that his urine was not dark. At no time was there diarrhoea. The patient was referred to the Medical Outpatient Department of Edinburgh Royal Infirmary in January 1954 and was admitted to Ward 26 because he was anaemic. He was pale and icteric, and the liver was found to be palpable two finger breadths below the costal margin on inspiration. The spleen did not appear to be enlarged. The tongue was furred and dirty and in this respect/
respect differed from the usual lingual appearance in pernicious anaemia. No abnormality was found in the nervous system or elsewhere.

There was histamine fast achlorhydria, and the blood levels were:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin</td>
<td>4.4 g. per 100 ml.</td>
</tr>
<tr>
<td>Red cells</td>
<td>1,250,000 per c.mm.</td>
</tr>
<tr>
<td>C.I.</td>
<td>1.2</td>
</tr>
<tr>
<td>PCV</td>
<td>13.5%</td>
</tr>
<tr>
<td>MCV</td>
<td>108 µµ.</td>
</tr>
<tr>
<td>MCHC</td>
<td>32.6%</td>
</tr>
<tr>
<td>White cells</td>
<td>12,200 per c.mm.</td>
</tr>
<tr>
<td>Reticulocytes</td>
<td>&lt; 1%</td>
</tr>
<tr>
<td>Platelets</td>
<td>50,000 per c.mm.</td>
</tr>
</tbody>
</table>

The marrow was megaloblastic.

There seemed to be evidence of hepatic disorder in that the cephalin cholesterol flocculation test was +3 as is shown above, but the problem of diagnosis remained. The chief possibilities appeared to be:

- Hepatic Cirrhosis with megaloblastic anaemia due to vitamin B₁₂ deficiency.
- Hepatic Cirrhosis with megaloblastic anaemia due to deficiency of folic acid substances.
- Hepatic Cirrhosis complicated by pernicious anaemia or some other form of megaloblastic anaemia.
- Pernicious Anaemia alone.
- Idiopathic Steatorrhea alone.

The most important test to carry out first was the serum vitamin B₁₂ level, and this was found to be 50 µµg./ml., consistent with a diagnosis of pernicious anaemia, or of vitamin B₁₂ deficiency from another cause. The patient required immediate treatment, and 100 µg. of cyanocobalamin were given. To this there was a satisfactory response. It was thought that/
that at this stage a differential urinary folic acid excretion test might merely obscure the diagnosis since there might be a haemopoietic response to the pteroylglutamic acid given in the test. As a test of vitamin $B_{12}$ absorption it was thought that 100 $\mu$g. of cyanocobalamin might be given by mouth and the serum levels measured, but that this should not be done until the serum level had returned to subnormal figures after the rise produced by the 100 $\mu$g. injection. The results were of no value because although no rise of serum vitamin $B_{12}$ level occurred after the oral dose, the same was true in a patient with rheumatoid arthritis who had hydrochloric acid in the gastric juice.

**DISCUSSION**

These results do not add much to our knowledge of megaloblastic anaemia in association with hepatic cirrhosis but merely demonstrate some of the problems that may arise. It is true that we have shown that it is possible for a patient to have such advanced hepatic cirrhosis that death ensues and yet have a relatively high content of haemopoietic factors in the liver, but this is the result in only one case and does not necessarily indicate what may happen in others. It does show, however, that it is possible to have macrocytic anaemia in chronic liver disease without having deficiency of vitamin $B_{12}$ or folic acid substances. The difficulty of obtaining suitable material is that few patients die of cirrhosis without first receiving treatment with liver or cyanocobalamin injections.
The two cases of megaloblastic anaemia described in this chapter show different aspects of the subject – in one instance there was a complex problem with multiple pathology where renal impairment rendered a folic acid excretion test of little value and in the other there was a more simple diagnostic problem where a serum vitamin B₁₂ estimation assisted in the diagnosis. The rarity of cases of megaloblastic anaemia associated with hepatic cirrhosis means that our progress in investigating the metabolic disorders involved is likely to be slow.

**SUMMARY**

In a patient who died of advanced hepatic cirrhosis the liver contained a normal amount of vitamin B₁₂ and folic acid substances. An account is given of two cases which illustrate the problems of diagnosis that may be involved where there is suspected hepatic cirrhosis in association with megaloblastic anaemia.
CHAPTER 18

THE EXCRETION OF A TEST DOSE OF FOLIC ACID IN CASES OF MALIGNANT DISEASE AND IN ALLIED CONDITIONS

It has already been shown in previous chapters that when 5 mg. of pteroylglutamic acid is injected in a normal individual, a significant proportion is promptly excreted in the urine. Most of this excretion occurs within a few hours, but it is our practice to collect the urine for 24 hours after the injection. It has been shown, too, in Chapters 7 and 9 that the urinary excretion of folic acid after a test dose is diminished in many cases of pernicious anaemia, and in other chapters that there is a lowered output in many instances in other forms of megaloblastic anaemia. At first we followed the practice of Bethell et al. (1947) in using non-anaemic controls for these studies, but soon decided that patients with non-megaloblastic forms of anaemia should be used. Accordingly the present investigation was started as a study of folic acid excretion in various types of anaemia. At an early stage, however, it was found that, of eight patients with iron deficiency anaemia, folic acid excretion was diminished in four. All of these four were found to be suffering from advanced malignant disease. It was, therefore, decided to extend further the urinary folic acid excretion studies to patients suffering from malignant disease and from various non-neoplastic conditions. About this time it was reported (Swendseid et al., 1952) that folic acid excretion was impaired in leukaemia.

The patients in the present investigation were all given a single 5 mg. injection of pteroylglutamic acid subcutaneously, and/
and the urine was collected for 24 hours and tested for its folic acid content by the *S. faecalis* method. The method employed and precautions taken were similar to those described in Chapter 9 which deals with the differential urinary folic acid excretion test. As before we prefer to give to the substance injected the name pteroylglutamic acid and to the substance excreted the name folic acid, since although the substance excreted has similar microbiological properties to that injected this is not absolute proof that the two are completely identical. No antibiotics, sulphonamides or vitamin preparations were given during the course of the test or immediately before it.

The 'resting' urinary content of folic acid is so small that no correction requires to be made for it in the folic acid readings. The proportion of folic acid substance excreted as citrovorum factor is very small, as is shown in the tables that follow, and the 'folic acid' figures include any growth due to citrovorum factor. At the time of these assays, 1 µg. of citrovorum factor was showing falsely as about 0.5 - 0.7 µg. of pteroylglutamic acid in the *S. faecalis* assay, so it can be seen that correction for this would not cause any alteration in the results obtained.

The citrovorum factor readings (*L. citrovorum* assay) have not been corrected for the 'resting' urinary content of citrovorum factor. This on the average is of the order of 0.3 µg./24 hours, but may vary slightly from day to day.

RESULTS

The number of patients studied was so large that it is necessary/
necessary to give the results in tabular form. A few illustrative cases will be given in more detail after the tables have been presented. Three groups will be considered:

(1) Patients without malignant disease or chronic infection.

(2) Patients with malignant disease and those suspected of having it.

(3) Patients with chronic infections and allied conditions.

**FOLIC ACID EXCRETION IN PATIENTS NOT SUFFERING FROM MALIGNANT DISEASE, MEgaloblastic Anaemia, Intestinal Malabsorption or Prolonged Infection**

The results of the urinary excretion of folic acid in a number of patients with various non-malignant diseases are given in Table 59. It will be seen that the only patient in whom the excretion was less than 30% of the dose administered (less than 1.5 mg.) was a man with severe congestive cardiac failure and oedema (Case 453). Two patients, one with a large pleural effusion and the other with massive ascites, are not included; in both instances folic acid excretion was impaired, but it was possible to show that much of the vitamin was present in the effusion, and that urinary excretion took place over several days. These results suggest that in a patient with normal renal function and without oedema or a large effusion the 24 hour urinary excretion of a 5 mg. dose of folic acid should be greater than 1.5 mg., and indeed an excretion of less than 2 mg. in a normal person suggests that the urinary collection may not have been absolutely complete.

It will be seen from Table 59 that anaemia in itself, even if severe, does not cause an abnormally low folic acid excretion following a 5 mg. test dose. The same is true of hepatic/
hepatic cirrhosis. Case 452 had frank untreated scurvy but a normoblastic marrow. The surprising feature in this case was the high output of citrovorum factor after the test dose of pteroylglutamic acid. No explanation can be given for this.
### Folic Acid Excretion after 5 mg. Test Dose in Patients without Evidence of Malignant Disease

<table>
<thead>
<tr>
<th>No.</th>
<th>Sex</th>
<th>Age</th>
<th>Diagnosis</th>
<th>Hb</th>
<th>Rbc</th>
<th>Folic Acid</th>
<th>Cit. Excn.</th>
</tr>
</thead>
<tbody>
<tr>
<td>400</td>
<td>M</td>
<td>27</td>
<td>Target cell anaemia</td>
<td>14.2</td>
<td>4.72</td>
<td>4.83</td>
<td>-</td>
</tr>
<tr>
<td>401</td>
<td>F</td>
<td>52</td>
<td>Neurosis</td>
<td>12.5</td>
<td>4.92</td>
<td>4.80</td>
<td>-</td>
</tr>
<tr>
<td>402</td>
<td>F</td>
<td>65</td>
<td>Neurosis</td>
<td>14.6</td>
<td>5.01</td>
<td>4.02</td>
<td>5.5</td>
</tr>
<tr>
<td>403</td>
<td>M</td>
<td>35</td>
<td>Normal</td>
<td>15.2</td>
<td>5.36</td>
<td>3.61</td>
<td>-</td>
</tr>
<tr>
<td>404</td>
<td>F</td>
<td>34</td>
<td>Agranulocytosis</td>
<td>11.0</td>
<td>5.11</td>
<td>3.75</td>
<td>-</td>
</tr>
<tr>
<td>405</td>
<td>M</td>
<td>57</td>
<td>Ankylosing spondylitis</td>
<td>8.0</td>
<td>2.7</td>
<td>3.72</td>
<td>6.2</td>
</tr>
<tr>
<td>406</td>
<td>F</td>
<td>34</td>
<td>Erythema nodosum: mitral stenosis</td>
<td>12.3</td>
<td>-</td>
<td>3.56</td>
<td>-</td>
</tr>
<tr>
<td>407</td>
<td>M</td>
<td>30</td>
<td>Normal</td>
<td>15.4</td>
<td>-</td>
<td>3.56</td>
<td>-</td>
</tr>
<tr>
<td>408</td>
<td>M</td>
<td>35</td>
<td>Bleeding duodenal ulcer</td>
<td>13.2</td>
<td>-</td>
<td>3.52</td>
<td>-</td>
</tr>
<tr>
<td>409</td>
<td>F</td>
<td>46</td>
<td>Portal cirrhosis: portal hypertension</td>
<td>10.0</td>
<td>3.15</td>
<td>3.41</td>
<td>5.1</td>
</tr>
<tr>
<td>410</td>
<td>F</td>
<td>48</td>
<td>Acquired haemolytic anaemia</td>
<td>10.3</td>
<td>3.53</td>
<td>3.32</td>
<td>3.7</td>
</tr>
<tr>
<td>411</td>
<td>M</td>
<td>42</td>
<td>Lobar pneumonia</td>
<td>14.8</td>
<td>5.16</td>
<td>3.30</td>
<td>9.6</td>
</tr>
<tr>
<td>412</td>
<td>M</td>
<td>77</td>
<td>V. strict vegetarian with neurological complications</td>
<td>12.5</td>
<td>4.22</td>
<td>3.20</td>
<td>0.8</td>
</tr>
<tr>
<td>413</td>
<td>M</td>
<td>26</td>
<td>Bleeding duodenal ulcer</td>
<td>11.8</td>
<td>-</td>
<td>3.20</td>
<td>-</td>
</tr>
<tr>
<td>414</td>
<td>F</td>
<td>32</td>
<td>Brachial neuritis</td>
<td>14.2</td>
<td>-</td>
<td>3.19</td>
<td>-</td>
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<tr>
<td>415</td>
<td>M</td>
<td>49</td>
<td>Bleeding duodenal ulcer</td>
<td>11.6</td>
<td>-</td>
<td>3.19</td>
<td>-</td>
</tr>
<tr>
<td>416</td>
<td>F</td>
<td>36</td>
<td>Iron Defic. anaemia</td>
<td>3.8</td>
<td>2.60</td>
<td>3.16</td>
<td>10.8</td>
</tr>
<tr>
<td>417</td>
<td>M</td>
<td>63</td>
<td>Hypertension; heart failure</td>
<td>15.4</td>
<td>-</td>
<td>3.16</td>
<td>2.4</td>
</tr>
<tr>
<td>418</td>
<td>F</td>
<td>42</td>
<td>Iron Def. anaemia</td>
<td>8.1</td>
<td>4.21</td>
<td>3.04</td>
<td>-</td>
</tr>
<tr>
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<td>7.1</td>
<td>4.98</td>
<td>5.02</td>
<td>4.1</td>
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<td>F</td>
<td>45</td>
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<td>4.18</td>
<td>2.96</td>
<td>1.6</td>
</tr>
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<td>M</td>
<td>48</td>
<td>Chronic amoebiasis</td>
<td>11.2</td>
<td>5.98</td>
<td>2.96</td>
<td>-</td>
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<tr>
<td>422</td>
<td>F</td>
<td>49</td>
<td>Portal cirrhosis; portal hypertension</td>
<td>8.9</td>
<td>4.07</td>
<td>2.90</td>
<td>7.4</td>
</tr>
<tr>
<td>423</td>
<td>F</td>
<td>34</td>
<td>Peptic ulcer; mitral stenosis</td>
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<td>-</td>
<td>2.89</td>
<td>2.0</td>
</tr>
<tr>
<td>424</td>
<td>F</td>
<td>70</td>
<td>Coronary thrombosis; pulmonary oedema; oesophageal hernia</td>
<td>11.2</td>
<td>5.5</td>
<td>2.89</td>
<td>-</td>
</tr>
<tr>
<td>425</td>
<td>M</td>
<td>55</td>
<td>Portal cirrhosis: diarrhoea</td>
<td>14.8</td>
<td>-</td>
<td>2.89</td>
<td>6.6</td>
</tr>
<tr>
<td>426</td>
<td>M</td>
<td>42</td>
<td>Anxiety state: diarrhoea</td>
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<td>2.86</td>
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<td>No.</td>
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<td>Diagnosis</td>
<td>Hb.</td>
<td>Rbc.</td>
<td>F.A.</td>
<td>C.F.</td>
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<td>------</td>
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<td>427</td>
<td>F</td>
<td>59</td>
<td>Psychoneurosis</td>
<td>12.5</td>
<td>4.01</td>
<td>2.82</td>
<td>-</td>
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<tr>
<td>428</td>
<td>F</td>
<td>17</td>
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<td>5.5</td>
<td>2.71</td>
<td>5.4</td>
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<tr>
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<td>F</td>
<td>26</td>
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<td>4.98</td>
<td>2.70</td>
<td>2.0</td>
</tr>
<tr>
<td>430</td>
<td>M</td>
<td>57</td>
<td>Polycythemia</td>
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<td>6.91</td>
<td>2.68</td>
<td>9.0</td>
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<tr>
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<td>M</td>
<td>52</td>
<td>Gastric ulcer</td>
<td>15.3</td>
<td>-</td>
<td>2.66</td>
<td>-</td>
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<td>F</td>
<td>62</td>
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<td>3.35</td>
<td>2.63</td>
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<tr>
<td>433</td>
<td>F</td>
<td>66</td>
<td>Iron Defic. anaemia</td>
<td>7.9</td>
<td>3.65</td>
<td>2.58</td>
<td>-</td>
</tr>
<tr>
<td>434</td>
<td>F</td>
<td>84</td>
<td>Thyrotoxicosis</td>
<td>14.8</td>
<td>4.99</td>
<td>2.57</td>
<td>-</td>
</tr>
<tr>
<td>435</td>
<td>F</td>
<td>84</td>
<td>Aplastic anaemia</td>
<td>8.0</td>
<td>2.71</td>
<td>2.56</td>
<td>2.0</td>
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<td>F</td>
<td>72</td>
<td>Portal cirrhosis</td>
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<td>4.40</td>
<td>2.55</td>
<td>3.3</td>
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<td>437</td>
<td>M</td>
<td>56</td>
<td>Bleeding duoden. ulcer</td>
<td>9.7</td>
<td>4.91</td>
<td>2.50</td>
<td>1.9</td>
</tr>
<tr>
<td>438</td>
<td>F</td>
<td>45</td>
<td>Portal cirrhosis</td>
<td>9.6</td>
<td>3.85</td>
<td>2.50</td>
<td>2.9</td>
</tr>
<tr>
<td>439</td>
<td>F</td>
<td>69</td>
<td>Myelosclerosis</td>
<td>8.1</td>
<td>3.15</td>
<td>2.47</td>
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<td>42</td>
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<td>4.64</td>
<td>2.42</td>
<td>2.1</td>
</tr>
<tr>
<td>441</td>
<td>F</td>
<td>36</td>
<td>Sciatica</td>
<td>11.9</td>
<td>-</td>
<td>2.51</td>
<td>-</td>
</tr>
<tr>
<td>442</td>
<td>M</td>
<td>42</td>
<td>Gastric ulcer</td>
<td>14.6</td>
<td>-</td>
<td>2.27</td>
<td>-</td>
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<td>F</td>
<td>55</td>
<td>Para-oesophageal hernia</td>
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<td>3.13</td>
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<td>6.2</td>
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<tr>
<td>444</td>
<td>F</td>
<td>32</td>
<td>Dyspepsia</td>
<td>13.9</td>
<td>-</td>
<td>2.17</td>
<td>3.1</td>
</tr>
<tr>
<td>445</td>
<td>F</td>
<td>48</td>
<td>Bronchial asthma</td>
<td>12.5</td>
<td>-</td>
<td>2.05</td>
<td>-</td>
</tr>
<tr>
<td>446</td>
<td>F</td>
<td>48</td>
<td>Gastric neurosis</td>
<td>15.3</td>
<td>5.01</td>
<td>2.02</td>
<td>1.4</td>
</tr>
<tr>
<td>447</td>
<td>F</td>
<td>46</td>
<td>Iron Defic. anaemia</td>
<td>8.1</td>
<td>4.67</td>
<td>1.85</td>
<td>7.4</td>
</tr>
<tr>
<td>448</td>
<td>F</td>
<td>42</td>
<td>Sciatica</td>
<td>14.2</td>
<td>-</td>
<td>1.73</td>
<td>-</td>
</tr>
<tr>
<td>449</td>
<td>F</td>
<td>66</td>
<td>Chronic alcoholic bronchopneumonia, malnutrition</td>
<td>15.0</td>
<td>-</td>
<td>1.73</td>
<td>-</td>
</tr>
<tr>
<td>450</td>
<td>F</td>
<td>68</td>
<td>Malnutrition</td>
<td>9.3</td>
<td>3.6</td>
<td>1.52</td>
<td>1.5</td>
</tr>
<tr>
<td>451</td>
<td>F</td>
<td>84</td>
<td>Malnutrition</td>
<td>4.0</td>
<td>3.1</td>
<td>1.52</td>
<td>2.8</td>
</tr>
<tr>
<td>452</td>
<td>M</td>
<td>79</td>
<td>Scurvy</td>
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<td>2.7</td>
<td>1.50</td>
<td>12.0</td>
</tr>
<tr>
<td>453</td>
<td>M</td>
<td>66</td>
<td>Hypertensive cardiac failure with oedema</td>
<td>15.2</td>
<td>-</td>
<td>0.79</td>
<td>1.6</td>
</tr>
</tbody>
</table>
FOLIC ACID EXCRETION IN PATIENTS CONSIDERED TO BE SUFFERING FROM MALIGNANT DISEASE AND ALLIED CONDITIONS

In Table 60 there are given the urinary folic acid excretion results following the test dose in a number of persons suspected of having malignant disease (including the leukaemias and reticulosés) or found as a result of hospital investigations to be so afflicted. The table is constructed so as to show in parenthesis the provisional diagnosis when the patient was first seen in connection with the present investigation. Thus in many instances it was not known whether or not malignancy was present, and it will be seen that in some cases it was fortunately possible to show that no neoplastic disease existed. In the cases included in this table the final diagnoses had been definitely established, in most instances by biopsy or laparotomy. Most of the patients included in Table 60 had had no therapy, but Cases 503, 507, 525, 536 and 532 had received X-ray therapy, and the last mentioned had also been given oral nitrogen mustards, as had Case 517.
TABLE 60.

<table>
<thead>
<tr>
<th>No.</th>
<th>Sex</th>
<th>Age</th>
<th>DIAGNOSIS</th>
<th>Hb</th>
<th>Rbc</th>
<th>ESR</th>
<th>F.A.</th>
<th>C.F.</th>
<th>Gen.*</th>
<th>Cond.&amp;</th>
<th>Course*</th>
</tr>
</thead>
<tbody>
<tr>
<td>454</td>
<td>M</td>
<td>64</td>
<td>Gastric ulcer (Gastric carcinoma)</td>
<td>10.0</td>
<td>3.8</td>
<td>45</td>
<td>4.05</td>
<td>-</td>
<td>Good</td>
<td></td>
<td></td>
</tr>
<tr>
<td>455</td>
<td>M</td>
<td>57</td>
<td>Spondylitis ankylopoiet. (Carcinomatosis)</td>
<td>8.0</td>
<td>2.7</td>
<td>28</td>
<td>3.72</td>
<td>6.2</td>
<td>Good</td>
<td></td>
<td></td>
</tr>
<tr>
<td>456</td>
<td>F</td>
<td>48</td>
<td>Carc. of cervix (local) (Carcinomatosis)</td>
<td>13.6</td>
<td>4.5</td>
<td>-</td>
<td>3.70</td>
<td>-</td>
<td>Good</td>
<td>S(T)</td>
<td></td>
</tr>
<tr>
<td>457</td>
<td>F</td>
<td>52</td>
<td>Brain stem thrombosis (Carcinomatosis)</td>
<td>14.8</td>
<td>3</td>
<td>3.70</td>
<td>1.8</td>
<td>-</td>
<td>Good</td>
<td></td>
<td></td>
</tr>
<tr>
<td>458</td>
<td>F</td>
<td>50</td>
<td>Gastric ulcer (Gastric carcinoma)</td>
<td>12.2</td>
<td>5.3</td>
<td>50</td>
<td>3.70</td>
<td>-</td>
<td>Fair</td>
<td></td>
<td></td>
</tr>
<tr>
<td>459</td>
<td>F</td>
<td>72</td>
<td>Gastric ulcer (Gastric carcinoma)</td>
<td>12.6</td>
<td>5.3</td>
<td>50</td>
<td>3.70</td>
<td>-</td>
<td>Fair</td>
<td></td>
<td></td>
</tr>
<tr>
<td>460</td>
<td>F</td>
<td>18</td>
<td>Subacute lymphatic leukemia. WBC 10,400 (Acute rheumatism)</td>
<td>7.7</td>
<td>2.44</td>
<td>3.70</td>
<td>-</td>
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* Subsequent course.
R = Rapid downhill course R(T) if with treatment.
S = Very slow downhill course without treatment.
S(T) = Very slow downhill course with treatment.
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<td>M</td>
<td>43</td>
<td>Acute lymphatic leukaemia WBC 15,000</td>
<td>6.0</td>
<td>1.6</td>
<td>168</td>
<td>0.23</td>
<td>5.8</td>
<td>Poor</td>
</tr>
<tr>
<td>527</td>
<td>F</td>
<td>22</td>
<td>Lymphadenoma</td>
<td>7.5</td>
<td>2.4</td>
<td>110</td>
<td>0.22</td>
<td>-</td>
<td>R(T)</td>
</tr>
<tr>
<td>528</td>
<td>F</td>
<td>50</td>
<td>Bronchial Ca. Extensive hepatic spread. (Shoulder pains)</td>
<td>11.8</td>
<td>-</td>
<td>86</td>
<td>0.18</td>
<td>5.1</td>
<td>Good</td>
</tr>
<tr>
<td>529</td>
<td>F</td>
<td>36</td>
<td>Acute lymphatic leukaemia WBC 26,000</td>
<td>5.3</td>
<td>2.1</td>
<td>132</td>
<td>0.18</td>
<td>-</td>
<td>Poor</td>
</tr>
<tr>
<td>530</td>
<td>M</td>
<td>34</td>
<td>Lymphadenoma (Glandular enlargement)</td>
<td>14.8</td>
<td>-</td>
<td>47</td>
<td>0.14</td>
<td>5.8</td>
<td>Poor</td>
</tr>
<tr>
<td>531</td>
<td>F</td>
<td>32</td>
<td>Multiple myeloma</td>
<td>12.3</td>
<td>-</td>
<td>57</td>
<td>0.13</td>
<td>2.9</td>
<td>Good</td>
</tr>
<tr>
<td>532</td>
<td>M</td>
<td>37</td>
<td>Lymphadenoma</td>
<td>8.5</td>
<td>3.1</td>
<td>-</td>
<td>0.13</td>
<td>-</td>
<td>Poor</td>
</tr>
<tr>
<td>533</td>
<td>F</td>
<td>52</td>
<td>Multiple myeloma</td>
<td>12.6</td>
<td>-</td>
<td>57</td>
<td>0.12</td>
<td>2.9</td>
<td>Good</td>
</tr>
<tr>
<td>534</td>
<td>F</td>
<td>42</td>
<td>Carcinomatosis (Emaciation)</td>
<td>10.2</td>
<td>3.6</td>
<td>120</td>
<td>0.10</td>
<td>8.4</td>
<td>Poor</td>
</tr>
<tr>
<td>535</td>
<td>F</td>
<td>66</td>
<td>Lymphosarcoma (Anaemia)</td>
<td>6.6</td>
<td>2.8</td>
<td>8</td>
<td>0.08</td>
<td>3.4</td>
<td>Good</td>
</tr>
<tr>
<td>536</td>
<td>M</td>
<td>66</td>
<td>Chronic lymphatic leukaemia; heart failure</td>
<td>11.1</td>
<td>3.4</td>
<td>-</td>
<td>negl.</td>
<td>negl.</td>
<td>Poor</td>
</tr>
<tr>
<td>537</td>
<td>M</td>
<td>46</td>
<td>Acute termination of chronic myeloid leukaemia</td>
<td>5.5</td>
<td>1.7</td>
<td>-</td>
<td>negl.</td>
<td>negl.</td>
<td>Poor</td>
</tr>
</tbody>
</table>

(The above cases had (n) anaemia presenting symptoms were available that before the presence of anaemia was established by history and physical examination, biopsy or laboratory. Various weight losses were not invariably present in patients with a positive test, and conversely some of the patients in Table 50 with marked weight loss had a negative test. Photographs of a few of the patients are given later in this chapter, but an indication is given in Table 50 of the general anaemia treatment of the time of the first, and the variety of the subsequent developments.)
It will be seen from Table 60 that a normal folic acid excretion (1.5 mg. or more after the subcutaneous injection of 5 mg.) is of no value in excluding the presence of malignancy. Twenty-one patients with carcinoma, leukaemia, lymphadenoma or some other form of reticulosis had a normal folic acid excretion. It is true that in most of these cases the general condition was good and there was chronic disease rather than acute, or localised carcinoma rather than a widely disseminated condition, but such was not invariably the case. For example, Cases 460 (a nurse with subacute lymphatic leukaemia), 468 (a lady with secondary deposits in the neck from an unknown primary) and 473 (a man with hypernephroma and secondary deposits in the lungs and bones) had widespread disease.

Inspection of the records in Table 60 of cases who excreted less than 1.5 mg. of folic acid shows that all of them (except Case 513 who had subacute bacterial endocarditis probably of long duration) suffered from malignant disease. In some instances the folic acid excretion results were available some time before the presence of malignancy was established by X-ray examination, biopsy or laparotomy. Obvious weight loss was not invariably present in patients with a positive test, and conversely some of the patients in Table 59 with marked weight loss had a negative test. Photographs of a few of the patients are given later in this chapter, and an indication is given in Table 60 of the general condition at the time of the test, and the rapidity of the subsequent downhill cause. Where the latter is recorded as being rapid it means that death/
death occurred within nine months.

It will be seen, too, that patients could have advanced malignancy with a markedly positive folic acid excretion test and yet have a normal sedimentation rate (e.g., Cases 514, 523 and 535).

The output of citrovorum factor after the test dose was not necessarily diminished in the cases with a positive folic acid excretion test. Indeed if Case 465 be omitted because of its peculiar nature (apparent spontaneous 'cure' of lymphosarcoma) and also Cases 536 and 537 because the urinary output of haemopoietic factors was too small to be measured, there are left 26 cases in Table 60 who excreted more than 1.5 mg. of folic acid after the test dose, and had the urinary output of citrovorum factor estimated, and 29 cases that excreted less than 1.5 mg. of folic acid and had the citrovorum factor output measured. In the former group the mean output of citrovorum factor was 4.35 µg. in 24 hours and in the latter it was 4.51 µg. in 24 hours. The difference is not significant.

It would be of great benefit to clinicians if this test were of assistance in the assessment of the prognosis in any individual patient. Unfortunately this does not appear to be so. The cases considered here form too heterogeneous a collection for statistical analysis, particularly since various methods of treatment were tried in different patients.

In Table 61, however, a rough analysis has been made of the patients according to the diagnosis, the result of the folic acid excretion test, the general condition at the time of the test, and the rapidity of the subsequent downhill course.
## TABLE 61.
### ANALYSIS OF MALIGNANT CASES IN TABLE 60
(No. of patients)

<table>
<thead>
<tr>
<th>Type of Disease</th>
<th>Folic Acid Output more than 1.5 mg.</th>
<th>Folic Acid Output less than 1.5 mg.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>General Condition</td>
<td>Subsequent Downhill Course</td>
</tr>
<tr>
<td></td>
<td>Good or Fair</td>
<td>Rapid</td>
</tr>
<tr>
<td>Local Carcinoma</td>
<td>8 1</td>
<td>3 6</td>
</tr>
<tr>
<td>General Carcinoma</td>
<td>2 1</td>
<td>3 0</td>
</tr>
<tr>
<td>Acute Leukaemia</td>
<td>1 0</td>
<td>1 0</td>
</tr>
<tr>
<td>Chronic Leukaemia</td>
<td>2 0</td>
<td>1 1</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>2 1</td>
<td>1 2</td>
</tr>
<tr>
<td>Lymphadenoma and other reticuloses</td>
<td>1 0</td>
<td>1 0</td>
</tr>
<tr>
<td>Multiple Myeloma</td>
<td>1 0</td>
<td>0 1</td>
</tr>
<tr>
<td>Totals</td>
<td>17 3</td>
<td>10 10</td>
</tr>
</tbody>
</table>

It would seem that if the patient has carcinoma with a positive folic acid test (and it is reasonably certain that the carcinoma is not producing the latter by interfering mechanically with absorption or that severe malnutrition is not a feature) then the carcinoma is likely to be widely disseminated and the subsequent downhill course will be rapid. In acute leukaemia the downhill course is not likely to be slow whatever the/
the result may be; it is possible to have chronic leukaemia with a normal or abnormal result of the test, and the subsequent response to therapy may be good or poor. The presence of a markedly positive test in lymphosarcoma, lymphadenoma and the other reticuloses, or multiple myeloma does not necessarily indicate that the patient has only a few months to live - unfortunately, however, the presence of a negative test does not always mean that life will be prolonged. Case 482 who had lymphadenoma died within three months of the test despite treatment with nitrogen mustards. A folic acid excretion survey of a very large number of cases subsequently treated by various methods would be required to show whether or not this test is of any value as a guide to the best form of therapy that should be employed in individual patients.

**FOLIC ACID EXCRETION IN CHRONIC INFECTIONS AND ASSOCIATED CONDITIONS**

There are occasions on which it would be helpful to have a test to determine whether a patient suffers from chronic infection or from malignancy. For this reason a separate table (Table 62) has been constructed to include untreated patients with chronic infections and collagen diseases. In all the cases of pulmonary tuberculosis the disease was extensive, but except in Cases 548 and 557 the general condition under a sanatorium regime was good.
## TABLE 62.

Folic Acid Excretion after Test Dose in
Prolonged Infections and Associated Conditions

<table>
<thead>
<tr>
<th>No.</th>
<th>Sex</th>
<th>Age</th>
<th>Diagnosis &amp; Remarks</th>
<th>Hb.</th>
<th>Rbc.</th>
<th>ESR</th>
<th>Folic Cit.</th>
<th>Acid Fact.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>including duration.</td>
<td>G/</td>
<td>M/</td>
<td>mm/</td>
<td>100</td>
<td>mg.</td>
</tr>
<tr>
<td>538</td>
<td>F</td>
<td>27</td>
<td>Rheumatoid arthritis (6 months)</td>
<td>8.6</td>
<td>-</td>
<td>60</td>
<td>4.23</td>
<td>-</td>
</tr>
<tr>
<td>539</td>
<td>M</td>
<td>23</td>
<td>Pulmonary tuberculosis (6 yrs.) Extensive bilateral</td>
<td>10.8</td>
<td>-</td>
<td>5</td>
<td>5.49</td>
<td>-</td>
</tr>
<tr>
<td>540</td>
<td>F</td>
<td>26</td>
<td>P.U.O. Temp. to 103°F. (1 1/2 yrs.)</td>
<td>12.5</td>
<td>4.9</td>
<td>5</td>
<td>5.48</td>
<td>5.7</td>
</tr>
<tr>
<td>541</td>
<td>M</td>
<td>37</td>
<td>Pulmonary tuberculosis (2 yrs.) Bilateral</td>
<td>13.9</td>
<td>-</td>
<td>52</td>
<td>3.34</td>
<td>-</td>
</tr>
<tr>
<td>542</td>
<td>F</td>
<td>23</td>
<td>P.U.O. (3 months) Temp. to 99.5°F. (3 yrs.) Large cavity</td>
<td>12.4</td>
<td>4.1</td>
<td>18</td>
<td>3.23</td>
<td>6.5</td>
</tr>
<tr>
<td>543</td>
<td>M</td>
<td>47</td>
<td>Pulmonary tuberculosis (3 yrs.)</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>5.01</td>
<td>-</td>
</tr>
<tr>
<td>544</td>
<td>F</td>
<td>52</td>
<td>Chronic bronchitis (20 yrs.) Acute recurrence</td>
<td>14.0</td>
<td>-</td>
<td>100</td>
<td>2.98</td>
<td>-</td>
</tr>
<tr>
<td>545</td>
<td>M</td>
<td>20</td>
<td>Glandular fever (3 months)</td>
<td>15.2</td>
<td>-</td>
<td>5</td>
<td>2.98</td>
<td>12.7</td>
</tr>
<tr>
<td>546</td>
<td>M</td>
<td>62</td>
<td>Pulmonary tuberculosis (1 1/2 yrs.) Bilateral</td>
<td>-</td>
<td>-</td>
<td>35</td>
<td>2.62</td>
<td>-</td>
</tr>
<tr>
<td>547</td>
<td>M</td>
<td>53</td>
<td>Pulmonary tuberculosis (2 yrs.) Bilateral</td>
<td>-</td>
<td>-</td>
<td>47</td>
<td>2.59</td>
<td>-</td>
</tr>
<tr>
<td>548</td>
<td>M</td>
<td>67</td>
<td>Pulmonary tuberculosis (Many yrs.) Extensive unilateral</td>
<td>14.2</td>
<td>-</td>
<td>26</td>
<td>2.43</td>
<td>4.1</td>
</tr>
<tr>
<td>549</td>
<td>F</td>
<td>21</td>
<td>Undulant Fever (2 months)</td>
<td>15.2</td>
<td>-</td>
<td>12</td>
<td>2.38</td>
<td>11.2</td>
</tr>
<tr>
<td>550</td>
<td>F</td>
<td>43</td>
<td>P.U.O. (1 1/2 yrs.) 3 st. weight loss</td>
<td>9.8</td>
<td>-</td>
<td>22</td>
<td>2.38</td>
<td>-</td>
</tr>
<tr>
<td>551</td>
<td>F</td>
<td>63</td>
<td>Rheumatoid arthritis (3 yrs.)</td>
<td>9.9</td>
<td>3.4</td>
<td>5</td>
<td>2.30</td>
<td>2.8</td>
</tr>
<tr>
<td>552</td>
<td>F</td>
<td>70</td>
<td>Severe chronic bronchitis (Many yrs.) Heart failure</td>
<td>13.0</td>
<td>4.5</td>
<td>40</td>
<td>2.20</td>
<td>-</td>
</tr>
<tr>
<td>553</td>
<td>M</td>
<td>48</td>
<td>Pulmonary tuberculosis (1 yr.) Extensive bilateral</td>
<td>-</td>
<td>-</td>
<td>40</td>
<td>2.14</td>
<td>-</td>
</tr>
<tr>
<td>554</td>
<td>M</td>
<td>52</td>
<td>P.U.O. (4 months) Temp. to 101.5°F.</td>
<td>13.4</td>
<td>5.8</td>
<td>130</td>
<td>1.79</td>
<td>-</td>
</tr>
<tr>
<td>555</td>
<td>F</td>
<td>55</td>
<td>Chronic pneumonia (1 1/2 yrs.) Eosinophilia</td>
<td>11.0</td>
<td>3.9</td>
<td>74</td>
<td>1.74</td>
<td>-</td>
</tr>
<tr>
<td>556</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No.</td>
<td>Sex</td>
<td>Age</td>
<td>Diagnosis &amp; Remarks</td>
<td>Hb.</td>
<td>Rbc.</td>
<td>ESR</td>
<td>Folic Acid</td>
<td>Cit. Acid</td>
</tr>
<tr>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>----------------------------------------</td>
<td>-----</td>
<td>------</td>
<td>-----</td>
<td>------------</td>
<td>----------</td>
</tr>
<tr>
<td>556</td>
<td>M</td>
<td>46</td>
<td>Sarcoïdosis - recent, No symptoms. Large spleen</td>
<td>14.6</td>
<td>5.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>557</td>
<td>F</td>
<td>45</td>
<td>Pulmonary tuberculosis (13 yrs.) Amyloid liver</td>
<td>-</td>
<td>-</td>
<td>55</td>
<td>1.14</td>
<td>6.1</td>
</tr>
<tr>
<td>558</td>
<td>F</td>
<td>50</td>
<td>Lupus erythematosus disseminatus (2 yrs.)</td>
<td>7.4</td>
<td>3.6</td>
<td>110</td>
<td>0.95</td>
<td>-</td>
</tr>
<tr>
<td>559</td>
<td>F</td>
<td>50</td>
<td>Rheumatoid arthritis (2 months) No response to salicylates</td>
<td>14.6</td>
<td>-</td>
<td>35</td>
<td>0.83</td>
<td>-</td>
</tr>
<tr>
<td>560</td>
<td>M</td>
<td>26</td>
<td>Subacute bacterial endo- carditis (1 yr.) untreated</td>
<td>11.0</td>
<td>3.6</td>
<td>76</td>
<td>0.14</td>
<td>-</td>
</tr>
</tbody>
</table>
Case 560 illustrates most clearly that prolonged generalised bacterial infection can give a diminished output of folic acid after a test dose. No treatment had been given during the year that the patient had been ill: the practitioner had not suspected subacute bacterial endocarditis.

In Case 557 the possibility that amyloid tissue has an affinity for folic acid would require consideration.

It is possible that the diagnosis of rheumatoid arthritis in Case 559 was incorrect, as there was an increase in plasma cells in the marrow, and the symptoms and fever did not respond to salicylates.

Case 556 (sarcoidosis) is of interest in that the patient's general condition was good, and the only obvious abnormality was splenic enlargement. Nevertheless the test was positive. The diagnosis was proved by splenectomy.

Four patients with pyrexia, the cause of which was not established, are included to show that prolonged fever will not itself necessarily lead to a diminished excretion of folic acid. In this respect Case 540 is of interest in that quite high fever - rising to 103° F. - had been present intermittently for eighteen months without evidence of general deterioration. The diagnosis of Case 550 was uncertain, but, although emaciation was severe, folic acid excretion was normal. Case 558 (lupus erythematosus disseminatus) was also much wasted, and, although cortisone therapy led to marked clinical improvement, the folic acid excretion remained abnormal in this patient.
FOLIC ACID EXCRETION IN PERNICIOUS ANAEMIA

This is not being considered in detail here because it has been referred to on pp. 293 and 332. Of 20 pernicious anaemia patients in relapse, 9 had an output of less than 1.5 mg. after the test dose.

SUMMARIES OF ILLUSTRATIVE CASES

Case 528. A case of malignant disease that was at first puzzling, but had a markedly 'positive' folic acid excretion test. The diagnosis soon became obvious.

Miss E.C., a 50 year old patient, was admitted to hospital in August 1953 with pain in the shoulders, back and neck of three weeks' duration. She felt listless and was troubled by sweating. Her doctor thought that the symptoms, which followed a caravan holiday, might be explained by a diagnosis of acute fibrositis.

At the time of admission the general condition was good and no localising features were found. The pulse rate varied from 80 to 90 per minute and the temperature from 96.5° to 99°. The sedimentation rate was 86 mm./hr. and the white cell count was 7,600 per c.mm., with no abnormality in the film. The haemoglobin level was 11.8 G. per 100 ml., and a sternal puncture revealed no abnormality in the bone marrow. Blood cultures and agglutination tests for enteric fever, undulant fever and glandular fever were negative.

A folic acid excretion test after a 5 mg. test dose of pteroylglutamic acid showed an excretion of 0.18 mg.

The resident doctor has recorded in the case notes that a/
a diagnosis of "something very nasty" was made because of this finding.

An X-ray of the chest showed that mediastinal glands were prominent, and in addition the liver rapidly became enlarged. The patient's condition soon deteriorated and she died a month after she was admitted to hospital. At post mortem there was a bronchogenic carcinoma of the right lung with spread to the mediastinal lymph glands and the liver. The cells were highly undifferentiated, being round or spindle shaped, with hyperchromatic nuclei.

**Case 519.**
A patient who had been suspected of exaggerating his symptoms of pain for which no cause had been found. Markedly positive folic acid test. Death from carcinomatosis.

A 44 year old man who had been in the regular Army and who had been a heavy drinker was admitted to hospital in July 1953 with a two year old history of pain in the epigastrium, and in the left shoulder and buttock. He had been investigated in the Medical Out-patient Department of Edinburgh Royal Infirmary. No abnormality had been found on physical examination and a barium meal had been negative.

At the time of admission no localising features were found and the general condition was good. The haemoglobin level was 13.6 G. per 100 ml., red cells 4,800,000 per c.mm., but the sedimentation rate was 49 mm./hr. On occasion the temperature rose to 100° F. but it was usually normal. A folic acid excretion test revealed that only 0.5 mg. was excreted from the 5 mg. test dose, so that it was taken as certain/
certain that the patient was not exaggerating his symptoms as had first been suspected. The bone marrow was normal as were the results of barium meal examinations. Blood cultures and agglutination tests revealed no abnormality.

The liver became enlarged to one and a half finger-breaths below the costal margin and a gland was felt in the left supraclavicular region. A biopsy of this was performed and meantime further X-rays showed osteolytic areas in the neck of the left femur and right scapula, and a fracture of one of the ribs.

The gland from the neck was found to be largely replaced by an anaplastic carcinoma showing a good deal of variation in cellular and nuclear structure. Active growth was evidenced by numerous mitoses, while the fibrous framework was scanty and infiltrated with lymphocytes and plasma cells. It was thought that a primary bronchial carcinoma was likely but the site of the primary could not be suggested with any degree of certainty. The X-ray of the chest had shown only an ill-defined soft tissue opacity to the right of the upper mediastinum.

The patient rapidly became worse and died six weeks after admission. Permission for post mortem examination was refused.

Case 517.
Operation for suspected gastric carcinoma stopped because of investigations that followed the finding of a positive folic acid excretion test.
spleen or lymph glands. The iliac puncture findings were consistent with a diagnosis of lymphosarcoma.

Radiotherapy was given to the spleen and later to the stomach area. However, the patient's condition deteriorated rapidly and she died two months after the carrying out of the folic acid test.

At post mortem the stomach was found to be completely normal. The spleen weighed 325 G. and the liver 1,250 G. There was infiltration of the thoracic and abdominal lymph nodes, bone marrow and possibly spleen with cells which could not be satisfactorily identified (because, it was stated, of the X-ray therapy), but they were thought to be cells of lymphosarcoma or lymphadenoma.

Case 482. Death from lymphadenoma despite a normal folic acid excretion test result.

In May 1952 a 51 year old man was admitted to Ward 26 of Edinburgh Royal Infirmary with a nine months' history of pyrexia and weakness. There were no previous illnesses of note, and no enlargement of lymph glands, liver or spleen, or other abnormality was found on physical examination. The general condition was reasonably good, but there certainly was fever of a Pel Ebstein type, the temperature rising as high as 104° F. The haemoglobin level was 8.7 G. per 100 ml., red cells 3,200,000 per c.mm., and the bone marrow was normal. The ESR was 127 mm./hr. Agglutination tests for enteric, abortus and glandular fevers were negative, as were blood cultures. The white cell count was 5,200 per c.mm., and there/
there were 63% polymorphs, 21% lymphocytes, 14% monocytes, 1% eosinophils and 1% basophils.

A folic acid excretion test showed that 2.42 mg. of the 5 mg. test dose was excreted. The plasma albumin level was 2.32 G. per 100 ml., globulin 3.38 G. per 100 ml., alkaline phosphatase 22 units, cephalin cholesterol flocculation test + 1. Subsequent examinations did not confirm this reversal of the albumin/globulin ratio, although the alkaline phosphatase level remained high.

There was no response to treatment with sulphonamides or antibiotics, and it was thought the diagnosis must be one of lymphadenoma. Treatment was with blood transfusion and the patient was transferred to Inverness for intravenous nitrogen mustard therapy. Despite this the patient died within three months and at post mortem there was found to be lymphadenoma involving the liver, spleen and aortic glands.

This case is included to make it plain that the folic acid excretion test may be very misleading as a guide to prognosis. In this instance the output after the test dose was normal, but the patient soon died.

Case 404. A case in which there was doubt as to whether the diagnosis was one of agranulocytosis or of aleukaemic leukaemia; a positive folic acid excretion test would probably have suggested the latter, but a negative result was not helpful.

A 34 year old female patient was admitted to Stockton hospital in August 1952 because a blood count done prior to dental extraction had shown the haemoglobin level to be 11.2 G. per/
per 100 ml. and the white cell count to be 2,000 per c.mm.
The diagnosis appeared to lie between idiopathic agranulocytosis
and aleukaemic leukaemia, and the patient was transferred to
Ward 27, Edinburgh Royal Infirmary.

There was no history of the recent taking of any drugs
that might have caused agranulocytosis. No abnormality was
found on physical examination, and the patient appeared to be
in the best of health. The temperature was normal. Liver
function tests revealed no abnormality but the ESR was raised,
the highest reading being 90 mm./hr. The white cell count
was 1,000 per c.mm., the differential count being 22% poly-
morphs, 60% lymphocytes, 16% monocytes, 2% basophils. The
cells were not abnormal in appearance. The haemoglobin level
was 11.0 G. per 100 ml., red cells 5,110,000 per c.mm. and the
platelet count was 205,000 per c.mm. The bone marrow
contained an increased number of myelocytes and metamyelocytes,
and a smaller proportion than usual of later stages of granulo-
cytes. There was no increase of myeloblasts.

A folic acid excretion test showed that 3.75 mg. was
excreted after the 5 mg. test dose of pteroylglutamic acid.
Since there was absolutely nothing to suggest that the patient
suffered from a severe prolonged chronic infection, it had been
considered that a low output after the test dose of pteroyl-
glutamic acid would favour a diagnosis of leukaemia. As it
was, however, the result was not abnormal, hence the test was
not helpful because, for example, Case 460 certainly had
leukaemia and yet had a normal excretion of folic acid after
the/
the test dose.

The patient was treated with cortisone since this might give benefit whichever diagnosis was correct. No immediate improvement occurred and the patient returned to Stockton. Three months later it was ascertained from the physician at the hospital there that the blood count was normal. The patient was seen again in Edinburgh seven months after the cortisone therapy was stopped. No abnormality was found and the blood levels were haemoglobin 12.7 G. per 100 ml., red cells 4,710,000 per c.mm., white cells 3,400 per c.mm., platelets 204,000 per c.mm., polymorphs 61%, lymphocytes 29%, monocytes 8%, eosinophils 2%. The bone marrow obtained by sternal puncture was completely normal.

The following case is a sharp contrast to the above.

Case 513. Chronic Infection, with a positive folic acid excretion test.

A 57 year old patient was admitted to Ward 27, Edinburgh Royal Infirmary, in May 1953 with a history of pallor, breathlessness, loss of energy and pain in the left side of the chest of seven weeks' duration. She had been seen at the Blood Clinic in 1948 because of tiredness, weight loss and ankle swelling. At that time the blood pressure was 190/102 and the blood levels were haemoglobin 8.6 G. per 100 ml., red cells 2,710,000 per c.mm., Colour index 1.07. It was particularly noted then that the film was not typical of pernicious anaemia in that anisocytosis, poikilocytosis and ovalocytosis/
ovalocytosis were not marked. The patient would not come into hospital for investigation and liver injections were given. There was rapid improvement and in March 1950 the levels were haemoglobin 15.5 G. per 100 ml., red cells 5,120,000 per c.mm. It was not until October 1952 that any significant deterioration in blood levels was noted.

When the patient was admitted eight months later, there were harsh aortic systolic and diastolic murmurs, the liver and spleen were palpable, and there was intermittent fever to 100°F. The blood levels were haemoglobin 7.4 G. per 100 ml., red cells 3,400,000 per c.mm., ESR 42 mm./hr., white cells 7,400 per c.mm., polymorphs 83%, lymphocytes 15%, monocytes 2%. The marrow contained large numbers of myelocytes and metamyelocytes and a diagnosis of aleukaemic myeloid leukaemia or of chronic infection with a hold up in granulocyte maturation was considered. A folic acid excretion test showed an excretion of 0.74 mg. after the 5 mg. test dose. In view of the findings in Case 560 (subacute bacterial endocarditis) and in cases of leukaemia, this was of no value as an aid to diagnosis. A test meal showed free hydrochloric acid to be present in the gastric juice, so the original possible diagnosis of pernicious anaemia was untenable. Non-haemolytic streptococci were isolated from the blood stream and a diagnosis of subacute bacterial endocarditis was made. There was a good response to treatment with antibiotics. The cause of the original anaemia in 1948 is obscure.

These/
These last three cases, in contrast to the preceding ones, show the lack of clinical value of the test.

DISCUSSION

The aims of this part of the investigation were to see whether the folic acid excretion test was of any value as an aid to diagnosis or prognosis, and to carry out a limited survey of the results of the test in a variety of diseases. It may be said that the value of the test as an aid to diagnosis is very limited. If the patient excretes more than 1.5 mg. of folic acid following the 5 mg. subcutaneous dose of pteroylglutamic acid, malignant disease (using the term in its broadest sense) may or may not be present. If a patient who is not suffering from intestinal malabsorption, megaloblastic anaemia, a large effusion or general oedema and who does not have impaired renal function, excretes less than 1.5 mg., then widespread malignant disease is likely to be present. A similar result may be found in prolonged chronic infection, but usually such infection is obvious clinically. On the other hand, widespread malignant disease is also usually obvious, and it is only in an occasional patient like Case 519 that information of any value is obtained. (This was the patient with pains of two years' duration in various sites who was at one time thought to be exaggerating his symptoms, but who, in fact, had carcinoma probably arising in the lung and involving bones, liver, glands and perhaps other areas.) It is reason-
reasonable to say that the result in this instance indicated widespread disease, and this was known before the other findings were available. From the point of view of prognosis in any individual patient the test cannot be claimed to be of real value, and therefore it would appear to be more of biochemical interest than practical use.

It cannot be stated with certainty what is being measured by this test. In nutritional megaloblastic anaemia and in untreated cases of intestinal malabsorption, the presence of megaloblasts in the marrow is taken to indicate deficiency of folic acid or vitamin B₁₂. We have seen (pp.362 and 364) that a normal output may occur after a test dose even in patients with normal serum vitamin B₁₂ levels who have megaloblastic anaemia responding to pteroylglutamic acid. In untreated pernicious anaemia, where the primary deficiency is of vitamin B₁₂, folic acid excretion following the test dose is frequently low but may be normal. Moreover, transitional erythroblasts rather than true megaloblasts may be found in association with either a normal output or abnormal output of folic acid. Now we are faced with the finding that in malignant disease there is frequently a low output after the test dose, but megaloblasts are only very rarely found in the marrow. They were not seen in any of the cases reported here.

On the whole the diminished output of folic acid occurred in patients with widespread rapidly growing tumour cells, and it would be reasonable to assume that the tumour tissues compete/
compete with the host for folic acid or its derivatives, but not to the extent of depriving the red cell precursors of enough folic acid for maturation along normoblastic lines. The factors that may be involved in this include the rate of excretion of folic acid by the kidneys, the extent of tumour tissue in the body, its vascularity, the nature of the tumour cells and their metabolic requirements, the mechanism of uptake of folic acid by these cells and possibly the extent to which other substances can substitute for folic acid. It does appear that the red cell precursors are able to obtain sufficient folic acid for normoblastic development even although bodily depletion of the substance as assessed by this test is great.

Other modes of production of the apparent folic acid deficiency in cases of malignant disease that merit consideration are deficient intake due to anaemia with resulting primary malnutrition, and diminished absorption produced by widespread metastases, or by other means.

It seems unlikely that either of these mechanisms could operate without significant weight loss occurring, and there was little or no weight loss in many of the patients whose condition is referred to in Table 60 as being good at the time of the test. Moreover, in Cases 506, 520 and 556 there was no evidence at operation of any cause for intestinal malabsorption. This was true also at post mortem examination in Cases 524, 529 and 535.

In contrast there was very severe primary malnutrition
of many years' duration in Cases 449, 450 and 451 as established by a detailed dietetic investigation. An important source of calories for Case 449 was whisky. Case 450 weighed only 4 st. 12 lbs.: a fat balance test in this patient revealed normal fat absorption. A post mortem examination of Case 451 revealed no abnormality other than generalised wasting.

Nevertheless these patients, while excreting less of the administered folic acid than did most of the other control cases, were able to excrete 1.5 mg. or more.

In Chapter 9 which deals with the differential urinary folic acid excretion test, some of the control cases are patients with malignant disease, and there is no evidence of malabsorption of folic acid as measured by the test.

One further possibility to explain our results might be that a substance that acts as a folic acid antagonist in the *S. faecalis* assay is produced and excreted in advanced malignant disease. However, in two patients with acute leukaemia and one with widespread carcinomatosis, and who were subsequently shown to have 'positive' folic acid excretion tests, the admixture of the urine with the pteroylglutamic acid used in the standard for the assay did not impair its ability to support the growth of *S. faecalis*.

**ADDENDUM**

In February 1954, after the above had been written, we received a letter from Ludes & Haehner in Cologne, confirming our results which had been published in abstract form after a Biochemical/
Biochemical Society meeting. These German workers were more optimistic than ourselves about the value of the test as an aid to diagnosis.

**SUMMARY**

A test dose of 5 mg. of pteroylglutamic acid was given subcutaneously to 161 patients not suffering from megaloblastic anaemia. The urinary excretion of folic acid should exceed 30% of the test dose, and an excretion of less than 1.5 mg. is recorded as a 'positive' test. Where renal function is good and the patient does not suffer from megaloblastic anaemia, intestinal malabsorption, generalised oedema, a large effusion or severe infection of long duration, a positive test is likely to indicate the presence of widespread, rapidly growing malignant disease (including the reticuloses). However, it is possible to have advanced malignancy with a negative folic acid test, and since the test is not very helpful as a guide to prognosis, it is more of biochemical interest than of value to the clinician.
ILLUSTRATIVE CASES TO SHOW LACK OF RELATIONSHIP BETWEEN AMOUNT OF WASTING AND RESULT OF URINARY FOLIC ACID EXCRETION TEST.

Case 496. Carcinoma of rectum with hepatic spread. Folic acid excretion 1.55 mg.

Case 500. Hypernephroma with pulmonary and glandular secondaries. Folic acid excretion 1.19 mg.

(Normal renal function)
Folic acid excretion. 2.38 mg.

Case 556. Sarcoidosis with good general condition. 
Folic acid excretion. 1.15 mg.
Case 509. Lymphadenoma.
Folic acid excretion. 0.91 mg.

Case 540. Pyrexia of unknown origin without weight loss.
Folic acid excretion. 3.48 mg.
CHAPTER 19.

A NEW CLASSIFICATION OF THE MEGALOBLASTIC ANAEMIAS AND OF OTHER CONDITIONS ASSOCIATED WITH BIOCHEMICAL CHANGES SUGGESTING DEFICIENCY OF ANTIMEGALOBLASTIC SUBSTANCES

In the light of the conclusions reached in the previous chapters it is possible to suggest that there exist the following types of megaloblastic anaemias and related conditions. It is not, of course, intended that this complex classification should find its way into textbooks of medicine. It is intended rather as a jumping off point for further research.

A. Primary Nutritional Macrocytic Anaemia

Due to deficiency of folic acid substances (? includes Wills' factor deficiency)

Due to vitamin B12 deficiency

Due to deficiency of both

Due to deficiency of either or both and complicated by other conditions, particularly

- pregnancy
- malaria
- sprue
- chronic diarrhoea
- ankylostomiasis.

The importance of changes in intestinal flora, of the influence of prolonged severe exertion or of hypothetical antimetabolites of antimegaloblastic substances is uncertain.

B. Pernicious Anaemia

With a low serum vitamin B12 level

Occasionally/
Occasionally with an apparently normal serum vitamin B12 level

With biochemical evidence suggesting folic acid depletion

Without biochemical evidence of folic acid depletion

Complicated by other forms of megaloblastic anaemia

C. Megaloblastic Anaemia associated with structural abnormalities of the alimentary tract.

Due to gastric carcinoma

Following total gastrectomy

Following partial gastrectomy

Due to other pathology destroying the intrinsic factor bearing area

Associated with intestinal short circuits

Due to disease of the small intestine

Due to disease of the mesenteric glands

Associated with stagnant loops

Due to a combination of such factors

D. Diphyllobothrium latum Anaemia.

E. Megaloblastic anaemia associated with intestinal malabsorption of antimegaloblastic substances.

(Some of the cases included under C. might be classified under this heading.)

Due to idiopathic steatorrhoea with diarrhoea

Due to idiopathic steatorrhoea without diarrhoea, but with a positive fat balance test

Without evidence of steatorrhoea

In coeliac disease

In tropical sprue

In regional jejunitis

Due to other disease of the small intestine or mesenteric glands, e.g. tuberculosis or neoplasm

With/
With malabsorption only of folic acid substances

? With malabsorption only of vitamin B₁₂

With malabsorption of both

With atrophy of the small intestine as a secondary result of malnutrition caused by the primary malabsorption

With atrophy of the small intestine as a secondary result of primary malnutrition

Folic acid depletion without anaemia

F. **Macrocytic anaemia associated with liver disease.**

Without depletion of folic acid or vitamin B₁₂

With depletion of vitamin B₁₂

? With depletion of folic acid

Other forms of megaloblastic anaemia complicated by chronic liver disease

G. **Megaloblastic anaemia of infancy.**

Responding to cyanocobalamin

Not responding to cyanocobalamin, but responding to folic acid substances

H. **Megaloblastic anaemia of pregnancy and the puerperium.**

Nutritional macrocytic anaemia complicated by pregnancy

  due to deficiency of vitamin B₁₂
  due to deficiency of folic acid substances
  due to deficiency of both

Megaloblastic anaemia of pregnancy with biochemical evidence of folic acid depletion

Megaloblastic anaemia of pregnancy without biochemical evidence of folic acid depletion — probably due to disordered metabolism of folic acid substances

A combination of this with nutritional megaloblastic anaemia

Idiopathic steatorrhoea or tropical sprue complicated by pregnancy

Pernicious anaemia complicated by pregnancy

Other forms of megaloblastic anaemia complicated by pregnancy.
I. **Megaloblastic anaemia associated with leukaemia.**

This is rarely seen; it sometimes follows therapy with 4-aminopteroylglutamic acid.

J. **Megaloblastic anaemia associated with haemolytic anaemia.**

This is rare and is associated with other factors such as pregnancy or malnutrition.

K. **Refractory megaloblastic anaemia.**

Cyanocobalamin-refractory megaloblastic anaemia

Due to gastric carcinoma or other malignant disease.
Due to unsuspected intestinal malabsorption of folic acid
? idiopathic forms
? due to myxoedema*

Pteroylglutamic acid-refractory megaloblastic anaemia

Due to prolonged treatment with pteroylglutamic acid where the primary deficiency is of vitamin B₁₂

L. **Vitamin B₁₂ deficiency without megaloblastic anaemia.**

With subacute combined degeneration of the cord
Without subacute combined degeneration of the cord

M. **Folic acid deficiency without megaloblastic anaemia.**

In conditions such as idiopathic steatorrhoea before megaloblastic anaemia develops
In malignant disease
In prolonged chronic infections.

**Summary.**

A new classification of the megaloblastic anaemias and related conditions is made in the light of present knowledge.

* It is uncertain whether megaloblastic anaemia occasionally occurs as a result of Simmonds' disease or myxoedema.
CHAPTER 20.

A BRIEF CONSIDERATION OF THE INFORMATION GAINED FROM THE PRESENT INVESTIGATIONS.

This is in the nature of a postscript — a bird’s eye view of the information obtained from the study of approximately 1,350 patients recorded in this thesis and of the many others for whom representative case reports are given, together with investigations carried out on guinea pigs and on gastro-intestinal bacteria.

So far as these animal and microbial studies are concerned, a period of about a year was spent in attempting to produce megaloblastic anaemia in the guinea pig, and another year in investigating the effects of gastro-intestinal bacteria on haemopoietic factors in culture medium. The results of these two pieces of investigation were to a certain extent related in that succinylsulphathiazole together with 4-aminopteroylglutamic acid produced a condition more akin to megaloblastic anaemia in the guinea pig than did 4-aminopteroylglutamic acid alone. This may have been due to an effect on intestinal microorganisms.

The results of the investigations on gastro-intestinal bacteria are complex and difficult to relate to clinical medicine. There is no doubt that certain organisms can absorb vitamin B₁₂ and it is possible that some can convert this into other substances which are growth factors for L. leichmannii. Our data suggested that certain gastro-intestinal/
gastro-intestinal organisms could synthesise folic acid, but the majority could not do so under our experimental conditions, and we could not demonstrate clearly the synthesis of vitamin B12 itself. The stools contained relatively large amounts of growth factors for \textit{L. leichmannii} and \textit{S. faecalis} but much of this activity may have been due to substances liberated from dead bacteria and in any case we do not know the haemopoietic activity, if any, of growth factors for \textit{L. leichmannii} other than vitamin B12 compounds themselves.

On the clinical side, examples have been given of many subdivisions of the various main types of megaloblastic anaemia, and the response to various therapeutic agents has been considered. In the main, however, the intention has been to combine clinical research with new biochemical tests that have been devised to meet the various problems that have arisen, and particularly in the case of the nutritional macrocytic anaemias, to consider past data in the light of present knowledge. From the information thus obtained it has been suggested in Chapter 19 that the subject of the megaloblastic anaemias and related conditions is even more complex than has been realised.

In the nutritional megaloblastic anaemias and the megaloblastic anaemias of pregnancy in particular, it is felt that our data indicate that we are dealing with groups of disorders rather than individual syndromes. It seems, too, that the clinico-biochemical approach is of value in establishing the pathogenesis of macrocytic anaemia in diseases of the alimentary tract, including both the conditions with structural/
structural changes such as intestinal fistulae, and those with evidence only of disordered physiology, such as idiopathic steatorrhoea. Moreover, the introduction of cyanocobalamin therapy, of the new differential urinary folic acid excretion test, and of the Birmingham workers' fat balance test makes the investigation of cases of suspected idiopathic refractory megaloblastic anaemia a relatively simple matter. The differential urinary folic acid excretion test and the serum vitamin B12 assay can in many instances be carried out without the patient even having to be admitted to hospital.

In Chapter 12 the metabolism of the antimegaloblastic substances has been dealt with in some detail, and in Chapter 13 the metabolic functions of these substances has been considered. Accordingly, such matters need not be discussed further.

On the other hand it may be useful to put forward the following sketch which demonstrates the main methods of investigation that we have attempted and the main conclusions that we have reached about the metabolism of vitamin B12, folic acid, citrovorum factor and related substances.
Content of haemopoietic factors may be measured directly at post mortem by microbiological assay.

Citrovorum Folic Factor & Acid & Conjugates

Hematopoietic factors may be measured directly Conjugates Substances

Intrinsic Factor

Measured by radioactive vitamin B₁₂ studies on a P.A. case...

Bacteriological assessment a future possibility.

Serum vitamin B₁₂ studies possible.
Serum folic acid or citrovorum factor studies not helpful.

Malignant tissue may utilise folic acid & lead to bodily depletion; measured by folic acid excretion test.

Pregnancy may interfere with folic acid metabolism.

Malabsorption measured by differential urinary folic acid excretion test.

Malabsorption might be measured by radioactive vitamin B₁₂ studies.

Exact role of bacteria uncertain Many can absorb vitamin B₁₂

If renal function normal, urinary excretion test after a test dose may be a valuable test of bodily desaturation of folic acid substances. Vitamin B₁₂ depletion cannot be measured this way.

Stools contain growth factors for L. Leichmannii, S. faecalis, L. citrovorum, etc., largely derived from bacteria.

Marrow is megaloblastic in late stages of deficiency of vitamin B₁₂, folic acid substances, or both.
In conclusion it must be said that now that haematological research involves biochemical rather than purely morphological studies, it becomes increasingly obvious that many discoveries have yet to be made. In our researches we have crossed the paths of the workers investigating the mode of action of antibiotics and sulphonamides and have seen that there is an abnormality of folic acid metabolism in malignant disease. Perhaps this, the end, is just the beginning.
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Table 51.
(Duplicate)
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<th>Column 5</th>
</tr>
</thead>
<tbody>
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<td>Data 2</td>
<td>Data 3</td>
<td>Data 4</td>
<td>Data 5</td>
</tr>
<tr>
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<td>Data 8</td>
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<td>Data 10</td>
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<tr>
<td>Data 11</td>
<td>Data 12</td>
<td>Data 13</td>
<td>Data 14</td>
<td>Data 15</td>
</tr>
</tbody>
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